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# INDIAN PHARMACOPOEIA 2010

## Volume II




Government of India
Ministry of Health & Family Welfare

PUBLISHED BY
THE INDIAN PHARMACOPOEIA COMMISSION, GHAZIABAD




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The Secretary-cum-Scientific Director
INDIAN PHARMACOPOEIA COMMISSION
Sector-23, Raj Nagar,
Ghaziabad-201 002, India
Tel: (91-120) – 2783401
Fax: (91-120) – 2783311
Website: www.ipc.gov.in
E.mail: ipclab@vsnl.net

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# General Notices

## General Statements

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

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

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

**Name**. The full name or title of this book, including addenda thereto, is Indian Pharmacopoeia 2010, abbreviated to IP 2010. In the texts, the term "Pharmacopoeia" or "IP" without qualification means the Indian Pharmacopoeia 2010 and any addenda thereto.

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

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

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

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

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

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

The active pharmaceutical ingredients (drug substances), excipients (pharmaceutical aids), pharmaceutical preparations (dosage forms) and other articles described in the monographs are intended for human and veterinary use (unless explicitly restricted to one of these uses).

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

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

**Added Substances**. An official substance, as distinguished from an official preparation, contains no added substances except when specifically permitted in the individual monograph. Unless otherwise specified in the individual monograph, or elsewhere in the General Notices, suitable substances may be added to an official preparation to enhance its stability, usefulness or elegance, or to facilitate its preparation. Such auxiliary substances shall be harmless in the amounts used, shall not exceed the minimum quantity required to provide their intended effect, shall not impair the therapeutic efficacy or the bioavailability or safety of the preparation and shall not interfere with the tests and assays prescribed for determining compliance with the official standards. Particular care should be taken to ensure that such substances are free from harmful organisms. The freedom to the manufacturers to add auxiliary substances imposes on them the responsibility of satisfying the licensing authorities on the purpose of the addition and the innocuity of such substances.

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

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



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**Meanings of Terms**

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

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

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

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

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

**Freshly prepared**. Made not more than 24 hours before it is issued for use.

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

**Negligible**. A quantity not exceeding 0.50 mg.

**Solution**. Where the name of the solvent is not stated, "solution" implies a solution in water. The water used complies with the requirements of the monograph on Purified Water. The term 'distilled water' indicates Purified Water prepared by distillation.

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

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

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

**Provisions Applicable To Monographs and Test Methods**

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

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

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

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

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

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

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

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

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

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

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

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

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



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provides the method to be followed and that the values specified are the applicable limits.

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

## Monographs

### General Monographs

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

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

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

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

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

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

### Individual Monographs

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

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

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

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

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

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

**Definition**. The opening statement of a monograph is one that constitutes an official definition of the substance, preparation or other article that is the subject of the



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monograph. In certain monographs for pharmaceutical preparations the statement is given in terms of the principal ingredient(s).

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

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

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

**Category**. The statement of category is provided for information and is indicative of the medical or pharmaceutical basis for recognition in the Pharmacopoeia. It generally represents an application of the best known pharmacological action of the article or of its active ingredient. In the case of pharmaceutical aids it may indicate the more common usage of the article. The statement is not intended to limit in any way the choice or use of the article nor to indicate that it has no other activity or use.

**Dose**. Doses mentioned in the Pharmacopoeia are intended merely for general guidance and represent, unless otherwise stated, the average range of quantities which are generally regarded as suitable for adults when administered by mouth. They are not to be regarded as binding upon the prescribers. The medical practitioner will exercise his own judgment and act on his own responsibility in respect of the amount of any therapeutic agent he may prescribe or administer or the frequency of its administration. If it is usual to administer a drug by a method other than by mouth, the single dose suitable for that method of administration is mentioned. In the case of some preparations notes have been given below the statement of doses to show the approximate quantities of active ingredients contained in the maximal doses as information for the prescriber.

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

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

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

### Test Methods

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

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

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

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

### Tests and Assays

The tests and assays are the official methods upon which the standards of the Pharmacopoeia depend. The requirements are not framed to take into account all possible impurities. It is not to be presumed, for example, that an impurity that is not detectable by means of the prescribed tests is tolerated. Material found to contain such an impurity is not of pharmacopoeial quality if the nature or amount of the impurity found is incompatible with good pharmaceutical practice.

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

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

Where it is directed that an analytical operation is to be carried out 'in subdued light', precautions should be taken to avoid exposure to direct sunlight or other strong light. Where a procedure is directed to be performed 'protected from light' precautions should be taken to exclude actinic light by the



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use of low-actinic glassware, working in a dark room or similar procedures.

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

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

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

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

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

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

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

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

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

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

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

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

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

IP Reference Substances, abbreviated to IPRS (and referred to as RS in the individual monographs) are issued by the Indian Pharmacopoeia Commission (IPC). They are the official standards to be used in cases of arbitration. Secondary Standards (Working Standards) may be used for routine analysis, provided they are standardized at regular intervals against the Reference Substances

Biological Reference Substances, also abbreviated to IPRS and Standard Preparations of antibiotics are issued by agencies authorised by the IPC. They are standardized against the International Standards and Reference Preparations established by the World Health Organization (WHO). The potency of these preparations is expressed in International Units.

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

**Test Animals**. Unless otherwise directed, animals used in a test or an assay shall be healthy and are drawn from a uniform stock, and have not previously been treated with any material that will interfere with the test or the assay.

**Calculation of Results**. In determining compliance with a numerical limit in assay or test, the result should be calculated



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to one decimal place more than the significant figures stated and then rounded up or down as follows: if the last figure calculated is 5 to 9, the preceding figure is increased by 1; if it is 4 or less, the preceding figure is left unchanged.

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

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

— Store in a dry, well-ventilated place at a temperature not exceeding 30°

— Store in a refrigerator (2° to 8°). Do not freeze

— Store in a freezer (-2° to -18°)

— Store in a deep freezer (Below -18°)

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

— Store protected from light

— Store protected from light and moisture

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

**Storage Containers**. The requirements, guidance and information on containers for pharmaceutical use are given in the chapter entitled Containers (6.1).

In general, an article should be packed in a well-closed container i.e. one that protects the contents from contamination by extraneous solids, liquids or vapours and from loss of the article under normal conditions of handling and storage.

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

In certain cases, special requirements of pack have been indicated in some monographs under Storage, using expressions that have been defined in chapter 6.1.

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



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# DOSAGE FORMS


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## General Requirements

The Pharmacopoeia provides monographs of dosage forms for most of the pharmacopoeial drug substances. Additionally, the general requirements including the processes for the preparation of many of them and the tests of a general nature applicable to each type of dosage form are given in the following pages. In addition to defining the dosage forms, this section presents the general principles involved in the production of some of them.

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

## Capsules

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

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

The contents of capsules other than Modified-release (Sustained-release) Capsules do not contain any added colouring agent.

**Hard Gelatin Capsules.** Hard gelatin capsules contain the medicament(s) in the solid form. Where two mutually incompatible drugs are present in the mixture, one of the drugs can be put as a tablet or pellet or in small capsule and then enclosed with the other drug in a large capsule.

### Production

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

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

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

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

The contents of soft capsules usually consist of liquids or solids dissolved or dispersed in suitable excipients to give a paste-like consistency. With suitable equipment, powders, granules and other dry solids also may be filled into soft-shell capsules. As soft gelatin shells contain appreciable amounts of water, migration of capsule contents, particularly of water-soluble ingredients, may occur.

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

**Enteric Capsules** (Gastro-resistant Capsules). Enteric Capsules are hard or soft capsules prepared in such a manner that the shell resists the action of the gastric fluid but is attacked by the intestinal fluid to release the contents.

During manufacture, packaging, storage and distribution of capsules, suitable means shall be taken to ensure their microbial quality; acceptance criteria for microbial quality are given in Chapter 5.9.

### Tests

**Content of active ingredients.** Determine the amount of active ingredient(s) by the method described in the Assay and calculate the amount of active ingredient(s) in each capsule. The result lies within the range for the content of active ingredient(s) stated in the monograph. This range is based on



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the requirement that 20 capsules, or such other number as may be indicated in the monograph, are used in the Assay. Where 20 capsules cannot be obtained, a smaller number, which must not be less than 5, may be used, but to allow for sampling errors the tolerances are widened in accordance with Table 1. The requirements of Table 1 apply when the stated limits are between 90 and 110 per cent. For limits other than 90 to 110 per cent, proportionately smaller or larger allowances should be made

Table 1

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

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

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

Table 2

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

**Uniformity of content**. This test is applicable to capsules that contain less than 10 mg or less than 10 per cent w/w of active ingredient. For capsules containing more than one active ingredient carry out the test for each active ingredient that corresponds to the afore-mentioned conditions.

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

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

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

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

**Hard Capsules**. Comply with the disintegration test (2.5.1). Unless otherwise directed in the individual monograph use *water* as the medium. If the capsules float on the surface of the medium, a disc may be added. If the capsules adhere to the discs, attach a removable piece of stainless steel woven gauze with mesh aperture of 2.00 mm to the upper plate of the basket rack assembly and carry out the test omitting the discs. Operate the apparatus for 30 minutes unless otherwise directed.

**Soft Capsules**. Comply with the disintegration test (2.5.1). Unless otherwise directed in the individual monograph use *water* as the medium and add a disc to each tube. Operate the apparatus for 60 minutes unless otherwise directed.

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

**Enteric Capsules**. Use the apparatus described under disintegration test (2.5.1), using one capsule in each tube. Operate the apparatus for 2 hours without the discs in *0.1 M hydrochloric acid*. No capsule shows signs of disintegration or of rupture permitting the escape of the contents. Replace the medium in the vessel with *mixed phosphate buffer pH 6.8*, add a disc to each tube and operate the apparatus for a further 60 minutes. Remove the apparatus from the medium and examine the capsules. They pass the test if no residue remains on the screen or on the underside of the discs, or, if a residue remains, it consists of fragments of shell or of a soft mass with no palpable, unmoistened core.

**Storage**. Store at a temperature not exceeding 30°.

**Labelling**. The label states the name of any added antimicrobial preservative.



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# Creams

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

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

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

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

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

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

## Production

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

During manufacture, packaging, storage and distribution of creams, suitable means shall be taken to ensure their microbial quality; acceptance criteria for microbial quality are given in Chapter 5.9.

## Tests

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

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

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

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

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

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

# Ear Drops

Otic Drops; Otic Solutions

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

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

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

During manufacture, packaging, storage and distribution of ear drops, suitable means shall be taken to ensure their microbial quality; acceptance criteria for microbial quality are given in Chapter 5.9.



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

### Production

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

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

### Tests

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

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

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

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

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

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

## Eye Drops

### Ophthalmic Drops

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

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

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

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

**Containers**. Eye Drops should be packed in tamper-evident containers. Containers should be made from materials that do



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not cause deterioration of the preparation as a result of diffusion into or across the material of the container or by yielding foreign substances to the preparation.

The container and package of a single dose preparation should be such as to maintain sterility of the contents and the applicator up to the time of use. Containers for multiple application preparations should be fitted with an integral dropper or with a sterile screw cap of suitable materials incorporating a dropper and plastic or rubber teat. Alternatively, such a cap assembly may be packed separately after it is sterilised. Containers of multiple application preparations should permit the withdrawal of successive doses of the preparation. Such containers should normally hold not more than 10 ml.

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

### Tests

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

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

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

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

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

## Eye Ointments

### Ophthalmic Ointments

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

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

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

### Tests

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

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

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

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

## Gels

Gels are homogeneous, semi-solid preparations usually consisting of solutions or dispersions of one or more medicaments in suitable hydrophilic or hydrophobic bases.



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They are normally prepared with the aid of suitable gelling agents. They are intended to be applied to the skin or certain mucous membranes for protective, prophylactic or therapeutic purposes. Gels may contain suitable added substances such as antioxidants, stabilisers and antimicrobial preservatives.

During manufacture, packaging, storage and distribution of gels, suitable means shall be taken to ensure their microbial quality; acceptance criteria for microbial quality are given in Chapter 5.9.

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

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

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

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

### Tests

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

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

## Inhalation Preparations

Inhalation Preparations are liquid or solid dosage forms intended for administration as vapours or aerosols to the lung in order to obtain a local or systemic effect. They contain solutions or dispersions of one or more active ingredients which may be dissolved or dispersed in a suitable vehicle.

Inhalation Preparations may, depending on the type of preparation, contain propellants, diluents, antimicrobial agents, solubilising and stabilising agents etc. depending on the type of preparation. They are available in single-dose or multidose containers.

Inhalation Preparations intended to be administered as aerosols (dispersions of solid or liquid particles of active ingredient(s) in a gas) are administered by pressurized metered-dose inhalers or by powder inhalers.

### Production

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

During the development of a preparation that contains an antimicrobial preservative, the effectiveness of the preservative selected, shall be determined as described in chapter 2.2.2 (Efficacy of antimicrobial preservation).

The size of aerosol particles shall be controlled so that a significant fraction is deposited in the lung.

The most commonly used method of preparation involves filling under pressure and sometimes by filling after refrigeration to temperatures below 0°. In filling under pressure, the requisite volume of the concentrate of the active ingredient(s) is filled in the container and either the propellant is forced under pressure through the valve orifice after the valve is sealed, or the propellant is allowed to flow under the valve cap and the valve assembly is sealed. In either case, the air in the container must be evacuated by means of vacuum or displacement with a small amount of the propellant.

During production, strict control should be exercised by process controls that include propellant and medicament fill weights, pressure test and leak test of the finished product.

For preparations adversely affected by water present in quantities beyond certain limits, care should be taken to protect the products from moisture.

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

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

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

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

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



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Pressurised metered-dose preparations are solutions, suspensions or emulsions supplied in containers equipped with a metering valve and which are held under pressure with suitable propellants or mixtures of liquefied propellants.

Pressurised Metered Dose Inhalers are dosage forms containing therapeutically active ingredients that are packaged under pressure in a sealed container and are released as a fine mist of spray upon activation of a suitable valve system.

The basic components of an aerosol system are the container, the propellant, the concentrate containing the active ingredient(s), the valve and the actuator.

Pressurised metered dose preparations are of two types, the two-phase system consisting of gas and liquid or the three-phase system consisting of gas, liquid and solid or liquid. The two-phase preparation comprises a solution of active ingredient(s) in liquefied propellant and the vaporised propellant. The solvent is usually the propellant or a mixture of the propellant and co-solvents such as ethanol, propylene glycol and polyethylene glycols. The three-phase preparation consists of a suspension or emulsion of the active ingredient(s) and the vaporised propellants. In the suspension the ingredient(s) may be dispersed in the propellant system with the aid of suitable pharmaceutical aids such as wetting agents, solubilising agents, emulsifying agents, suspending agents and lubricating agents to prevent clogging of valves.

**Active ingredients**. For satisfactory bioavailability the active ingredient(s) should have the majority of particles under 10 µm in size in the case of inhalation aerosols and not more than 100 µm for other types of aerosols.

**Propellants**. For pressurised metered dose inhalations propellants perform the essential function of expelling the material from the container by supplying the necessary pressure within the aerosol system. They are liquefied or compounded gases having vapour pressures exceeding atmospheric pressure. The commonly used propellants in aerosol systems are hydrocarbons, especially the fluorochloro-derivatives of methane and ethane, the butanes and pentanes and compressed gases such as nitrogen and carbon dioxide. Mixtures of propellants are often employed to obtain the necessary delivery and spray characteristics of the aerosol.

**Valves**. The valve regulates the flow of the active ingredient(s) and propellant from the container and determines the spray characteristics of the aerosol. It must be manufactured from materials which are inert to the contents of the aerosol. The commonly used materials are rubber, plastic, aluminium and stainless steel.

Products for oral or nasal inhalation require metered-dose valves which ensure delivery of a uniform quantity of spray and an accurate dose of the active ingredient(s), both within specified tolerances, with each activation of the valve. Metered valves may need priming before use if the aerosol packages have not been stored properly or have not been used for long periods of time.

**Actuators**. The actuator or adaptor which is fitted to the aerosol valve stem is a device which on depression or any other required movement opens the valve and directs the spray to the desired area. The design of the actuator which incorporates an orifice of varying size and shape and expansion chamber is very important in influencing the physical characteristics of the spray or foam, particularly in the case of inhalation aerosols, where the active ingredient(s) must be delivered in the proper particle size range. A proportion of the active ingredient(s) is usually deposited on the inner surface of the actuator; the amount available is therefore less than the amount released by actuation of the valve.

**Containers**. Aerosol containers are made of metal (stainless steel, aluminum or tin-plated steel), glass or plastic or a combination of these materials. The containers must be so designed that they provide the maximum in pressure safety and impact resistance.

## Tests

### Pressurised Metered-dose Preparations

### Content of active ingredient delivered per actuation.

#### Apparatus

A small sample vessel suitable for shaking. The size of the vessel is such that when the aerosol is discharged into the specified volume of solvent under the conditions described in the Method below, the discharge takes place not less than 25 mm below the surface of the solvent. A stainless steel base plate with 3 legs and a central circular indentation with a hole about 1.5 mm in diameter is placed in the sample vessel. The arrangement should prevent particle entrapment and side-of-stem leakage during the delivery of the sample.

#### Procedure

Remove the pressurised container from the actuator and remove all labels and markings which may be present on the container with a suitable solvent. Dry the container, replace in its actuator, shake for about 30 seconds and holding it in an inverted position actuate the valve by discharging about 5 sprays to waste. Remove the pressurised container from its actuator, clean the valve stem (internally and externally) and valve ferrule by washing with a suitable solvent. Dry the complete valve assembly using an air-supply line fitted with an appropriate narrow jet to ensure that all solvent is removed from the inside of the valve stem. Wash the actuator after the initial discharge of 5 sprays to waste, with a suitable solvent and allow it to dry.



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For test solution add to the sample vessel a volume of solvent or solvent mixture specified in the monograph so that the final concentration of the active ingredient in the test solutin corresponds to the reference solution. Shake the pressurised container for about 30 seconds and place it inverted in the vessel. Discharge 10 deliveries below the surface of the solvent actuating the valve at intervals of not less than 5 seconds, maintaining the pressurised container in the vertical plane and discharging the aerosol through the hole in the centre of the base plate. With some preparations it may be necessary to shake the pressurised container between each actuation of the valve; in such cases shaking should be carried out without removing the pressurised container from its inverted position in the vessel. Remove the pressurised container, wash it with the specified solvent and dilute the combined solution and washings to the volume specified in the monograph. Determine the amount of active ingredient by the method described under Assay in the individual monograph This amount of active ingredient is referred as metered dose assay (A) for metered dose inhalers.

Fit the washed and dried actuator to the pressurised container and actuate the valve 10 times at intervals of not less than 5 seconds. Remove the actuator carefully from the pressurised container and wash it with small quantities of the specified solvent or solvent mixture. Dilute the combined washings suitably and on the resulting solution determine the amount of active ingredient as per the method given in the individual monograph under the test for 'Content of active ingredient delivered per actuation' and calculate the amount of active ingredient per actuation of the valve. This amount of active ingredient is referred to as actuator retention (B) for metered dose inhalers.

Calculate the content of active ingredient delivered per actuation from the expression A – B.

**Uniformity of delivered dose**

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

The test is applicable to inhalation preparations containing the drug formulation (e.g., solution, suspension, or powder) either in reservoirs or in premetered dosage units, and for drug formulations packaged in reservoirs or in premetered dosage units where these containers are labeled for use with a named inhalation device.

**Apparatus**

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

For all the cases, prepare the inhaler as directed in the instructions to the patient and connect to a dose collection apparatus, which must be capable of quantitatively capturing the delivered dose (see Fig.1).

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

The filter disk and other materials used in the construction of the apparatus must be compatible with the active substance and solvents that are used to extract the active substance from the filter.

One end of the collection tube is designed to hold the filter disk tightly against the filter-support base. When assembled, the joints between the components of the apparatus are airtight so that when a vacuum is applied to the base of the filter, all of the air drawn through the collection tube passes through the inhaler.

**Procedure**

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

Repeat the procedure for a further 2 doses.

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

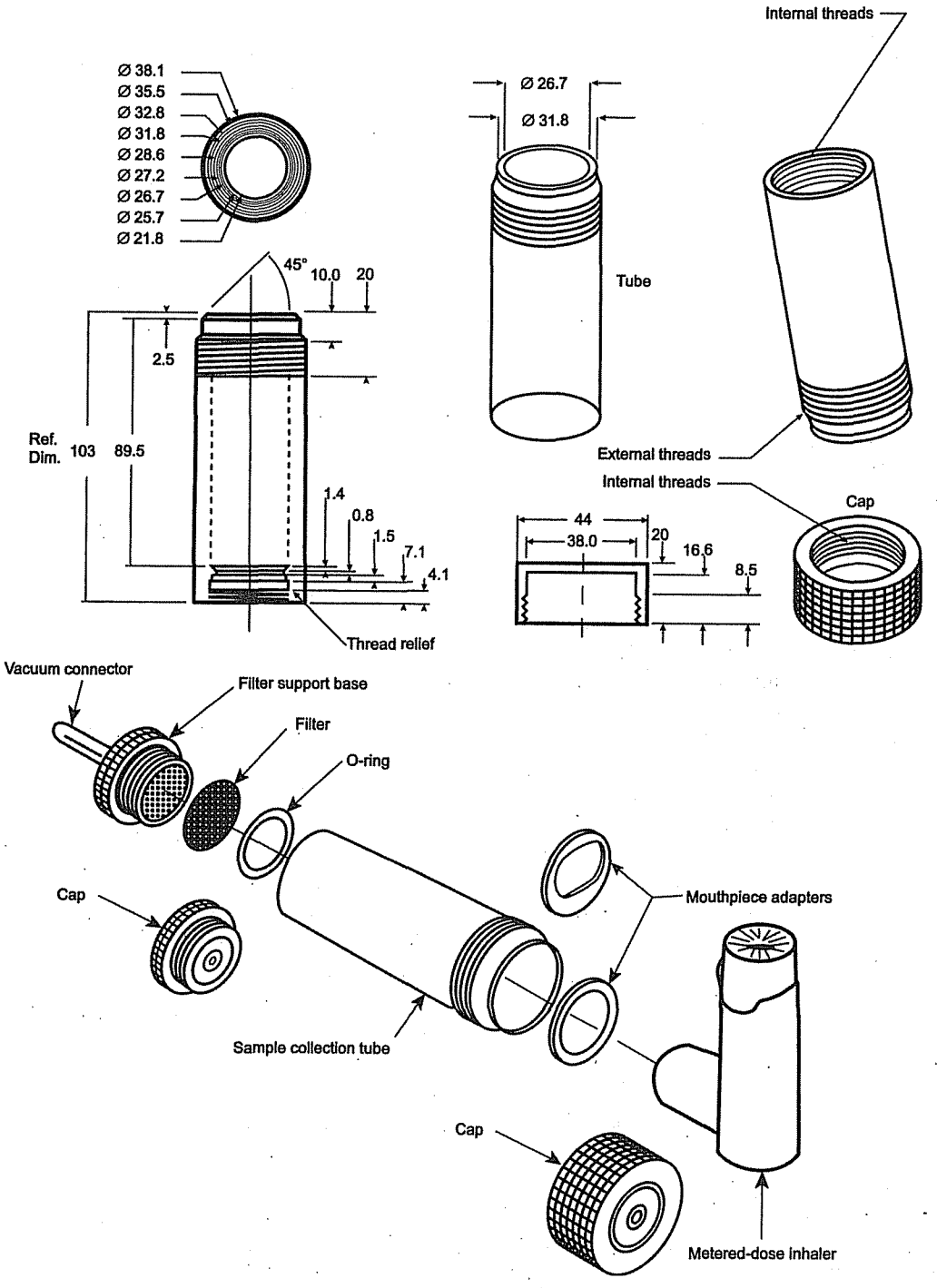
Discharge the device to waste, waiting not less than 5 seconds between actuations until 3 doses remain. Collect these 3 doses using the procedure described above.



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*Dimensions in millimeters*

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

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

**Acceptance criteria**

Unless otherwise justified and authorised, the preparation complies with the test if 9 out of 10 results lie between 75 per



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cent and 125 per cent of the average value and all lie between 65 per cent and 135 per cent. If 2 or 3 values lie outside the limits of 75 per cent to 125 per cent, repeat the test for 2 more inhalers. Not more than 3 of the 30 values lie outside the limits of 75 per cent to 125 per cent and no value lies outside the limits of 65 per cent to 135 per cent.

**Particle size**

*NOTE — Carry out the test in a laminar flow cabinet. Filter all solvents through an appropriately sized filter before use.*

Assemble a suitable membrane filtration apparatus. Use a filter holder fitted with an input chamber designed to prevent any loss of material when the actuator mouthpiece of the aerosol is inserted and the valve actuated. Before assembly wash all parts of the membrane filter holder with *water* and *methanol* and dry in a stream of nitrogen or allow to dry in a laminar flow cabinet. Use a membrane filter with a nominal pore size not greater than 5 µm and with the filtering surface free from foreign particles when examined microscopically using a magnification of not less than × 40.

Discharge 50 deliveries from the pressurised container into the orifice of the input chamber, actuating the valve at intervals of not less than 5 seconds and washing down the particles deposited in the input chamber with successive 10-ml quantities of *light petroleum (40° to 60°)*, *ethanol (95 per cent)* and *water* after 20, 40 and 50 actuations of the valve. Remove the pressurised container and dry the membrane filter. Examine its entire filtering surface microscopically using a magnification of not less than x40. Record the number and size of all individual particles (not agglomerates) more than 10 µm in length measured along the longest axis. The number of particles longer than 20 µm does not exceed 50 and no particle exceeds 100 µm in length.

**Number of deliveries per container**. Take the pressurised container used in the test for Particle size and discharge the remaining contents to waste, actuating the valve at intervals of not less than 5 seconds. Record the number of deliveries discharged. The total number of deliveries so discharged in the test for Particle size is not less than the number stated on the label.

**Leak test**. Select 12 pressurised containers at random, and record the date and time to the nearest half-hour. Weigh each container to the nearest mg, and record the weight, in mg, of each as $W_1$. Allow the container to stand in an upright position at room temperature for not less than 3 days, and again weigh each container, recording the weight, in mg, of each as $W_2$ and recording the date and time to the nearest half-hour. Determine the time, $T$, in hours, during which the containers were under test. Calculate the leakage rate, in mg per year, of each container from the expression $365 \times 24/T \times (W_1 - W_2)$.

Empty the contents of each container tested by chilling to reduce the internal pressure, removing the valve and pouring. Remove any residual contents by rinsing with suitable solvents, then rinse with a few portions of *methanol*. Retain as a unit the container, the valve, and all associated parts, and heat them at 100° for 5 minutes. Cool, weigh and record the weight as $W_3$, and determine the net fill weight $(W_1-W_3)$ for each container tested.

The requirements are met if the average leakage rate of the 12 containers is not more than 3.5 per cent of the net fill weight per year and none of the containers leaks more than 5.0 per cent of the net fill weight per year. If 1 container leaks more than 5.0 per cent per year, and if none of the containers leaks more than 7.0 per cent per year, determine the leakage rate of an additional 24 containers as directed herein. Not more than 2 of the 36 containers leak more than 7.0 per cent of the net fill weight per year.

Where the net fill weight is less than 15 g the requirements are met if the average leakage rate of the 12 containers is not more than 525 mg per year and none of the container leaks more than 750 mg per year. If 1 container leaks more than 750 mg per year but not more than 1.1 g per year, determine the leakage rate of an additional 24 containers as directed herein. Not more than 2 of the 36 containers leak more than 750 mg per year and none of the 36 containers leaks more than 1.1 g per year.

**Deposition of the emitted dose**

The deposition of the emitted dose is a measure of the drug deposition during inhalation. This test is used to determine the fine particle characteristics of the aerosol clouds generated by preparations for inhalation and may be expected to correlate with the drug dose or that fraction of the drug dose that penetrates the lung during inhalation. Individual monographs may also define the emitted fractions of the delivered dose in more than one particle size range.

*Stage Mensuration.* Manufacturers of cascade impaction devices provide a definitive calibration for the separation characteristics of each impaction stage in terms of the relationship between the stage collection efficiency and the aerodynamic diameter of particles and droplets passing through it as an aerosol. Calibration is a property of the jet dimensions, the spatial arrangement of the jet and its collection surface, and the airflow rate passing through it. Because jets can corrode and wear over time, the critical dimensions of each stage, which define that impaction stage's calibration, must be measured on a regular basis. This process, known as stage mensuration, replaces the need for repetitive calibration (using standard aerosols) and ensures that only devices that conform to specifications are used for testing inhaler output. The process involves the measurement and adjustment of the critical dimensions of the instrument.

*Re-entrainment (for apparatus B).* To ensure efficient particle capture, coat each plate with glycerol, silicone oil or similar



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high viscosity liquid, typically deposited from a volatile solvent. Plate coating must be part of method validation and may be omitted where justified and authorised.

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

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

**Apparatus A. Glass impinger**

The apparatus is shown in Fig. 2 and the dimensions are given in Table 1.

**Procedure**

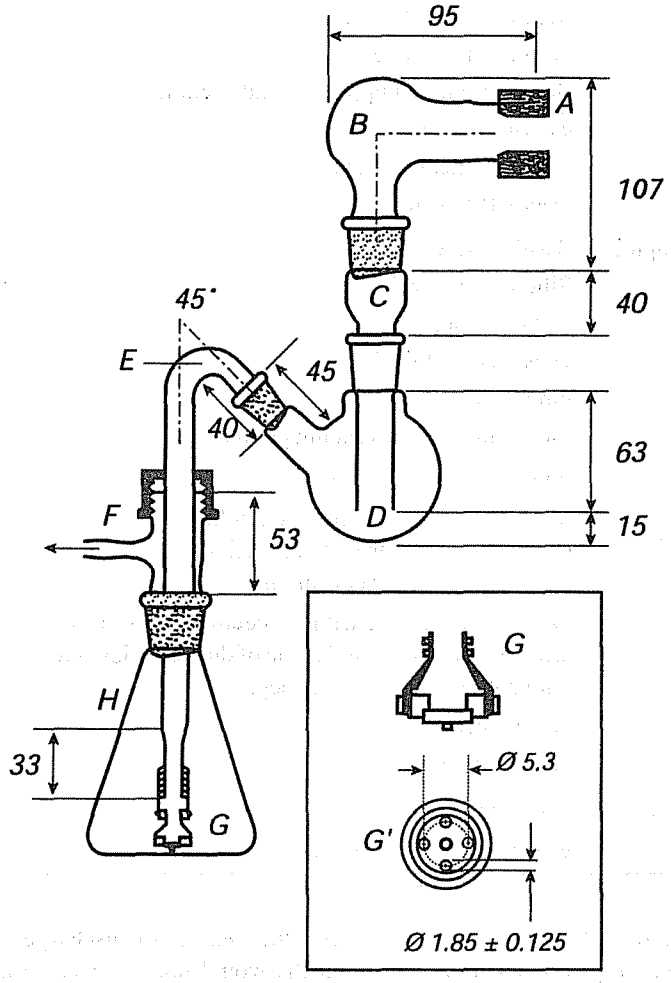
Place the actuator adapter in position at the end of the throat so that the mouthpiece end of the actuator, when inserted to a depth of about 10 mm, lines up along the horizontal axis of the throat and the open end of the actuator, which accepts the pressurised container, is uppermost and in the same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

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

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

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

Shake for about 5 seconds, switch on the pump to the apparatus and locate the mouthpiece end of the actuator in




*Dimensions in millimeters (tolerances ± 1 mm, unless otherwise specified)*

Fig. 2: Apparatus A. Glass impinger



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Table 1

| Code | Item | Description | Dimensions |
|---|---|---|---|
| A | Mouthpiece adaptor | Moulded rubber adapter for actuator mouthpiece. | |
| B. | Throat | Modified round-bottomed flask: | 50 ml |
| | | ground-glass inlet socket | 29/32 |
| | | ground-glass outlet cone | 24/29 |
| C. | Neck | Modified glass adapter: | |
| | | ground-glass inlet socket | 24/29 |
| | | ground-glass outlet cone | 24/29 |
| | | Lower outlet section of precision-bore glass tubing: | |
| | | bore diameter | 14 |
| | | Selected bore light-wall glass tubing: | |
| | | external diameter | 17 |
| D. | Upper impingement chamber | Modified round-bottomed flask | 100 ml |
| | | ground-glass inlet socket | 24/29 |
| | | ground-glass outlet cone | 24/29 |
| E. | Coupling tube | Medium-wall glass tubing: | |
| | | ground-glass cone | 14/23 |
| | | Bent section and upper vertical section: | |
| | | external diameter | 13 |
| | | Lower vertical section: | |
| | | external diameter | 8 |
| F. | Screw thread, side-arm adaptor | Plastic screw cap | 28/13 |
| | | Silicone rubber ring | 28/11 |
| | | PTFE washer | 28/11 |
| | | Glass screw thread: | |
| | | thread size | 28 |
| | | Side-arm outlet to vacuum pump: | |
| | | minimum bore diameter | 5 |
| G | Lower jet assembly | Modified polypropylene filter holder connected to lower vertical section of coupling tube by PTFE tubing | See Figure 1 |
| | | Acetal circular disc with the centres of four jets arranged on a projected circle of diameter 5.3 mm with an integral jet spacer peg: | 10 |
| | | peg diameter | 2 |
| | | peg protrusion | 2 |
| H. | Lower impingement chamber | Conical flask | 250 ml |
| | | ground-glass inlet socket | 24/29 |

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



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

**Apparatus B. Andersen Cascade impactor**

The Andersen 1 ACFM non-viable cascade impactor consists of 8 stages together with a final filter. Material of construction may be aluminium, stainless steel or other suitable material. The stages are clamped together and sealed with O-rings. Critical dimensions applied by the manufacturer of apparatus B are provided in Table 2. In use, some occlusion and wear of holes will occur. In-use mensuration tolerances need to be justified. In the configuration used for pressurised inhalers (Fig. 3) the entry cone of the impactor is connected to an induction port (see Fig. 4). A suitable mouthpiece adapter is used to provide an airtight seal between the inhaler and the induction port. The front face of the inhaler mouthpiece must be flush with the front face of the induction port.

Table 2 - Critical dimensions for Apparatus B

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

In the configuration for powder inhalers, a pre-separator is placed above the top stage to collect large masses of non-

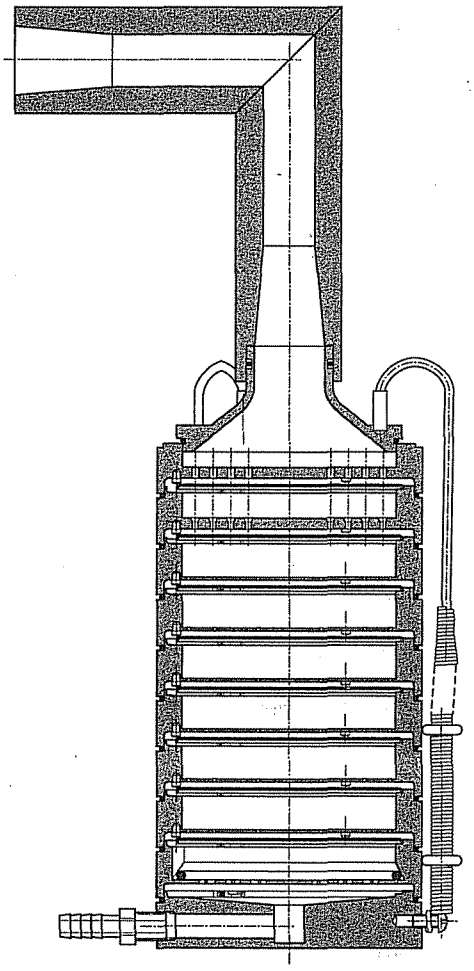


Fig. 3: Apparatus B. Anderson cascade impactor



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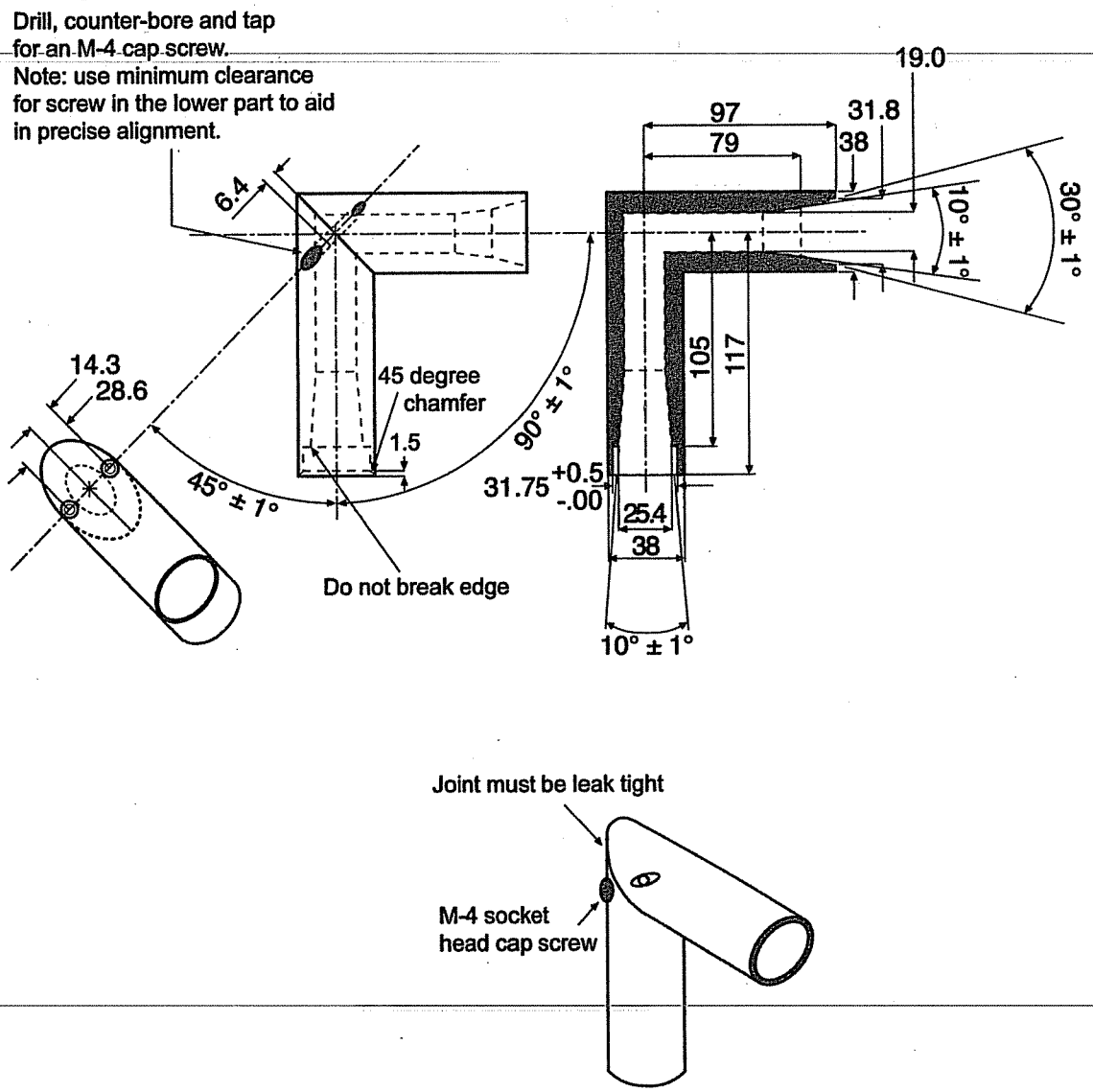
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respirable powder. It is connected to the induction port as shown in Fig. 5. To accommodate high flow rates through the impactor, the outlet nipple, used to connect the impactor to the vacuum system is enlarged to have an internal diameter of greater than or equal to 8 mm.

**Procedure**

Assemble the Andersen impactor with a suitable filter in place. Ensure that the system is airtight. In that respect, follow the manufacturer's instructions. Place a suitable mouthpiece




**Note:**

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

*Dimensions in millimeters unless otherwise stated*

Fig.4: Induction port



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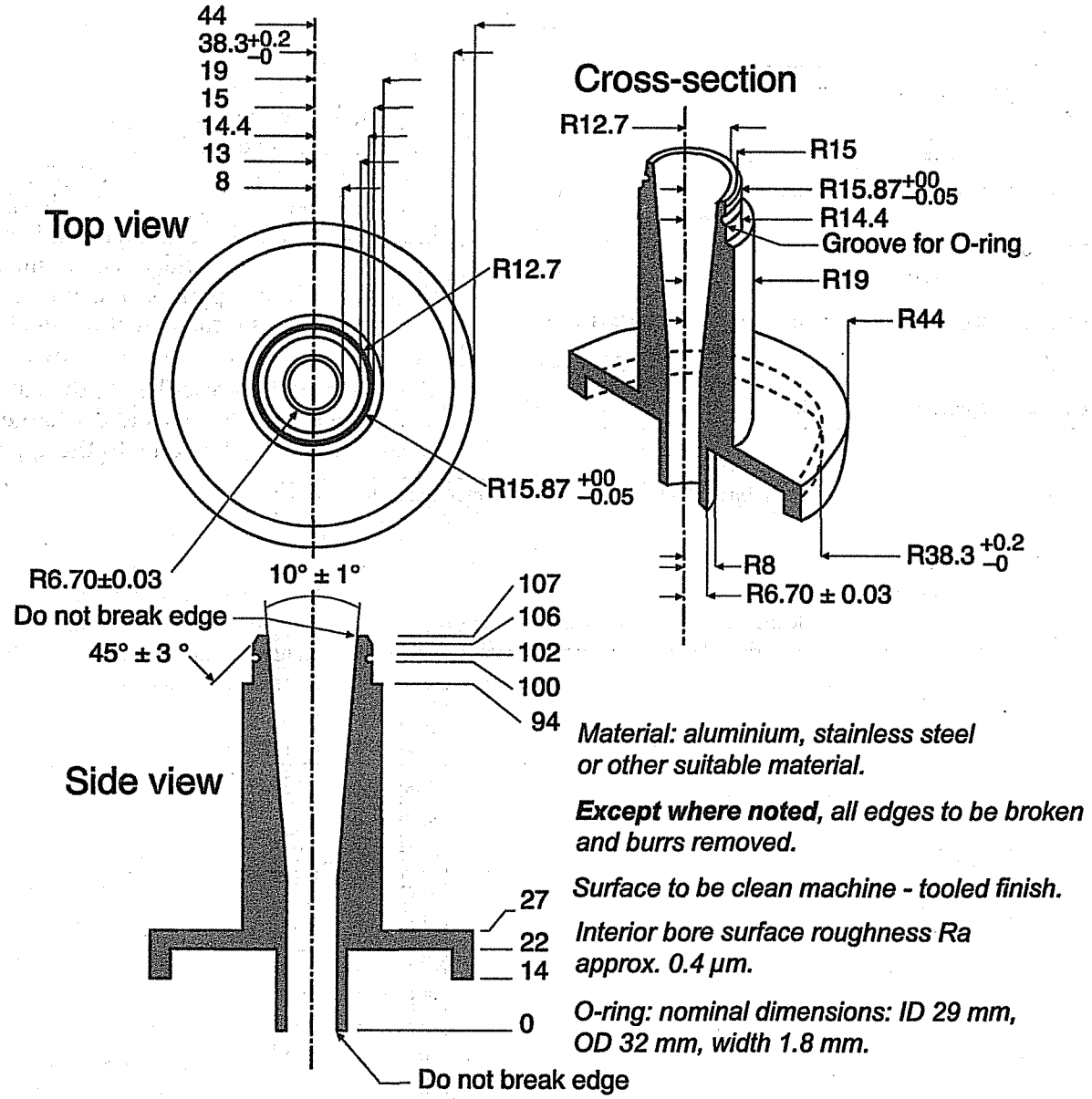
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adapter in position at the end of the induction port so that the mouthpiece end of the actuator, when inserted, lines up along the horizontal axis of the induction port and the inhaler unit is positioned in the same orientation as the intended use. Connect a suitable pump to the outlet of the apparatus and adjust the air flow through the apparatus, as measured at the inlet to the induction port, to 28.3 litres per minute (± 5 per cent). Switch off the pump.

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

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




*(Dimensions are in millimeters unless otherwise stated)*

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



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

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

Calculate the fine particle dose as described below.

### Calculations

From the analysis of the solutions, calculate the mass of active substance deposited on each stage per discharge and the mass of active substance per discharge deposited in the induction port, mouthpiece adapter and when used, the pre-separator.

Starting at the final collection site (filter or MOC), derive a table of cumulative mass versus cut-off diameter of the respective stage (see Table 3). Calculate by interpolation the mass of the active substance less than 5 µm. This is the Fine Particle Dose (FPD).

If necessary, and where appropriate (e.g., where there is a log-normal distribution), plot the cumulative fraction of active substance versus cut-off diameter (see Table 4) on log probability paper, and use this plot to determine values for the Mass Median Aerodynamic Diameter (MMAD) and Geometric Standard Deviation (GSD) as appropriate. Appropriate computational methods may also be used.

## Powders for Inhalation

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

They are intended either for inhalation for local action in the lungs or for systemic absorption through the alveoli or for topical application to the skin or various body orifices. Inhalation aerosols are metered dose preparations which provide controlled amounts of the active ingredient(s).

### Tests

### Uniformity of delivered dose

### Procedure

Prepare the inhaler as directed in the instructions to the patient. The dose collection apparatus must be capable of quantitatively capturing the delivered dose. A dose collection apparatus similar to that described for the evaluation of pressurised metered-dose inhalers may be used provided that the dimensions of the tube and the filter can accommodate the measured flow rate. A suitable tube is defined in Table 4. Connect the tube to a flow system according to the scheme specified in Fig. 6 and Table 4.

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

Prepare the inhaler for use and connect it to the inlet of the apparatus using a mouthpiece adapter to ensure an airtight seal. Use a mouthpiece adapter which ensures that the front face of the inhaler mouthpiece fits with the front face of the sample collection tube. Connect one port of a differential pressure meter to the pressure reading point, P1, in Figure 6

Table 3 – Calculations for apparatus D when used at a flow rate of 28.3 litres/minute

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



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Table 4 – Specifications of the apparatus shown in Fig. 6

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

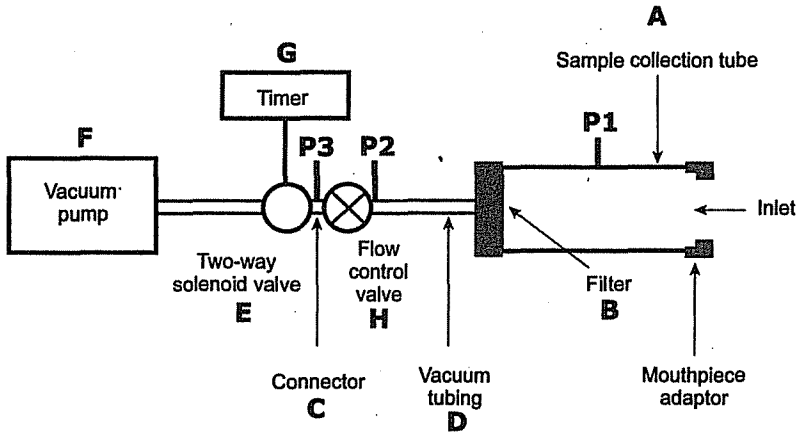



Fig. 6: Apparatus for measuring the uniformity of delivered dose for powders for inhalation



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Table 5 – Component specification for set-up in Fig. 7

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

and let the other be open to the atmosphere. Switch on the pump, open the 2-way solenoid valve and adjust the flow control valve until the pressure drop across the inhaler is 4.0 kPa (40.8 cm $H_2O$) as indicated by the differential pressure meter. Remove the inhaler from the mouthpiece adapter and without touching the flow control valve, connect a flowmeter to the inlet of the sampling apparatus. Use a flowmeter calibrated for the volumetric flow leaving the meter, or calculate the volumetric flow leaving the meter ($Q_{out}$) using the ideal gas law. For a meter calibrated for the entering volumetric flow ($Q_{in}$), use the following expression:

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

$P_0$ = Atmospheric pressure.

DP= Pressure drop over the meter.

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

Ensure that critical flow occurs in the flow control valve by the following procedure; with the inhaler in place and the test flow rate Qout, measure the absolute pressure on both sides of the control valve (pressure reading points P2 and P3 in Figure 6). A ratio P3/P2 of less than or equal to 0.5 indicates critical flow. Switch to a more powerful pump and re-measure the test flow rate if critical flow is not indicated.

*Predispensed systems*: Prepare the inhaler as directed in the instructions to the patient and connect it to the apparatus using an adapter which ensures a good seal. Draw air through the inhaler using the predetermined conditions. Repeat the procedure until the number of deliveries which constitute the minimum recommended dose have been sampled. Quantitatively collect the contents of the apparatus and determine the amount of active substance.

Repeat the procedure for a further 9 doses.

*Reservoir systems*: Prepare the inhaler as directed in the instructions to the patient and connect it to the apparatus using an adapter which ensures a good seal. Draw air through the inhaler under the predetermined conditions. Repeat the procedure until the number of deliveries which constitute the minimum recommended dose have been sampled. Quantitatively collect the contents of the apparatus and determine the amount of active substance.



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Repeat the procedure for a further 2 doses.

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

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

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

**Acceptance criteria**

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

In justified and authorised cases, these ranges may be extended but no value should be greater than 150 per cent or less than 50 per cent of the average value.

Deposition of emitted dose and fine particle dose

**Apparatus.**Use the apparatus described under Pressurised metered-dose Preparations.

**Procedure**

The aerodynamic cut-off diameters of the individual stages of this apparatus are currently not well-established at flow rates other than 28.3 litres per minute. Users must justify and validate the use of the impactor in the chosen conditions, when flow rates different from 28.3 litres per minute are selected.

Assemble the Andersen impactor with the pre-separator and a suitable filter in place and ensure that the system is airtight. Depending on the product characteristics, the pre-separator may be omitted, where justified and authorised. Stages 6 and 7 may also be omitted at high flow rates, if justified. The pre-separator may be coated in the same way as the plates or may contain 10 ml of a suitable solvent. Connect the apparatus to a flow system according to the scheme specified in Figure 7 and Table 5.

Unless otherwise defined, conduct the test at the flow rate, $Q_{out}$, used in the test for uniformity of delivered dose drawing 4 litres of air from the mouthpiece of the inhaler and through the apparatus.

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

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

$P_0$ = atmospheric pressure,

DP = pressure drop over the meter.

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

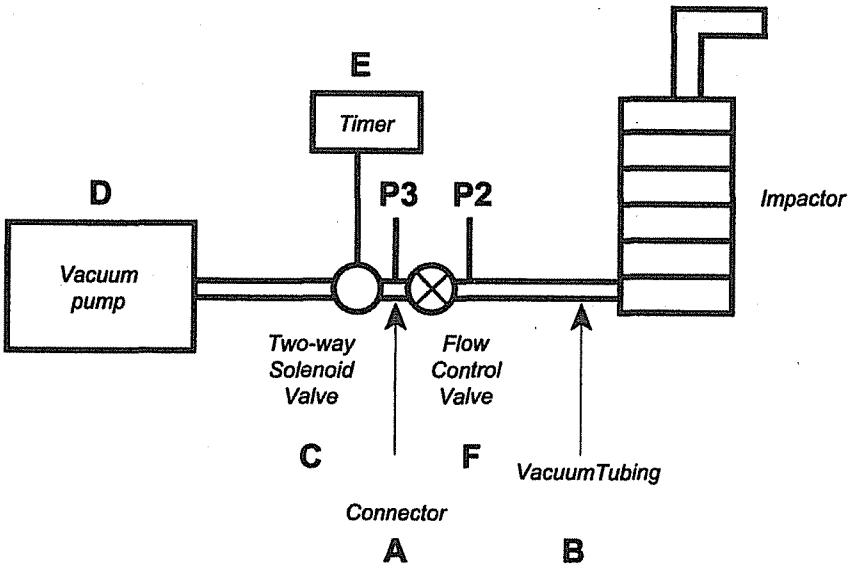



Fig. 7: Experimental set-up for testing powder inhalers



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With the inhaler in place and the test flow rate established, measure the absolute pressure on both sides of the control valve (pressure reading points P2 and P3 in Figure 7). A ratio P3/P2 of less than or equal to 0.5 indicates critical flow. Switch to a more powerful pump and re-measure the test flow rate if critical flow is not indicated.

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

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

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

Calculate the fine particle dose as given under Calculations for Pressurised Metered-dose Preparations.

**Uniformity of Content.** For dry powder inhalers in premetered dosage units, carry out the test for uniformity of content of the contents as given in Capsules.

**Number of deliveries per container.** Discharge doses from the inhaler until empty, at the predetermined flow rate. Record the deliveries discharged. The total number of doses delivered is not less than the number stated on the label.

**Microbial contamination** (2.2.9). Total viable aerobic bacterial count. Not more than 100 cfu per g of the powder.

*E. coli*. Absent in 10 g of the powder.

Salmonella. absent in 50 g of the powder.

*Staphylococcus aureus*. Absent in 10 g of the powder.

*Psedomonas aeruginosa*. Absent in 10 g of the powder.

# Insulin Preparations

## Introduction

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

## Production

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

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

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

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

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

## Tests

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

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

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

Impurities with molecular masses greater than that of insulin

Determine by size-exclusion chromatography (2.4.16).



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

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

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

Chromatographic system

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

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

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

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

**Related proteins**

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

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

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

Inject 20 µl of the test solution and 20 µl of either reference solution (a), for insulin preparations containing 100 IU/ml, or reference solution (b), for insulin preparations containing 40 IU/ml. If necessary, adjust the injection volume to a volume between 10 µl and 20 µl in accordance with the results obtained in the test for linearity as described under Assay. Record the chromatograms for approximately 50 min. If necessary, make further adjustments to the mobile phase in order to ensure that the antimicrobial preservatives present in the test solution are well separated from the insulin and show a shorter retention time. A small reduction in the concentration of acetonitrile increases the retention time of the insulin peaks relatively more than those of the preservatives. In the chromatogram obtained with either reference solution (a), or reference solution (b), as appropriate, A21 desamido insulin appears as a small peak after the principal peak and has a retention time of about



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1.3 relative to the principal peak, due to insulin. In the chromatogram obtained with the test solution the area of the peak due to A21 desamido insulin is not greater than 5.0 per cent of the total area of the peaks; the sum of the areas of any other peaks, apart from those due to insulin and A21 desamido insulin is not greater than 6.0 per cent of the total area of the peaks. Disregard the peaks due to the preservatives and protamine (early eluting peaks).

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

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

B. Determine by atomic absorption spectrometry (2.4.2).

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

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

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

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

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

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

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

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

# Liposomal Preparations

## Liposomal Injectable Preparations

### Introduction

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

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

### Tests

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

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

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

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

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

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

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

## Powders for Liposomal Injection

### Definition

Powders for Liposomal Injection are solid, sterile substances distributed in their final containers and which, when shaken



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with the prescribed volume of a prescribed sterile liquid rapidly form translucent to opalescent dispersion and practically particle-free uniform dispersions.

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

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

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

# Nasal Preparations

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

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

During manufacture, packaging, storage and distribution of nasal preparations, suitable means shall be taken to ensure their microbial quality; acceptance criteria for microbial quality are given in Chapter 5.9.

## Tests

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

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

## Nasal Drops, Solutions and Sprays

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

## Nasal Powders

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

**Storage.** Store protected from light and moisture.

## Tests

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

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

# Ointments

Ointments are homogeneous, semi-solid preparations intended for external application to the skin or certain mucous membranes for emollient, protective, therapeutic or prophylactic purposes where a degree of occlusion is desired. They usually consist of solutions or dispersions of one or more medicaments in suitable bases. They are formulated using hydrophobic, hydrophillic or water-emulsifying bases to provide preparations that are immiscible, miscible or emulsifiable with the skin secretion, respectively. The base should not produce irritation or sensitisation of the skin, nor should it retard wound healing; it should be smooth, inert, odourless or almost odourless, physically and chemically stable and compatible with the skin and with incorporated medicaments. The proportions of the base ingredients should be such that the ointment is not too soft or too hard for convenient use. The consistency should be such that the ointment spreads and softens when stress is applied.

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

During manufacture, packaging, storage and distribution of ointments, suitable means shall be taken to ensure their microbial quality; acceptance criteria for microbial quality are given in Chapter 5.9.

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

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



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### Tests

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

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

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

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

## Oral Liquids

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

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

During manufacture, packaging, storage and distribution of oral liquids, suitable means shall be taken to ensure their microbial quality; acceptance criteria for microbial quality are given in Chapter 5.9.

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

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

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

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

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

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

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

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

**Oral Suspensions**. Oral Suspensions are Oral Liquids containing one or more active ingredients suspended in a suitable vehicle. Suspended solids may slowly separate on keeping but are easily redispersed.

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

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

**Containers**. Oral Liquids may be supplied in multiple dose or single dose containers. Oral Emulsions and Oral Suspensions should be packed in bottles sufficiently wide-mouthed to facilitate the flow of the contents. They are administered either in volumes such as 5 ml, or multiples of 5 ml, or in small volumes



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(drops). Each dose of a multiple dose Oral Liquid is administered by means of a suitable measuring device which is usually provided with the container.

### Tests

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

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

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

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

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

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

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

## Oral Powders

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

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

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

In the manufacture of oral powders, means are taken to ensure a suitable particle size with regard to the intended use of the product. During manufacture, packaging, storage and distribution of oral powders, suitable means shall be taken to ensure their microbial quality; acceptance criteria for microbial quality are given in Chapter 5.9.

**Storage.** Store Oral Powders in containers protected from moisture.

### Tests

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

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



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

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

**Uniformity of weight**. Unless otherwise specified, Oral Powders presented in single dose containers comply with the test for contents of packaged dosage forms (2.5.6).

# Parenteral Preparations

## Injectable Preparations

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

### Introduction

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

### Production

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

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

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

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

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

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

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

**Containers**. Containers for Parenteral Preparations are made as far as possible from materials that (1) are sufficiently transparent to permit visual inspection of the contents, except



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

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

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

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

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

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

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

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

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

In the case of Parenteral Preparations like Powders for Injection and Concentrated Solutions for Injection wherein a diluent is intended to be added before use, the label also states (1) the composition of the recommended diluent; (2) the conditions under which the constituted preparation should be stored; (3) the period within which the constituted solution should be used if it has been stored under the recommended conditions of storage after constitution. In the case of Powders for Injection, the label also states the amount of diluent to be used to attain a specific concentration of the active ingredient in the solution or suspension so obtained whereas in the case of Concentrated Solutions for Injection, the amount of diluent to be used to attain a specific concentration and the final volume of the solution or suspension so obtained.

## Injections

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



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

## Tests

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

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

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

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

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

**Extractable volume**. Where the nominal volume does not exceed 5 ml, the containers comply with the requirements of Method 1 and where the nominal volume is greater than 5 ml, the containers comply with the requirements of Method 2. Suspensions should be shaken before the contents are withdrawn; oily injections may be warmed but should be cooled to 25° before carrying out the test.

*Method 1* — Use 6 containers, 5 for the test and 1 for rinsing the syringe used. Inspect the 5 containers to be used in the test visually and ensure that each contains approximately the same volume of the preparation.

Using a syringe with a capacity not exceeding twice the volume to be measured and fitted with a suitable needle, take up a small quantity of the liquid under examination from the container reserved for rinsing the syringe, and discharge it from the syringe whilst the needle is pointing upwards so as to expel any air. Withdraw as much as possible the contents of one of the containers reserved for the test and transfer, without emptying the needle, to a dry graduated cylinder of such capacity that the total combined volume to be measured occupies not less than 40 per cent of the nominal volume of the cylinder. Repeat the procedure until the contents of the 5 containers have been transferred and measure the volume. The content of each container is not less than the nominal volume and the average content of 5 containers is not more than 115 per cent of the nominal volume.

*Method 2* — Transfer the contents of not less than 3 containers separately to dry graduated cylinders such that the volume to be measured occupies not less than 40 per cent of the nominal volume of the cylinder and measure the volume transferred. The contents of each container is not less than the nominal volume and not more than 110 per cent of the nominal volume.

Multiple dose containers labelled to yield a specific number of doses shall contain a sufficient excess to permit the withdrawal of the designated number of doses.

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

**Pyrogens**. Unless otherwise stated in the individual monograph, when the volume to be injected in a single dose is 10 ml or more, Injections comply with the test for pyrogens (2.2.8), unless the test for bacterial endotoxins (2.2.3), is prescribed.

## Infusions

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



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### Tests

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

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

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

**Pyrogens**. Where no test for bacterial endotoxins (2.2.3) is prescribed, Intravenous Infusions comply with the test for pyrogens (2.2.8). Unless otherwise stated in the individual monograph inject 10 ml per kg of body weight into each animal.

## Powders for injection

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

### Tests

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

**Uniformity of content**. Unless otherwise stated in the individual monograph, Powders for injection that contain 10 mg or less than 10 mg or less than 10 per cent of active ingredient or that have a unit weight equal to or less than 50 mg comply with the test for Uniformity of content described under Injections. For Powders for injection containing more than one active ingredient carry out the test for each active ingredient that corresponds to the above conditions. The test is not applicable to Powders for injection containing multivitamins and trace elements.

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

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

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

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

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

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

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

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

## Concentrated Solutions for injection

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

### Tests

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

### Implants

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

### Tests

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



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# Pessaries

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

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

During manufacture, packaging, storage and distribution of pessaries, suitable means shall be taken to ensure their microbial quality; acceptance criteria for microbial quality are given in Chapter 5.9.

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

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

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

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

**Storage**. Store in ventilated containers.

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

**Storage**. Store in well-closed containers.

## Tests

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

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

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

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

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

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

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

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

# Suppositories

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

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

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

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

Moulded Suppositories have the characteristics of Moulded Pessaries.

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



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Shell Suppositories have the characteristics of Shell Pessaries.

During manufacture, packaging, storage and distribution of suppositories, suitable means shall be taken to ensure their microbial quality; acceptance criteria for microbial quality are given in Chapter 5.9.

### Tests

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

**Storage**. Store in well-closed containers.

## Tablets

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

### Introduction

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

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

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

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

### Production

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

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

During manufacture, packaging, storage and distribution of tablets, suitable means shall be taken to ensure their microbial quality; acceptance criteria for microbial quality are given in Chapter 5.9.

### Tests

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

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

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

Table 1

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

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



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w/w of active ingredient. For tablets containing more than one active ingredient carry out the test for each active ingredient that corresponds to the aforementioned conditions. The test is also applicable to coated tablets other than film-coated tablets, irrespective of their content of active substance(s).

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

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

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

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

## Uncoated Tablets

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

The addition of flavouring agents to uncoated tablets other than multi-layer tablets is not official unless permitted in the individual monograph. Uncoated Tablets have the general characteristics of tablets. When a broken section of an uncoated tablet is examined under a lens, either a relatively uniform texture (single-layer tablets) or a stratified structure (multi-layer tablets) is seen; there are no signs of coating.

### Tests

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

*The test does not apply to chewable tablets.*

## Coated Tablets

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

Coated tablets may contain flavouring agents.

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

### Tests

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

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

## Film-coated Tablets

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

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

*The test does not apply to chewable tablets.*

## Dispersible Tablets

Dispersible tablets are uncoated or film-coated tablets that produce a uniform dispersion in water and may contain permitted flavouring and sweetening agents. However, if saccharin, including its sodium and potassium salts, is used as a sweetening agent, its concentration in dispersible tablets



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meant for paediatric use should be restricted so as to limit its intake to 5 mg/kg of body weight.

### Tests

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

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

## Effervescent Tablets

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

### Tests

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

## Modified-release Tablets

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

Modified-release tablets include enteric-coated tablets, prolonged-release tablets and delayed-release tablets.

## Enteric-coated Tablets

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

Enteric-coated tablets have the characteristics of Coated Tablets.

### Tests

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

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

## Prolonged- release Tablets

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

### Tests

**Dissolution** (2.5.2). The test should be designed to demonstrate the appropriate release of the active substance(s). The manufacturer is expected to give specifications for drug release at 3 or more test-time points. The first point should be set after a testing period corresponding to a dissolved amount of typically 20 per cent to 30 per cent. The second point should define the dissolution pattern and should be set at around 50 per cent release. The final point should ensure almost complete release that is generally understood as more than 80 per cent release.

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

## Soluble Tablets

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



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### Tests

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

## Tablets for Use in the Mouth

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

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

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



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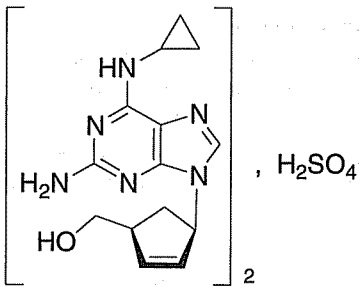


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# Abacavir Sulphate



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

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

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

**Category**. Antiretroviral.

**Dose**. 300 mg twice daily.

**Description**. A white or almost white, crystalline powder.

## Identification

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

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

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

## Tests

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

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

*Test solution.* A 0.05 per cent w/v solution of the substance under examination in the mobile phase.

*Reference solution.* Dilute 1 ml of the test solution to 200 ml with the mobile phase.

Inject the test solution and the reference solution. Calculate the content of each impurity in the chromatogram obtained with the test solution by comparing the peak area of each peak with the area of the principal peak in the chromatogram obtained with the reference solution. The content of any individual impurity is not greater than 0.5 per cent and the sum of all the impurities is not greater than 1.5 per cent.

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

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

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

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* A 0.01 per cent w/v solution of the substance under examination in the mobile phase.

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

Chromatographic system

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

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

Inject the test solution and the reference solution.

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

**Storage**. Store at a temperature not exceeding 30°.

# Abacavir Oral Solution

Abacavir Sulphate Oral Solution

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

**Usual strength**. 20 mg per ml.



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### Identification

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

### Tests

**pH** (2.4.24). 4.6 to 5.0.

**Related substances**. Determine by liquid chromatography (2.4.14).

*NOTE — Prepare the solutions immediately before use.*

*Test solution*. Weigh a quantity of the oral solution containing 50 mg of abacavir, dissolve in 100 ml of the mobile phase and mix.

*Reference solution (a)*. A 0.05 per cent w/v solution of *abacavir sulphate RS* in the mobile phase.

*Reference solution (b)*. Dilute 1 ml of reference solution (a) to 100 ml with the mobile phase.

Chromatographic system

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

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

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

**Other tests**. Complies with the tests stated under Oral liquids.

**Assay**. Determine by liquid chromatography (2.4.14).

*NOTE — Prepare the solutions immediately before use.*

*Test solution*. Weigh accurately a quantity of the oral solution containing 60 mg of abacavir, dissolve in 100.0 ml of the mobile phase and mix. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

*Reference solution*. A 0.06 per cent w/v solution of *abacavir sulphate RS* in the mobile phase and filter. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Chromatographic system

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

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

Inject the test solution and the reference solution.

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

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

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

## Abacavir Tablets

Abacavir Sulphate Tablets

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

**Usual strength**. 300 mg.

### Identification

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

### Tests

**Dissolution** (2.5.2).

Apparatus. No 1,
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 75 rpm and 15 minutes.



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Withdraw a suitable volume of the medium and filter, discarding the first few ml of the filtrate.

Determine by liquid chromatography (2.4.14).

*Test solution*. Dilute the filtrate, if necessary, with the dissolution medium.

*Reference solution*. A 0.075 per cent w/v solution of *abacavir sulphate RS* in the dissolution medium. Dilute 5 ml of the solution to 10 ml with the dissolution medium.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 40°,
- mobile phase: a mixture of 85 volumes of a buffer solution prepared by dissolving 1.15 g of *ammonium dihydrogen phosphate* and 2 g of *tetrabutyl ammonium hydrogen sulphate* in 1000 ml of *water* and adjusting the pH to 6.0 with *triethylamine*, and 15 volumes of *acetonitrile*.
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 214 nm,
- injection volume. 10 µl.

Inject the test solution and the reference solution.

D. Not less than 80 per cent of the stated amount of $C_{14}H_{18}N_6O$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 50 mg of abacavir, disperse in 100.0 ml of the mobile phase and filter.

*Reference solution (a)*. A solution of *abacavir sulphate RS* containing 0.05 per cent w/v of abacavir in the mobile phase.

*Reference solution (b)*. Dilute 1.0 ml of reference solution (a) to 100 ml with the mobile phase.

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

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

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

**Other tests**. Comply with the tests stated under Tablets.

**Water** (2.3.43). Not more than 5.0 per cent, determined on 0.5 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 50 mg of abacavir, disperse in 100.0 ml of the mobile phase and filter. Dilute 5.0 ml of the filtrate to 50.0 ml with the mobile phase.

*Reference solution*. A 0.060 per cent w/v solution of *abacavir sulphate RS* in the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 40°,
- mobile phase: a mixture of 85 volumes of a buffer solution prepared by dissolving 1.15 g of *ammonium dihydrogen phosphate* and 2 g of *tetrabutyl ammonium hydrogen sulphate* in 1000 ml of *water* and adjusting the pH to 6.0 with *triethylamine*, and 15 volumes of *acetonitrile*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 214 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, the column efficiency in not less than 2000 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{14}H_{18}N_6O$.

**Storage**. Store protected from moisture, at a temperature not exceeding 30°.

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

# Abacavir and Lamivudine Tablets

## Abacavir Sulphate and Lamivudine Tablets

Abacavir and Lamivudine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of abacavir, $C_{14}H_{18}N_6O$ and lamivudine, $C_8H_{11}N_3O_3S$.

**Usual strengths**. 600 mg abacavir and 300 mg Lamivudine.



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## Identification

In the Assay, the principal peaks in the chromatogram obtained with the test solution correspond to the principal peaks in the chromatogram obtained with the reference solution.

## Tests

**Dissolution** (2.5.2).

Apparatus. No 1,
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14)

*Test solution.* The filtrate obtained as given above. Dilute the filtrate if necessary, with the dissolution medium.

*Reference solution.* Dissolve 75 mg of *abacavir sulphate RS* and 30 mg of *lamivudine RS* in 10 ml of *methanol* and dilute to 100 ml with the dissolution medium.

Use the chromatographic system described under Assay.

Inject the reference solution. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

D. Not less than 80 per cent of the stated amounts of $C_{14}H_{18}N_6O$ and $C_8H_{11}N_3O_3S$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture.* 95 volumes of mobile phase A and 5 volumes of mobile phase B.

*Test solution.* Weigh accurately a quantity of the powdered tablets containing 100 mg of abacavir and disperse in 100 ml of the solvent mixture and filter.

*Reference solution (a).* A 0.05 per cent w/v solution of *lamivudine RS* in the solvent mixture.

*Reference solution (b).* Dilute 1 ml of reference solution (a) to 100 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to silica (5 µm),
- column temperature. 40°,
- mobile phase: A. a buffer solution prepared by dissolving 1.9 g of *ammonium acetate* in 900 ml of *water*, adjusting the pH to 3.8 with *glacial acetic acid* and diluting to 1000 ml with *water*;

  B. *methanol*,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 277 nm,
- injection volume. 20 µl.

| Time (in min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0 | 95 | 5 |
| 20 | 95 | 5 |
| 40 | 30 | 70 |
| 45 | 95 | 5 |
| 50 | 95 | 5 |

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

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

**Other tests**. Comply with the tests stated under Tablets.

**Water** (2.3.43). Not more than 3.0 per cent, determined on 0.5 g.

**Assay**. Determine by liquid chromatography (2.4.14)

*Test solution.* Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 60 mg of abacavir, dissolve in 20 ml of *0.1 M hydrochloric acid* and dilute to 100.0 ml with *methanol.* Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

*Reference solution.* Dissolve 35 mg of *abacavir RS* and 15 mg of *lamivudine RS* in 15 ml of *0.1 M hydrochloric acid* and dilute to 50.0 ml with *methanol.* Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 40°,
- mobile phase: a mixture of 50 volumes of a buffer solution prepared by dissolving 7.66 g of *ammonium acetate* in 1000 ml of a 0.5 per cent w/v solution of *glacial acetic acid* and 50 volumes of *methanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 282 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.



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Inject the test solution and the reference solution.

Calculate the contents of $C_{14}H_{18}N_6O$ and $C_8H_{11}N_3O_3S$ in the tablets.

**Storage**. Store protected from moisture, at a temperature not exceeding 30°.

## Abacavir, Lamivudine and Zidovudine Tablets

Abacavir, Lamivudine and Zidovudine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of abacavir, $C_{14}H_{18}N_6O$ lamivudine, $C_8H_{11}N_3O_3S$ and zidovudine, $C_{10}H_{13}N_5O_4$.

**Usual strength**. 300 mg Abacavir, 150 mg Lamivudine and 300 mg Zidovudine.

### Identification

In the Assay, the principal peaks in the chromatogram obtained with the test solution correspond to the peaks in the chromatogram obtained with the reference solution.

### Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

*Test solution*. Use the filtrate obtained as given above.

*Reference solution*. A solution containing 0.035 per cent w/v of *abacavir sulphate RS*, 0.015 per cent w/v *lamivudine RS* and 0.03 per cent w/v of *zidovudine RS* in the dissolution medium.

Chromatographic system
- a stainless steel column 5 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm) (such as Restek's Pinnacle II C-18),
- column temperature. 50°,
- mobile phase: a mixture of 88 volumes of a buffer solution prepared by dissolving 1 g of *octanesulphonic acid* and 1 ml of *triethylamine* in 1000 ml of *water* and adjusting the pH to 2.5 with *orthophosphoric acid*, and 12 volumes of *acetonitrile*,
- flow rate. 2.5 ml per minute.
- spectrophotometer set at 272 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the resolution between lamivudine and zidovudine peaks is not less than 2.5, the column efficiency determined from lamivudine, zidovudine and abacavir peaks is not less than 700, 1200 and 2000 theoretical plates respectively, the tailing factor for lamivudine, zidovudine and abacavir peaks is not more than 2.0 and the relative standard deviation of replicate injections is not more than 2.0 per cent for each component.

Inject the test solution and the reference solution.

Calculate the contents of $C_{14}H_{18}N_6O$, $C_8H_{11}N_3O_3S$ and $C_{10}H_{13}N_5O_4$.

D. Not less than 70 per cent of the stated amounts of $C_{14}H_{18}N_6O$, $C_8H_{11}N_3O_3S$ and $C_{10}H_{13}N_5O_4$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*NOTE — Prepare the solutions immediately before use.*

*Solvent mixture*. A 0.2 per cent v/v solution of *orthophosphoric acid* in a mixture of 70 volumes of *water* and 30 volumes of *methanol*.

*Test solution*. Weigh a quantity of the powdered tablets containing 75 mg of Lamivudine, disperse in 100 ml of the solvent mixture and filter.

*Reference solution (a)*. A 0.075 per cent w/v solution of *lamivudine RS* in the solvent mixture.

*Reference solution (b)*. Dilute 1.0 ml of the solution to 100 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm),
- mobile phase: A. a mixture of 70 volumes of *methanol*, 30 volumes of *acetonitrile* and 0.4 volume of *tetrahydrofuran*,
  B. a buffer solution pH 3.0 prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, adjusting the pH to 3.0 with *orthophosphoric acid* and filtering,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 225 nm,
- injection volume.10 µl.

| Time (in min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) | Flow rate ml per minute |
|---|---|---|---|
| 0 | 2 | 98 | 1 |
| 10 | 2 | 98 | 1 |
| 25 | 20 | 80 | 1 |
| 28 | 20 | 80 | 1 |
| 50 | 30 | 70 | 1 |
| 60 | 35 | 65 | 1.3 |
| 63 | 35 | 65 | 1.3 |
| 66 | 2 | 98 | 1 |
| 80 | 2 | 98 | 1 |



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Inject reference solution (a). The test is not valid unless the column efficiency is not less than 3000 and the tailing factor is not more than 1.5 for each component.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 3 times the area of the peak in the chromatogram obtained with reference solution (3.0 per cent) and the sum of all the secondary peaks is not more than 5 times the area of the peak in the chromatogram obtained with reference solution (5.0 per cent).

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. 50 volumes of *water* and 50 volumes of *methanol*.

*Test solution*. Weigh accurately a quantity of the powdered tablets containing 150 mg of abacavir, dissolve in 100 ml of *water*, add 80 ml of *methanol* and dilute to 200.0 ml with *methanol*. Dilute 10.0 ml of the solution to 25.0 ml with the solvent mixture and filter.

*Reference solution*. A solution containing 0.35 per cent w/v of *abacavir sulphate RS*, 0.15 per cent w/v *lamivudine RS* and 0.30 per cent w/v of *zidovudine RS* in the solvent mixture. Dilute 5.0 ml of the solution to 50.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm), (such as Kromasil C-18),
- column temperature 50°,
- mobile phase: a mixture of 65 volumes of a buffer solution prepared by dissolving 1 g of *octane sulphonic acid* and 1 ml of *triethylamine* in 1000 ml of *water*, adjusting the pH to 4.5 with *orthophosphoric acid* and filtering, and 35 volumes of *methanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 272 nm,
- injection volume.10 µl.

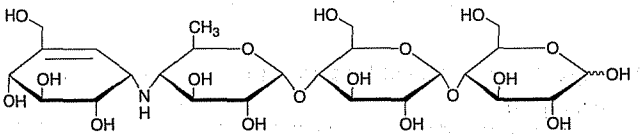
Inject the reference solution. The test is not valid unless the column efficiency determined from the peak due to lamivudine is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 for each component and the relative standard deviation of replicate injections is not more than 2.0 per cent for each component.

Inject the test solution and the reference solution.

Calculate the contents of $(C_{14}H_{18}N_6O)_2,H_2SO_4$, $C_8H_{11}N_3O_3S$ and $C_{10}H_{13}N_5O_4$ in the tablets.

**Storage**. Store protected from moisture, at a temperature not exceeding 30°.

# Acarbose



$C_{25}H_{43}NO_{18}$ Mol. Wt. 646.0

Acarbose is *O*-4,6-dideoxy-4-[[(1*S*,4*R*, 5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino-α-D-glucopyranosyl-(1→4)-*O*-α-D- glucopyranosyl-(1→4)-D-glucopyranose, which is produced by certain strains of *Actinoplanes utahensis*.

Acarbose contains not less than 95.0 per cent and not more than 102.0 per cent of $C_{25}H_{43}NO_{18}$, calculated on the anhydrous basis.

**Category**. Antidiabetic.

**Description**. A white or yellowish, amorphous powder, hygroscopic.

## Identification

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

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

## Tests

**pH** (2.4.24). 5.5 to 7.5, determined in 5.0 per cent w/v solution in *carbon dioxide-free water* (solution A).

**Specific optical rotation** (2.4.22). +168° to +183°, dilute 2 ml of solution A to 10 ml with *water*.

**Light absorption** (2.4.7). Absorbance of solution A at 425 nm, not more than 0.15.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 0.2 g of the substance under examination in 100 ml of *water*.

*Reference solution (a)*. A 0.2 per cent w/v solution of *acarbose RS* in *water*.

*Reference solution (b)*. Dilute 1 ml of reference solution (a) to 100 ml with *water*.

Chromatographic system as described under Assay.

Inject reference solution (a). Test is not valid unless the column effciency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.



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Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 1.5 times the area of the peak in the chromatogram obtained with reference solution (b) (1.5 per cent) and the sum of areas of all the secondary peaks is not more than thrice the area of the peak in the chromatogram obtained with reference solution (b) (3.0 per cent).

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

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Water** (2.3.43). Not more than 4.0 per cent, determined on 0.3 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 10 mg of the substance under examination in 50.0 ml of *water*. Dilute 5.0 ml of the solution to 50.0 ml with *water*.

*Reference solution*. A 0.002 per cent w/v solution of *acarbose RS* in *water*.

Chromatographic system
- a stainless steel column 25 cm x 4 mm packed with aminopropylsilyl silica (5 µm),
- mobile phase: a mixture of 75 volumes of *acetonitrile* and 25 volumes of a solution containing 0.06 per cent w/v of *potassium dihydrogen phosphate* and 0.035 per cent w/v of *disodium hydrogen phosphate dihydrate,*
- flow rate. 2 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume.10 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{25}H_{43}NO_{18}$.

**Storage**. Store protected from moisture.

# Acarbose Tablets

Acarbose Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of acarbose, $C_{25}H_{43}NO_{18}$.

**Usual strengths**. 50 mg; 100 mg.

## Identification

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

## Tests

**Dissolution** (2.5.2).

Apparatus No. 1,

Medium. 500 ml of a phosphate buffer prepared by dissolving 1.36 g of *potassium dihydrogen orthophosphate* and 2 ml of *triethylamine* in 1000 ml of *water* and adjusting the pH to 3.0 with *orthophosphoric acid,*

Speed and time. 100 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

*Solvent mixture*. 15 volumes of the phosphate buffer and 85 volumes of *acetonitrile*.

*Test solution*. The filtrate diluted with solvent mixture to produce a 0.002 per cent w/v solution.

*Reference solution*. A 0.002 per cent w/v solution of *acarbose RS* in the solvent mixture.

Use the chromatographic system described under Assay.

Calculate the content of $C_{25}H_{43}NO_{18}$.

D. Not less than 70 per cent of the stated amount of $C_{25}H_{43}NO_{18}$.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 50 mg of Acarbose in the mobile phase by shaking mechanically, dilute to 250.0 ml with the mobile phase and filter.

*Reference solution*. A 0.02 per cent w/v solution of *acarbose RS* in the mobile phase

Charomatographic system
- a stainless steel column 25cm x 4.6 mm, packed with amino groups chemically bonded to porous silica particles (5 µm)
- mobile phase: a mixture of 40 volumes of a buffer solution prepared by dissolving 0.6 g of *potassium dihydrogen orthophosphate* and 0.35 g of *sodium dihydrogen phosphate* in 1000 ml of *water* and 60 volumes of *acetonitrile,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{25}H_{43}NO_{18}$ in the tablets.

**Storage**. Store protected from light and moisture.

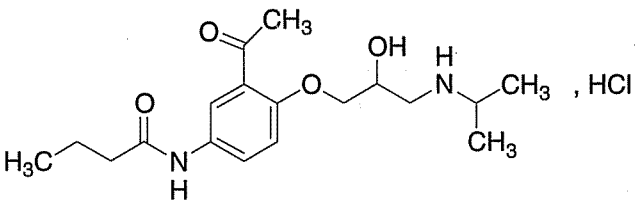


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# Acebutolol Hydrochloride



$C_{18}H_{28}N_2O_4,HCl$ Mol. Wt. 372.9

Acebutolol Hydrochloride is (*RS*)-3'-acetyl-4'-(2-hydroxy-3-isopropylaminopropoxy)butyranilide hydrochloride.

Acebutolol Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{18}H_{28}N_2O_4$, HCl, calculated on the dried basis.

**Category**. $\beta_1$-receptor antagonist; antihypertensive; antianginal; antiarrhythmic.

**Dose**. As antihypertensive, initially, 400 mg once daily or 200 mg twice daily, increased after 2 weeks to 400 mg twice daily. As antianginal, initially, 400 mg once daily or 200 mg twice daily; in severe angina, 300 mg thrice daily; maintenance dose, upto 1.2 g daily. As antiarrhythmic, 400 mg to 1.2 g daily, in 2 to 3 divided doses.

**Description**. A white or almost white, crystalline powder.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *acebutolol hydrochloride RS* or with the reference spectrum of acebutolol hydrochloride.

B. When examined in the range 220 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in 0.1 per cent v/v solution of *hydrochloric acid* shows absorption maxima at about 233 nm and 322 nm; absorbance at 233 nm, 0.55 to 0.61.

C. Determine by thin layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 60 volumes of *water*, 40 volumes of *methanol* and 0.5 volumes of *perchloric acid*.

*Test solution*. Dissolve 0.1 g of the substance under examination in 100 ml of *methanol*.

*Reference solution (a)*. A 0.1 per cent w/v solution of *acebutolol hydrochloride RS* in *methanol*.

*Reference solution (b)*. A mixture of equal volumes of reference solution (a) and a 0.1 per cent w/v solution of *pindolol RS* in *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of warm air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

D. A 5 per cent w/v solution gives reaction A of chlorides (2.3.1).

## Tests

**Appearance of solution**. A 5.0 per cent w/v solution is not more opalescent than opalescence standard OS2 (2.4.1) and not more intensely coloured than reference solution BYS5 (2.4.1).

**pH** (2.4.24). 5.0 to 7.0, determined in a 1.0 per cent w/v solution.

**Related substances**. Determine by thin layer chromatography (2.4.17), coating two plates with *silica gel GF254*.

*Mobile phase (a)*. The upper layer obtained by shaking together 50 volumes of *water*, 40 volumes of *1-butanol* and 10 volumes of *glacial acetic acid*.

*Mobile phase (b)*. A mixture of 90 volumes of *2-propanol* and 10 volumes of *glacial acetic acid*.

*Test solution*. Shake 0.5 g of the substance under examination with 30 ml of *methanol* in a 50-ml volumetric flask for 15 minutes, dilute to volume with *methanol*.

*Reference solution (a)*. Dilute 1 ml of the test solution to 10 ml with *methanol*.

*Reference solution (b)*. Dilute 3 ml of reference solution (a) to 100 ml with *methanol*.

*Reference solution (c)*. A 0.1 per cent w/v solution of *acebutolol hydrochloride RS* in *methanol*.

*Reference solution (d)*. Dilute 1 volume of reference solution (c) to 3 volumes with *methanol*.

Apply 20 µl of each solution on each plate. Develop two chromatograms using separately the two mobile phases. After development, dry the plates in a current of warm air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatograms obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b) and not more than two such spots are more intense than the spot in the chromatograms obtained with reference solution (d). Ignore any spot at the point of application.



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

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

**Assay**. Weigh accurately about 0.3 g, dissolve in 50 ml of *ethanol (95 per cent)* and add 1 ml of *0.1 M hydrochloric acid*. Titrate with *0.1 M sodium hydroxide*, determining the end point potentiometrically (2.4.25). Read the volumes added between the two points of inflection.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.03729 g of $C_{18}H_{28}N_2O_4$,HCl.

**Storage**. Store protected from light.

# Acebutolol Tablets

Acebutolol HydrochlorideTablets

Acebutolol Tablets contain Acebutolol Hydrochloride.

Acebutolol Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of acebutolol hydrochloride, $C_{18}H_{28}N_2O_4$, HCl.

**Usual strengths**. 200 mg; 400 mg.

## Identification

A. When examined in the range 220 nm to 360 nm (2.4.7), the solution obtained in the Assay, shows an absorption maximum at about 233 nm.

B. Determine by thin layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 60 volumes of *water*, 40 volumes of *methanol* and 0.5 volumes of *perchloric acid*.

*Test solution*. Shake a quantity of the powdered tablets containing 0.5 g of Acebutolol Hydrochloride with 30 ml of *methanol* in a 50-ml volumetric flask for 15 minutes, dilute to volume with *methanol*, centrifuge and use the clear supernatant liquid.

*Reference solution (a)*. A 0.1 per cent w/v solution of *acebutolol hydrochloride RS* in *methanol*.

*Reference solution (b)*. A mixture of equal volumes of reference solution (a) and a 0.1 per cent w/v solution of *pindolol RS* in *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of warm air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

## Tests

**Related substances**. Determine by thin layer chromatography (2.4.17), coating two plates with *silica gel GF 254*.

*Mobile phase (a)*. The upper layer obtained by shaking together 50 volumes of *water*, 40 volumes of *1-butanol* and 10 volumes of *glacial acetic acid*.

*Mobile phase (b)*. A mixture of 90 volumes of *2-propanol* and 10 volumes of *glacial acetic acid*.

*Test solution*. Shake a quantity of the powdered tablets containing 0.5 g of Acebutolol Hydrochloride with 30 ml of *methanol* in a 50-ml volumetric flask for 15 minutes, dilute to volume with *methanol*, centrifuge and use the clear supernatant liquid.

*Reference solution (a)*. Dilute 1 ml of the test solution to 10 ml with *methanol*.

*Reference solution (b)*. Dilute 3 ml of reference solution (a) to 100 ml with *methanol*.

*Reference solution (c)*. A 0.1 per cent w/v solution of *acebutolol hydrochloride RS* in *methanol*.

*Reference solution (d)*. Dilute 1 volume of reference solution (c) to 3 volumes with *methanol*.

Apply 20 µl of each solution on each plate. Develop two chromatograms using separately the two mobile phases. After development, dry the plates in a current of warm air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatograms obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b) and not more than two such spots are more intense than the spot in the chromatograms obtained with reference solution (d). Ignore any spot at the point of application.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.1 g of Acebutolol Hydrochloride, shake with 40 ml of *0.1M hydrochloric acid* and add sufficient *water* to produce 100.0 ml, filter and dilute 10.0 ml of the filtrate to 100.0 ml with *water*. Dilute 10.0 ml of this solution to 100.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 233 nm (2.4.7).

Calculate the content of $C_{18}H_{28}N_2O_4$,HCl taking 580 as the specific absorbance at 233 nm.

**Storage**. Store protected from light.

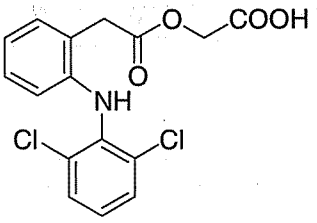


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# Aceclofenac




$C_{16}H_{13}Cl_2NO_4$ Mol. Wt. 354.2

Aceclofenac is 2-[(2,6-dichlorophenyl)amino] phenylacetoxyacetic acid.

Aceclofenac contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{16}H_{13}Cl_2NO_4$, calculated on the dried basis.

**Category**. Nonsteroidal antiinflammatory.

**Description**. A white or almost white, crystalline powder.

## Identification

*Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.*

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

B. When examined in the range 220 nm to 370 nm (2.4.7), the 0.002 per cent w/v solution in *methanol* shows an absorption maximum at 275 nm.

C. Dissolve about 10 mg in 10 ml of *ethanol*. To 1 ml of the solution, add 0.2 ml of a mixture, prepared immediately before use, of equal volumes of a 0.6 per cent solution of *potassium ferricyanide* and a 0.9 per cent solution of *ferric chloride*. Allow to stand protected from light for 5 minutes. Add 3 ml of a 1 per cent solution of *hydrochloric acid*. Allow to stand protected from light for 15 minutes. A blue colour develops and a precipitate is formed.

## Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. A mixture of 30 volumes of mobile phase A and 70 volumes of mobile phase B.

*Test solution*. Dissolve 50 mg of the substance under examination in 25 ml in solvent mixture.

*Reference solution (a)*. A 0.2 per cent w/v solution of *aceclofenac RS* in solvent mixture.

*Reference solution (b)*. Dilute 1 ml of reference solution (a) to 100 ml with solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with spherical end-capped octadecylsilane bonded to porous silica (5 µm), with a pore size of 10 µm and carbon loading of 19 per cent,
- mobile phase: A. a 0.112 per cent w/v solution of *orthophosphoric acid* adjusted to pH 7.0 using a 4.2 per cent solution of *sodium hydroxide*,
  B. 1 volume of *water* and 9 volumes of *acetonitrile*,
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 275 nm,
- injection volume.10 µl.

| Time (in min) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0 | 70 | 30 |
| 25 | 50 | 50 |
| 30 | 20 | 80 |
| 50 | 20 | 80 |
| 52 | 70 | 30 |
| 65 | 70 | 30 |

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with the reference solution (b) (2.0 per cent).

**Heavy metals** (2.3.13). 2.0 g complies with limit test for heavy metals, Method B ( 10 ppm ).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.3 g and dissolve in 40 ml of *methanol*. Titrate with *0.1 M sodium hydroxide*. Determine the end point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.03542 g of $C_{16}H_{13}Cl_2NO_4$.

**Storage**. Store protected from light, at a temperature not exceeding 30°.

# Aceclofenac Tablets

Aceclofenac Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of aceclofenac, $C_{16}H_{13}Cl_2NO_4$.



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**Usual strength**. 100 mg.

## Identification

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

## Tests

**Dissoluton** (2.5.2).

Apparatus. No. 1,
Medium: 900 ml of *phosphate buffer pH 7.5, 0.33 M Mixed*,
Speed and time. 50 rpm for 45 minutes.

Withdraw a suitable volume of the medium and filter. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate with dissolution medium. Measure the absorbance of the resulting solution at the maximum at about 273 nm (2.4.7). Calculate the content of aceclofenac, $C_{16}H_{13}Cl_2NO_4$ in the medium from the absorbance obtained from a solution of known concentration of *aceclofenac RS*.

D. Not less than 70 per cent of the stated amount of $C_{16}H_{13}Cl_2NO_4$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. A mixture of 50 volumes of *acetonitrile* and 50 volumes of *water*.

*Test solution*. Weigh accurately a quantity of powdered tablet containing 100 mg of Aceclofenac, disperse in 100 ml of the solvent mixture and filter.

*Reference solution (a)*. A 0.1 per cent w/v solution of *aceclofenac RS* in the solvent mixture.

*Reference solution (b)*. Weigh accurately a quantity of *diclofenac sodium RS* equivalent to 25 mg of diclofenac, dissolve in 25.0 ml of the solvent mixture. Dilute 1 ml of the solution and 1 ml of reference solution (a) to 100 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with dimethyloctylsilane (C8 alkyl chain) fully endcapped stationary phase (5 µm) (such as Hypersil MOS),
- mobile phase: a mixture of 55 volumes of buffer pH 3.5 prepared by adding 1.2 ml of *glacial acetic acid* in 1000 ml of *water*. Adjust the pH to 3.5 with *dilute sodium hydroxide* and filter, 22.5 volumes of *acetonitrile* and 22.5 volumes of *tetrahydrofuran*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 275 nm,
- injection volume. 20 µl.

Inject reference solution (b). Test is not valid unless the resolution between aceclofenac and diclofenac is not less than 5.0 and the column efficiency is not less than 2000 theoretical plates for peak due to aceclofenac.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of peak due to diclofenac is not more than five times the area of the peak due to diclofenac in the chromatogram obtained with reference solution (b) (5.0 per cent), the area of any secondary peak other than diclofenac is not more than the area of the peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of areas of all the secondary peaks excluding diclofenac peak is not more than twice the area of the peak in the chromatogram obtained with the reference solution (b) (2.0 per cent).

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. A mixture of 55 volumes of *acetonitrile* and 45 volumes of *water*.

*Test solution*. Weigh and powder 20 tablets. Weigh accurately a quantity of powdered tablet containing 100 mg of Aceclofenac, add about 60 ml of *acetonitrile* and sonicate for 10 minutes. Make up the volume to 100.0 ml with *acetonitrile*. Dilute 5.0 ml of the solution to 50.0 ml with solvent mixture.

*Reference solution*. Weigh about 25 mg of *aceclofenac RS* and add *acetonitrile* to dissolve and make up the volume to 25.0 ml with *acetonitrile*. Dilute 5.0 ml of the above solution to 50.0 ml with solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm packed with octadecylsilane stationary phase (5 µm) (such as Hypersil ODS),
- mobile phase: a mixture of 55 volumes of buffer solution prepared by adding 1.0 ml of *glacial acetic acid* in 1000 ml of *water* and 45 volumes of *acetonitrile*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 275 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2500 theoretical plates. The tailing factor is not more than 2.0.The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{16}H_{13}Cl_2NO_4$.

**Storage**. Store protected from light and moisture, at a temperature not exceeding 30°.

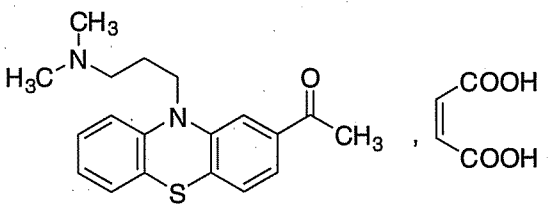


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# Acepromazine Maleate



$C_{19}H_{22}N_2OS,C_4H_4O_4$ Mol. Wt. 442.5

Acepromazine Maleate is 2-acetyl-10-(3-dimethylaminopropyl) phenothiazine hydrogen maleate.

Acepromazine Maleate contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{19}H_{22}N_2OS,C_4H_4O_4$, calculated on the dried basis.

**Category**. Antipsychotic.

**Description**. A yellow coloured, crystalline powder.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *acepromazine maleate RS* or with the reference spectrum of acepromazine maleate.

B. Complies with the test for identification of phenothiazine (2.3.3).

C. Dissolve 0.2 g in a mixture of 3 ml of *water* and 2 ml of *5 M sodium hydroxide* and shake with three 3 ml of *ether*. Add to the aqueous solution 2 ml of *bromine solution*, warm in a water-bath for 10 minutes, heat to boiling, cool and add 0.25 ml to a solution of 10 mg of *resorcinol* in 3 ml of *sulphuric acid*; a bluish black colour develops on heating for 15 minutes in a water-bath.

## Tests

**pH** (2.4.24). 4.0 to 5.5, determined in a 1.0 per cent w/v solution.

**Related substances**. Complies with the test for related substances in phenothiazines (2.3.5), but using a mixture of 75 volumes of *n-hexane*, 17 volumes of *butan-2-one* and 8 volumes of *diethylamine* as the mobile phase.

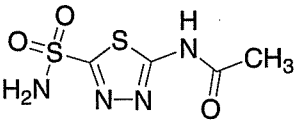
**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° at a pressure not exceeding 0.7 kPa for 16 hours.

**Assay**. Weigh accurately about 0.4 g, dissolve in 50 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04425 g of $C_{19}H_{22}N_2OS,C_4H_4O_4$.

# Acetazolamide



$C_4H_6N_4O_3S_2$ Mol. Wt. 222.2

Acetazolamide is *N*-(5-sulphamoyl-1,3,4-thiadiazol-2-yl) acetamide.

Acetazolamide contains not less than 98.5 per cent and not more than 101.0 per cent of $C_4H_6N_4O_3S_2$, calculated on the dried basis.

**Category**. Carbonic anhydrase inhibitor; used in the treatment of glaucoma.

**Dose**. Initial dose, 500 mg; subsequent doses, 250 mg every six hours.

**Description**. A white to faintly yellowish-white, crystalline powder; odourless.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests C and D may be omitted if tests A and B are carried out.*

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

B. When examined in the range 230 nm to 260 nm (2.4.7), a 0.003 per cent w/v solution in *0.01 M sodium hydroxide* shows an absorption maximum at about 240 nm; absorbance at about 240 nm, 0.49 to 0.53. When examined in the range 260 nm to 360 nm (2.4.7), a 0.00075 per cent w/v solution in *0.01 M sodium hydroxide* shows an absorption maximum at about 292 nm; absorbance at about 292 nm, 0.43 to 0.47.

C. To about 20 mg in a test-tube add 4 ml of *2 M hydrochloric acid* and 0.2 g of *zinc powder* and immediately place a piece of *lead acetate paper* over the mouth of the tube; the paper exhibits a brownish-black colour.

D. To about 25 mg add 5 ml of *water*, 4 drops of *1 M sodium hydroxide* and 2 drops of *cupric sulphate solution*; a bluish-green colour or precipitate is produced.

## Tests

**Silver-reducing substances**. Mix 5 g with 25 ml of *ethanol (95 per cent)*, add 125 ml of *water*, 10 ml of *nitric acid* and 5 ml



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of *0.1 M silver nitrate*, stir for 30 minutes and filter. Wash the residue with *water*, mix the filtrate and washings and titrate the excess of silver nitrate in the mixture with *0.05 M ammonium thiocyanate* using *ferric ammonium sulphate solution* as indicator; not less than 9.5 ml of *0.05 M ammonium thiocyanate* is required.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A freshly prepared mixture of 50 volumes of *2-propanol*, 30 volumes of *ethyl acetate* and 20 volumes of *strong ammonia solution*.

*Test solution*. Dissolve 0.5 g of the substance under examination in 100 ml of a mixture of equal volumes of *ethanol (95 per cent)* and *ethyl acetate*.

*Reference solution*. A 0.005 per cent w/v solution of the substance under examination in a mixture of equal volumes of *ethanol (95 per cent)* and *ethyl acetate*.

Apply to the plate 20 µl of each solution. Do not line the walls of the tank. Allow to saturate for 1 hour before development. After development, dry the plate in a current of warm air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Heavy metals** (2.3.13). 1.0 g dissolved in a mixture of 10 ml of *1 M sodium hydroxide* and 15 ml of *water* complies with the limit test for heavy metals, Method C (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 2.5 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.4 g of the substance under examination and dissolve in 90 ml of *dimethylformamide*. Titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end-point potentiometrically (2.4.25). Take precautions to prevent absorption of atmospheric carbon dioxide. Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.02222 g of $C_4H_6N_4O_3S_2$.

**Storage**. Store protected from light.

# Acetazolamide Tablets

Acetazolamide Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of acetazolamide, $C_4H_6N_4O_3S_2$.

**Usual strength**. 250 mg.

## Identification

A. To a quantity of the powdered tablets containing 0.5 g of Acetazolamide add 2 ml of *1 M sodium hydroxide*, shake thoroughly and filter. Neutralise the filtrate with *glacial acetic acid*, filter and dry the resulting precipitate at 105°. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *acetazolamide RS* or with the reference spectrum of acetazolamide.

B. Triturate a quantity of the powdered tablets containing 0.5 g of Acetazolamide with a mixture of 5 ml of *water* and 1 ml of *1 M sodium hydroxide*, transfer to a test-tube, add 0.2 g of *zinc powder*, add 0.5 ml of *hydrochloric acid* and immediately place a piece of *lead acetate paper* over the mouth of the tube; the paper exhibits a brownish-black colour.

C. To a quantity of the powdered tablets containing 25 mg of Acetazolamide add 5 ml of *water*, 3 drops of *1 M sodium hydroxide* and 2 drops of *cupric sulphate solution*; a bluish-green colour or precipitate is produced.

## Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A freshly prepared mixture of 50 volumes of *2-propanol*, 30 volumes of *ethyl acetate* and 20 volumes of *strong ammonia solution*.

*Solvent mixture*. A mixture of equal volumes of *ethanol (95 per cent)* and *ethyl acetate*.

*Test solution*. Shake a quantity of the powdered tablets containing 50 mg of Acetazolamide for 20 minutes with 10 ml of solvent mixture, filter and use the filtrate.

*Reference solution*. Dilute 1 volume of the test solution to 100 volumes with the same solvent mixture.

Apply to the plate 20 µl of each solution. Do not line the walls of the tank. Allow to saturate for 1 hour before development. After development, dry the plate in a current of warm air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.4 g of Acetazolamide and add 90 ml of *dimethylformamide*. Titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.



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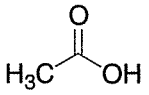
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1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.02222 g of $C_4H_6N_4O_3S_2$.

**Storage**. Store protected from light.

# Glacial Acetic Acid



$C_2H_4O_2$ Mol. Wt. 60.1

Glacial Acetic acid contains not less than 99.0 per cent w/w and not more than 100.5 per cent w/w of $C_2H_4O_2$.

**Category**. Acidifying agent; buffering agent; pharmaceutical aid (analytical reagent).

**Description**. A crystalline mass or clear, colourless, volatile liquid.

## Identification

A. A 10 per cent w/v solution is strongly acidic.

B. To 0.03 ml add 3 ml of *water* and neutralize with *2 M sodium hydroxide*; the solution gives reaction C of acetates (2.3.1).

## Tests

**Freezing point** (2.4.11). Not less than 14.8°.

**Residue on evaporation**. Not more than 0.01 per cent, determined on 20.0 g by evaporating to dryness on a water-bath and drying at 105°.

**Reducing substances**. To 5 ml add 10 ml of *water* and mix. To 5 ml of the resulting solution add 6 ml of *sulphuric acid* and cool. Add 2 ml of *0.0167 M potassium dichromate*, allow to stand for 1 minute and add 25 ml of *water* and 1 ml of freshly prepared *dilute potassium iodide solution*.Titrate with *0.1 M sodium thiosulphate* using 1 ml of *starch solution* as indicator. Not less than 1.0 ml of *0.1 M sodium thiosulphate* is required.

**Heavy metals** (2.3.13). Dissolve the residue obtained in the test for Residue on evaporation by heating with two quantities, each of 15 ml, of *water* and add sufficient *water* to produce 50 ml (solution A). The solution complies with the limit test for heavy metals, Method D (5 ppm). Use 10 ml of *lead standard solution (2 ppm Pb)* to prepare the standard.

**Iron** (2.3.14). 5 ml of solution A diluted to 10 ml with *water* complies with the limit test for iron (5 ppm). Use 1.0 ml of *iron standard solution (10 ppm Fe)* to prepare the standard.

**Chlorides** (2.3.12). To 20 ml add sufficient *water* to produce 100 ml (solution B). 10 ml of solution B diluted to 15 ml with *water* complies with the limit test for chlorides (25 ppm). Use 10 ml of *chloride standard solution (5 ppm Cl)* to prepare the standard.

**Sulphates** (2.3.17). 15 ml of solution B complies with the limit test for sulphates (50 ppm).

**Assay**. Weigh accurately a conical flask with a ground-glass stopper containing 25 ml of *water*, add 1 ml of the substance under examination and reweigh. Titrate with *1 M sodium hydroxide* using 0.5 ml of *phenolphthalein solution* of indicator.

1 ml of *1 M sodium hydroxide* is equivalent to 0.06005 g of $C_2H_4O_2$.

**Storage**. Store protected from light and moisture.

# Acetic Acid Ear Drops

Acetic Acid Otic Solution

Acetic Acid Ear Drops is a solution of Glacial Acetic Acid in a suitable non-aqueous solvent.

Acetic Acid Ear Drops contain not less than 85.0 per cent and not more than 130.0 per cent of the stated amount of acetic acid, $C_2H_4O_2$.

## Identification

A. Dilute 5 ml with 10 ml of *water* and adjust to a pH of about 7 with *1 M sodium hydroxide*. Add *ferric chloride test solution*, a deep red colour is produced, which is decolorized on the addition of *hydrochloric acid*.

B.Warm the solution with *sulphuric acid* and *ethanol (95 per cent)*; a characteristic odour of *ethyl acetate* is evolved.

## Tests

**pH** (2.4.24). 2.0 to 4.0, determined in a 50.0 per cent v/v solution.

**Other tests**. Comply with the tests stated under Ear Drops.

**Assay**. Transfer a volume containing about 0.1 g of Glacial Acetic Acid to a conical flask, add 5 ml of *sodium chloride solution* and about 40 ml of *water*. Titrate with *0.1 M sodium hydroxide*, using 0.15 ml of *phenolphthalein solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.006005 g of $C_2H_4O_2$.

**Storage**. Store protected from light and moisture.



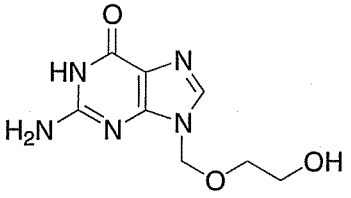
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## Aciclovir

Acyclovir



$C_8H_{11}N_5O_3$ Mol. Wt. 225.2

Aciclovir is 2-amino-9-[(2-hydroxyethoxy)methyl]-1,9-dihydro-6*H*-purin-6-one.

Aciclovir contains not less than 98.5 per cent and not more than 101.0 per cent of $C_8H_{11}N_5O_3$, calculated on the anhydrous basis.

**Category**. Antiviral.

**Dose**. 200 to 800 mg 4 to 5 times daily. By intravenous infusion, 5 to 10 mg per kg of body weight every 8 hours. By topical application as cream or eye ointment, as appropriate, every 4 hours.

**Description**. A white or almost white, crystalline powder.

### Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *aciclovir RS*.

### Tests

**Appearance of solution**. A 1.0 per cent w/v solution in *0.1 M sodium hydroxide* is clear (2.4.1), and not more intensely coloured than reference solution YS7 (2.4.1).

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 80 volumes of *dichloromethane*, 20 volumes of *methanol* and 2 volumes of *strong ammonia solution*.

*Prepare the following solutions immediately before use.*

*Test solution*. Dissolve 1.0 g of the substance under examination in 100 ml of *dimethyl sulphoxide*.

*Reference solution*. A 0.005 per cent w/v solution of *aciclovir impurity A RS* in *dimethyl sulphoxide*.

Apply to the plate 10 µl of each solution. Keep the spots compact by drying in a current of warm air and allow the plate to cool. Allow the mobile phase to rise 10 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot with Rf value greater than that of the principal spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 6.0 per cent, determined on 0.5 g.

**Assay**. Weigh accurately about 0.15 g and dissolve in 60 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02252 g of $C_8H_{11}N_5O_3$.

**Storage**. Store protected from light and moisture.

## Aciclovir Intravenous Infusion

Acyclovir Intravenous Infusion; Acyclovir Sodium Intravenous Infusion

Aciclovir Intravenous Infusion is a sterile material consisting of aciclovir sodium, prepared from Aciclovir with the aid of a suitable alkali, with or without auxiliary substances. It is filled in a sealed container.

The infusion is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

*The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).*

**Storage**. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Aciclovir Intravenous Infusion contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of aciclovir, $C_8H_{11}N_5O_3$.

**Usual strength**. 500 mg per vial.

**Description**. A white or almost white, crystalline powder.

*The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injections) and with the following requirements.*

### Identification

A. When examined in the range 230 nm to 360 nm (2.4.7), the solution prepared in the Assay shows an absorption maximum at about 255 nm and a broad shoulder at about 274 nm.

B. In the test for Guanine, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

C. Gives reaction A of sodium salts (2.3.1).



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### Tests

**Appearance of solution**. Dissolve the contents of a sealed container in sufficient *water for injection* to produce a solution containing the equivalent of 2.5 per cent w/v solution of Aciclovir (solution A). The solution is not more opalescent than opalescence standard OS2 (2.4.1), and not more intensely coloured than reference solution BYS5 (2.4.1).

**pH** (2.4.24). 10.7 to 11.7, determined in solution A.

**Guanine**. Determine by thin-layer chromatography (2.4.17), coating the plate with *cellulose F254* (Merck celllulose F plates are suitable).

*Mobile phase*. A mixture of 10 volumes of *1-propanol*, 30 volumes of *strong ammonia solution* and 60 volumes of a 5 per cent w/v solution of *ammonium sulphate*.

*Test solution (a)*. Dissolve a suitable quantity of the substance under examination in sufficient *0.1 M sodium hydroxide* to produce a solution containing 0.5 per cent of Aciclovir.

*Test solution (b)*. Dilute 1 volume of test solution (a) to 10 volumes with *0.1 M sodium hydroxide*.

*Reference solution (a)*. A 0.05 per cent w/v solution of *aciclovir RS* in *0.1 M sodium hydroxide*.

*Reference solution (b)*. A 0.005 per cent w/v solution of *guanine* in *0.1 M sodium hydroxide*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the pate in a current of warm air and examine in ultraviolet light at 254 nm. Any secondary spot corresponding to guanine in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b) (1per cent).

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 80 volumes of *dichloromethane*, 20 volumes of *methanol* and 2 volumes of *strong ammonia solution*.

*Prepare the following solutions immediately before use.*

*Test solution*. Dissolve a suitable quantity of the substance under examination in *dimethyl sulphoxide* to produce a solution containing 2.5 per cent of aciclovir.

*Reference solution*. Dilute 1 volume of the test solution to 200 volumes with *dimethyl sulphoxide*.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in a current of warm air and examine in ultraviolet light at 254 nm. Any secondary spot with an Rf value greater than that of the principal spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (0.5 per cent).

**Bacterial endotoxins** (2.2.3). Not more than 0.174 Endotoxin Units per mg of acyclovir.

**Assay**. Dissolve a quantity of the mixed contents of 10 containers containing 0.10 g of Aciclovir in sufficient *0.1 M hydrochloric acid* to produce 500.0 ml. Dilute 5.0 ml of the resulting solution to 100.0 ml with *0.1 M hydrochloric acid*. Measure the absorbance of the resulting solution at the maximum at about 255 nm (2.4.7). Calculate the content of $C_8H_{11}N_5O_3$ taking 560 as the specific absorbance at 255 nm.

**Storage**. Store protected from moisture, in a sterile, tamper-evident container sealed so as to exclude micro-organisms, at a temperature not exceeding 30°.

**Labelling**. The label states (1) the quantity of aciclovir sodium in the sealed container in terms of the equivalent amount of Aciclovir; (2) the strength of the constituted solution in terms of the equivalent amount of Aciclovir in a suitable dose-volume.

## Aciclovir Tablets

Acyclovir Tablets

Aciclovir Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of aciclovir, $C_8H_{11}N_5O_3$.

**Usual strengths**. 200 mg; 400 mg; 800 mg.

### Identification

A. When examined in the range 230 nm to 360 nm (2.4.7), the solution prepared in the Assay shows an absorption maximum at about 255 nm and a broad shoulder at about 274 nm.

B. In the test for Guanine, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

### Tests

**Guanine**. Determine by thin-layer chromatography (2.4.17), coating the plate with *cellulose F254*. (such as Merck celllulose F plates).

*Mobile phase*. A mixture of 10 volumes of *1- propanol*, 30 volumes of *strong ammonia solution* and 60 volumes of a 5 per cent w/v solution of *ammonium sulphate*.

*Test solution (a)*. Shake a quantity of the powdered tablets containing 0.25 g of Aciclovir with 25 ml of *0.1 M sodium hydroxide* for 10 minutes. Add a sufficient quantity of *0.1 M sodium hydroxide* to produce 50 ml. Allow to stand and allow



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any undissolved material to settle before application to the plate.

*Test solution (b)*. Dilute 1 volume of test solution (a) to 10 volumes with *0.1 M sodium hydroxide*.

*Reference solution (a)*. A 0.05 per cent w/v solution of *aciclovir RS* in *0.1 M sodium hydroxide*.

*Reference solution (b)*. A 0.005 per cent w/v solution of *guanine* in *0.1 M sodium hydroxide*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air and examine in ultraviolet light at 254 nm. Any secondary spot corresponding to guanine in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b) (1 per cent).

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 80 volumes of *dichloromethane*, 20 volumes of *methanol* and 2 volumes of *strong ammonia solution*.

*Prepare the following solutions immediately before use.*

*Test solution*. Shake a quantity of the powdered tablets containing 0.25 g of Aciclovir with 10 ml of *dimethyl sulphoxide* for 15 minutes and filter.

*Reference solution*. Dilute 0.7 volume of the test solution to 100 volumes with *dimethyl sulphoxide*.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in a current of warm air and examine in ultraviolet light at 254 nm. Any secondary spot with an Rf value greater than that of the principal spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (0.7 per cent).

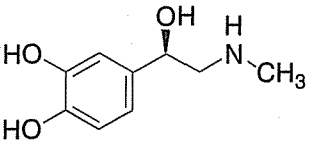
**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.1 g of Aciclovir, add 60 ml of *0.1 M sodium hydroxide* and disperse with the aid of ultrasound for 15 minutes. Add sufficient quantity of *0.1 M sodium hydroxide* to produce 100.0 ml, mix well and to the filtrate add 50 ml of *water*, 5.8 ml of *2 M hydrochloric acid* and sufficient *water* to produce 100.0 ml. To 5.0 ml of the resulting solution add sufficient *0.1 M hydrochloric acid* to produce 50.0 ml and mix well. Measure the absorbance of the solution at the maximum at about 255 nm (2.4.7), using *0.1 M hydrochloric* acid as the blank. Calculate the content of $C_8H_{11}N_5O_3$ taking 560 as the specific absorbance at 255 nm.

**Storage**. Store protected from light.

# Adrenaline

Epinephrine




$C_9H_{13}NO_3$ Mol. Wt. 183.2

Adrenaline is (*R*)-1-(3,4-dihydroxyphenyl)-2-methylaminoethanol

Adrenaline contains not less than 98.5 per cent and not more than 101.0 per cent of $C_9H_{13}NO_3$, calculated on the dried basis.

**Category**. Sympathomimetic.

**Dose**. By subcutaneous or intramuscular injection, 200 to 500 µg as a single dose.

**Description**. A white or creamy-white, microcrystalline powder or granules. It gradually darkens on exposure to light and air, decomposition being faster in the presence of moisture and at higher temperatures.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Test C may be omitted if tests A, B and D are carried out.*

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

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.003 per cent w/v solution in *0.01 M hydrochloric acid* shows an absorption maximum at about 280 nm; absorbance at about 280 nm, about 0.45.

C. To 1 ml of a neutral or faintly acid solution add dropwise a 0.25 per cent w/v solution of *ferric chloride* until an emerald-green colour is produced. Add *sodium bicarbonate solution* gradually; the solution changes first to blue and then to red.

D. To 1 ml of a 0.1 per cent w/v solution add 1 ml of a 1.0 per cent v/v solution of *2,5-diethoxytetrahydrofuran* in *glacial acetic acid*. Heat at 80° for 2 minutes, cool in ice and add 3 ml of a 2.0 per cent w/v solution of *4-dimethylaminobenzaldehyde* in a mixture of 19 volumes of *glacial acetic* acid and 1 volume of *hydrochloric acid*. Mix and allow to stand for 2 minutes. The solution becomes yellow and is similar to the one obtained by performing the test in the same manner but omitting the substance under examination (distinction from noradrenaline).

## Tests

**Specific optical rotation** (2.4.22). –50.0° to –53.5°, determined in a freshly prepared 4.0 per cent w/v solution in *1 M hydrochloric acid*.



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**Phenones**. Absorbance of a 0.2 per cent w/v solution in *0.1 M hydrochloric acid* at the maximum at about 310 nm, not greater than 0.20, calculated on the dried basis (2.4.7).

**Noradrenaline**. Dissolve 5 mg in 1 ml of a 0.5 per cent w/v solution of *tartaric acid*, add 4 ml of *buffer pH 9.6*, mix, add 1 ml of a freshly prepared 0.5 per cent w/v solution of *sodium 1,2-naphthaquinone-4-sulphonate*, mix and allow to stand for 30 minutes. Add 0.2 ml of a 1 per cent v/v solution of *benzalkonium chloride solution*, mix, add 15 ml of *toluene* previously washed with *buffer pH 9.6* and filtered through a dry filter paper, shake for 30 minutes and allow to separate, centrifuging if necessary. Any red or purple colour in the toluene layer is not more intense than that produced by treating a solution of 0.40 mg of *noradrenaline acid tartrate* and 9 mg of *noradrenaline-free adrenaline acid tartrate* in 1 ml of *water* in a similar manner.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 18 hours.

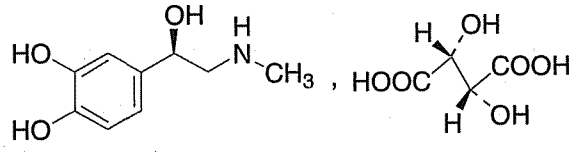
**Assay**. Weigh accurately about 0.3 g and dissolve in 50 ml of *anhydrous glacial acetic acid*, warming slightly, if necessary, to effect solution. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01832 g of $C_9H_{13}NO_3$.

**Storage**. Store protected from light in containers preferably filled with nitrogen.

# Adrenaline Tartrate

Adrenaline Acid Tartrate; Adrenaline Bitartrate; Epinephrine Bitartrate.



$C_9H_{13}NO_3,C_4H_6O_6$ Mol. Wt. 333.3

Adrenaline tartrate is (*R*)-1-(3,4-dihydroxyphenyl)-2-methylaminoethanol hydrogen tartrate.

Adrenaline Tartrate contains not less than 98.0 per cent and not more than 101.0 per cent of $C_9H_{13}NO_3,C_4H_6O_6$, calculated on the dried basis.

**Category**. Sympathomimetic.

**Dose**. By subcutaneous injection, 400 µg to 1 mg, as a single dose.

**Description**. A white or greyish-white, crystalline powder; odourless. It darkens on exposure to air and light, decomposition being faster in the presence of moisture and at higher temperatures.

## Identification

Dissolve about 1 g in 10 ml of *water* containing 0.1 g of *sodium metabisulphite*, add a slight excess of *dilute ammonia solution* and allow to stand at about 4° for 1 hour, filter and reserve the filtrate for test C. Wash the precipitate with three successive quantities, each of 2 ml, of cold *water*, then with 5 ml of cold *ethanol (95 per cent)* and finally with 5 ml of cold *ether* and dry over *silica gel* at a pressure of 1.5 to 2.5 kPa for 3 hours. The residue comply with the following tests.

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

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.005 per cent w/v solution in *0.01 M hydrochloric acid* shows an absorption maximum only at about 279 nm; absorbance at about 279 nm, about 0.4.

C. The filtrate reserved above gives the reactions of tartrates (2.3.1).

## Tests

**Appearance of solution**. A 5.0 per cent w/v solution examined immediately after preparation is not more opalescent than opalescence standard OS2 (2.4.1) and not more intensely coloured than reference solution BYS4 (2.4.1).

**Specific optical rotation** (2.4.22). −50.0° to −54.0°, determined in a freshly prepared 4.0 per cent w/v solution in *1 M hydrochloric acid*.

**pH** (2.4.24). 2.8 to 4.0, determined in a 1.0 per cent w/v solution.

**Phenones**. Absorbance of a 0.2 per cent w/v solution in *0.1 M hydrochloric acid* at the maximum at about 310 nm, not more than 0.10, calculated on the dried basis (2.4.7).

**Noradrenaline**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 100 volumes of *acetone*, 100 volumes of *dichloromethane* and 1 volume of *anhydrous formic acid*.

*Test solution*. Dissolve 2.5 g of the substance under examination in 100 ml of *water*.



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*Reference solution (a)*. A freshly prepared 0.125 per cent w/v solution of *noradrenaline bitartrate RS* in *water*.

*Reference solution (b)*. A freshly prepared 0.025 per cent w/v solution of *noradrenaline bitartrate RS* in *water*.

*Reference solution (c)*. A mixture of equal volumes of the test solution and reference solution (b).

Apply to the plate 6 µl of each of the test solution and reference solutions (a) and (b) and 12 µl of reference solution (c) as bands 20 mm by 2 mm.

Allow the applied bands to dry and spray them with a saturated solution of *sodium bicarbonate*. Allow the plate to dry in air, spray the applied bands twice with *acetic anhydride*, drying the plate between the two sprayings and heat the plate at 50° for 90 minutes and develop the chromatograms. After removal of the plate, allow it to dry in air and spray with a freshly prepared mixture of 8 volumes of *methanol*, 2 volumes of *ethylenediamine* and 2 volumes of a 0.5 per cent w/v solution of *potassium ferricyanide*. Dry the plate at 60° for 10 minutes and examine in ultraviolet light at 254 nm and 365 nm. Any band situated between the two most intense bands in the chromatogram obtained with the test solution is not more intense than the corresponding band in the chromatogram obtained with reference solution (b). The test is not valid unless the chromatogram obtained with reference solution (c) exhibits between the two most intense bands a clearly separated band corresponding to the most intense band in the chromatogram obtained with reference solution (a).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying over *phosphorus pentoxide* at a pressure of 1.5 to 2.5 kPa for 18 hours.

**Assay**. Weigh accurately about 0.3 g and dissolve in 50 ml of *anhydrous glacial acetic acid*, warming slightly, if necessary, to effect solution. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03333 g of $C_9H_{13}NO_3,C_4H_6O_6$.

**Storage**. Store protected from light in containers preferably filled with nitrogen.

# Adrenaline Injection

Adrenaline Bitartrate Injection; Adrenaline Acid Tartrate Injection; Adrenaline Tartrate Injection; Epinephrine Tartrate Injection

Adrenaline Injection is a sterile, isotonic solution containing 0.18 per cent w/v of Adrenaline Tartrate in Water for Injection.

Adrenaline Injection contains the equivalent of not less than 0.09 per cent and not more than 0.115 per cent w/v of adrenaline, $C_9H_{13}NO_3$.

**Usual strength**. Adrenaline, 1 in 1000 (1 mg per ml) as Adrenaline Bitartrate.

**Description**. A clear, colourless or almost colourless solution.

## Identification

A. To an appropriate quantity add sufficient *0.01M hydrochloric acid* to produce a solution containing 0.005 per cent w/v of adrenaline. When examined in the range 230 nm to 360 nm (2.4.7), the solution shows an absorption maximum at about 279 nm; absorbance at about 279 nm, about 0.4.

B. To 1 ml add dropwise a 0.25 per cent w/v solution of *ferric chloride* until an emerald-green colour is produced. Add *sodium bicarbonate solution* gradually; the solution changes first to blue and then to red.

C. To 10 ml add 2 ml of *disodium hydrogen phosphate solution* and sufficient *iodine solution* to produce a brown colour. Add *0.1 M sodium thiosulphate* dropwise until excess iodine is removed; a red colour is produced.

## Tests

**Appearance of solution**. Examine the injection in a clear glass test-tube against a white background; it is not pinkish and does not contain a precipitate. If any yellow colour is observed, it is not more intense than a reference solution prepared by diluting 0.4 ml of *0.1 M iodine* to 100 ml with *water*, when viewed similarly.

**pH** (2.4.24). 2.8 to 3.6.

**Noradrenaline**. Determine by liquid chromatography (2.4.14).

*Test solution*. Substance under examination.

*Reference solution (a)*. A 0.0018 per cent w/v solution of *noradrenaline acid tartrate* in the mobile phase.

*Reference solution (b)*. A solution containing 0.0018 per cent w/v of *noradrenaline-free adrenaline acid tartrate* and 0.0018 per cent w/v of *noradrenaline acid tartrate* in the mobile phase.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles 5 to 10 µm (such as Nucleosil ODS),
- mobile phase: Dissolve 4.0 g of *tetramethylammonium hydrogen sulphate*, 1.1 g of *sodium heptanesulphonate* and 2 ml of *0.1 M disodium edetate* in 1000 ml of 5 per cent v/v solution of *methanol*, with pH adjusted to 3.5 to 3.6 with *1 M sodium hydroxide*,



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- flow rate. 2 ml per minute,
- spectrophotometer set at 205 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution factor between the two principal peaks is not less than 2.0.

Inject the test solution and reference solution (a). The area of any peak corresponding to noradrenaline is not greater than the area of the principal peak in the chromatogram obtained with reference solution (a).

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Accurately measured volume of the injection containing 20 mg of Adrenaline Tartrate diluted to 100.0 ml with the mobile phase.

*Reference solution (a).* A 0.02 per cent w/v solution of *adrenaline acid tartrate RS* in the mobile phase.

*Reference solution (b).* A solution containing 0.02 per cent w/v of *adrenaline acid tartrate RS* and 0.02 per cent w/v of *noradrenaline acid tartrate* in the mobile phase.

Chromatographic system

- a stainless steel column 10 cm × 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm) (Such as Nucleosil C18),
- mobile phase: a solution prepared by adding 4.0 g of *tetramethylammonium hydrogen sulphate*, 1.1 g of *sodium heptanesulphonate* and 2 ml of *0.1 M disodium edetate* to a mixture of 950 volumes of *water* and 50 volumes of *methanol* and adjusting the pH of the mixture to 3.5 with *1 M sodium hydroxide*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 205 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to adrenaline acid tartrate and noradrenaline acid tartrate in the chromatogram obtained is not less than 2.0.

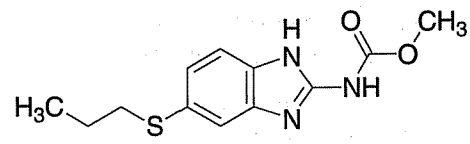
Inject the test solution and reference solution (a).

Calculate the content of $C_9H_{13}NO_3$ in the injection.

**Storage**. Store protected from light, in a single dose or multiple dose container.

**Labelling**. The label states (1) the quantity of active ingredient in parts per 1000 or mg per ml in terms of equivalent amount of adrenaline; (2) that the injection should not be used if it is pinkish or darker than slightly yellow.

# Albendazole



$C_{12}H_{15}N_3O_2S$ Mol. Wt. 265.3

Albendazole is methyl 5-propylthio-1*H*-benzimidazol-2-yl-carbamate.

Albendazole contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{12}H_{15}N_3O_2S$, calculated on the dried basis.

**Category**. Anthelmintic.

**Dose**. Nematodal infestation, 400 mg as a single dose; cestodal infestation, 400 mg daily for three consecutive days; strongyloidiasis, 400 mg daily for three consecutive days.

**Description**. A white to pale buff-coloured powder.

## Identification

*Test A may be omitted if tests B and C are carried out. Test B may be omitted if tests A and C* are carried out.

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

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

C. Melting point (2.4.21). 208° to 210°.

## Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase.* A mixture of 60 volumes of *chloroform*, 10 volumes of *ether* and 10 volumes of *glacial acetic acid.*

*Test solution (a).* Dissolve 0.2 g of the substance under examination in sufficient *glacial acetic acid* to produce 10 ml.

*Test solution (b).* Dilute 1 ml of test solution (a) to 4 ml with *glacial acetic acid.*

*Reference solution (a).* Dilute 1 ml of test solution (a) to 200 ml with *glacial acetic acid.*

*Reference solution (b).* Dissolve 25 mg of *albendazole RS* in sufficient *glacial acetic acid* to produce 5 ml.

Apply to the plate 10 µl of each solution. After development, dry in a current of warm air and examine in ultraviolet light at



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254 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the principal spot in the chromatogram obtained with reference solution (a).

**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm)

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 4 hours.

**Assay**. Weigh accurately about 0.5 g and dissolve in 80 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02653 g of $C_{12}H_{15}N_3O_2S$

**Storage**. Store protected from light.

## Albendazole Tablets

Albendazole Tablets contain Albendazole. The tablets may contain permitted flavouring agents.

Albendazole Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of albendazole, $C_{12}H_{15}N_3O_2S$.

**Usual strength**. 400 mg.

### Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 60 volumes of *chloroform*, 10 volumes of *ether* and 10 volumes of *glacial acetic acid*.

*Test solution*. Add a quantity of the powdered tablets containing 200 mg of Albendazole to 20 ml of a mixture of 18 volumes of *chloroform* and 1 volume of *formic acid*, warm the suspension on a water-bath for 15 minutes, cool and filter. Dilute 10 ml of the filtrate with an equal volume of *glacial acetic acid*.

*Reference solution*. Dissolve 25 mg of *albendazole RS* in sufficient *glacial acetic acid* to produce 5 ml.

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of warm air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. Extract a quantity of the powdered tablets containing 100 mg of Albendazole with 100 ml of *0.1 M methanolic hydrochloric acid*, filter and dilute 1 ml of the filtrate to 100 ml with *0.1 M sodium hydroxide*. The absorbance of the resulting solution at the maximum at about 309 nm, about 0.74 (2.4.7).

### Tests

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.1 g of Albendazole, add about 150 ml *0.1 M methanolic hydrochloric acid*, shake for 15 minutes and dilute to 250.0 ml with *0.1 M methanolic hydrochloric acid*. Mix, filter and dilute 5.0 ml of the filtrate to 250.0 ml with *0.1 M sodium hydroxide*. Measure the absorbance of the resulting solution at the maximum at about 309 nm (2.4.7). Calculate the content of $C_{12}H_{15}N_3O_2S$ taking 742 as the specific absorbance at 309 nm.

**Storage**. Store protected from light.

**Labelling**. The label states that the tablets should be chewed before swallowing.

## Alginic Acid

Polymannuronic Acid

Alginic acid is a hydrophilic colloidal mixture of polyuronic acids, $[(C_6H_8O_6)_n]$, composed of residues of D-mannuronic acid and L-guluronic acid extracted with dilute alkali from various species of brown seaweeds (Fam. Phaeophyceae).

Alginic Acid contains not less than 19.0 per cent and not more than 25.0 per cent of carboxylic acid groups (COOH), calculated on the dried basis.

**Category**. Pharmaceutical aid.

**Description**. A white to yellowish-white, fibrous powder; odourless.

### Identification

A. To 5 ml of a 0.75 per cent w/v solution in *0.1 M sodium hydroxide* add 1 ml of *calcium chloride solution*; a gelatinous precipitate is formed.

B. To 5 ml of the solution obtained in test A add 1 ml of *2 M sulphuric acid*; a gelatinous precipitate is formed.

C. To about 5 mg in a test-tube add 5 ml of *water*, 1 ml of a freshly-prepared 1 per cent w/v solution of *1,3-naphthalenediol* in *ethanol (95 per cent)* and 5 ml of *hydrochloric acid*. Heat the mixture to boiling, boil gently for 3 minutes and cool to about 15°. Transfer the contents of the test-tube to a small separator with the aid of 5 ml of *water* and extract with 15 ml of *di-isopropyl ether*; the di-isopropyl ether extract exhibits a deep purple colour which is more intense than that exhibited by a blank prepared in the same manner without the substance under examination.



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## Tests

**pH** (2.4.24). 1.5 to 3.5, determined in a 3.0 per cent w/v dispersion in *water*.

**Arsenic** (2.3.10). Mix 2.0 g with 5 ml of *sulphuric acid*, add a few glass beads and digest at a temperature not exceeding 120° until charring begins. Additional sulphuric acid may be added if necessary but the total volume of acid added should not exceed 10 ml. Add cautiously, dropwise, *hydrogen peroxide solution (100 vol)* allowing the reaction to subside and again heating between addition of drops. Discontinue heating if foaming becomes excessive. When the reaction has abated, heat cautiously rotating the flask occasionally. Maintain oxidising conditions at all times during the digestion by adding small quantities of the hydrogen peroxide solution whenever the mixture turns brown or darkens. Continue the digestion until the organic matter has been destroyed, gradually raising the temperature until fumes of sulphur trioxide are copiously evolved and the solution becomes colourless or has only a light straw colour. Cool, add cautiously 10 ml of *water*, mix, and again evaporate till there is strong fuming, repeating this procedure to remove any trace of hydrogen peroxide. Cool, add cautiously 10 ml of *water*, wash the sides of the flask with a few ml of *water* and dilute with *water* to 35 ml. The resulting solution complies with the limit test for arsenic (5 ppm).

**Heavy metals** (2.3.13). 0.5 g complies with the limit test for heavy metals, Method B (40 ppm). Use *nitric acid Sp.* in place of *sulphuric acid Sp.* to wet the sample.

**Acid value**. Not less than 230, calculated on the dried basis and determined in the following manner. Weigh accurately about 1.0 g and suspend in a mixture of 50 ml of *water* and 30 ml of a 4.4 per cent w/v solution of *calcium acetate*. Shake vigorously, allow the mixture to stand for 1 hour, add *phenolphthalein solution* and titrate the liberated acetic acid with *0.1 M sodium hydroxide*. Carry out a blank titration.

Calculate the acid value from the expression 5.611 *A/W*, where *A* is the volume, in ml, of *0.1 M sodium hydroxide* consumed and W is the weight, in g, of the sample.

**Microbial contamination** (2.2.9). 1 g is free from *Escherichia coli* and 10 g is free from *salmonellae*.

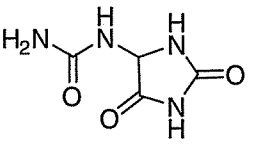
**Total ash** (2.3.19). Not more than 4.0 per cent, determined on 0.5 g by Method B.

**Loss on drying** (2.4.19). Not more than 15.0 per cent, determined on 0.1 g by drying in an oven at 105° for 4 hours.

**Assay**. Weigh accurately about 0.25 g, add 25 ml of *water* and 25.0 ml of *0.1 M sodium hydroxide* and titrate with *0.1 M hydrochloric acid* using 0.2 ml of *dilute phenolphthalein solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.004502 g of carboxylic acid groups (COOH).

# Allantoin



$C_4H_6N_4O_3$ Mol. Wt. 158.1

Allantoin is (*RS*)-(2,5-dioxo-4-imidazolidinyl)urea.

Allantoin contains not less than 98.5 per cent and not more than 101.0 per cent of $C_4H_6N_4O_3$, calculated on the dried basis.

**Category**. Astringent.

**Description**. A white or almost white, crystalline powder.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

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

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

C. Boil 20 mg with a mixture of 1 ml each of *dilute sodium hydroxide solution* and *water*, allow to cool. Add 1 ml of *dilute hydrochloric acid*. To 0.1 ml of the solution add 0.1 ml of a 10 per cent w/v solution of *potassium bromide*, 0.1 ml of a 2 per cent w/v solution of *resorcinol* and 3 ml of *sulphuric acid*. Heat for 10 minutes on a water bath; a dark blue colour develops, which becomes red after cooling and pouring into about 10 ml of *water*.

D. Heat about 0.5 g, ammonia vapour is evolved, which turns *red litmus paper* blue.

## Tests

**Melting point** (2.4.21). about 225°.

**Acidity or alkalinity**. To 5 ml of a 0.5 per cent w/v solution in *carbon dioxide-free water* with heating if necessary (solution A), add 5 ml of *carbon dioxide-free water*, 0.1 ml of *methyl red solution* and 0.2 ml of *0.01 M sodium hydroxide*, the solution is yellow. Add 0.4 ml of *0.01 M hydrochloric acid*, the solution is red.

**Optical rotation** (2.4.22). − 0.10° to + 0.10°, determined on solution A.

**Reducing substances**. Shake 1.0 g with 10 ml of *water* for 2 minutes, filter. Add 1.5 ml of *0.02 M potassium permanganate*. The solution must remain violet for at least 10 minutes.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *cellulose*.



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*Mobile phase*. A mixture of 15 volumes of *glacial acetic acid*, 25 volumes of *water* and 60 volumes of *butanol*.

*Test solution (a)*. Dissolve 0.1 g of the substance under examination in 5.0 ml of *water* with heating and allow to cool. Dilute to 10 ml with *methanol* (*Use the solution immediately after preparation*).

*Test solution (b)*. Dilute 1 ml of test solution (a) to 10 ml with a mixture of 1 volume of *methanol* and 1 volume of *water*.

*Reference solution (a)*. A 0.1 per cent w/v solution of *allantoin RS* in a mixture of 1 volume of *methanol* and 1 volume of *water*.

*Reference solution (b)*. Dissolve 10 mg of *urea* in 10 ml of *water*. Dilute 1 ml of this solution to 10 ml with *methanol*.

*Reference solution (c)*. Mix 1 ml each of reference solution (a) and reference solution (b).

Apply to the plate 10 µl of test solution (a) and 5 µl each of test solution (b), reference solution (a), (b) and (c). Allow the mobile phase to rise 10 cm. Dry the plate in air, and spray with a 0.5 per cent w/v solution of *dimethylaminobenzaldehyde* in a mixture of 1 volume of *hydrochloric acid* and 3 volumes of *methanol*. Dry the plate in a current of hot air. Examine in daylight after 30 minutes. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the principal spot in the chromatogram obtained with reference solution (b) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated principal spots.

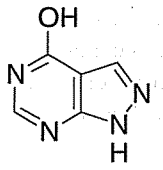
**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.1 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Dissolve about 120 mg in 40 ml of *water*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.01581 g of $C_4H_6N_4O_3$.

# Allopurinol




$C_5H_4N_4O$ Mol. Wt. 136.1

Allopurinol is a tautomeric mixture of 1*H*-pyrazolo[3,4-*d*] pyrimidin-4-ol and 1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one.

Allopurinol contains not less than 98.0 per cent and not more than 101.0 per cent of $C_5H_4N_4O$, calculated on the dried basis.

**Category**. Uricosuric agent.

**Dose**. Initially, 100 mg daily as a single dose gradually increased to 300 mg daily. Usual maintenance dose, 200 to 400 mg daily, in divided doses in moderate and severe gout.

**Description**. A white or almost white, crystalline powder.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *allopurinol RS*.

B. Dissolve 0.1 g in 10 ml of *0.1 M sodium hydroxide* and add sufficient *0.1 M hydrochloric acid* to produce 100.0 ml; dilute 10.0 ml to 100.0 ml with *0.1 M hydrochloric acid* and dilute 10.0 ml of this solution to 100.0 ml with *0.1 M hydrochloric acid*. When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum at about 250 nm and a minimum at about 231 nm; ratio of the absorbance at the minimum at about 231 nm to that at the maximum at about 250 nm, 0.52 to 0.62.

C. Dissolve 50 mg in 5 ml of *dilute sodium hydroxide solution*, add 1 ml of *alkaline potassium mercuri-iodide solution*, heat to boiling and allow to stand; a flocculent yellow precipitate is produced.

D. Shake about 0.1 g with 5 ml of *dilute sodium hydroxide solution*, add 3 ml of *lithium and sodium molybdophosphotungstate solution* and 5 ml of a 20 per cent w/v solution of *sodium carbonate*; a grey-blue colour is produced.

## Tests

**Appearance of solution**. A 5.0 per cent w/v solution in *2 M sodium hydroxide* is clear, (2.4.1), and not more intensely coloured than reference solution YS6 or GYS4 (2.4.1).

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 60 volumes of *2-butanone*, 20 volumes of *2-methoxyethanol* and 20 volumes of *strong ammonia solution*.

*Test solution*. A 2.5 per cent w/v solution of the substance under examination in *strong ammonia solution*.

*Reference solution*. A 0.005 per cent w/v solution of *5-aminopyrazole-4-carboxamide hemisulphate RS* in *strong ammonia solution*.

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of warm air and examine in ultraviolet



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light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Heavy metals** (2.3.13). Mix carefully 1.0 g in a silica crucible with 4 ml of a 25 per cent w/v solution of *magnesium sulphate* in *1 M sulphuric acid* and heat cautiously to dryness. Ignite the residue at a temperature not exceeding 800° and continue heating until a white or greyish residue is obtained. Allow to cool, moisten with 0.2 ml of *1 M sulphuric acid*, evaporate, ignite again and allow to cool. The total ignition period should be less than 2 hours. Dissolve the residue with two quantities, each of 5 ml, of *2 M hydrochloric acid*. Add 2 drops of *dilute phenolphthalein solution* and *strong ammonia solution* dropwise until a pink colour is produced. Cool, add *glacial acetic acid* until the solution gets decolorised and add a further 0.5 ml. Filter, if necessary, and dilute the solution to 20 ml with *water*. The resulting solution complies with the limit test for heavy metals, Method D (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.2 g and dissolve with gentle heating, if necessary, in 50 ml of *dimethylformamide*. Titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.01361 g of $C_5H_4N_4O$.

## Allopurinol Tablets

Allopurinol Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of allopurinol, $C_5H_4N_4O$.

**Usual strengths**. 100 mg; 300 mg.

### Identification

A. When examined in the range 230 nm to 360 nm (2.4.7), the solution obtained in the Assay shows an absorption maximum only at about 250 nm.

B. Shake a quantity of the powdered tablets containing about 0.1 g of Allopurinol with 5 ml of *dilute sodium hydroxide solution*, add 3 ml of *lithium and sodium molybdophosphotungstate solution* and 5 ml of a 20 per cent w/v solution of *sodium carbonate*; a grey-blue colour is produced.

### Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 60 volumes of *2-butanone*, 20 volumes of *2-methoxyethanol* and 20 volumes of *strong ammonia solution*.

*Test solution*. Shake a quantity of the powdered tablets containing about 0.25 g of Allopurinol with 10 ml of *strong ammonia solution* and filter.

*Reference solution*. A 0.005 per cent w/v solution of *5-aminopyrazole-4-carboxamide hemisulphate RS* in *strong ammonia solution*

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of warm air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Disintegration** (2.5.1). 30 minutes.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.1 g of Allopurinol and shake with 20 ml of *0.05 M sodium hydroxide* for 15 to 20 minutes, add 75 ml of *0.1 M hydrochloric acid* shake for 10 minutes, add sufficient *0.1 M hydrochloric acid* to produce 250.0 ml, filter and dilute 5.0 ml of the filtrate to 250.0 ml with *0.1 M hydrochloric acid*. Measure the absorbance of the resulting solution at the maximum at about 250 nm (2.4.7) using *0.1 M hydrochloric acid* as the blank.

Calculate the content of $C_5H_4N_4O$, taking 563 as the specific absorbance at 250 nm.

## Aloes

Aloes is the dried juice of the leaves of *Aloe barbadensis* Miller (*A. vera* Linn), known in commerce as Curacao Aloes or Barbados Aloes, or of *A. ferox* Miller and hybrids of this species with *A. africana* Miller and *A. spicata* Baker, known in commerce as Cape Aloes (Fam. Liliaceae). Indian Aloes of commerce is obtained from *A. barbadensis*.

Aloes contains not less than 50.0 per cent of water-soluble extractive. Curacao Aloes contains not less than 18.0 per cent and Cape Aloes not less than 28.0 per cent of hydroxyanthracene derivatives, calculated as anhydrous barbaloin.

**Category**. Laxative.

**Description**. *Unground Curacao Aloes* — Brownish-black, opaque masses; fractured surface uneven, waxy and somewhat resinous; odour, strong and characteristic.



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*Unground Cape Aloes* — Dark-brown or greenish-brown to olive-brown masses; fractured surface shiny and conchoidal; odour, strong and characteristic.

## Identification

Mix 0.5 g with 50 ml of *water*, boil until nearly dissolved, cool, add 0.5 g of *silica gel* and filter. On the filtrate carry out the following tests.

A. Heat 5 ml with 0.2 g of *borax* until dissolved, add a few drops of this solution to a test-tube nearly filled with *water*; a green fluorescence is produced.

B. Mix 2 ml with 2 ml of *bromine water*; a pale yellow precipitate is produced. The supernatant liquid is violet with Curacao Aloes; no such violet colour appears with Cape Aloes.

C. Mix 5 ml with 2 ml of *nitric acid*; with Cape Aloes a reddish-yellow colour is produced; with Socotrine Aloes a pale brownish-yellow colour is produced; with Cape Aloes a yellowish-brown colour passing rapidly to green is produced.

D. Determine by thin-layer chromatography (2.4.17), coating the plate with silica *gel G*.

*Mobile phase*. A mixture of 100 volumes of *ethyl acetate*, 17 volumes of *methanol* and 13 volumes of *water*.

*Test solution*. Heat 0.5 g, in powder, with 20 ml *methanol* to boiling on a water-bath, shake well, decant the supernatant liquid, keep at 4° and use within 24 hours.

*Reference solution*. Dissolve 50 mg of *barbaloin* in 10 ml *methanol*.

Apply to the plate 5 µl of each solution as bands 20 mm x 3 mm. Allow the mobile phase to rise 15 cm. Dry the plate in a current of air, spray with a 10 per cent w/v solution of *potassium hydroxide* in *methanol* and examine in ultraviolet light at 365 nm. The chromatogram obtained with the reference solution shows a yellow band with an Rf value of 0.4 to 0.5. In the case of Curacao Aloes, the chromatogram obtained with the test solution shows a yellow fluorescent band corresponding to that due to barbaloin in the chromatogram obtained with the reference solution and in the lower part a light blue fluorescent band (corresponding to aloesine). In the case of Cape Aloes, the test solution shows a yellow fluorescent band corresponding to that due to barbaloin in the chromatogram obtained with the reference solution and in the lower part two yellow fluorescent bands (due to aloinosides A and B) as well as a blue fluorescent band (due to aloesine). Heat the plate at 110° for 5 minutes. In the case of Curacao Aloes, with the test solution a violet fluorescent band appears just below the yellow band corresponding to barbaloin while in the case of Cape Aloes no such violet band appears.

## Tests

**Ethanol-insoluble substances**. Weigh accurately about 1.0 g, in fine powder, and add to 50 ml of *ethanol (95 per cent)* in a flask. Reflux the mixture for 15 minutes. Remove the source of heat and set aside for 1 hour, shaking frequently, filter through a small dried and tared filter paper or suitable filtering crucible and wash the residue on the filter with *ethanol (95 per cent)* till the washings are colourless. The residue after drying to constant weight at 105° weighs not more than 0.1 g.

**Water-soluble extractive**. Weigh accurately about 2.0 g, in fine powder, and macerate with about 60 to 70 ml of *water* in a flask. Shake the mixture at 30-minute intervals for 8 hours and allow to stand for a further 16 hours without shaking. Filter, wash the flask and the residue with small portions of *water*, passing the washings through the filter until the filtrate measures 100 ml. Evaporate 50 ml of this filtrate to dryness in a tared dish on a water-bath and dry at 105° for 3 hours; the residue weighs not less than 0.5 g.

**Total ash** (2.3.19). Not more than 0.5 per cent, determined on 1.0 g by Method A.

**Loss on drying** (2.4.19). Not more than 12 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Moisten 0.2 g, in fine powder, with 2 ml of *methanol*, add 5 ml of *water* at about 60°, mix, add a further 75 ml of *water* at about 60°, shake for 30 minutes, cool, filter through a filter paper, washing the flask with 20 ml of *water* and add sufficient *water* to the combined filtrate and washings to produce 1000.0 ml. Transfer 10.0 ml of the solution to a flask containing 1 ml of a 60 per cent w/v solution of *ferric chloride hexahydrate* and 6 ml of *hydrochloric acid*, heat in a water-bath under a reflux condenser for 4 hours so that the water level is always above that of the liquid in the flask, cool, transfer the solution to a separating funnel, rinsing the flask successively with 4 ml of *1 M sodium hydroxide* and 4 ml of *water* and adding the rinsings to the contents of the separating funnel. Extract with three quantities, each of 20 ml, of *carbon tetrachloride* and wash the combined carbon tetrachloride layers with two quantities, each of 100 ml, of *water*, discarding the washings. Dilute the organic phase to 100.0 ml with *carbon tetrachloride*, evaporate 20.0 ml carefully to dryness on a water-bath and dissolve the residue in 10.0 ml of *1 M sodium hydroxide*. Immediately measure the absorbance of the resulting solution at the maximum at about 440 nm and at about 500 nm (2.4.7) Calculate the content of anhydrous barbaloin, taking 200 as the specific absorbance at 500 nm. The result of the Assay is not valid unless the ratio of the absorbance at about 500 nm to that at about 440 nm is not less than 1.9.

**Storage**. Store protected from light and moisture.

**Labelling**. The label states whether the material is Curacao Aloes or Cape Aloes.

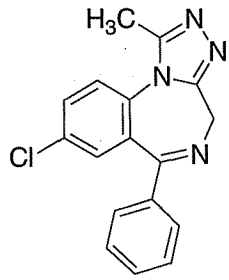


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# Alprazolam



$C_{17}H_{13}ClN_4$ Mol. Wt. 308.8

Alprazolam is 8-chloro-1-methyl-6-phenyl-4*H*-1,2,4-triazolo[4,3-*a*][1,4]benzodiazepine.

Alprazolam contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{17}H_{13}ClN_4$, calculated on the dried basis.

**Category**. Anxiolytic.

**Dose**. 0.25 mg to 1 mg daily.

**Description**. A white to off-white, crystalline powder.

## Identification

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

B. Dissolve 10.0 mg in *water* and dilute to 500.0 ml with the same solvent. Dilute 20.0 ml of this solution to 100.0 ml with *water*. When examined in the range 210 nm to 360 nm (2.4.7), the solution shows an absorption maximum at about 220 nm.

C. Melts at about 225° (2.4.21).

## Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 0.1 g of the substance under examination in 10 ml of *dimethylformamide*.

*Reference solution*. Dilute 5 ml of the test solution to 100 ml with *dimethylformamide*. Dilute 0.5 ml of this solution to 10 ml with *dimethyformamide*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature 40°,
- mobile phase: A. a mixture of 44 volumes of buffer solution prepared by dissolving 7.7 g of *ammonium acetate* in 1000 ml of *water*, adjust pH to 4.2 with *glacial acetic acid* and 56 volumes of *methanol*, B. a mixture of 5 volumes of buffer solution prepared by dissolving 7.7 g of *ammonium acetate* in 1000 ml of *water*, adjust pH to 4.2 with *glacial acetic acid* and 95 volumes of methanol,
- a linear gradient programme using the conditions given below,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

| Time (min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0-15 | 98 | 2 |
| 15-35 | 98-1 | 2-99 |
| 35-40 | 1 | 99 |

Equilibrate the column for at least 30 minutes with the initial eluent composition. For subsequent chromatographs equilibrate the column for 10 minutes with the same eluent. Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram of the reference solution is not less than 50 per cent of the full scale of the recorder. The retention time of the principal peak is about 10 minutes.

Inject the test solution and the reference solution. In the chromatogram obtained with the test solution, the sum of areas of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with the reference solution (0.25 per cent). Ignore any peaks with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

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

**Sulphated ash** (2.3.18). Not more than 0.5 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa for 16 hours.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh accurately about 25 mg and dissolve in sufficient *acetonitrile* to produce 100.0 ml. Dilute 10.0 ml of this solution to 100.0 ml with *acetonitrile*.

*Reference solution*. A solution containing 0.0025 per cent w/v of *alprazolam RS* in *acetonitrile*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with porous silica particles 3 to 10 µm,
- mobile phase: a mixture of 850 volumes of *acetonitrile*, 80 volumes of *chloroform*, 50 volumes of *1-butanol*, 20 volumes of *water* and 0.5 volume of *glacial acetic acid*,
- flow rate. 2 ml per minute,



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- spectrophotometer set at 254 nm,
- injection volume. 10 µl or 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the percentage content of $C_{17}H_{13}ClN_4$.

**Storage**. Store protected from light.

# Alprazolam Tablets

Alprazolam Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of alprazolam, $C_{17}H_{13}ClN_4$.

**Usual strengths**. 0.25 mg; 0.5 mg; 1 mg.

## Identification

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

## Tests

**Dissolution** (2.5.2).

Apparatus No.1
Medium. 500 ml of buffer solution prepared by dissolving 0.8 gm of *monobasic potassium phosphate* and 0.2 gm of *dibasic potassium phosphate* in 1000 ml of *water;* adjust the pH to 6.0 with *orthophosphoric acid.*
Speed and time. 100 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

*Test solution.* The filtrate obtained as given above. Dilute the filtrate if necessary, with the dissolution medium.

*Reference solution.* A 0.005 per cent w/v solution of *alprazolam RS* in *methanol.* Dilute the solution with the dissolution medium to obtain a solution of about the same concentration as the test solution.

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase. a mixture of 60 volumes of buffer solution, 35 volumes of *acetonitrile* and 5 volumes of *tetrahydrofuran,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 500 theoretical plates, and the relative standard deviation for replicate injections is not more than 3.0 per cent

Inject the reference solution and the test solution.

Calculate the content of $C_{17}H_{13}ClN_4$.

D. Not less than 80 per cent of the stated amount of $C_{17}H_{13}ClN_4$.

**Uniformity of content**. Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14).

*Test solution.* Transfer one tablet to a container, add 0.4 ml of *water* on to the tablet, allow the tablet to stand for 2 minutes and swirl the container to disperse the tablet. Add sufficient *acetonitrile* to produce a solution containing 0.0025 per cent w/v of alprazolam. Shake to mix and centrifuge, if necessary.

*Reference solution.* A solution containing 0.0025 per cent w/v of *alprazolam RS* in *acetonitrile.*

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with porous silica particles, 5 to 10 µm in diameter,
- mobile phase: a mixture of 850 volumes of *acetonitrile,* 80 volumes of *chloroform,* 50 volumes of *1-butanol,* 20 volumes of *water* and 0.5 volume of *glacial acetic acid.*
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl or 20 µl.

Calculate the content of $C_{17}H_{13}ClN_4$ in the tablet.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Place 5 tablets in a flask, add 2 ml of *water* and swirl to disperse the tablets. Add sufficient *acetonitrile* to produce 25.0 ml. Shake for 10 to 15 minutes and centrifuge if necessary. Dilute a portion of the clear solution with *acetonitrile* to produce a solution containing 0.0025 per cent w/v of alprazolam.

*Reference solution.* A 0.0025 per cent w/v solution of *alprazolam RS* in *acetonitrile.*

Chromatographic system as described under Uniformity of content.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_{17}H_{13}ClN_4$ in the tablets.

**Storage**. Store protected from light.



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## Aluminium Acetate Ear Drops

Aluminium Acetate Otic Drops; Aluminium Acetate Solution; Burow's Solution.

| | | |
|---|---|---|
| Aluminium Sulphate | 255 | g |
| Calcium Carbonate | 100 | g |
| Tartaric Acid | 45 | g |
| Glacial Acetic Acid | 82.5 | ml |
| Purified Water sufficient to produce | 1000 | ml |

Dissolve the Aluminium Sulphate in 600 ml of Purified Water, add Glacial Acetic Acid followed by Calcium Carbonate mixed with the remainder of the Purified Water and allow to stand for not less than 24 hours in a cool place, stirring occasionally. Filter, add the Tartaric Acid to the filtrate and mix.

Aluminium Acetate Ear Drops contain not less than 1.7 per cent w/v and not more than 1.9 per cent w/v of aluminium, Al.

**Description**. A clear solution.

### Tests

**Weight per ml** (2.4.29). 1.06 g to 1.08 g.

**Other tests**. Comply with the tests stated under Ear Drops.

**Assay**. Dilute 10.0 ml to 100.0 ml with *water*. To 10.0 ml of the resulting solution add 40.0 ml of *0.05 M disodium edetate*, 90 ml of *water* and 0.15 ml of *methyl red solution*. Neutralise by the addition of *1 M sodium hydroxide* dropwise and warm on a water-bath for 30 minutes. Cool, add 1 ml of *2 M nitric acid* and 5 g of *hexamine* and titrate with *0.05 M lead nitrate* using 0.5 ml of *xylenol orange solution* as indicator.

1 ml of *0.05 M disodium edetate* is equivalent to 0.001349 g of Al.

**Storage**. Store protected from light, in well-filled containers.

## Aluminium Hydroxide Gel

Aluminium Hydroxide Suspension; Aluminium Hydroxide Mixture

Aluminium Hydroxide Gel is an aqueous suspension of hydrated aluminium oxide together with varying quantities of basic aluminium carbonate and bicarbonate. It may contain Glycerin, Sorbitol, Sucrose or Saccharin as sweetening agents and Peppermint Oil or other suitable flavours. It may also contain suitable antimicrobial agents.

Aluminium Hydroxide Gel contains not less than 3.5 per cent and not more than 4.4 per cent w/w of $Al_2O_3$.

**Category**. Antacid.

**Dose**. 7.5 to 15 ml.

**Description**. A white, viscous suspension, translucent in thin layers; small amounts of clear liquid may separate on standing.

### Identification

A solution in *dilute hydrochloric acid* gives the reactions of aluminium salts (2.3.1).

### Tests

**pH** (2.4.24). 5.5 to 8.0.

**Arsenic** (2.3.10). Dissolve 10.0 g in 18 ml of *brominated hydrochloric acid*, add 42 ml of *water* and remove the excess bromine with a few drops of *stannous chloride solution AsT*. The resulting solution complies with the limit test for arsenic (1 ppm).

**Heavy metals** (2.3.13). Dissolve 5.0 g in 10 ml of *dilute hydrochloric acid*, filter if necessary, and dilute to 25 ml with *water*. The resulting solution complies with the limit test for heavy metals, Method A (10 ppm).

**Chlorides** (2.3.12). Dissolve 0.5 g in 5 ml of *dilute nitric acid*, boil, cool, dilute to 100 ml with *water* and filter. 20 ml of the filtrate complies with the limit test for chlorides (0.25 per cent).

**Sulphates** (2.3.17). Dissolve 1.0 g in 5 ml of *dilute hydrochloric acid* with the aid of heat. Cool and dilute to 100 ml with *water*. Mix well and filter, if necessary. To 5 ml of the filtrate add 2 ml of *dilute hydrochloric acid*; the solution complies with the limit test for sulphates (0.3 per cent).

**Neutralising capacity**. Disperse 5.0 g in 100 ml of *water*, heat to 37°, add 100.0 ml of *0.1 M hydrochloric acid* previously heated to 37°and stir continuously, maintaining the temperature at 37°; the pH of the solution, at 37°, after 10, 15 and 20 minutes, is not less than 1.8, 2.3 and 3.0 respectively and at no time is more than 4.5. Add 10.0 ml of *0.5 M hydrochloric acid* previously heated to 37°, stir continuously for 1 hour maintaining the temperature at 37° and titrate with *0.1 M sodium hydroxide* to pH 3.5.

Not more than 50.0 ml of *0.1 M sodium hydroxide* is required.

**Microbial contamination** (2.2.9). Total viable aerobic count, not more than 100 micro-organisms per ml, determined by plate count. 1 ml is free from *Escherichia coli*.

**Assay**. Weigh accurately about 5.0 g and dissolve in 3 ml of *hydrochloric acid* by warming on a water-bath; cool to below 20° and dilute to 100.0 ml with *water*. To 20.0 ml of this solution, add 40.0 ml of *0.05 M disodium edetate*, 80 ml of *water*, and 0.15 ml of *methyl red solution* and neutralise by the dropwise addition of *1 M sodium hydroxide*. Warm on a water-bath for 30 minutes, add 3 g of *hexamine* and titrate with *0.05 M lead nitrate* using 0.5 ml of *xylenol orange solution* as indicator.



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1 ml of *0.05 M disodium edetate* is equivalent to 0.002549 g of $Al_2O_3$.

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

## Dried Aluminium Hydroxide Gel

Dried Aluminium Hydroxide; Hydrated Aluminium Oxide

Dried Aluminium Hydroxide Gel consists largely of hydrated aluminium oxide together with varying quantities of basic aluminium carbonate and bicarbonate.

Dried Aluminium Hydroxide Gel contains not less than 47.0 per cent and not more than 60.0 per cent of $Al_2O_3$.

**Dose**. 500 mg to 1g.

**Description**. A white, light, amorphous powder containing some aggregates; odourless; tasteless.

### Identification

A solution in *dilute hydrochloric acid* gives the reactions of aluminium salts (2.3.1).

### Tests

**pH** (2.4.24). Not more than 10.0, determined in a 4.0 per cent w/v suspension in *carbon dioxide-free water*.

**Arsenic** (2.3.10). Dissolve 2 g in 18 ml of *brominated hydrochloric acid*, add 42 ml of *water* and remove the excess of bromine with a few drops of *stannous chloride solution AsT*. The resulting solution complies with the limit test for arsenic (5 ppm).

**Heavy metals** (2.3.13). Dissolve 0.33 g in 10 ml of *dilute hydrochloric acid* with the aid of heat, filter if necessary, and dilute to 25 ml with *water*. The resulting solution complies with the limit test for heavy metals Method A (60 ppm).

**Chlorides** (2.3.12). Dissolve 0.1 g in 10 ml of *dilute nitric acid*, boil, cool, dilute to 100 ml with *water* and filter. 20 ml of the filtrate complies with the limit test for chlorides (1.25 per cent).

**Sulphates** (2.3.17). Dissolve 0.5 g in 5 ml of *dilute hydrochloric acid*, boil, cool, dilute to 200 ml with *water* and filter. 5 ml of the filtrate complies with the limit test for sulphates (1.2 per cent).

**Neutralising capacity**. Pass a sufficient quantity, triturated if necessary, through a sieve of nominal mesh aperture of 150 µm. Weigh accurately 0.5 g of the sifted material and add to 200.0 ml of *0.05 M hydrochloric acid* previously heated to 37° and stir continuously, maintaining the temperature at 37°; the pH of the solution, at 37°, after 10, 15 and 20 minutes, is not less than 1.8, 2.3 and 3.0 respectively and at no time is more than 4.5. Add 10.0 ml of *0.5 M hydrochloric acid* previously heated to 37°, stir continuously for 1 hour maintaining the temperature at 37° and titrate with *0.1 M sodium hydroxide* to pH 3.5.

Not more than 35.0 ml of *0.1 M sodium hydroxide* is required and the pH of the solution at 37° at no time is more than 4.5.

**Microbial contamination** (2.2.9). 1 g is free from *Escherichia coli*.

**Assay**. Weigh accurately about 0.4 g and dissolve in a mixture of 3 ml of *hydrochloric acid* and 3 ml of *water* by warming on a water-bath, cool to below 20° and dilute to 100.0 ml with *water*. To 20.0 ml of this solution, add 40.0 ml of *0.05 M disodium edetate*, 80 ml of *water*, and 0.15 ml of *methyl red solution* and neutralise by the dropwise addition of *1 M sodium hydroxide*. Warm on a water-bath for 30 minutes, add 3 g of *hexamine* and titrate with *0.05 M lead nitrate* using 0.5 ml of *xylenol orange solution* as indicator.

1 ml of *0.05 M disodium edetate* is equivalent to 0.002549 g of $Al_2O_3$.

**Storage**. Store protected from moisture.

## Aluminium Magnesium Silicate

$Al_2MgO_8Si_2$ Mol. Wt. 262.4

Aluminium Magnesium Silicate is mixture of particles with colloidal particle size of montmorillonite and saponite, free from grit and non-swellable ore.

Aluminium Magnesium Silicate contains not less than 95.0 per cent and not more than 105.0 per cent each of the stated amount of aluminium, Al and magnesium, Mg, calculated on the dried basis.

**Category**. Pharmaceutical aid.

**Description**. A almost white powder, granules or plates.

### Identification

A. Fuse 1 g with 2 g of *anhydrous sodium carbonate*. Warm the residue with *water* and filter. Acidify the filtrate with *hydrochloric acid* and evaporate to dryness on a water bath. About 0.25 g of the residue gives the reaction of silicates (2.3.1).

B. Dissolve the remainder of the residue obtained in identification test A in a mixture of 5 ml of *dilute hydrochloric acid* and 10 ml of *water*. Filter and add *ammonium chloride buffer solution pH 10*. A white, gelatinous precipitate is formed. Centrifuge and keep the supernatant for identification C. Dissolve the remaining precipitate in *dilute hydrochloric acid*; gives the reaction of aluminium (2.3.1).



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C. The supernatant liquid obtained after centrifugation in identification test B gives the reaction of magnesium (2.3.1).

## Tests

**pH** (2.4.24). 9.0 to 10.0, determined in a 5.0 per cent w/v solution in *carbon dioxide-free water*.

**Arsenic** (2.3.10). Dissolve 16.6 g of the substance under examination in 100 ml of *dilute hydrochloric acid* in a 250-ml beaker. Mix, cover with a watch glass and boil gently, with occasional stirring, for 15 minutes, allow the insoluble matter to settle and decant the supernatant liquid through a rapid-flow filter paper into a 250-ml volumetric flask, retaining as much sediment as possible in the beaker. To the residue in the beaker, add 25 ml of hot *dilute hydrochloric acid*, stir, heat to boiling, allow the insoluble matter to settle and decant the supernatant liquid through the filter into the volumetric flask. Repeat the extraction with 4 additional quantities, each of 25 ml, of hot *dilute hydrochloric acid*, decanting each supernatant liquid through the filter into the volumetric flask. At the last extraction, transfer as much of the insoluble matter as possible onto the filter. Allow the combined filtrates to cool to room temperature and dilute to 250.0 ml with *dilute hydrochloric acid*. Dilute 5.0 ml of this solution to 25.0 ml with *dilute hydrochloric acid*. The resulting solution complies with the limit test for arsenic (3 ppm).

**Lead**. Not more than 15 ppm, determine by atomic absorption spectrophotometry (2.4.2), measuring at 217 nm using a oxidising air-acetylene flame.

*Test solution*. Dissolve 10 g of the substance under examination in 100 ml of *dilute hydrochloric acid* in a 250-ml beaker. Mix, cover with a watch glass and boil for 15 minutes, cool to room temperature, allow the insoluble matter to settle. Decant the supernatant liquid through a rapid-flow filter paper into a 400 ml beaker. To the insoluble matter in the 250-ml beaker, add 25 ml of hot *water*. Stir, allow the insoluble matter to settle and decant the supernatant liquid through the filter into the 400 ml beaker. Repeat the extraction with 2 additional quantities, each of 25 ml of *water*, decanting each time the supernatant liquid through the filter into the 400-ml beaker. Wash the filter with 25 ml of hot *water*, collecting this filtrate in the 400-ml beaker. Concentrate the combined filtrates to about 20 ml by gently boiling. If a precipitate appears, add about 0.1 ml of *nitric acid*, heat to boiling and allow to cool to room temperature. Filter the concentrated extracts through a rapid-flow filter paper into a 50-ml volumetric flask. Transfer the remaining contents of the 400-ml beaker through the filter paper and into the flask with *water*. Dilute this solution to 50.0 ml with *water*.

*Reference solution*. Prepare the reference solution using *lead standard solution AAS (10 ppm Pb)*, diluted if necessary with *water*.

**Loss on drying** (2.4.19). Not more than 8.0 per cent, determined on 1.0 g by drying in an oven at 105°.

**Microbial contamination** (2.2.9). Total viable aerobic count is not more than $10^3$ micro-organisms per gram, determined by plate count. It complies with the test for *Escherichia coli*.

**Assay**. *For Aluminium*—Determine by atomic absorption spectrophotometry (2.4.2), measuring at 309 nm using a oxidising acetylene-nitrous oxide flame and aluminium hollow-cathode lamp.

*Test solution*. Mix 0.2 g with 1.0 g of *lithium metaborate* in a platinum crucible. Heat slowly at first and ignite at 1000 to 1200° for 15 minutes, cool, then place the crucible in a 100-ml beaker containing 25 ml of *dilute nitric acid* and add an additional 50 ml of *dilute nitric acid*, filling and submerging the crucible. Place a polytetrafluoroethylene-coated magnetic stirring bar in the crucible and stir gently with a magnetic stirrer until dissolution is complete. Pour the contents into a 250-ml beaker and remove the crucible. Warm the solution and transfer through a rapid-flow filter paper into a 250-ml volumetric flask, wash the filter and beaker with *water* and dilute to 250.0 ml with *water* (solution A). To 20.0 ml of solution A, add 20 ml of a 1.0 per cent w/v solution of *sodium chloride* and dilute to 100.0 ml with *water*.

*Reference solution*. Dissolve, with gentle heating, 1.0 g of *aluminium* in a mixture of 10 ml of *hydrochloric acid* and 10 ml of *water*, cool. Dilute to 1000.0 ml with *water* (1 mg of aluminium per millilitre). Into 3 identical volumetric flasks, each containing 0.2 g of *sodium chloride*, introduce 2.0 ml, 5.0 ml and 10.0 ml of this solution respectively, and dilute to 100.0 ml with *water*.

*For Magnesium*—Determine by atomic absorption spectrophotometry (2.4.2), measuring at 285 nm using a reducing air acetylene flame and magnesium hollow-cathode lamp.

*Test solution*. Dilute 25.0 ml of solution A, prepared in the assay for aluminium, to 50.0 ml with *water*. To 5.0 ml of this solution add 20.0 ml of *lanthanum nitrate solution* and dilute to 100.0 ml with *water*.

*Reference solution*. Place 1.0 g of *magnesium* in a 250-ml beaker containing 20 ml of *water* and carefully add 20 ml of *hydrochloric acid*, warming if necessary to dissolve. Transfer the solution to a volumetric flask and dilute to 1000.0 ml with *water* (1 mg of magnesium per millilitre). Dilute 5.0 ml of this solution to 250.0 ml with *water*. Into 4 identical volumetric flasks, introduce 5.0 ml, 10.0 ml, 15.0 ml and 20.0 ml of the solution respectively. To each flask add 20.0 ml of *lanthanum nitrate solution* and dilute to 100.0 ml with *water*.

**Labelling**. The label states the content of aluminium and magnesium.

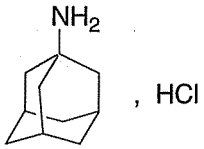


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# Amantadine Hydrochloride




$C_{10}H_{17}N,HCl$ Mol. Wt. 187.7

Amantadine Hydrochloride is tricyclo[3.3.1.1$^{3,7}$]dec-1-ylamine hydrochloride.

Amantadine Hydrochloride contains not less then 98.5 per cent and not more than 101.0 per cent of $C_{10}H_{17}N,HCl$, calculated on the anhydrous basis.

**Category**. Antiviral; antiparkinsonian.

**Dose**. 100 mg daily, increased if necessary to 200 mg daily, in two divided doses.

**Description**. A white or almost white, crystalline powder; sublimes when heated.

## Identification

*Test A may be omitted if tests B and C are carried out. Test B may be omitted if tests A and C are carried out.*

A. Dissolve 0.1 g in 5 ml of *water*, add 0.5 ml of *5 M sodium hydroxide*, extract with 5 ml of *dichloromethane*, filter the dichloromethane layer through *anhydrous sodium sulphate* with 2 ml of *dichloromethane* and evaporate the solution to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *amantadine hydrochloride RS* treated in the same manner or with the reference spectrum of amantadine.

B. Dissolve 0.2 g in 1 ml of *0.1 M hydrochloric acid* and add 1 ml of a 50 per cent w/v solution of *sodium nitrite*; a white precipitate is produced.

C. 1 ml of a 10 per cent w/v solution in *carbon dioxide-free water* gives reaction A of chlorides (2.3.1).

## Tests

**Appearance of solution**. A 10.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1) and not more intensely coloured than reference solution YS7 (2.4.1).

**pH** (2.4.24). 3.0 to 5.5, determined in a 20.0 per cent w/v solution.

**Heavy metals** (2.3.13). A solution prepared by dissolving 1.0 g in 1 ml of *dilute acetic acid* and sufficient *water* to produce 25 ml complies with the limit test for heavy metals, Method A (20 ppm).

**Related substances**. Determine by gas chromatography (2.4.13).

*Test solution*. Dissolve 0.1 g of the substance under examination in 2 ml of *water*, add 2 ml of a 20 per cent w/v solution of *sodium hydroxide* and 2 ml of *chloroform* and shake for 10 minutes. Separate the chloroform layer, dry over *anhydrous sodium sulphate* and filter.

Chromatographic system

- a glass column 1.8 m x 2 mm, packed with material prepared in the following manner. Mix 19.5 g of silanised diatomaceous support (such as Chromosorb G/AW/DMCS) with 60 ml of a 0.33 per cent w/v solution of *potassium hydroxide* in *methanol* and evaporate the solvent under reduced pressure while slowly rotating the mixture. Dissolve over a 5-hour period 0.4 g of low-vapour pressure hydrocarbons (type L) (such as Apiezon L) in 60 ml of *toluene*, add this solution to the prepared silanised diatomaceous support and evaporate the solvent under reduced pressure while slowly rotating the mixture,
- temperature:
  column. Allow the temperature to increase from 100° to 200° at a constant rate of 6° per minute,
  inlet port. 220°,
  detector. 300°,
- flow rate. 30 ml per minute of the carrier gas.

Inject 1 µl or other suitable volume of the test solution. Record the chromatogram for at least 2.5 times the retention time of the principal peak.

The area of any secondary peak is not greater than 0.3 per cent and the sum of the areas of any secondary peaks is not greater than 1 per cent by normalisation.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 0.5 per cent, determined on 2.0 g.

**Assay**. Weigh accurately about 0.15 g, dissolve in a mixture of 5.0 ml of *0.01 M hydrochloric acid* and 50 ml of *ethanol (95 per cent)* and titrate with *0.1 M sodium hydroxide* determining the end-point potentiometrically (2.4.25). Record the volume used between the two inflections.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.01877 g of $C_{10}H_{17}N,HCl$.

# Amantadine Capsules

## Amantadine Hydrochloride Capsules

Amantadine Capsules contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of amantadine hydrochloride, $C_{10}H_{17}N,HCl$.

**Usual strength**. 100 mg.



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### Identification

Dissolve the contents of capsules containing about 0.1 g of Amantadine Hydrochloride in 5 ml of *water*, add 0.5 ml of *5 M sodium hydroxide*, extract with 5 ml of *dichloromethane*, filter the dichloromethane layer through *anhydrous sodium sulphate* with 2 ml of *dichloromethane* and evaporate the solution to dryness. The residue complies with the following test.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *amantadine hydrochloride RS* treated in the same manner or with the reference spectrum of amantadine.

B. Dissolve 0.2 g in 1 ml of *0.1 M hydrochloric acid* and add 1 ml of a 50 per cent w/v solution of *sodium nitrite*; a white precipitate is produced.

### Tests

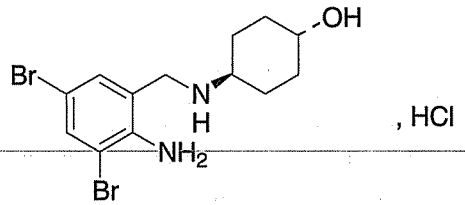
**Other tests**. Comply with the tests stated under Capsules.

**Assay**. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 0.12 g of Amantadine Hydrochloride and warm in a mixture of 30 ml of *anhydrous glacial acetic acid* and 10 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01877 g of $C_{10}H_{17}N,HCl$.

**Storage**. Store protected from moisture at a temperature not exceeding 30°.

## Ambroxol Hydrochloride




, HCl

$C_{13}H_{18}Br_2N_2O,HCl$ Mol. Wt. 414.6

Ambroxol hydrochloride is *trans*-4-[(2-amino-3,5-dibromobenzyl)amino]cyclohexanol hydrochloride.

Ambroxol Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{13}H_{18}Br_2N_2O,HCl$, calculated on the dried basis.

**Category**. Mucolytic.

**Description**. A white or yellowish crystalline powder.

### Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ambroxol hydrochloride RS* or with the reference spectrum of ambroxol hydrochloride.

B. Dissolve 25 mg in 2.5 ml of *water*, add 1.0 ml of *dilute ammonia* and allow to stand for 5 minutes. Acidify the aqueous layer with *dilute nitric acid* and filter. The filtrate gives reaction A of chlorides (2.3.1).

### Tests

**pH** (2.4.24). 4.5 to 6.0, determined in a 1.0 per cent w/v solution in *carbon dioxide-free water*.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 50 mg of the substance under examination in *water* and dilute to 50 ml with the same solvent.

*Reference solution (a)*. Dissolve 5 mg of *ambroxol hydrochloride RS* in 250 ml of *water*. Dilute 5 ml of the solution to 100 ml with the mobile phase.

*Reference solution (b)*. Dissolve 5 mg of the substance under examination in 0.2 ml of *methanol* and add 0.04 ml of a mixture of 1 volume of *formaldehyde solution* and 99 volumes of *water*. Heat at 60° for 5 minutes. Evaporate to dryness under a current of nitrogen. Dissolve the residue in 5 ml of *water* and dilute to 20 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of equal volumes of *acetonitrile* and a buffer solution prepared by dissolving 1.32 g of *ammonium phosphate* in 900 ml of *water*, adjusting the pH to 7.0 with *phosphoric acid* and diluting to 1000 ml with *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 248 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the secondary peak (*trans*-4-(6,8-dibromo-1,4-dihydroquinazolin-3(2*H*)-yl)cyclohexanol) and the ambroxol peak is at least 4.0.

Inject the test solution and reference solution (a). Continue the chromatography for 3 times the retention time of the principal peak in the chromatogram obtained with the test solution. The area of any secondary peak in the chromatogram obtained with the test solution is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). The sum of the areas of all the secondary peaks is not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.01 per cent).



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

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

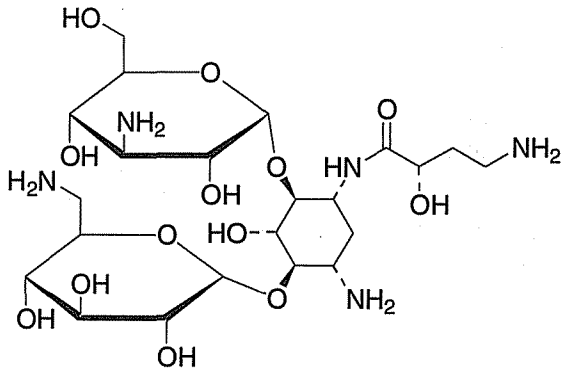
**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Dissolve 0.3 g in 70 ml of *ethanol*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.04146 g of $C_{13}H_{18}Br_2N_2O$, HCl.

**Storage**. Store protected from light.

## Amikacin



$C_{22}H_{43}N_5O_{13}$ Mol. Wt. 585.6

Amikacin is (*S*)-*O*-3-amino-3-deoxy-α-D-glucopyranosyl-(1→6)-*O*-[6-amino-6-deoxy-α-D-glucopyranosyl(1→4)]-*N*$^1$-(4-amino-2-hydroxy-1-oxobutyl)-2-deoxy-D-streptamine.

Amikacin contains not less than 900 µg of $C_{22}H_{43}N_5O_{13}$ per mg, calculated on the anhydrous basis.

**Category**. Antibacterial.

**Dose**. By intramuscular or slow intravenous injection or by infusion, upto 1.5 g daily, in two divided doses.

**Description**. A white crystalline powder; almost odourless.

### Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 60 volumes of *methanol*, 30 volumes of *strong ammonia solution* and 25 volumes of *chloroform*.

*Test solution*. A 0.6 per cent w/v solution of the substance under examination.

*Reference solution (a)*. A 0.6 per cent w/v solution of *amikacin RS*.

*Reference solution (b)*. A mixture of equal volumes of the test solution and reference solution (a).

Apply to the plate 3 µl of each solution. After development, allow the plate to dry in air, heat it at 110° for 15 minutes and immediately spray it with a 1 per cent w/v solution of *ninhydrin* in a mixture of 100 volumes of 1-*butanol* and 1 volume of *pyridine*. The principal pink-coloured spot in the chromatogram obtained with the test solution corresponds to those in the chromatograms obtained with reference solutions (a) and (b).

B. To 1 ml of a 1 per cent w/v solution add 1 ml of *2 M sodium hydroxide*, mix and add 2 ml of a 1 per cent w/v solution of *cobalt nitrate*; a violet colour is produced.

C. To a solution of 50 mg in 5 ml of *water* add 4 ml of a 0.035 per cent w/v solution of *anthrone* in *sulphuric acid*; a bluish-violet colour is produced.

### Tests

**pH** (2.4.24). 9.5 to 11.5, determined in a 1.0 per cent w/v solution in *carbon dioxide-free water*.

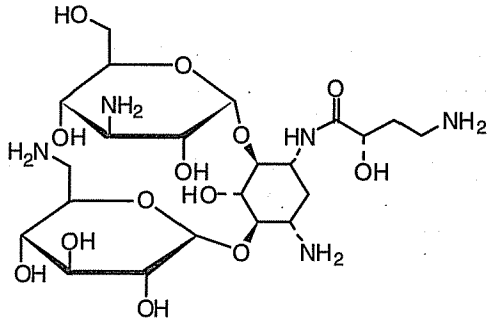
**Specific optical rotation** (2.4.22). +97° to +105°, determined in a 2.0 per cent w/v solution.

**Sulphated ash** (2.3.18). Not more than 1.0 per cent, the charred residue being moistened with 2 ml of *nitric acid* and 5 drops of *sulphuric acid*.

**Water** (2.3.43). Not more than 8.5 per cent, determined on 0.2 g.

**Assay**. Determine by the microbiological assay of antibiotics, Method B (2.2.10), and express the result in µg of Amikacin, $C_{22}H_{43}N_5O_{13}$, per mg.

## Amikacin Sulphate



$C_{22}H_{43}N_5O_{13},1.8H_2SO_4$ Mol. Wt.762.1
$C_{22}H_{43}N_5O_{13},2H_2SO_4$ Mol. Wt. 781.8

Amikacin Sulphate is (*S*)-*O*-3-amino-3-deoxy-α-D-glucopyranosyl-(1→6)-*O*-[6-amino-6-deoxy-α-D-



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glucopyranosyl(1→4)]-$N^1$-(4-amino-2-hydroxy-1-oxobutyl)-2-deoxy-D-streptamine sulphate (1:2 or 1:1.8)(salt).

Amikacin Sulphate having a molar ratio of Amikacin to $H_2SO_4$ of 1:2 contains the equivalent of not less than 674 µg and not more than 786 µg of $C_{22}H_{43}N_5O_{13}$ per mg, calculated on the dried basis. Amikacin Sulphate having a molar ratio of Amikacin to $H_2SO_4$ of 1:1.8 contains the equivalent of not less than 691µg and not more than 806 µg of $C_{22}H_{43}N_5O_{13}$ per mg, calculated on the dried basis.

**Category**. Antibacterial.

**Dose**. By intramuscular or slow intravenous injection or by infusion, upto 1.5 g daily, in two divided doses.

**Description**. A white to yellowish-white crystalline powder; almost odourless.

### Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 60 volumes of *methanol*, 30 volumes of *strong ammonia solution* and 25 volumes of *chloroform*.

*Test solution*. A 0.6 per cent w/v solution of the substance under examination.

*Reference solution (a)*. A 0.6 per cent w/v solution of *amikacin RS*.

*Reference solution (b)*. A mixture of equal volumes of the test solution and reference solution (a).

Apply to the plate 3 µl of each solution. After development, allow the plate to dry in air, heat it at 110° for 15 minutes and immediately spray it with a 1 per cent w/v solution of *ninhydrin* in a mixture of 100 volumes of 1-*butanol* and 1 volume of *pyridine*. The principal pink-coloured spot in the chromatogram obtained with the test solution corresponds to those in the chromatograms obtained with reference solutions (a) and (b).

B. To 1 ml of a 1 per cent w/v solution add 1 ml of 2 *M sodium hydroxide*, mix and add 2 ml of a 1 per cent w/v solution of *cobalt nitrate*; a violet colour is produced.

C. To a solution of 50 mg in 5 ml of *water* add 4 ml of a 0.035 per cent w/v solution of *anthrone* in *sulphuric acid*; a bluish-violet colour is produced.

### Tests

**pH** (2.4.24). 2.0 to 4.0 (1:2 salt), or 6.0 to 7.3 (1:1.8 salt), determined in a 1.0 per cent w/v solution in *carbon dioxide-free water*.

**Specific optical rotation** (2.4.22). +76.0° to +84.0°, determined in a 2.0 per cent w/v solution.

**Sulphated ash** (2.3.18). Not more than 1.0 per cent, the charred residue being moistened with 2 ml of *nitric acid* and 5 drops of *sulphuric acid*.

**Loss on drying** (2.4.19). Not more than 13.0 per cent, determined on 0.1 g by drying in an oven over *phosphorus pentoxide* at 110° at a pressure not exceeding 0.7 kPa for 3 hours.

**Assay**. Determine by the microbiological assay of antibiotics, Method B (2.2.10), and express the result in µg of amikacin, $C_{22}H_{43}N_5O_{13}$, per mg.

**Labelling**. The label states (1) whether the molar ratio of amikacin to $H_2SO_4$ of the contents is 1:2 or 1:1.8; (2) whether the material is intended for use in the manufacture of parenteral preparations.

## Amikacin Injection

Amikacin Sulphate Injection

Amikacin Injection is a sterile solution of Amikacin Sulphate in Water for Injections or of Amikacin in Water for Injections prepared with the aid of Sulphuric Acid.

Amikacin Injection contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of amikacin, $C_{22}H_{43}N_5O_{13}$.

**Usual strengths**. The equivalent of 100 mg or 500 mg of amikacin in 2 ml.

### Identification

Dilute the injection to obtain a solution containing 6 mg of amikacin per ml (test solution). The test solution complies with the following tests.

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 60 volumes of *methanol*, 30 volumes of *strong ammonia solution* and 25 volumes of *chloroform*.

*Reference solution (a)*. A 0.6 per cent w/v solution of *amikacin RS*.

*Reference solution (b)*. A mixture of equal volumes of the test solution and reference solution (a).

Apply to the plate 3 µl of each solution. After development, allow the plate to dry in air, heat it at 110° for 15 minutes and immediately spray it with a 1 per cent w/v solution of *ninhydrin* in a mixture of 100 volumes of 1-*butanol* and 1 volume of *pyridine*. The principal pink-coloured spot in the chromatogram obtained with the test solution corresponds to those in the chromatograms obtained with reference solutions (a) and (b).



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B. To 1.5 ml of the test solution add 1 ml of *2 M sodium hydroxide*, mix and add 2 ml of a 1 per cent w/v solution of *cobalt nitrate*; a violet colour is produced.

C. To 1.5 ml of the test solution add 3.5 ml of *water* mix and add 4 ml of a 0.035 per cent w/v solution of *anthrone* in *sulphuric acid*; a bluish-violet colour is produced.

### Tests

**pH** (2.4.24). 3.5 to 5.5.

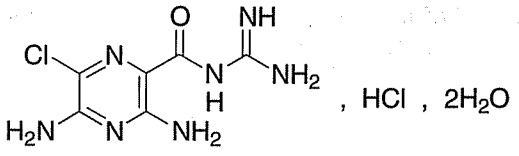
**Bacterial Endotoxins** (2.2.3). Not more than 0.33 Endotoxin unit per mg of amikacin.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. Dilute the injection to obtain a solution containing 1 mg of amikacin per ml. Determine by the microbiological assay of antibiotics, Method B, (2.2.10) and express the result in mg of amikacin, $C_{22}H_{43}N_5O_{13}$ per ml.

**Labelling**. The label states the quantity of Amikacin Sulphate contained in the sealed container in terms of the equivalent amount of amikacin.

## Amiloride Hydrochloride




$C_6H_8ClN_7O,HCl,2H_2O$ Mol. Wt. 302.1

Amiloride Hydrochloride is *N*-amidino-3,5-diamino-6-chloropyrazine-2-carboxamide hydrochloride dihydrate.

Amiloride Hydrochloride contains not less than 98.0 per cent and not more than 101.0 per cent of $C_6H_8ClN_7O,HCl$, calculated on the anhydrous basis.

**Category**. Diuretic.

**Dose**. Initially, 5 to 10 mg daily; maximum 20 mg daily.

**Description**. A pale yellow to greenish-yellow powder.

### Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.*

A. Determine by infrared absorption spectrphotometry (2.4.6). Compare the spectrum with that obtained with *amiloride hydrochloride RS* or with the reference spectrum of amiloride hydrochloride.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with a suitable silica gel.

*Mobile phase*. A freshly prepared mixture of 88 volumes of *dioxan*, 6 volumes of *dilute ammonia solution* and 6 volumes of *water*.

*Test solution*. Dissolve 0.2 g of the substance under examination in sufficient *methanol* to produce 50 ml.

*Reference solution*. A 0.4 per cent w/v solution of *amiloride hydrochloride RS* in *methanol*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air and examine in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

C. Dissolve 10 mg in 10 ml of *water* and add 10 ml of a 20 per cent w/v solution of *cetrimide*, 0.25 ml of *2 M sodium hydroxide* and 1 ml of *bromine water*; a greenish-yellow colour is produced. Add 2 ml of *2 M hydrochloric acid*; the solution becomes deep yellow and exhibits a blue fluorescence in ultraviolet light at 365 nm.

D. A 5 per cent w/v solution gives the reactions of chlorides (2.3.1).

### Tests

**Free acid**. Dissolve 1.0 g in 100 ml of a mixture of equal volumes of *methanol* and *water* and titrate with *0.1 M sodium hydroxide* determining the end-point potentiometrically (2.4.25); not more than 0.3 ml is required.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. A mixture of 1 volume of *acetonitrile* and 3 volumes of *water*.

*Test solution* (a) Dissolve 0.2 g of the substance under examination in 100 ml of solvent mixture.

*Test solution (b)*. Dilute 1 ml of test solution (a) to 100 ml with the same solvent mixture.

*Test solution (c)*. Dilute 10 ml of test solution (b) to 100 ml with the same solvent mixture.

*Reference solution*. A 0.001 per cent w/v solution of *methyl 3,5-diamino- 6-chloropyrazine-2-carboxylate RS* in the same solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm) (such as Nucleosil C18),
- mobile phase: a mixture of 745 volumes of *water*, 250 volumes of *acetonitrile* and 5 volumes of



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*tetramethylammonium hydroxide solution* (10 per cent), the pH of the mixture being adjusted to 7.0 with a mixture of 1 volume of *phosphoric acid* and 9 volumes of *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject the reference solution and adjust the concentration of *acetonitrile* so that the retention time of methyl 3,5-diamino-6-chloropyrazine-2-carboxylate is 5 to 6 minutes (an increase in the concentration of *acetonitrile* reduces the retention time). Inject 20 µl of test solution (b) and adjust the concentrations of *tetramethylammonium hydroxide* and *orthophosphoric acid* so that the retention time of amiloride is 9 to 12 minutes keeping the pH at 7.0 (an increase in the concentrations reduces the retention time).

Inject each of test solution (a) and the reference solution and allow the chromatography to proceed for 5 times the retention time of amiloride. In the chromatogram obtained with test solution (a) the sum of the areas of any secondary peaks is not greater than the area of the peak due to methyl 3,5-diamino-6-chloro- pyrazine-2-carboxylate in the chromatogram obtained with the reference solution. Ignore any peak with an area less than 10 per cent of the area of the peak due to methyl 3,5-diamino-6- chloropyrazine-2-carboxylate in the chromatogram obtained with the reference solution.

Inject test solution (c). The test is not valid if the signal-to-noise ratio of the peak due to amiloride in the chromatogram obtained with this solution is less than 5.0.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). 11.0 to 13.0 per cent, determined on 0.2 g.

**Assay**. Weigh accurately about 0.25 g and dissolve in a mixture of 100 ml of *anhydrous glacial acetic acid* and 15 ml of *dioxan* and add 10 ml of *mercuric acetate solution* Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02661 g of $C_6H_8ClN_7O,HCl$.

**Storage**. Store protected from light.

# Amiloride Tablets

## Amiloride Hydrochloride Tablets

Amiloride Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of anhydrous amiloride hydrochloride, $C_6H_8ClN_7O,HCl$.

**Usual strength**. 5 mg.

### Identification

A. Extract a quantity of the powdered tablets containing 0.5 mg of anhydrous amiloride hydrochloride with 100 ml of *0.1 M hydrochloric acid* and filter. When examined in the range 230 nm to 380 nm (2.4.7), the solution shows absorption maxima at about 285 nm and at about 363 nm.

B. Carry out the method described under Related substances using the following solutions.

*Test solution*. Shake a quantity of the powdered tablets containing 10 mg of anhydrous amiloride hydrochloride with 10 ml of *methanol* and centrifuge.

*Reference solution*. A 0.1 per cent w/v solution of *amiloride hydrochloride RS* in *methanol*.

The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

### Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), using a precoated silica gel plate (such as Merck silica gel 60 plates).

*Mobile phase*. A freshly prepared mixture of 90 volumes of *dioxan* and 12 volumes of *3 M ammonia*.

*Test solution*. Shake a quantity of the powdered tablets containing 17.5 mg of anhydrous amiloride hydrochloride with 5 ml of *methanol* and centrifuge.

Reference solution (a). A 0.002 per cent w/v solution of *methyl 3,5-diamino-6-chloropyrazine-2-carboxylate RS* in *methanol*.

Reference solution (b). A 0.0008 per cent w/v solution of *methyl 3,5-diamino-6-chloropyrazine-2- carboxylate RS* in *methanol*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 365 nm. Any spot corresponding to methyl 3,5-diamino-6-chloropyrazine-2-carboxylate in the chromatogram obtained with the test solution is not more intense than the spot in chromatogram obtained with reference solution (a). Any other secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

**Uniformity of content**. Comply with the test stated under Tablets.

Powder one tablet and transfer to a 100-ml volumetric flask, add 60 ml of *0.1 M hydrochloric acid*, and shake by mechanical means for 30 minutes. Dilute with *0.1 M*



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*hydrochloric acid* to volume, mix, and centrifuge a portion of the mixture. Dilute an accurately measured portion of the clear supernatant liquid quantitatively to obtain a solution containing about 10 µg of amiloride hydrochloride per ml. Measure the absorbance of the resulting solution at the maximum at about 363 nm (2.4.7).

Calculate the content of $C_6H_8ClN_7O$, HCl taking 692 as the specific absorbance at 363 nm.

**Other tests**. Comply with the tests stated under Tablets.

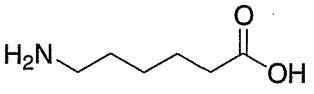
**Assay**. Weigh and finely powder 20 tablets. Weigh accurately a quantity of the powder containing about 10 mg of anhydrous amiloride hydrochloride, transfer to a 100-ml volumetric flask, add 60 ml of *0.1 M hydrochloric acid*, and shake by mechanical means for 30 minutes. Dilute with *0.1 M hydrochloric acid* to volume, mix, and centrifuge a portion of the mixture. Dilute an accurately measured portion of the clear supernatant liquid quantitatively to obtain a solution containing about 10 µg of amiloride hydrochloride per ml. Measure the absorbance of the resulting solution at the maximum at about 363 nm (2.4.7).

Calculate the content of $C_6H_8ClN_7O$,HCl taking 692 as the specific absorbance at 363 nm.

**Storage**. Store protected from light.

**Labelling**. The label states the strength in terms of the equivalent amount of anhydrous amiloride hydrochloride.

# Aminocaproic Acid




$C_6H_{13}NO_2$ Mol. Wt. 131.2

Aminocaproic Acid is 6-aminohexanoic acid.

Aminocaproic Acid contains not less than 98.5 per cent and not more than 101.0 per cent of $C_6H_{13}NO_2$, calculated on the dried basis.

**Category**. Haemostatic; antifibrinolytic.

**Dose**. Orally and by slow intravenous infusion, initially 5 g followed by 1 to 1.25 g every hour until bleeding is under control; not more than 30 g per 24-hour period.

**Description**. Colourless crystals or a white, crystalline powder.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *aminocaproic acid RS*.

B. Determine by thin-layer chromatography (2.4.7), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 25 volumes of *ethanol (95 per cent)*, 3 volumes of *water* and 4 volumes of *strong ammonia solution*.

*Test solution*. Dissolve 0.25 g of the substance under examination in 100 ml of *water*.

*Reference solution*. A 0.25 per cent w/v solution of *aminocaproic acid RS*.

Apply to the plate 2 µl of each solution. After development, remove the plate, spray it with a 0.25 per cent w/v solution of *ninhydrin* in a mixture of equal volumes of *methanol* and *pyridine* and heat at 105° for 2 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

## Tests

**Appearance of solution**. A 20.0 per cent w/v solution remains clear for 24 hours (2.4.1), and is colourless (2.4.1).

**pH** (2.4.24). 7.5 to 8.0, determined in a 20.0 per cent w/v solution.

**Stability**. Place 20.0 g evenly spread in a shallow dish about 9 cm in diameter, cover and allow to stand at 100° ± 2° for 72 hours. Dissolve in sufficient *water* to produce 100.0 ml. Prepare a 20.0 per cent w/v solution of the substance under examination but without the above treatment. Measure the absorbances (2.4.7) of the two solutions at the maximum at about 287 nm and at about 450 nm. Absorbance of the solution prepared from the exposed substance being examined at the maximum at about 287 nm is not more than 0.15 and of the solution of the substance under examination without the above treatment, at the maximum at about 287 nm is not more than 0.10. Absorbance of both solutions at the maximum at about 450 nm is not more than 0.03.

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (10 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.2 g, dissolve in about 100 ml of *anhydrous glacial acetic acid* with gentle heat to effect solution, cool and add 15 ml of *mercuric acetate* solution. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01312 g of $C_6H_{13}NO_2$.



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# Aminocaproic Acid Injection

Aminocaproic Acid Injection is a sterile solution of Aminocaproic Acid in Water for Injections.

Aminocaproic Acid Injection contains not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of aminocaproic acid, $C_6H_{13}NO_2$.

**Usual strength**. 400 mg per ml.

## Identification

To a volume containing 0.4 g of Aminocaproic Acid add 2 ml of *ether*, stir, add 2 ml of *methanol*, stir again and allow to stand; the crystals after drying on a water-bath comply with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *aminocaproic acid RS*.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 25 volumes of *ethanol (95 per cent)*, 3 volumes of *water* and 4 volumes of *strong ammonia solution*.

*Test solution*. Dissolve 0.25 g of the substance under examination in 100 ml of *water*.

*Reference solution*. A 0.25 per cent w/v solution of *aminocaproic acid RS*.

Apply to the plate 2 µl of each solution. After development, remove the plate, spray it with a 0.25 per cent w/v solution of *ninhydrin* in a mixture of equal volumes of *methanol* and *pyridine* and heat at 105° for 2 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

## Tests

**pH** (2.4.24). 6.0 to 7.6.

**Bacterial endotoxins** (2.2.3). Not more than 0.05 Endotoxin Unit per mg of aminocaproic acid.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. To a volume containing 0.2 g of Aminocaproic Acid add 10 ml of *ethanol* and evaporate to dryness on a water-bath. Dissolve the residue in 100 ml of *anhydrous glacial acetic acid* by gentle heating, if necessary, cool and add 15 ml of *mercuric acetate* solution. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01312 g of $C_6H_{13}NO_2$.

# Aminocaproic Acid Tablets

Aminocaproic Acid Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of aminocaproic acid, $C_6H_{13}NO_2$.

**Usual strength**. 500 mg.

## Identification

Triturate 2 tablets with 10 ml of *water* and filter into 100 ml of *acetone*. Swirl the mixture and allow to stand for 15 minutes to complete crystallisation. Filter through a medium porosity, sintered-glass filter and wash the crystals with 25 ml of *acetone*. Apply vacuum to remove the solvent, dry at 105° for 30 minutes and cool. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *aminocaproic acid RS*.

B. Determine by thin-layer chromatography (2.4.7), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 25 volumes of *ethanol (95 per cent)*, 3 volumes of *water* and 4 volumes of *strong ammonia solution*.

*Test solution*. Dissolve 0.25 g of the substance under examination in 100 ml of *water*.

*Reference solution*. A 0.25 per cent w/v solution of *aminocaproic acid RS*.

Apply to the plate 2 µl of each solution. After development, remove the plate, spray it with a 0.25 per cent w/v solution of *ninhydrin* in a mixture of equal volumes of *methanol* and *pyridine* and heat at 105° for 2 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

## Tests

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.2 g of Aminocaproic Acid, add about 100 ml of *anhydrous glacial acetic acid*, heat gently to effect solution, cool and add 15 ml of *mercuric acetate* solution. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1ml of *0.1 M perchloric acid* is equivalent to 0.01312 g of $C_6H_{13}NO_2$.



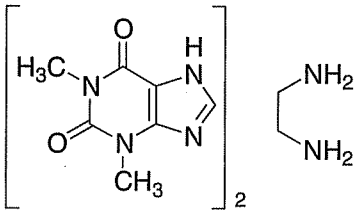
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# Aminophylline

Theophylline and Ethylenediamine



$(C_7H_8N_4O_2)_2,C_2H_8N_2$ Mol. Wt. 420.4 (anhydrous)

Aminophylline is a stable mixture or combination of theophylline and ethylenediamine. It may be anhydrous or may contain not more than two molecules of water of hydration.

Aminophylline contains the equivalent of not less than 84.0 per cent and not more than 87.4 per cent of theophylline, $C_7H_8N_4O_2$, and the equivalent of not less than 13.5 per cent and not more than 15.0 per cent of ethylenediamine, $C_2H_8N_2$, both calculated on the anhydrous basis.

**Category**. Bronchodilator.

**Dose**. Orally, 100 to 300 mg; by slow intravenous injection, 250 to 500 mg.

**Description**. A white or slightly yellowish granules or powder; odour, slightly ammoniacal. On exposure to air it gradually loses ethylenediamine and absorbs carbon dioxide with liberation of free theophylline. Even in the absence of light, it is gradually decomposed on exposure to a humid environment, the degradation being faster at higher temperatures.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

Dissolve 1 g in 10 ml of *water* and add 2 ml of *dilute hydrochloric acid* dropwise, with shaking. Separate the precipitate by filtration and reserve the filtrate for test D. Wash the precipitate with successive small quantities of cold *water*, recrystallise from hot *water* and dry at 100° to 105°. The residue complies with the following tests.

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

B. To 10 mg of the residue obtained in test A add 1 ml of *hydrochloric acid* in a porcelain dish and 0.1 g of *potassium chlorate* and evaporate to dryness on a water-bath; invert the dish over a vessel containing a few drops of *dilute ammonia solution*; the residue acquires a purple colour. Add a few drops of *dilute sodium hydroxide solution*; the colour is discharged.

C. Saturate in *water* a portion of the residue obtained in test A and add *tannic acid solution*; a precipitate soluble in excess of the reagent is produced.

D. The filtrate complies with the following test.

To the filtrate reserved above add 0.2 ml of *benzoyl chloride*, make alkaline with *2 M sodium hydroxide* and shake vigorously. Filter, wash the precipitate with 10 ml of *water*, dissolve in 5 ml of hot *ethanol (95 per cent)* and add 5 ml of *water*. The precipitate, after washing with *water* and drying at 100° to 105° melts at 248° to 252° (2.4.21).

## Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17) coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 40 volumes of *1-butanol*, 30 volumes of *acetone*, 30 volumes of *chloroform* and 10 volumes of *strong ammonia solution*.

*Test solution*. Dissolve 0.2 g of the substance under examination in 2 ml of *water* with the aid of heat and dilute to 10 ml with *methanol*.

*Reference solution*. Dilute 1 volume of the test solution to 200 volumes with *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Heavy metals** (2.3.13). A 8.0 per cent w/v solution complies with the limit test for heavy metals, Method A (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 1.5 per cent (for anhydrous), determined on 2.0 g dissolved in 20 ml of *pyridine*. 3.0 to 8.0 per cent (for hydrate), determined on 0.5 g.

**Assay**. *For theophylline* — Determine by liquid chromatography (2.4.14).

*Solvent mixture*. A mixture of 80 volumes of *water* and 20 volumes of *methanol*.

*Test solution*. Dissolve 24 mg of the substance under examination in 250 ml with the solvent mixture.

*Reference solution (a)*. A 0.008 per cent w/v solution of *theophylline RS* in the solvent mixture.

*Reference solution (b)*. A 0.008 per cent w/v solution of theobromine in reference solution (a). Dilute 20.0 ml of this solution to 25 ml with the solvent mixture.



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Chromatographic system
- a stainless steel column 15 cm x 3.9 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 200 ml of *methanol* and 960 mg of *sodium 1-pentanesulphonate*, diluted to 1000 ml with *water*, adjust the pH to 2.9 with *glacial acetic acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Inject reference solution (b). The relative retention time for theobromine with respect to theophylline is about 0.65. The test is not valid unless the tailing factor for theophylline is not more than 2.0, the resolution between the peaks due to theobromine and theophylline is not less than 3.0 and the relative standard deviation for the replicate injection is not more than 2.0 per cent.

Inject the test solution and reference solution (a).

Calculate the content of the theophylline ($C_7H_8N_4O_2$).

*For ethylenediamine* — Weigh accurately about 0.25 g and dissolve in 30 ml of *water*. Titrate with *0.1 M hydrochloric acid* using *methyl orange solution* as indicator.

1 ml of *0.1 M hydrochloric acid* is equivalent to 0.003005 g of $C_2H_8N_2$.

**Storage**. Store protected from light and from atmospheric carbon dioxide.

# Aminophylline Injection

## Theophylline and Ethylenediamine Injection

Aminophylline Injection is a sterile solution of Aminophylline in Water for Injections or is a sterile solution of Theophylline in a solution of Ethylenediamine Hydrate in Water for Injections free from carbon dioxide. Aminophylline Injection may contain an excess of ethylenediamine but no other substance may be added.

Aminophylline Injection contains theophylline, $C_7H_8N_4O_2$, equivalent to not less than 73.25 per cent and not more than 88.25 per cent of the stated amount of aminophylline, and not more than 0.295 g of ethylenediamine, $C_2H_8N_2$ for each g of anhydrous theophylline, $C_7H_8N_4O_2$, determined in the Assay for theophylline.

**Usual strengths**. 250 mg in 10 ml; 500 mg in 2 ml.

### Identification

Dilute a volume containing about 0.5 g of aminophylline with *water* to about 25 ml and add 1 ml of *dilute hydrochloric acid* with constant stirring. Separate the precipitate by filtration and reserve the filtrate for test D. Wash the precipitate with a small portion of cold *water*, recrystallise from hot *water* and dry at 100° to 105°. The crystalline powder complies with the following tests.

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

B. To 10 mg add 1 ml of *hydrochloric acid* in a porcelain dish and 0.1 g of *potassium chlorate* and evaporate to dryness on a water-bath; invert the dish over a vessel containing a few drops of *dilute ammonia solution*; the residue acquires a purple colour. Add a few drops of *dilute sodium hydroxide solution*; the colour is discharged.

C. Saturate a portion in *water* and add *tannic acid solution*; a precipitate soluble in excess of the reagent is produced.

D. The filtrate complies with the following test.

Add 0.2 ml of *benzoyl chloride*, make alkaline with *2 M sodium hydroxide* and shake vigorously. Filter, wash the precipitate with 10 ml of *water*, dissolve in 5 ml of hot *ethanol (95 per cent)* and add 5 ml of *water*. The precipitate, after washing with *water* and drying at 100° to 105° melts at 248° to 252° (2.4.21).

### Tests

**pH** (2.4.24). 8.8 to 10.0.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. *For theophylline* — Determine by liquid chromatography (2.4.14).

*Solvent mixture*. A mixture of 80 volumes of *water* and 20 volumes of *methanol*.

*Test solution*. Measure accurately a volume containing about 100 mg of theophylline to 100 ml with solvent mixture. Dilute 4.0 ml of this solution to 50.0 ml with the solvent mixture.

*Reference solution (a)*. A 0.008 per cent w/v solution of *theophylline RS* in the solvent mixture.

*Reference solution (b)*. A 0.008 per cent w/v solution of theobromine in reference solution (a). Dilute 20.0 ml of this solution to 25 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 3.9 mm, packed with octylsilane bonded to porous silica (5µm),
- mobile phase: a mixture of 200 ml of *methanol* and 960 mg of *sodium 1-pentanesulfonate*, diluted to 1000 ml with *water*, adjust the pH to 2.9 with *glacial acetic acid*,
- flow rate. 1 ml per minute,



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– spectrophotometer set at 254 nm
– injection volume. 10 µl.

Inject reference solution (b). The relative retention time for theobromine with respect to theophylline is about 0.65. The test is not valid unless the tailing factor for theophylline is not more than 2.0, the resolution between the peaks due to theobromine and theophylline is not less than 3.0 and the relative standard deviation for the replicate injection is not more than 2.0 per cent.

Inject the test solution and reference solution (a).

Calculate the content of the theophylline ($C_7H_8N_4O_2$).

*For ethylenediamine* — To a volume containing about 0.25 g of aminophylline, add sufficient *water* to produce 30 ml. Titrate with *0.1 M hydrochloric acid* using *methyl orange solution* as indicator.

1 ml of *0.1 M hydrochloric acid* is equivalent to 0.003005 g of $C_2H_8N_2$.

**Storage**. Store in single dose containers, from which carbon dioxide has been excluded. Do not allow contact with metals.

**Labelling**. The label states (1) the strength in terms of the equivalent amount of anhydrous aminophylline in a suitable dose-volume; (2) the route of injection; (3) that the injection is not to be used if crystals have separated.

# Aminophylline Tablets

Theophylline and Ethylenediamine Tablets

Aminophylline Tablets contain theophylline, $C_7H_8N_4O_2$, equivalent to not less than 80.6 per cent and not more than 90.8 per cent of the stated amount of aminophylline, and ethylenediamine, $C_2H_8N_2$, equivalent to not less than 10.9 per cent of the stated amount of aminophylline.

**Usual strength**. 100 mg.

## Identification

Shake a quantity of the powdered tablets containing about 0.5 g of aminophylline with 25 ml of *water* and filter. To the filtrate add 1 ml of *dilute hydrochloric acid* with constant stirring. Separate the precipitate by filtration and reserve the filtrate. Wash the precipitate with a small portion of cold *water*, recrystallise from hot *water* and dry at 100° to 105°. The crystalline powder complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *theophylline RS*.

B. To 10 mg add 1 ml of *hydrochloric acid* in a porcelain dish and 0.1 g of *potassium chlorate* and evaporate to dryness on a water-bath; invert the dish over a vessel containing a few drops of *dilute ammonia solution*; the residue acquires a purple colour. Add a few drops of *dilute sodium hydroxide solution*; the colour is discharged.

C. Saturate a portion in *water* and add *tannic acid solution*; a precipitate soluble in excess of the reagent is produced.

The filtrate complies with the following test.

Add 0.2 ml of *benzoyl chloride*, make alkaline with *2 M sodium hydroxide* and shake vigorously. Filter, wash the precipitate with 10 ml of *water*, dissolve in 5 ml of hot *ethanol (95 per cent)* and add 5 ml of *water*. The precipitate, after washing with *water* and drying at 100° to 105° melts at 248° to 252° (2.4.21).

## Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *water*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with *water* if necessary, at the maximum at about 269 nm (2.4.7). Calculate the content of $C_7H_8N_4O_2$ in the medium from the absorbance obtained from a known concentration of *theophylline RS* in the same medium.

D. Not less than 70 per cent of the stated amount of $C_7H_8N_4O_2$.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. *For theophylline* — Weigh and powder 20 tablets. Weigh accurately a quantity of the powdered tablets containing about 0.5 g of aminophylline, transfer to a 200-ml volumetric flask with the aid of a mixture of 50 ml of *water* and 15 ml of *dilute ammonia solution* and allow to stand for 30 minutes with frequent shaking, warming to about 50°, if necessary. Cool, add *water* to volume and mix. Centrifuge the mixture, and pipette a volume of the clear supernatant liquid equivalent to about 0.25 g of aminophylline into a flask, dilute with sufficient *water* to produce 40 ml and add 8 ml of *dilute ammonia solution*. Add 20.0 ml of *0.1 M silver nitrate*, mix and boil for 15 minutes. Cool to between 5° and 10° for 20 minutes, filter at a pressure not exceeding 2.75 kPa and wash the precipitate with three quantities, each of 10 ml, of *water*. Acidify the combined filtrate and washings with *nitric acid* and add an excess of 3 ml of the acid. Cool, add 2 ml of *ferric ammonium sulphate solution*, and titrate with *0.1 M ammonium thiocyanate*.

1 ml of *0.1 M silver nitrate* is equivalent to 0.01802 g of $C_7H_8N_4O_2$.

*For ethylenediamine* — Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.3 g of



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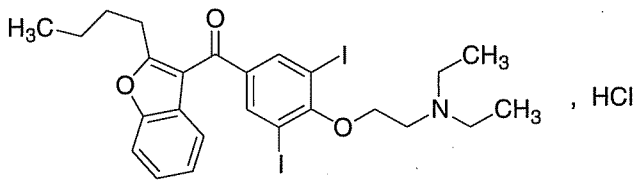
aminophylline, shake with 20 ml of *water*, heat at 50° for 30 minutes. Titrate with *0.1 M hydrochloric acid* using *methyl orange solution* as indicator.

1 ml of *0.1 M hydrochloric acid* is equivalent to 0.003005 g of $C_2H_8N_2$.

**Storage**. Store protected from light.

**Labelling**. The label states the strength in terms of the equivalent amount anhydrous aminophylline.

## Amiodarone Hydrochloride



$C_{25}H_{29}I_2NO_3,HCl$ Mol. Wt. 681.8

Amiodarone Hydrochloride is 2-butylbenzofuran-3-yl-4-(2-diethylaminoethoxy)-3,5-diiodophenyl ketone hydrochloride.

Amiodarone Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{25}H_{29}I_2NO_3,HCl$, calculated on the dried basis.

**Category**. Antiarrhythmic.

**Dose**. Initial dose, 200 mg three times daily for one week, reduced to 200 mg twice daily for a further week; usual maintenance dose, 200 mg daily.

**Description**. A white or almost white, fine crystalline powder.

### Identification

*Test B may be omitted if tests A and C are carried out. Test A may be omitted if tests B and C are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *amiodarone hydrochloride RS*.

B. In the test for Related substances the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

C. Gives reaction A of chlorides (2.3.1).

### Tests

**Appearance of solution**. A 5.0 per cent w/v solution is clear (2.4.1), and not more intensely coloured than reference solution GYS5 (2.4.1).

**pH** (2.4.24). 3.2 to 3.8, determined in 5.0 per cent w/v solution, prepared by dissolving in *carbon dioxide-free water* at 80° and cooling.

**Related substances**. Determine by thin layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*All the solutions should be protected from light and should be used immediately after preparation.*

*Mobile phase*. A mixture of 5 volumes of *anhydrous formic acid*, 10 volumes of *methanol* and 85 volumes of *dichloromethane*.

*Test solution (a)*. Dissolve 10 g of the substance under examination in 100 ml of *dichloromethane*.

*Test solution (b)*. Dissolve 0.5 g of the substance under examination in 100 ml of *dichloromethane*.

*Reference solution (a)*. A 0.5 per cent w/v solution of *amiodarone hydrochloride RS* in *dichloromethane*.

*Reference solution (b)*. A 0.05 per cent w/v solution of the substance under examination in *dichloromethane*.

*Reference solution (c)*. A 0.025 per cent w/v solution of the substance under examination in *dichloromethane*.

Reference solution (d). A 0.02 per cent w/v of *(2-chloroethyl) diethylamine hydrochloride RS* in *dichloromethane*.

Apply to the plate 5 µl of each solution. After development, dry in a current of cold air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (c) (0.25 per cent). Spray with *potassium iodobismuthate solution*. Examine immediately in daylight. Any spot corresponding to (2-chloroethyl) diethylamine hydrochloride in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (d) (0.2 per cent).

**Iodides**. Dissolve 1.5 g in 40 ml of water at 80° by shaking until completely dissolved. Cool and dilute to 50 ml with *water* (Solution A).

To 15 ml of solution A add 1 ml of *0.1 M hydrochloric acid* and 1ml of *0.05 M potassium iodate* and dilute to 20 ml with *water*. Allow to stand protected from light for 4 hours (Solution 1). To 15 ml of solution A add 1 ml of *0.1 M hydrochloric* acid, 1 ml of an 88.2 ppm solution of *potassium iodide* and 1 ml of *0.05 M potassium iodate* and dilute to 20 ml with *water*. Allow to stand protected from light for 4 hours (Solution 2). Measure the absorbances of solutions (1) and (2) at the maximum at about 420 nm, using as the blank a mixture of 15 ml of solution A and 1 ml of *0.1 M hydrochloric* acid diluted to 20 ml with



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*water* (2.4.7). The absorbance of solution (1) is not greater than half the absorbance of solution (2) (150 ppm).

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

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying at 100° at a pressure not exceeding 0.3 kPa for 4 hours.

**Assay**. Weigh accurately about 0.6 g and dissolve in a mixture of 5.0 ml of *0.01 M hydrochloric acid* and 75 ml of *ethanol (95 per cent)*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.06818 g of $C_{25}H_{29}I_2NO_3$, HCl.

**Storage**. Store protected from light, at a temperature not exceeding 30°.

# Amiodarone Tablets

Amiodarone Hydrochloride Tablets

Amiodarone Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of amiodarone hydrochloride, $C_{25}H_{29}I_2NO_3$, HCl.

**Usual strengths**. 100 mg; 200 mg.

## Identification

A. Shake a quantity of the powdered tablets containing about 0.3 g of Amiodarone Hydrochloride with 25 ml of *dichloromethane*, filter and evaporate the filtrate to dryness. To the residue, add 2 ml of *1 M sodium hydroxide* and extract with 25 ml of *ether*. Dry the extract over *anhydrous sodium sulphate*, filter and evaporate to dryness. Dry the residue obtained under reduced pressure over *phosphorus pentoxide* and dissolve in 2.5 ml of *dichloromethane*. The solution complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *amiodarone hydrochloride RS*, treated in the same manner.

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

## Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*

*Mobile phase*. A mixture of 85 volumes of *dichloromethane*, 10 volumes of *methanol* and 5 volumes of *anhydrous formic acid*.

*Test solution*. Shake a quantity of the powdered tablets containing about 50 mg of Amiodarone Hydrochloride with 20 ml of *methanol* and filter.

*Reference solution (a)*. Dilute 1 volume of the test solution to 200 volumes with *methanol*.

*Reference solution (b)*. A 0.00125 per cent w/v solution of *2-butyl-3-(4-hydroxy-3, 5-diiodobenzoyl) benzofuran RS* in *methanol*.

Apply separately to the plate (pre-washed with the mobile phase and dried in air before use) 10 µl of each solution After development, dry the plate in a current of warm air and examine in ultraviolet light at 254 nm. In the chromatogram obtained with the test solution any spot corresponding to 2-butyl-3 (4-hydroxy-3, 5-diiodobenzoyl)benzofuran is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent) and any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent).

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets.

Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh accurately a quantity of the powdered tablets containing about 0.1g of Amiodarone Hydrochloride, add 70 ml of *methanol*, mix with the aid of ultrasound for 15 minutes, cool and dilute to 100.0 ml with the same solvent and filter. Dilute 10.0 ml of the filtrate to 100.0 ml with the mobile phase.

*Reference solution*. Dissolve 0.1g of *amiodarone hydrochloride RS* in 70 ml of *methanol*, cool and dilute to 100.0 ml with the same solvent. Dilute 10.0 ml of the resulting solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 7.5 cm x 3.9 mm, packed with very finely divided silica gel consisting of porous spherical particles with chemically bonded nitrile group (4 µm), (such as Nova-Pack CNHP),
- mobile phase: a mixture of 45 volumes of *0.01 M sodium perchlorate* and 55 volumes of *acetonitrile*, adjusted to pH 3.0 with 2 *M orthophosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 244 nm,
- injection volume. 20 µl.

Inject the reference solution. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{25}H_{29}I_2NO_3$, HCl in the tablets.

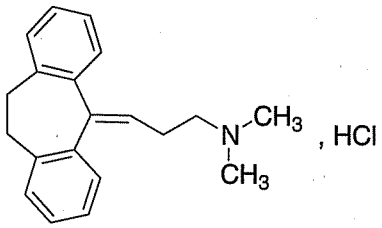


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# Amitriptyline Hydrochloride



$C_{20}H_{23}N,HCl$ Mol. Wt. 313.9

Amitriptyline Hydrochloride is 3-(10,11-dihydro-5*H*-dibenzo[*a,d*]cycloheptene-5-ylidene)propyldimethylamine hydrochloride.

Amitriptyline Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{20}H_{23}N$, HCl, calculated on the dried basis.

**Category**. Antidepressant.

**Dose**. 50 to 75 mg daily, in divided doses; maintenance dose, 50 to 100 mg daily, in divided doses.

**Description**. Colourless crystals or a white or almost white powder; almost odourless.

## Identification

*Test A may be omitted if tests B, C, and D are carried out. Tests B and C may be omitted if tests A and D are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *amitriptyline hydrochloride RS* or with the reference spectrum of amitriptyline hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.0012 per cent w/v solution in *methanol* shows an absorption maximum only at about 239 nm; absorbance at about 239 nm, about 0.55.

C. To about 50 mg dissolved in 3 ml of *water* add 1 drop of a 2.5 per cent w/v solution of *quinhydrone* in *methanol;* no red colour is produced within 15 minutes (distinction from nortriptyline).

D. Gives the reactions of chlorides (2.3.1).

## Tests

**Appearance of solution**. Dissolve 1.25 g in sufficient *water* to produce 25 ml. The solution is clear (2.4.1) and not more intensely coloured than reference solution BS8 (2.4.1).

**pH** (2.4.24). 4.5 to 6.0, determined in a 1.0 per cent w/v solution.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 100 mg of the substance under examination in 100 ml of the mobile phase.

*Reference solution*. A 0.0001 per cent w/v solution of *amitriptyline hydrochloride RS* in the mobile phase.

Chromatographic system as described under Assay.

The relative retention time with respect to amitriptyline hydrochloride for amitriptyline impurity A RS is about 0.35, for amitriptyline impurity B RS is about 0.52, for nortriptyline hydrochloride is about 0.60, for cyclobenzaprine hydrochloride is about 0.76.

Inject the test solution and the reference solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.2 times the area of the peak in the chromatogram obtained with the reference solution (0.2 per cent) and the sum of areas of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with the reference solution (1.0 per cent). Disregard any peak with an relative retention time of less than 0.22.

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

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 100 mg of the substance under examination in 100 ml of the mobile phase. Dilute 10 ml of this solution to 50 ml with the mobile phase.

*Reference solution*. A 0.02 per cent w/v solution of *amitriptyline hydrochloride RS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 45°,
- mobile phase: a mixture of 70 volumes of buffer solution prepared by dissolving 1.42 g of *dibasic sodium phosphate* in 1000 ml of *water*, adjust pH to 7.7 with *dilute orthophosphoric acid*, and 30 volumes of *methanol.*
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for the replicate injection is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{20}H_{23}N,HCl$.

**Storage**. Store protected from light.



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# Amitriptyline Tablets

Amitriptyline HydrochlorideTablets

Amitriptyline Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of amitriptyline hydrochloride, $C_{20}H_{23}N$, HCl. The tablets are coated.

**Usual strengths**. 10 mg; 25 mg; 50 mg.

## Identification

A. Shake a quantity of the powdered tablets containing about 5 mg of Amitriptyline Hydrochloride with 20 ml of *methanol* and filter. To 1 ml of the filtrate add 1 ml of a 2.5 per cent w/v solution of *sodium bicarbonate*, 1 ml of a 2 per cent w/v solution of *sodium periodate* and 1 ml of a 0.3 per cent w/v solution of *potassium permanganate*, allow to stand for 15 minutes, acidify with *dilute sulphuric acid* and extract with 10.0 ml of *2,2,4-trimethylpentane*. When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum only at about 265 nm.

B. Triturate a quantity of the powdered tablets containing 0.1 g of Amitriptyline Hydrochloride with 10 ml of *chloroform*, filter and evaporate the filtrate to a low volume. Add *ether* until a turbidity is produced and allow to stand. To about 50 mg of the precipitate dissolved in 3 ml of *water* add 1 drop of a 2.5 per cent w/v solution of *quinhydrone* in *methanol;* no red colour is produced within 15 minutes (distinction from nortriptyline).

C. The precipitate obtained in test B gives reaction A of chlorides (2.3.1).

## Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), protected from light, coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 85 volumes of *cyclohexane*, 15 volumes of *ethyl acetate* and 3 volumes of *diethylamine*.

*Test solution*. Extract a quantity of the powdered tablets containing about 20 mg of Amitriptyline Hydrochloride with 5 ml of a mixture of 9 volumes of *ethanol (95 per cent)* and 1 volume of *2 M hydrochloric acid* centrifuge and use the supernatant liquid, evaporated to dryness and dissolve in 10 ml of *chloroform*.

*Reference solution (a)*. A 0.001 per cent w/v solution of *dibenzosuberone RS* in *chloroform*.

*Reference solution (b)*. A 0.004 per cent w/v solution of *cyclobenzaprine hydrochloride RS* in *chloroform*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 14 cm in an unlined tank. Dry the plate in air until the odour of the solvent is no longer detectable, spray with a freshly prepared mixture of 4 volumes of *formaldehyde solution* and 96 volumes of *sulphuric acid*, heat at 105° for 10 minutes and examine in ultraviolet light at 365 nm. Any spots in the chromatogram obtained with the test solution corresponding to dibenzosuberone and cyclobenzaprine hydrochloride are not more intense than the spots in the chromatograms obtained with reference solutions (a) and (b) respectively and any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (b).

**Uniformity of content** *(For tablets containing 10 mg or less)*. Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14)

*Test solution*. Powder one tablet, shake with 2.5 ml of *0.1 M hydrochloric acid* until completely disintegrated, add 5 ml of *methanol*, shake for 30 minutes, dilute the suspension to 10 ml with *methanol*, centrifuge and use the clear supernatant liquid.

*Reference solution*. Weigh accurately 25.0 mg of *amitriptyline hydrochloride RS* dissolve in 10 ml of *methanol* and dilute to 25.0 ml with *methanol (50 per cent)*.

Chromatographic system

- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (10 µm),
- mobile phase: *0.03 M sodium hexanesulphonate* in a mixture of equal volumes of *acetonitrile* and *water*, adjusted to pH 4.5 by the addition of *glacial acetic acid,*
- flow rate. 2 ml per minute,
- spectrophotometer set at 239 nm,
- injection volume. 20 µl.

Calculate the content of $C_{20}H_{23}N$,HCl in the tablet.

**Dissolution** (2.5.2).

Apparatus. No 1,
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 239 nm (2.4.7). Calculate the content of $C_{20}H_{23}N$, HCl in the medium from the absorbance obtained from a solution of known concentration of *amitriptyline hydrochloride RS* in the same medium.

D. Not less than 75 per cent of the stated amount of $C_{20}H_{23}N$,HCl.

**Other tests**. Comply with the tests stated under Tablets.



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**Assay.** Determine by liquid chromatography (2.4.14).

*Test solution.* When tablets are film-coated, shake 20 tablets with 50 ml of *0.1 M hydrochloric acid* until completely disintegrated, add 100 ml of *methanol*, shake for 30 minutes, dilute the suspension to 200.0 ml with *methanol*, centrifuge and dilute a volume of the supernatant liquid equivalent to 25 mg of Amitriptyline Hydrochloride to 100.0 ml with *methanol (50 per cent)*.

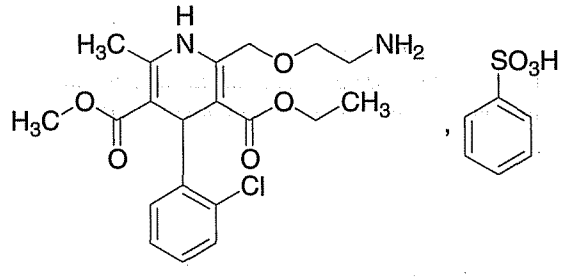
When tablets are sugar-coated, weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 50 mg of Amitriptyline Hydrochloride, shake with 50 ml of *0.1 M hydrochloric acid* for 30 minutes, add 100 ml of *methanol*, shake for 30 minutes, dilute the mixture to 200.0 ml with *water*, centrifuge and use the supernatant liquid.

*Reference solution.* Dissolve 50 mg of *amitriptyline hydrochloride RS* in 10 ml of *methanol* and dilute to 200.0 ml with *methanol (50 per cent)*.

Follow the procedure described under Uniformity of content.

Calculate the content of $C_{20}H_{23}N,HCl$ in the tablets.

# Amlodipine Besylate



$C_{26}H_{31}ClN_2O_8S$ Mol. Wt. 567.1

Amlodipine Besylate is 3-ethyl 5-methyl (4*RS*)-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate benzene sulphonate.

Amlodipine Besylate contains not less than 97.0 per cent and not more than 102.0 per cent of $C_{26}H_{31}ClN_2O_8S$, calculated on the anhydrous basis.

**Category.** Antihypertensive; antianginal.

**Description.** A white or almost white powder.

## Identification

*Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is* carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *amlodipine besylate RS or* with the reference spectrum of amlodipine besylate.

B. In test A for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

C. When examined in the range 300 nm to 400 nm (2.4.7), a 0.005 per cent w/v solution in a 1 per cent v/v solution of *0.1 M hydrochloric acid* in *methanol* shows an absorption maximum at about 360 nm. The specific absorbance at the maximum is 113 to 121.

## Tests

**Optical rotation** (2.4.22). -0.10° to +0.10°, determined in a 1.0 per cent w/v solution in *methanol.*

**Related substances.** A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

*Mobile phase.* The upper layer of a mixture of 25 volumes of *glacial acetic acid*, 25 volumes of *water* and 50 volumes of *methyl isobutyl ketone.*

*Test solution (a).* Dissolve 0.14 g of the substance under examination in 2 ml of *methanol.*

*Test solution (b).* Dilute 1 ml of test solution (a) to 10 ml with *methanol.*

*Reference solution (a).* Dissolve 70 mg of *amlodipine besylate RS* in 1 ml of *methanol.*

*Reference solution (b).* Dilute 1 ml of reference solution (a) to 10 ml with *methanol.*

*Reference solution (c).* Dilute 3 ml of test solution (b) to 100 ml with *methanol.*

*Reference solution (d).* Dilute 1 ml of test solution (b) to 100 ml with *methanol.*

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate at 80° for 15 minutes and examine in ultraviolet light at 254 nm and 365 nm. The chromatogram obtained with reference solution (a) shows two clearly separated minor spots with $R_f$ values of about 0.18 and 0.22. In the chromatogram obtained with test solution (a) any spot, other than the spots obtained with reference solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.3 per cent) and at most 2 spots are more intense than the spot in the chromatogram obtained with reference solution (d) (0.1 per cent).

B. Determine by liquid chromatography (2.4.14).

*Test solution (a).* Dissolve 50 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the mobile phase.



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*Test solution (b).* Dilute 5.0 ml of test solution (a) to 100.0 ml with the mobile phase.

*Reference solution (a).* A solution containing 0.005 per cent w/v of *amlodipine besylate RS* in the mobile phase.

*Reference solution (b).* Dilute 3 ml of test solution (a) to 100 ml with the mobile phase and dilute 5 ml of the solution to 50 ml with the mobile phase.

*Reference solution (c).* Dissolve 5 mg of the substance under examination in 5 ml of *strong hydrogen peroxide solution.* Heat at 70° for 45 minutes.

Chromatographic system

- a stainless steel column 15 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 15 volumes of *acetonitrile*, 35 volumes of *methanol* and 50 volumes of a solution prepared by dissolving 7.0 ml of *triethylamine* in 1000 ml of *water* and adjusting the pH to 3.0 with *phosphoric acid,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 237 nm,
- injection volume. 10 µl.

The relative retention time between amlodipine and 3-ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methylpyridine-3,5-dicarboxylate (amlodipine impurity D) is about 0.5.

Inject reference solution (c). The test is not valid unless the resolution between the peaks corresponding to amlodipine and impurity D is at least 4.5.

Inject test solution (a) and reference solutions (b) and (c).Continue the chromatography for 3 times the retention time of amlodipine. The area of any peak obtained due to impurity D multiplied by 2 is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent). The sum of the areas of all the other secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent). Ignore any peak due to benzene sulphonate (relative retention about 0.2) and any peak with an area 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.03 per cent).

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Water** (2.3.43). Not more than 0.5 per cent, determined on 3.0 g.

**Assay**. Determine by liquid chromatography (2.4.14) as described under Related substances.

Inject alternately test solution (b) and reference solution (a).

Calculate the content of $C_{26}H_{31}ClN_2O_8S$.

**Storage.** Store protected from moisture.

# Amlodipine Tablets

Amlodipine Besilate Tablets

Amlodipine Tablets contain Amlodipine Besilate.

Amlodipine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of amlodipine, $C_{20}H_{25}ClN_2O_5$.

**Usual strengths.** 5 mg; 10 mg.

## Identification

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

## Tests

**Related substances.** Determine by liquid chromatography (2.4.14).

*Test solution (a).* Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 50 mg amlodipine, dissolve in the mobile phase, dilute to 50.0 ml with the mobile phase and centrifuge.

*Test solution (b).* Dilute 5.0 ml of test solution (a) to 100.0 ml with the mobile phase.

*Reference solution (a).* A solution containing 0.005 per cent w/v of *amlodipine besilate RS* in the mobile phase.

*Reference solution (b).* Dilute 5 ml of test solution (a) to 100 ml with the mobile phase and dilute 5 ml of the solution to 50 ml with the mobile phase.

*Reference solution (c).* Dissolve 5 mg of *amlodipine besilate RS* in 5 ml of strong *hydrogen peroxide solution.* Heat at 70° for 45 minutes and centrifuge.

Chromatographic system

- a stainless steel column 15 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 15 volumes of *acetonitrile*, 35 volumes of *methanol* and 50 volumes of a solution prepared by dissolving 7.0 ml of *triethylamine* in 1000 ml of *water* and adjust to pH 3.0 with *phosphoric acid,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 237 nm,
- injection volume. 10 µl.

The relative retention time between amlodipine and 3-ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methylpyridine-3,5-dicarboxylate (amlodipine impurity D) is about 0.5.

Inject reference solution (c). The test is not valid unless the resolution between the peaks corresponding to amlodipine and impurity D is at least 4.5.



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Inject test solution (a) and reference solutions (b) and (c). Continue the chromatography for 3 times the retention time of amlodipine. The area of any peak corresponding to impurity D multiplied by 2 is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). The sum of the areas of all the other secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent) Ignore any peak due to benzene sulphonate (relative retention about 0.2) and any peak with an area 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.01 M hydrochloric acid*,
Speed and time. 75 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the dissolution medium if necessary, at the maximum at about 239 nm (2.4.7). Calculate the content of $C_{20}H_{25}ClN_2O_5$ in the medium from the absorbance obtained from a solution of known concentration of *amlodipine besilate RS* in the same medium.

D. Not less than 70 per cent of the stated amount of $C_{20}H_{25}ClN_2O_5$.

**Uniformity of content**. Comply with the test stated under Tablets.

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

*Test solution*. Powder one tablet and dissolve in 50 ml of the mobile phase, dilute with sufficient mobile phase get a solution containing 0.002 per cent w/v of amlodipine, shake for 10 minutes and filter through a glass-fibre filter paper.

*Reference solution*. A solution of *amlodipine besilate RS* in mobile phase equivalent to 0.002 per cent w/v of amlodipine.

Calculate the content of $C_{20}H_{25}ClN_2O_5$ in the tablet.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14) as described under Related substances.

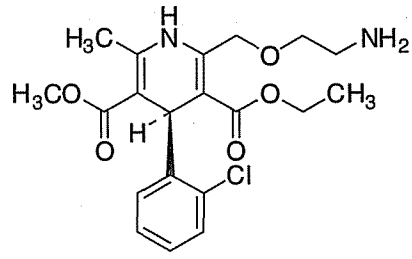
Inject alternately test solution (b) and reference solution (a).

Calculate the content of $C_{20}H_{25}ClN_2O_5$ in the tablets.

**Storage**. Store protected from moisture.

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

# *S*-Amlodipine Besylate



$C_{20}H_{25}ClN_2O_5,C_6H_6O_3S$ Mol. Wt. 567.1

*S*-Amlodipine Besylate is (*S*)-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylic acid 3-ethyl 5-methyl ester.

S-Amlodipine Besylate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{20}H_{25}ClN_2O_5,C_6H_6O_3S$, calculated on the anhydrous basis.

**Category**. Antihypertensive, antianginal.

**Description**. A white to pale yellow powder.

## Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *S-amlodipine besylate RS* or with the reference spectrum of S-amlodipine besylate.

## Tests

**Specific optical rotation** (2.4.22). -24.0° to -30.0°, determined in a 1.0 per cent w/v solution in *methanol*.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve about 100 mg of the substance under examination in 100.0 ml of the mobile phase. Dilute 5.0 ml of this solution to 100.0 ml with the mobile phase.

*Reference solution*. A 0.001 per cent w/v solution of *S-amlodipine besylate RS* in the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Use the chromatographic system as described under Assay.

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

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution the area of any secondary peak is not more than 2.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of the area of all the secondary peaks is not more than 5 times the area of the



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principal peak in the chromatogram obtained with the reference solution (1.0 per cent). Ignore any peak due to benzene sulphonic acid (Relative retention time is about 0.14).

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

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Water** (2.3.43). Not more than 8.0 per cent, determined on 0.1 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve about 100 mg of the substance under examination in 100.0 ml of the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase. Further dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

*Reference solution.* A 0.001 per cent w/v solution of *S-amlodipine besylate RS* in the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm), (such as Thermoquest),
- mobile phase: a mixture of 50 volumes of buffer solution pH 3.0 prepared by diluting 7 ml of *triethylamine* to 1000 ml with *water*, adjusted to pH 3.0 with *orthophosphoric acid*, 35 volumes of *methanol* and 15 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 237 nm,
- injection volume. 20 µl.

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

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{25}ClN_2O_5$,$C_6H_6O_3S$.

# *S*-Amlodipine Tablets

*S*- Amlodipine Besylate Tablets

*S*- Amlodipine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of *S*-amlodipine, $C_{20}H_{25}ClN_2O_5$.

**Usual strengths**. 5 mg; 10 mg.

## Identification

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

## Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 500 ml of *0.01 M hydrochloric acid*,
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Dilute the filtrate, if necessary with the dissolution medium . Measure the absorbance of the resulting solution at the maximum at about 239 nm (2.4.7). Calculate the content of $C_{20}H_{25}ClN_2O_5$ in the medium from the absorbance obtained from a solution of known concentration of *S-amlodipine besylate RS*.

D. Not less than 70 per cent of the stated amount of $C_{20}H_{25}ClN_2O_5$.

**Uniformity of content**. Comply with the test stated under tablets.

Determine by liquid chromatography (2.4.14), as described under Assay, using the following solution as the test solution.

*Test solution.* Disperse 1 tablet in the mobile phase, sonicate and dilute if necessary to obtain a solution containing 0.0025 per cent w/v of S- Amlodipine in the mobile phase.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 5 mg of S- Amlodipine with 200.0 ml of the mobile phase. Centrifuge 10.0 ml of the solution at 3500 rpm for 15minutes.

*Reference solution.* A solution of *S-amlodipine besylate RS* containing about 0.0025 per cent w/v of Amlodipine in the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of buffer solution pH 3.0 prepared by diluting 7 ml of *triethylamine* in 1000 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid*, 30 volumes of *acetonitrile* and 20 volumes of *methanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 237 nm,
- injection volume. 20 µl.

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

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{25}ClN_2O_5$ in the tablets.

**Labelling**. The label states the strength in terms of the equivalent amount of S-Amlodipine.



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# Ammonium Chloride

$NH_4Cl$ Mol. Wt. 53.5

Ammonium Chloride contains not less than 99.0 per cent and not more than 100.5 per cent of $NH_4Cl$, calculated on the dried basis.

**Category**. Expectorant; diuretic; systemic acidifier.

**Dose**. 3 to 6 g daily, in divided doses.

**Description**. Colourless crystals or a white, crystalline powder.

## Identification

Gives the reactions of ammonium salts and of chlorides (2.3.1).

## Tests

**Appearance of solution**. A 10.0 per cent solution is clear (2.4.1) and colourless (2.4.1).

**pH** (2.4.24). 4.5 to 6.0, determined in a 5.0 per cent solution.

**Arsenic** (2.3.10) Dissolve 2.5 g in 50 ml of *water* and add 10 ml of *stannated hydrochloric acid*. The resulting solution complies with the limit test for arsenic (4 ppm).

**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals, Method A (10 ppm).

**Iron** (2.3.14). 2.0 g complies with the limit test for iron (20 ppm).

**Calcium**. To 0.2 ml of *ethanolic calcium standard solution (100 ppm Ca)* add 1 ml of a 4 per cent w/v solution of *ammonium oxalate*. After 1 minute add 1 ml of *2 M acetic acid* and 15 ml of a solution made by diluting 5 ml of a 10 per cent solution of the substance under examination with 10 ml of *water* and shake. Compare any opalescence produced with that of a standard prepared in a similar manner but using a mixture of 10 ml of *calcium standard solution (10 ppm Ca)* and 5 ml of *water* instead of the solution of the substance under examination (200 ppm).

**Sulphates** (2.3.17). 1.0 g complies with the limit test for sulphates (150 ppm).

**Thiocyanate**. Acidify 10 ml of a 10 per cent w/v solution with *hydrochloric acid* and add a few drops of *ferric chloride solution*; no red colour is produced.

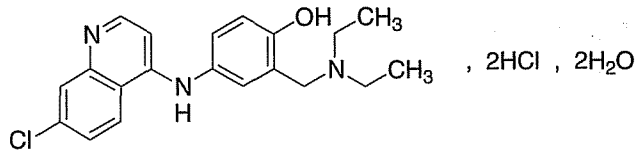
**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 1.0 per cent determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.1 g, dissolve in 20 ml of *water* and add a mixture of 5 ml of *formaldehyde solution*, previously neutralised to *dilute phenolphthalein solution*, and 20 ml of *water*. After 2 minutes, titrate slowly with *0.1 M sodium hydroxide* using a further 0.2 ml of *dilute phenolphthalein solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.005349 g of $NH_4Cl$.

# Amodiaquine Hydrochloride




$C_{20}H_{22}ClN_3O, 2HCl, 2H_2O$ Mol. Wt. 464.8

Amodiaquine Hydrochloride is 4-(7-chloro-4-quinolylamino)-2-(diethylaminomethyl)phenol dihydrochloride dihydrate.

Amodiaquine Hydrochloride contains not less than 98.0 per cent and not more than 101.5 per cent of $C_{20}H_{22}ClN_3O$, 2HCl, calculated on the anhydrous basis.

**Category**. Antimalarial.

**Dose**. Suppressive, the equivalent of 400 mg of amodiaquine weekly. Therapeutic, the equivalent of 400 to 600 mg of amodiaquine daily for three days.

**Description**. A yellow, crystalline powder; odourless or almost odourless.

## Identification

*Test A may be omitted if tests B, C, D and E are carried out. Tests C and D may be omitted if tests A, B and E are carried out.*

A. Dissolve 20 mg in 10 ml of *water* and add 1 ml *of strong ammonia solution*. Extract with two quantities, each of 25 ml, of *chloroform*, wash the combined *chloroform* extracts with *water*, dry with *anhydrous sodium sulphate*, evaporate the *chloroform* and dry the residue at 105° for 2 hours. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *amodiaquine RS*.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.0015 per cent w/v solution in *0.1 M hydrochloric acid* shows an absorption maximum at about 343 nm; absorbance at 343 nm, about 0.55.

C. To 1 ml of a 2 per cent w/v solution add 0.5 ml of *cobalt thiocyanate solution*; a green precipitate is produced.

D. To 20 ml of a 2 per cent w/v solution, add 1 ml of *dilute ammonia solution*. Shake and filter; the filtrate gives the reactions of chlorides (2.3.1).

E. The undried material melts at about 158° (2.4.21).



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### Tests

**pH** (2.4.24). 3.6 to 4.6, determined in a 2.0 per cent w/v solution.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 90 volumes of *chloroform* saturated with *strong ammonia solution* and 10 volumes of *ethanol*.

*Test solution*. Add to 200 mg of the substance under examination in a glass-stoppered test-tube 10 ml of *chloroform* saturated with *strong ammonia solution*, shake vigorously for 2 minutes, allow the solids to settle and decant the supernatant liquid.

*Reference solution (a)*. Prepare in the same manner as the test solution but using 200 mg of *amodiaquine hydrochloride RS* and 10 ml of *chloroform* saturated with *strong ammonia solution*.

*Reference solution (b)*. Dilute 1 volume of reference solution (a) with sufficient *chloroform* saturated with *strong ammonia solution* to obtain 200 volumes.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a) and no secondary spot in the chromatogram obtained with the test solution is more intense than the principal spot in the chromatogram obtained with reference solution (b).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). 6.0 to 10.0 per cent, determined on 0.5 g.

**Assay**. Weigh accurately about 0.3 g and dissolve in sufficient *0.1 M hydrochloric acid* to produce 200.0 ml. Dilute 10.0 ml to 1000.0 ml with *0.1 M hydrochloric acid*. Measure the absorbance of the resulting solution at the maximum at about 343 nm (2.4.7), using *0.1 M hydrochloric acid* as the blank.

Calculate the content of $C_{20}H_{22}ClN_3O$, 2HCl from the absorbance obtained by carrying out the Assay simultaneously on *amodiaquine hydrochloride RS*.

## Amodiaquine Tablets

### Amodiaquine Hydrochloride Tablets

Amodiaquine Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of amodiaquine, $C_{20}H_{22}ClN_3O$.

**Usual strength**. The equivalent of 200 mg of amodiaquine. (1 g of Amodiaquine Hydrochloride anhydrous is approximately equivalent to 0.83 g of amodiaquine).

### Identification

A. Extract the powdered tablets with *water* and filter. To 1 ml of the filtrate add 0.5 ml of *cobalt thiocyanate solution*; a green precipitate is produced.

B. The powdered tablets give the reactions of chlorides (2.3.1).

### Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *water*,
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with water if necessary, at the maximum at about 343 nm (2.4.7). Calculate the content of $C_{20}H_{22}ClN_3O$ in the medium from the absorbance obtained from a known concentration of *amodiaquine hydrochloride RS* in the same medium.

D. Not less than 70 per cent of the stated amount of $C_{20}H_{22}ClN_3O$.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 90 volumes of *chloroform* saturated with *strong ammonia solution* and 10 volumes of *ethanol*.

*Test solution*. Shake a quantity of the powdered tablets containing about 40 mg of Amodiaquine Hydrochloride with 20 ml of *water* for 1 minute, add 25 ml of *chloroform* and 1 ml of *strong ammonia solution* and shake vigorously for 2 minutes. Filter the chloroform extract through a cotton plug previously soaked in *chloroform*, evaporate the filtrate to dryness and dissolve the residue in 2 ml of *chloroform* saturated with *strong ammonia solution*.

*Reference solution (a)*. Prepare in the same manner as the test solution but using 200 mg of *amodiaquine hydrochloride RS* and 10 ml of *chloroform* saturated with *strong ammonia solution*

*Reference solution (b)*. Dilute 1 volume of reference solution (a) with sufficient *chloroform* saturated with *strong ammonia solution* to obtain 200 volumes.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a) and no secondary spot in the chromatogram obtained with the test solution is more intense than the principal spot in the chromatogram obtained with reference solution (b).

**Other tests**. Comply with the tests stated under Tablets.



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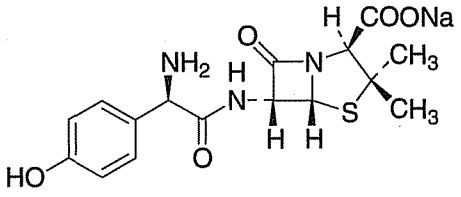


**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.3 g of amodiaquine, add 100 ml of *0.1 M hydrochloric acid* and heat on a water-bath for about 15 minutes with occasional stirring. Cool, transfer to a 200-ml graduated flask and dilute to volume with *0.1 M hydrochloric acid*. To 10.0 ml of the clear supernatant liquid in a separator, add 10 ml of *0.1 M hydrochloric acid* and extract with 20 ml of *chloroform*. Discard the chloroform extract. Add 4.5 ml of *1 M sodium hydroxide* and extract with four quantities, each of 25 ml, of *chloroform*. Extract the combined chloroform solutions with three quantities, each of 50 ml, of *0.1 M hydrochloric acid* and dilute with sufficient *0.1 M hydrochloric acid* to produce 200.0 ml. Dilute 10.0 ml with sufficient *0.1 M hydrochloric acid* to produce 100.0 ml. Measure the absorbance of the resulting solution at the maximum at about 343 nm (2.4.7), using *0.1 M hydrochloric acid* as the blank.

Calculate the content of $C_{20}H_{22}ClN_3O$, 2HCl from the absorbance obtained by carrying out the Assay simultaneously on *amodiaquine hydrochloride RS*. Multiply the result by 0.830 to get the equivalent quantity of $C_{20}H_{22}ClN_3O$.

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

## Amoxycillin Sodium




$C_{16}H_{18}N_3NaO_5S$ Mol. Wt. 387.4

Amoxycillin Sodium is sodium (6*R*)-6-(α-4-hydroxyphenyl-D-glycylamino)penicillanate.

Amoxycillin Sodium contains not less than 85.0 per cent and not more than 100.5 per cent of $C_{16}H_{18}N_3NaO_5S$, calculated on the anhydrous basis.

**Category**. Antibacterial.

**Dose**. By intramuscular or intravenous injection, the equivalent of 1 to 3 g of amoxycillin daily, in divided doses.

**Description**. A white or almost white powder; very hygroscopic.

### Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *amoxycillin sodium RS* or with the reference spectrum of amoxycillin sodium.

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

C. A 5 per cent w/v solution gives the reactions of sodium salts (2.3.1).

### Tests

**Appearance of solution**. A 10.0 per cent w/v solution is not more opalescent than opalescence standard OS2 (2.4.1) when examined immediately after preparation. The solution may initially show a pink colour and its absorbance after 5 minutes at about 430 nm is not more than 0.20 (2.4.7).

**pH** (2.4.24). 8.0 to 10.0, determined in a 10.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). +240° to +290°, determined in a 0.25 per cent w/v solution in a 0.4 per cent w/v solution of *potassium hydrogen phthalate*.

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

***N*,*N*-Dimethylaniline** (2.3.21). Not more than 20 ppm, determined by Method A.

**Sodium chloride**. Not more than 2.0 per cent, calculated on the anhydrous basis, determined by the following method. Weigh accurately about 1.0 g, dissolve in 50 ml of *distilled water*; add 10 ml of *2 M nitric acid* and titrate with *0.1 M silver nitrate*, determining the end-point potentiometrically (2.4.25) using a silver indicator electrode and a mercury-mercurous sulphate reference electrode or any other suitable electrode.

1 ml of *0.1 M silver nitrate* is equivalent to 0.005845 g of NaCl.

**2-Ethylhexanoic acid**. Not more than 2.0 per cent, determined by the following method.

Determine by gas chromatography (2.4.13).

*Test solution*. Prepare a 1.0 per cent w/v solution of *valeric acid* (internal standard) in *hexane* (solution A). Dissolve 1.0 g of the substance under examination in 5 ml of *water* in a glass-stoppered flask, add 3 ml of *2 M hydrochloric acid*, 1 ml of solution A and 5 ml of *hexane*, shake vigorously for 1 minute, centrifuge if necessary and use the clear supernatant layer.

*Reference solution (a)*. Prepare in the same manner as the test solution but using an extra 1 ml of *hexane* in place of solution A.

*Reference solution (b)*. Prepare in the same manner as the test solution but using 20 mg of *2-ethylhexanoic acid* suspended in 5 ml of *water* in place of the substance under examination.



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Chromatographic system
- a glass column 1.8 m x 4 mm, packed with a support impregnated with a stationary phase suitable for the separation of free fatty acids (such as a column containing 10 per cent of SP 1200 and 1 per cent of *phosphoric acid* on Chromosorb W AW, 80-100 mesh),
- temperature:
  column. 145°,
- inlet port and detector. 150°,
- flow rate. 45 ml per minute of the carrier gas.

**Water** (2.3.43). Not more than 4.0 per cent, determined on 0.4 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. Dissolve 6.8 g of *monobasic potassium phosphate* in 1000 ml of *water* and adjust the pH to about 5.0 with a 4.5 per cent w/v solution of *potassium hydroxide*.

*Test solution*. Transfer an accurately weighed quantity containing about 120 mg of Amoxycillin to a 100-ml volumetric flask, dissolve in the solvent mixture and dilute to 100.0 ml with the solvent mixture. Use this solution within 6 hours.

*Reference solution*. Weigh accurately a suitable quantity of *amoxycillin trihydrate RS*, dissolve in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound and dilute to obtain a solution having a known concentration of about 1.2 mg per ml. Use this solution within 6 hours.

Chromatographic system
- a stainless steel column 25 cm x 4.0 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: a mixture of 4 volumes of *acetonitrile* and 96 volumes of the solvent mixture,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the capacity factor is between 1.1 and 2.8, the column efficiency is not less than 1700 theoretical plates, the tailing factor is not more than 2.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the percentage content of $C_{16}H_{18}N_3NaO_5S$ by multiplying the percentage content of $C_{16}H_{19}N_3O_5S$ by 1.060.

*Amoxycillin Sodium intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.*

**Bacterial endotoxins** (2.2.3). Not more than 0.25 Endotoxin Unit per mg of amoxycillin.

*Amoxycillin Sodium intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.*

**Sterility** (2.2.11). Complies with the test for sterility.

**Storage**. Store protected from moisture, at a temperature not exceeding 30°. If it is intended for use in the manufacture of parenteral preparations, the container should be sterile, tamper-evident and sealed so as to exclude micro-organisms.

**Labelling**. The label states whether or not the material is intended for use in the manufacture of parenteral preparations.

# Amoxycillin Capsules

Amoxycillin Trihydrate Capsules; Amoxicillin Trihydrate Capsules; Amoxicillin Capsules

Amoxycillin Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of amoxycillin, $C_{16}H_{19}N_3O_5S$.

**Usual strengths**. The equivalent of 250 mg and 500 mg of amoxycillin.

## Identification

Shake a quantity of the contents of the capsules containing about 0.5 g of amoxycillin with 5 ml of *water* for 5 minutes, filter, wash the residue first with *ethanol* and then with *ether* and dry at a pressure not exceeding 0.7 kPa for 1 hour. The residue complies with the following tests.

*Test A may be omitted if test B is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *amoxycillin trihydrate RS* or with the reference spectrum of amoxycillin trihydrate.

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

## Tests

**Dissolution** (2.5.2).
Apparatus. No 1,
Medium. 900 ml of *water*,
Speed and time. 100 rpm and 60 minutes.

Use one capsule in the vessel for each test.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first 1 ml of the filtrate. Dilute the filtrate, if necessary, with the same solvent. Measure the absorbance of the resulting solution at the maximum at about 272 nm (2.4.7). Similarly measure the absorbance of a standard solution of known concentration of *amoxicillin*



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*tryhydrate RS* at about 272 nm and calculate the content of $C_{16}H_{19}N_3O_5S$.

D. Not less than 80 per cent of the stated amount of $C_{16}H_{19}N_3O_5S$.

**Other tests**. Comply with the tests stated under Capsules.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. Dissolve 6.8 g of *monobasic potassium phosphate* in 1000 ml of *water* and adjust the pH to 5.0 with a 4.5 per cent w/v solution of *potassium hydroxide*.

*Test solution*. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 100 mg of amoxicillin, add about 80 ml of the solvent mixture and dissolve by shaking for 15 minutes and mixing if necessary, with the aid of ultrasound. Dilute to 100.0 ml with the solvent mixture and filter. Use this solution within 6 hours.

*Reference solution*. Weigh accurately a suitable quantity of *amoxycillin trihydrate RS*, dissolve in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound and dilute to obtain a solution having a known concentration of about 1.2 mg per ml. Use this solution within 6 hours.

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica particles or ceramic microparticles (5 µm),
- mobile phase: a mixture of 4 volumes of *acetonitrile* and 96 volumes of the solvent mixture,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume.10 µl.

Inject the reference solution. The test is not valid unless the capacity factor is between 1.1 and 2.8, the column efficiency is not less than 1700 theoretical plates, the tailing factor is not more than 2.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_{16}H_{19}N_3O_5S$ in the capsules.

**Storage**. Store protected from moisture.

**Labelling**. The label states the quantity of the active ingredient in terms of the equivalent amount of amoxycillin.

# Amoxycillin Injection

Amoxicillin Sodium Injection; Amoxycillin Sodium Injection

Amoxycillin Injection is a sterile material consisting of Amoxycillin Sodium with or without excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

*The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).*

**Storage**. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Amoxycillin Injection contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of amoxycillin, $C_{16}H_{19}N_3O_5S$.

**Usual strengths**. The equivalent of 100 mg, 250 mg, 500 mg and 1 g of amoxycillin.

**Description**. A white or almost white powder; very hygroscopic.

*The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.*

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *amoxycillin sodium RS* or with the reference spectrum of amoxycillin sodium.

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

C. A 5 per cent w/v solution gives the reactions of sodium salts (2.3.1).

## Tests

**Appearance of solution**. A 10.0 per cent w/v solution is not more opalescent than opalescence standard OS2 (2.4.1) when examined immediately after preparation. The solution may initially show a pink colour and its absorbance after 5 minutes at about 430 nm is not more than 0.20 (2.4.7).

**pH** (2.4.24). 8.0 to 10.0, determined in a 10.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). +240° to +290°, determined in a 0.25 per cent w/v solution in a 0.4 per cent w/v solution of *potassium hydrogen phthalate*.

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

**N,N-Dimethylaniline** (2.3.21). Not more than 20 ppm, determined by Method A.

**Sodium chloride**. Not more than 2.0 per cent, calculated on the anhydrous basis, determined by the following method.



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Weigh accurately about 1.0 g, dissolve in 50 ml of *distilled water*; add 10 ml of *2 M nitric acid* and titrate with *0.1 M silver nitrate*, determining the end-point potentiometrically (2.4.25) using a silver indicator electrode and a mercury-mercurous sulphate reference electrode or any other suitable electrode.

1 ml of *0.1 M silver nitrate* is equivalent to 0.005845 g of NaCl.

**Bacterial endotoxins** (2.2.3). Not more than 0.25 Endotoxin Unit per mg of amoxycillin.

**Water** (2.3.43). Not more than 4.0 per cent, determined on 0.4 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. Dissolve 6.8 g of *monobasic potassium phosphate* in 1000 ml of *water* and adjust the pH to 5.0 with a 4.5 per cent w/v solution of *potassium hydroxide*.

*Test solution*. Determine the weight of the contents of 10 containers. Transfer an accurately weighed quantity of the mixed contents of the 10 containers containing about 100 mg of amoxycillin to a 100-ml volumetric flask, add 80 ml of the solvent mixture and dissolve by shaking and mixing if necessary, with the aid of ultrasound. Dilute to 100.0 ml with the solvent mixture and filter. Use this solution within 6 hours.

*Reference solution*. Dissolve an accurately weighed quantity of *amoxycillin trihydrate RS* in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound and dilute to obtain a solution having a known concentration of about 1.2 mg per ml. Use this solution within 6 hours.

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: a mixture of 4 volumes of *acetonitrile* and 96 volumes of the solvent mixture,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the capacity factor is between 1.1 and 2.8, the column efficiency is not less than 1700 theoretical plates, the tailing factor is not more than 2.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the percentage content of $C_{16}H_{19}N_3O_5S$ in the injection.

**Storage**. Store protected from moisture, in a sterile, tamper-evident container sealed so as to exclude micro-organisms, at a temperature not exceeding 30°.

**Labelling**. The label states the quantity of Amoxycillin Sodium contained in the sealed container in terms of the equivalent amount of amoxycillin.

# Amoxycillin Oral Suspension

Amoxicillin Oral Suspension

Amoxycillin Oral Suspension is a mixture consisting of Amoxycillin Trihydrate with buffering agents and other excipients. It contains a suitable flavouring agent. It is filled in a sealed container.

The suspension is constituted by dispersing the contents of the sealed container in the specified volume of Water just before issue.

Amoxycillin Oral Suspension contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of amoxicillin $C_{16}H_{19}N_3O_5S$.

When stored at the temperature and for the period stated on the label during which the constituted suspension may be expected to be satisfactory for use, it contains not less than 80.0 per cent of the stated amount of amoxycillin $C_{16}H_{19}N_3O_5S$.

**Storage**. Store protected from moisture at a temperature not exceeding 30°.

**Usual strengths**. Amoxycillin 125 mg per 5 ml; Amoxycillin 250 mg per 5 ml.

## Identification

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

*The constituted suspension complies with the tests stated under Oral liquids and with the following tests.*

## Tests

**pH** (2.4.24). 5.0 to 7.5.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. Dissolve 6.8 g of *monobasic potassium phosphate* in 1000 ml of *water* and adjust the pH to 4.5 with a 4.5 per cent w/v solution of *potassium hydroxide*.

*Test solution*. Transfer an accurately weighed quantity containing about 100 mg of amoxicillin to a 100-ml volumetric flask, dissolve in the solvent mixture and dilute to 100.0 ml with the solvent mixture and filter. Dilute 10.0 ml of the solution to 50.0 ml with the solvent mixture.

*Reference solution*. Dissolve an accurately weighed quantity of *amoxycillin trihydrate RS* in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound and dilute to obtain a solution having a known concentration of about 0.2 mg per ml of amoxycillin. Use this solution within 6 hours.

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm),



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- mobile phase: a mixture of 4 volumes of *acetonitrile* and 96 volumes of the solvent mixture,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume.10 µl.

Inject the reference solution. The test is not valid unless the capacity factor is between 1.1 and 2.8, the column efficiency is not less than 1700 theoretical plates, the tailing factor is not more than 2.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

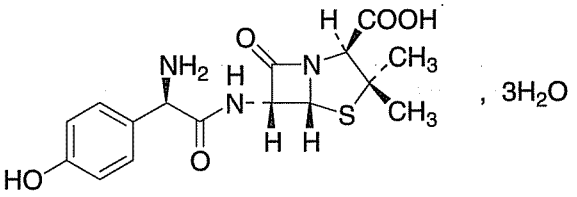
Inject alternately the test solution and the reference solution.

Determine the weight per ml of the oral suspension (2.4.29) and calculate the content of $C_{16}H_{19}N_3O_5S$ weight in volume.

Repeat the procedure using a portion of the constituted suspension that has been stored at the temperature and for the period stated on the label.

**Labelling**. The label states (1) the quantity of active ingredient in terms of the equivalent amount of amoxicillin; (2) the temperature of storage and the period during which the constituted suspension may be expected to be satisfactory for use.

# Amoxycillin Trihydrate




$C_{16}H_{19}N_3O_5S,3H_2O$ Mol. Wt. 419.4

Amoxycillin Trihydrate is (6*R*)-6-(α-4-hydroxyphenyl-D-glycylamino)penicillanic acid trihydrate.

Amoxycillin Trihydrate contains not less than 95.0 per cent and not more than 100.5 per cent of $C_{16}H_{19}N_3O_5S$, calculated on the anhydrous basis.

**Category**. Antibacterial.

**Dose**. The equivalent of 750 mg to 4.5 g of amoxycillin daily, in divided doses.

**Description**. A white or almost white, crystalline powder.

## Identification

*Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *amoxycillin trihydrate RS* or with the reference spectrum of amoxycillin trihydrate.

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

C. Place about 2 mg in a test-tube. Moisten with 0.05 ml of *water* and add 2 ml of *sulphuric acid-formaldehyde reagent*. Mix the contents of the tube by swirling; the solution is practically colourless. Place the tube in a water-bath for 1 minute; a dark yellow colour develops.

## Tests

**Appearance of solution**. Dissolve 1.0 g in 10 ml of *0.5 M hydrochloric acid*, and a further 1.0 g in a mixture of 3 ml of *dilute ammonia solution* and 7 ml of *water*. Both solutions when freshly prepared are not more opalescent than opalescence standard OS2 (2.4.1).

**pH** (2.4.24). 3.5 to 5.5, determined in a 0.2 per cent w/v solution.

**Specific optical rotation** (2.4.22). +290° to +315°, determined in a 0.2 per cent w/v solution in *carbon dioxide-free water*.

***N,N*-Dimethylaniline** (2.3.21). Not more than 20 ppm, determined by Method A.

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

**Sulphated ash** (2.3.18). Not more than 1.0 per cent.

**Water** (2.3.43). 11.5 to 14.5 per cent, determined on 0.1 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. Dissolve 6.8 g of *monobasic potassium phosphate* in 1000 ml of *water* and adjust the pH to 4.5 with a 4.5 per cent w/v solution of *potassium hydroxide*.

*Test solution*. Transfer an accurately weighed quantity of about 120 mg of the substance under examination to a 100-ml volumetric flask, dissolve in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound and dilute to 100.0 ml with the solvent mixture. Use this solution within 6 hours.

*Reference solution*. Weigh accurately a suitable quantity of *amoxycillin trihydrate RS*, dissolve in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound and dilute to obtain a solution having a known concentration of about 1.2 mg per ml. Use this solution within 6 hours.

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: a mixture of 4 volumes of *acetonitrile* and 96 volumes of the solvent mixture,



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- flow rate. 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the capacity factor is between 1.1 and 2.8, the column efficiency is not less than 1700 theoretical plates, the tailing factor is not more than 2.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the percentage content of $C_{16}H_{19}N_3O_5S$.

**Storage**. Store at a temperature not exceeding 30°.

**Labelling**. The label states (1) the strength in terms of the equivalent amount of amoxycillin; (2) that the tablets should be dispersed in water immediately before use.

## Amoxycillin Dispersible Tablets

Amoxycillin Trihydrate Dispersible Tablets; Dispersible Amoxicillin Tablets

Amoxycillin Dispersible Tablets contain Amoxycillin Trihydrate in a suitable dispersible base.

Amoxycillin Dispersible Tablets contain not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of amoxycillin, $C_{16}H_{19}N_3O_5S$.

**Usual strengths**. The equivalent of 125 mg and 250 mg of amoxycillin.

### Identification

Shake a quantity of the powdered tablets containing about 0.5 g of amoxycillin with 5 ml of *water* for 5 minutes, filter, wash the residue first with *ethanol* and then with *ether* and dry for 1 hour at a pressure not exceeding 0.7 kPa. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *amoxycillin trihydrate RS* or with the reference spectrum of amoxycillin trihydrate.

### Tests

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets.

Determine by liquid chromatography (2.4.14).

*Solvent mixture*. Dissolve 6.8 g of *monobasic potassium phosphate* in 1000 ml of *water* and adjust the pH to about 4.5 with a 4.5 per cent w/v solution of *potassium hydroxide*.

*Test solution*. Weigh accurately a quantity of the powdered tablets containing about 100 mg of amoxicillin and dissolve in the solvent mixture by shaking for 15 minutes and mixing if necessary, with the aid of ultrasound. Dilute to 100.0 ml with the solvent mixture and filter. Use this solution within 6 hours.

*Reference solution*. Weigh accurately a suitable quantity of *amoxycillin trihydrate RS*, dissolve in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound and dilute to obtain a solution having a known concentration of about 1.2 mg per ml. Use this solution within 6 hours.

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: a mixture of 4 volumes of *acetonitrile* and 96 volumes of the solvent mixture,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the capacity factor is between 1.1 and 2.8, the column efficiency is not less than 1700 theoretical plates, the tailing factor is not more than 2.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_{16}H_{19}N_3O_5S$ in the tablets.

**Storage**. Store protected from moisture at a temperature not exceeding 30°.

## Amoxycillin and Potassium Clavulanate Injection

Amoxicillin and Potassium Clavulanate injection

Amoxycillin and Potassium Clavulanate injection is a sterile material consisting of Amoxycillin Sodium and Potassium Clavulanate with or without excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

*The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).*

**Storage**. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Amoxycillin and Potassium Clavulanate Injection contains not less than 90.0 per cent and not more than 107.5 per cent of the



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stated amounts of amoxycillin, $C_{16}H_{19}N_3O_5S$ and of clavulanic acid, $C_8H_9NO_5$.

*The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.*

**Usual strengths**. 0.3 g per 10 ml; 0.6 g per 10 ml; 1.2 g per 10 ml.

### Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254* (such as Merck silica gel 60 GF254 plates).

*Mobile phase.* A mixture of 1 volume of *butan-1-ol*, 2 volumes of a 0.1 per cent w/v solution of *disodium edetate* in *mixed phosphate buffer pH 4.0*, 6 volumes of *glacial acetic acid* and 10 volumes of *butyl acetate*.

*Test solution.* Shake a quantity of the contents of the sealed container containing about 0.4 g of clavulanic acid in 100 ml of a mixture of 4 volumes of *methanol* and 6 volumes of *0.1 M mixed phosphate buffer pH 7.0* and filter.

*Reference solution.* A solution containing 0.4 per cent w/v of *lithium clavulanate RS* and 0.8 per cent w/v of *amoxycillin trihydrate RS* in a mixture of 4 volumes of *methanol* and 6 volumes of *0.1 M mixed phosphate buffer pH 7.0*.

Apply to the plate 1 µl of each of the solutions after impregnating the plate by spraying it with a 0.1 per cent w/v solution of *disodium edetate* in *mixed phosphate buffer pH 4.0* and allowing to dry overnight and activating the plate by heating at 105° for 1 hour just before use. After development, allow it to dry in air and examine in ultraviolet light at 254 nm. The principal spots in the chromatogram obtained with the test solution correspond to those in the chromatogram obtained with the reference solution.

B. In the Assay, the retention time of the two principal peaks in the chromatogram obtained with the test solution correspond to those in the chromatogram obtained with the reference solution.

### Tests

**pH** (2.4.24). 8.0 to 10.0, determined in a solution containing about 10 per cent w/v of amoxycillin.

**Bacterial endotoxins** (2.2.3). Not more than 0.25 Endotoxin Unit per mg of amoxycillin.

**Water** (2.3.43). Not more than 3.5 per cent, determined on 0.5 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Determine the weight of the contents of 10 containers. Dissolve, with shaking, a quantity of the mixed contents of the 10 containers containing about 60 mg of amoxycillin in *water* and dilute to 100.0 ml with the same solvent, mix and filter.

*Reference solution.* A solution containing 0.06 per cent w/v of *amoxycillin trihydrate RS* and 0.012 per cent w/v of *lithium clavulanate RS* in *water*.

Chromatographic system

- a stainless steel column 30 cm × 3.9 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 95 volumes of buffer solution prepared by dissolving 7.8 g of *monobasic sodium phosphate* in 900 ml of *water*, adjust the pH to 4.4 with *10 M sodium hydroxide or orthophosphoric acid* and 5 volumes of *methanol*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to amoxycillin and clavulanic acid is not less than 3.5, the tailing factor is not more than 1.5, the column efficiency is not less than 550 theoretical plates for both component and the relative standard deviation is not more than 2.0.
Inject alternately the test solution and the reference solution.

Calculate the content of $C_{16}H_{19}N_3O_5S$ and $C_8H_9NO_5$.

1 mg of $C_8H_8LiNO_5$ is equivalent to 0.9711 mg of $C_8H_9NO_5$.

**Labelling**. The label states the quantity of Amoxycillin Sodium contained in it, in terms of the equivalent amount of amoxycillin, and the quantity of Potassium Clavulanate, in terms of the equivalent amount of clavulanic acid.

## Amoxycillin and Potassium Clavulanate Oral Suspension

Amoxicillin and Potassium Clavulanate oral suspension

Amoxycillin and Potassium Clavulanate oral suspension is a mixture of Amoxycillin Trihydrate and Potassium Clavulanate or Potassium Clavulanate Diluted with buffering agents and other excipients. It contains a suitable flavouring agent.

The suspension is constituted by dispersing the contents of the sealed container in the specified volume of Water just before use.

Amoxycillin and Potassium Clavulanate Oral Suspension contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of amoxycillin, $C_{16}H_{19}N_3O_5S$ and not less than 90.0 per cent and not more than 125.0 per cent of the stated amount of clavulanic acid, $C_8H_9NO_5$.

When stored at the temperature and for the period stated on the label during which the constituted suspension may be



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expected to be satisfactory for use, it contains not less than 80.0 per cent of the stated amounts of amoxycillin, $C_{16}H_{19}N_3O_5S$ and clavulanic acid, $C_8H_9NO_5$.

**Usual strengths**. Amoxycillin 250 mg and Potassium Clavulanate 250 mg per 5 ml; Amoxycillin 400 mg and Potassium Clavulanate 400 mg per 5 ml.

### Identification

In the Assay, the retention time of the two principal peaks in the chromatogram obtained with the test solution correspond to those in the chromatogram obtained with the reference solution.

### Tests

**Water** (2.3.43). Not more than 7.5 per cent where the label indicates that after reconstitution as directed, the suspension contains an amount of amoxycillin that is less than 40 mg per ml; not more than 8.5 per cent where the label indicates that after reconstitution as directed, the suspension contains an amount of amoxicillin that is equal to or more than 40 mg per ml and is less than or equal to 50 mg per ml; not more than 11.0 per cent where the label indicates that after reconstitution as directed, the suspension contains an amount of amoxycillin that is more than 50 mg per ml and is less than or equal to 80 mg per ml; not more than 12.0 per cent where the label indicates that after reconstitution as directed, the suspension contains an amount of amoxycillin that is more than 80 mg per ml.

*The constituted suspension complies with the tests stated under Oral liquids and with the following tests.*

**pH** (2.4.24). 3.8 to 6.6.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Transfer an accurately weighed quantity containing about 50 mg of amoxycillin to a 100-ml volumetric flask, dissolve in *water*, dilute to 100.0 ml with the same solvent and filter. Use the filtrate as the test solution within 1 hour.

*Reference solution*. A solution containing 0.05 per cent w/v of *amoxycillin trihydrate RS* and 0.02 per cent w/v of *lithium clavulanate RS* in *water*.

Chromatographic system
- a stainless steel column 30 cm x 4 mm, packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a mixture of 95 volumes of *pH 4.4 sodium phosphate buffer* and 5 volumes of *methanol*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 20 µl.

Inject the reference solution. The relative retention times are about 0.5 for clavulanic acid and 1.0 for amoxycillin. The resolution between the amoxycillin and clavulanic acid peaks is not less than 3.5. The test is not valid unless the column efficiency determined from each analyte peak is not less than 550 theoretical plates, the tailing factor for each analyte peak is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Determine the weight per ml of the oral suspension (2.4.29) and calculate the content of $C_{16}H_{19}N_3O_5S$ and $C_8H_9NO_5$ weight in volume.

1 mg of $C_8H_8LiNO_5$ is equivalent to 0.9711 mg of $C_8H_9NO_5$.

Repeat the procedure using a portion of the constituted suspension that has been stored at the temperature and for the period stated on the label.

**Storage**. Store protected from moisture.

**Labelling**. The label states the quantity of Amoxycillin Trihydrate contained in it, in terms of the equivalent amount of amoxycillin, and the quantity of Potassium Clavulanate, in terms of the equivalent amount of clavulanic acid.

## Amoxycillin and Potassium Clavulanate Tablets

Amoxicillin and Potassium Clavulanate Tablets

Amoxycillin and Potassium Clavulanate Tablets contain Amoxycillin Trihydrate and Potassium Clavulanate. The tablets are coated.

Amoxycillin and Potassium Clavulanate Tablets contain not less than 90.0 per cent and not more than 120.0 per cent of the stated amounts of amoxycillin, $C_{16}H_{19}N_3O_5S$ and clavulanic acid, $C_8H_9NO_5$.

**Usual strengths**. 125 mg; 500 mg.

### Identification

In the Assay, the retention time of the two principal peaks in the chromatogram obtained with the test solution correspond to those in the chromatogram obtained with the reference solution.

### Tests

**Disintegration** (2.5.1). 30 minutes, for tablets labelled for veterinary use only, simulated gastric fluid being substituted for water in the test.

**Dissolution** (2.5.2). (Tablets labelled for veterinary use only are exempt from this requirement).
Apparatus. No 1,
Medium. 900 ml of *water*,



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Speed and time. 75 rpm and 30 minutes or 45 minutes where the Tablets are labelled as chewable.

Withdraw a suitable volume of the medium and filter. Carry out the method described under Assay.

D. Not less than 85 per cent of the stated amount of $C_{16}H_{19}N_3O_5S$ and not less than 80 per cent of the stated amount of $C_8H_9NO_5$.

For tablets labelled as chewable. Not less than 80 per cent of the stated amount of the $C_{16}H_{19}N_3O_5S$ and $C_8H_9NO_5$ is dissolved in 45 minutes.

**Uniformity of content**. Comply with the test stated under Tablets, determining the content of clavulanic acid in the tablets.

Follow the chromatographic procedure described under Assay using the following test solution.

Powder one tablet and transfer to a 100 ml flask. Dissolve in *water* and dilute to 100.0 ml with the same solvent and filter. Further dilute to obtain a solution containing 0.05 per cent w/v of amoxycillin. Use the solution within 1 hour.

Calculate the content of $C_8H_9NO_5$ in the tablet.

**Water** (2.3.43). Not more than 7.5 per cent, where the labelled amount of amoxycillin in each tablet is 250 mg or less; not more than 10.0 per cent where the labelled amount of amoxycillin in each tablet is more than 250 mg but less than or equal to 500 mg; not more than 11.0 per cent where the labelled amount of amoxycillin in each tablet is more than 500 mg. Where the tablets are labelled as chewable, not more than 6.0 per cent where the labelled amount of amoxycillin in each tablet is 125 mg or less; not more than 8.0 per cent where the labelled amount of amoxycillin in each tablet is more than 125 mg. Where the tablets are labelled for veterinary use only, not more than 10.0 per cent.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh and powder 20 tablets. Weigh accurately a quantity of the powdered tablet containing about 50 mg of amoxycillin, dissolve in *water*, dilute to 100.0 ml with *water* and filter. Use the filtrate as the test solution within 1 hour.

*Reference solution*. A solution containing 0.05 per cent w/v of *amoxycillin trihydrate RS* and 0.02 per cent w/v of *lithium clavulanate RS* in *water*.

Chromatographic system

- a stainless steel column 30 cm x 4 mm, packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a mixture of 95 volumes of 0.78 per cent w/v solution of *sodium phosphate*, adjusted to pH 4.4 with *orthophosphoric acid* and 5 volumes of *methanol*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 20 µl.

Inject the reference solution. The relative retention times are about 0.5 for clavulanic acid and 1.0 for amoxycillin. The resolution between the amoxycillin and clavulanic acid peaks is not less than 3.5. The test is not valid unless the column efficiency determined from each analyte peak is not less than 550 theoretical plates, the tailing factor for each analyte peak is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

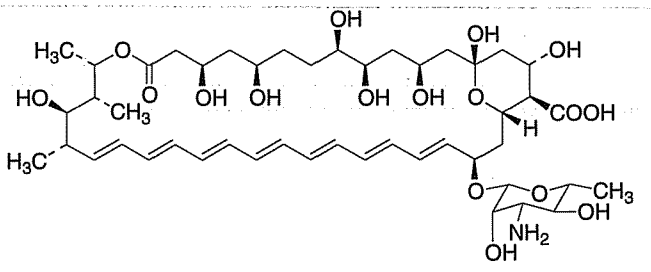
Calculate the content of $C_{16}H_{19}N_3O_5S$ and $C_8H_9NO_5$ in the tablets.

1 mg of $C_8H_8LiNO_5$ is equivalent to 0.9711 mg of $C_8H_9NO_5$.

**Storage**. Store protected from moisture.

**Labelling**. The label includes the word "chewable" in juxtaposition to the official name in the case of Chewable Tablets. The label also indicates that Chewable Tablets may be chewed before being swallowed or may be swallowed whole. Tablets intended for veterinary use only are so labelled.

## Amphotericin B



$C_{47}H_{73}NO_{17}$ Mol. Wt. 924.1

Amphotericin B is a mixture consisting mainly of amphotericin B which is (3*R*,5*R*,8*R*,9*R*,11*S*,13*R*,15*S*,16*R*,17*S*,19*R*,34*S*,35*R*,36*R*,37*S*)-19-(3-amino-3,6-dideoxy-β-D-mannopyranosyloxy)-16-carboxy-3,5,8,9,11,13,15, 35-octahydroxy-34,36-dimethyl-13,17-epoxyoctatriaconta- 20,22,24,26,28,30,32-heptaen-37-olide and other antifungal polyenes produced by the growth of certain strains of *Streptomyces nodosus* or by any other means.

Amphotericin B has a potency of not less than 750 Units per mg, calculated on the dried basis.

**Category**. Antifungal.

**Dose**. Orally, upto 200 mg every six hours; by slow intravenous injection, 250 mg per kg of body weight daily, increased to 1 mg per kg daily or 1.5 mg per kg on alternate days.



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**Description.** A yellow to orange powder; practically odourless. Even in the absence of light, it is gradually decomposed in a humid environment, degradation being faster at higher temperatures. In solutions, it is inactivated in the presence of light and at low pH values.

### Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *amphotericin B RS* or with the reference spectrum of amphotericin B.

B. Dissolve 25 mg in 5 ml of *dimethyl sulphoxide,* add sufficient *methanol* to produce 50 ml, and dilute 2 ml to 200 ml with *methanol.* When examined in the range 300 nm to 450 nm (2.4.7), the resulting solution shows absorption maxima at about 362 nm, 381 nm, and 405 nm. The ratio of the absorbance at the maximum at about 362 nm to the absorbance at the maximum at about 381 nm, 0.5 to 0.6; the ratio of the absorbance at the maximum at about 381 nm to the absorbance at the maximum at about 405 nm, about 0.9.

C. To 1 ml of a 0.05 per cent w/v solution in *dimethyl sulphoxide* add 5 ml of *phosphoric acid* to form a lower layer; a blue ring is immediately formed at the junction of the liquids. Mix; the mixture becomes intensely blue. Add 15 ml of *water* and mix; the solution becomes pale straw-coloured.

### Tests

**pH** (2.4.24). 6.0 to 8.0, determined in a 3.0 per cent w/v suspension in *water*; for parenteral use, 3.5 to 6.0.

**Tetraenes.** Not more than 15.0 per cent (for parenteral use, not more than 10.0 per cent), determined by the following method. Weigh accurately about 50 mg, dissolve in 5 ml of *dimethyl sulphoxide*, dilute to 50.0 ml with *methanol* and dilute 4.0 ml of the resulting solution to 50.0 ml with *methanol* (solution 1). Prepare solution (2) in a similar manner using 50 mg of *amphotericin B RS*, accurately weighed, instead of the substance under examination. For solution (3) dissolve 25 mg of *nystatin RS*, accurately weighed, in 25 ml of *dimethyl sulphoxide,* dilute to 250.0 ml with *methanol* and dilute 4.0 ml to 50.0 ml with *methanol.* Using as the blank a 0.8 per cent v/v solution of *dimethyl sulphoxide* in *methanol*, measure the absorbances of solutions (1), (2) and (3) at the maxima at about 282 nm and about 304 nm (2.4.7).

Calculate the specific absorbances for the substance under examination, *amphotericin B RS* and *nystatin RS* at both wavelengths and calculate the content of tetraenes from the expression

$$\frac{25\,W_N\,[(A_{B282}\times A_{U304})-(A_{B304}\times A_{U282})}{[(A_{B282}\times A_{N304})-(A_{B304}\times A_{N282})]\,W_U}$$

where $W_N$ is the weight, in mg, of nystatin RS, $A_{B282}$ and $A_{B304}$ are the specific absorbances of *amphotericin B RS* at about 282 nm and 304 nm, respectively, $A_{N282}$ and $A_{N304}$ are the specific absorbances of *nystatin RS* at about 282 nm and 304 nm respectively, $A_{U282}$ and $A_{U304}$ are the specific absorbances of the substance under examination at about 282 nm and 304 nm respectively and $W_U$ is the weight in mg of the sample taken.

**Sulphated ash** (2.3.18). Not more than 3.0 per cent; for parenteral use, not more than 0.5 per cent.

**Loss on drying** (2.4.19). Not more than 5.0 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa.

**Assay.** Determine by the microbiological assay of antibiotics, Method A (2.2.10) on a solution prepared in the following manner. Weigh accurately about 60 mg, triturate with *dimethylformamide* and add, with shaking, sufficient *dimethylformamide* to produce 100.0 ml. Dilute 10.0 ml to 100.0 ml with *dimethylformamide*. Express the result in Units per mg.

*Amphotericin B intended for use in the manufacture of parenteral preparations without a further appropriate procedure for removal of bacterial endotoxins complies with the following additional requirement.*

**Bacterial endotoxins** (2.2.3). Not more than 1.0 Endotoxin Unit per mg, using the supernatant liquid obtained after shaking 50 mg with 25 ml of *water BET* and centrifuging.

*Amphotericin B intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.*

**Sterility** (2.2.11). Complies with the test for sterility, using 50 mg from each container.

**Storage.** Store protected from light in a refrigerator (2° to 8°). Do not freeze.

**Labelling.** The label states (1) the number of Units per mg; (2) whether the material is intended for use in the manufacture of parenteral preparations.

## Amphotericin B Injection

Amphotericin B Injection is a sterile freeze dried mixture of Amphotericin B and deoxycholate sodium with one or more buffering agents. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

*The constituted solution complies with the requirements for Clarity of solution and Particulate Matter stated under Parenteral Preparations (Injections)*



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**Storage**. The constituted solution should be used immediately after preparation but, in any case within the period recommended by the manufacturer.

Amphotericin B Injection contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of amphotericin B, $C_{47}H_{73}NO_{17}$.

*The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.*

**Usual strength**. 50 mg per ml.

### Tests

**pH** (2.4.24). 7.2 to 8.0 determined in a solution containing 10 mg per ml of Amphotericin B.

**Bacterial Endotoxins** (2.2.3). Not more than 5.0 Endotoxin unit per mg of amphotericin B. For products used or labelled for intrathecal injection, not more than 0.9 Endotoxin unit per mg.

**Loss on drying** (2.4.19). Not more than 8.0 per cent, determined on 0.1 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa.

**Assay**. Determine by the microbiological assay of antibiotics, Method A (2.2.10) on a solution prepared in the following manner.

Mix the contents of 10 containers, dissolve in *dimethylformamide*. Express the results in mg per vial, taking each 1000 units found to be equivalent to 1 mg of amphotericin B.

**Storage**. Store in tightly closed containers between 2° to 8°, protected from light.

**Labeling**. Label it to state that it is intended for use by intravenous infusion to hospitalised patients only, and that the solution should be protected from light during administration.

## Liposomal Amphotericin B Injection

Liposomal Amphotericin B Injection is a sterile freeze dried mixture of Amphotericin B and along with phospholipids, cholesterol, antioxidant and one or more buffering agents. It is filled in a sealed container.

The injection is constituted with dissolving the content of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

*The constituted dispersion complies with the requirements for Particulate matter stated under Liposomal preparations.*

**Storage**. The constituted dispersion should be used immediately after preparation but, in any case within the period recommended by the manufacturer.

Liposomal Amphotericin B Injection contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of amphotericin B, $C_{47}H_{73}NO_{17}$.

*The contents of the sealed container comply with the requirements stated under Liposomal Preparations and with the following requirements.*

### Tests

**pH** (2.4.24). 4.5 to 6.5, determined on a dispersion containing 50 mg per ml of amphotericin B.

**Bacterial Endotoxins** (2.2.3). Not more than 5.0 Endotoxin unit per mg of amphotericin B.

**Water** (2.3.43). Not more than 2.0 per cent, determined on 0.5 g.

**Other tests**. Complies with the tests stated under Liposomal Preparations (Injections).

**Assay**. Determine by the microbiological assay of antibiotics, Method (a) (2.2.10) on a solution prepared in the following manner.

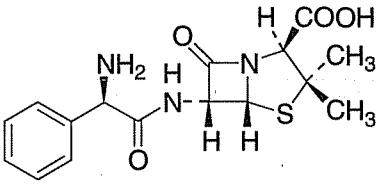
Mix the contents of 10 containers, dissolve in *dimethylformamide*. Express the result in mg per vial, taking each 1000 units found to be equivalent to 1 mg of amphotericin B.

**Vesicle size**. Determine the average vesicle size by light scattering method. The intensity- weighted average vesicle size for the injectable liposomal dispersion is not more than 150 nm, irrespective of the concentration of the dispersed phase.

**Storage**. Store protected from light and moisture, at a temperature between 2° to 8°.

**Labeling**. The label states that it is intended for use by intravenous infusion to hospitalized patients only and that the dispersion should be protected from light during administration.

## Ampicillin




$C_{16}H_{19}N_3O_4S$ Mol. Wt. 349.4

Ampicillin is (6*R*)-6-(α-phenyl-D-glycylamino)penicillanic acid.



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Ampicillin contains not less than 96.0 per cent and not more than 100.5 per cent of $C_{16}H_{19}N_3O_4S$, calculated on the anhydrous basis.

**Category**. Antibacterial.

**Dose**. 2 to 6 g daily, in divided doses.

**Description**. A white, crystalline powder.

### Identification

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

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

### Tests

**Appearance of solution**. Dissolve 1.0 g in 10 ml of *1 M hydrochloric acid* and a further 1.0 g in a mixture of 3 ml of *dilute ammonia solution* and 7 ml of *water*. Both solutions when freshly prepared are not more opalescent than opalescence standard OS2 (2.4.1).

**pH** (2.4.24). 3.5 to 5.5, determined in a 0.25 per cent w/v solution.

**Specific optical rotation** (2.4.22). +280° to +305°, determined in a 0.25 per cent w/v solution.

***N, N*-Dimethylaniline** (2.3.21). Not more than 20 ppm. determined by Method B.

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

**Sulphated ash** (2.3.18). Not more than 0.5 per cent.

**Water** (2.3.43). Not more than 2.0 per cent, determined on 0.3 g.

**Assay**. Determine by liquid chromatography (2. 4.14).

*Solvent mixture*. Mix 10 ml of *1 M monobasic potassium phosphate* and 1 ml of *1 M acetic acid* and dilute to 1000 ml with *water*.

*Test solution*. Transfer an accurately weighed quantity containing about 100 mg of ampicillin to a 100-ml volumetric flask, add about 80 ml of the solvent mixture, shake and mix with the aid of ultrasound if necessary to achieve complete dissolution and dilute to 100.0 ml with the solvent mixture. Use this solution promptly after preparation.

*Reference solution (a)*. Weigh accurately a suitable quantity of *ampicillin RS*, dissolve in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound to obtain a solution having a known concentration of about 1 mg per ml. Use this solution promptly after preparation.

*Reference solution (b)*. Dissolve *caffeine* in reference solution (a) to obtain a solution containing about 0.12 mg per ml.

Chromatographic system

- a stainless steel column 30 cm x 4.0 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: a mixture of 909 volumes of *water*, 80 volumes of *acetonitrile*, 10 volumes of *1 M monobasic potassium phosphate*, and 1 ml of *1 M acetic acid*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (b). The resolution between the caffeine and ampicillin peaks is not less than 2.0. The relative retention times are about 0.5 for ampicillin and 1.0 for caffeine.

Inject reference solution (a). The test is not valid unless the tailing factor is not more than 1.4 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and reference solution (a).

Calculate the percentage content of $C_{16}H_{19}N_3O_4S$.

**Storage**. Store protected from moisture at a temperature not exceeding 30°.

## Ampicillin Capsules

Ampicillin Capsules contain Ampicillin or Ampicillin Trihydrate equivalent to not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of ampicillin, $C_{16}H_{19}N_3O_4S$.

**Usual strengths**. 250 mg; 500 mg.

### Identification

The contents of the capsules comply with the following tests.

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

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

### Tests

**Dissolution** (2.5.2).

Apparatus. No 1,
Medium. 900 ml of *water*,
Speed and time. 100 rpm and 45 minutes.

Use one capsule in the vessel for each test.



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Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first 1 ml of the filtrate. Transfer an accurately measured portion of the filtrate, estimated to contain about 1 mg of ampicillin to a 50-ml volumetric flask, dilute with a 1 per cent v/v solution of *formaldehyde* in *0.3 M hydrochloric acid*. Heat the solution to 90° ± 5° in a constant temperature bath for 60 minutes. Measure the absorbance of the resulting solution at the maximum at about 352 nm (2.4.7). Calculate the content of $C_{16}H_{19}N_3O_4S$ in the medium from the absorbance obtained from a solution of known concentration of *ampicillin RS*.

D. Not less than 75 per cent of the stated amount of $C_{16}H_{19}N_3O_4S$.

**Other tests**. Comply with the tests stated under Capsules.

**Assay**. Determine by liquid chromatography (2. 4.14).

*Solvent mixture*. Mix 10 ml of *1 M monobasic potassium phosphate* and 1 ml of *1 M acetic acid* and dilute to 1000 ml with *water*.

*Test solution*. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 100 mg of ampicillin, add about 80 ml of the solvent mixture and dissolve by shaking for 15 minutes and mixing if necessary, with the aid of ultrasound. Dilute to 100.0 ml with the solvent mixture and filter. Use this solution promptly after preparation.

*Reference solution (a)*. Weigh accurately a suitable quantity of *ampicillin RS*, dissolve in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound to obtain a solution having a known concentration of about 1 mg per ml. Use this solution promptly after preparation.

*Reference solution (b)*. Dissolve *caffeine* in reference solution (a) to obtain a solution containing about 0.12 mg per ml.

Chromatographic system

- a stainless-steel column 30 cm x 4.0 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic micro particles (5 µm),
- mobile phase: a mixture of 900 volumes of *water*, 80 volumes of *acetonitrile*, 10 volumes of *1 M monobasic potassium phosphate*, and 1 ml of *1 M acetic acid*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (b). The resolution between the caffeine and ampicillin peaks is not less than 2.0. The relative retention times are about 0.5 for ampicillin and 1.0 for caffeine.

Inject reference solution (a). The capacity factor is not more than 2.5 and the tailing factor is not more than 1.4. The test is not valid unless the relative standard deviation for replicate injections is at most 2.0 per cent.

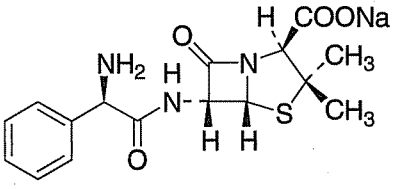
Inject alternately the test solution and reference solution (a).

Calculate the content of $C_{16}H_{19}N_3O_4S$ in the capsules.

**Storage**. Store protected from moisture at a temperature not exceeding 30°.

**Labelling**. The label states the strength in terms of the equivalent amount of ampicillin (when Ampicillin Trihydrate is used).

# Ampicillin Sodium




$C_{16}H_{18}N_3NaO_4S$ Mol. Wt. 371.4

Ampicillin Sodium is sodium (6*R*)-6-(α-phenyl-D-glycylamino)penicillinate

Ampicillin Sodium contains not less than 92.5 per cent and not more than 100.5 per cent of $C_{16}H_{18}N_3NaO_4S$, calculated on the anhydrous basis.

**Category**. Antibacterial.

**Dose**. By intramuscular or intravenous injection, the equivalent of 1 to 3 g of ampicillin daily, in divided doses.

**Description**. A white, crystalline powder; hygroscopic.

## Identification

A. Determine by infrared absorption spectophotometry (2.4.6). Compare the spectrum with that obtained with *ampicillin sodium RS* or with the reference spectrum of ampicillin sodium.

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

C. A 5 per cent w/v solution gives the reactions of sodium salts (2.3.1).

## Tests

**Appearance of solution**. A 10.0 per cent w/v solution is clear, when examined immediately after preparation (2.4.1), and the absorbance of the solution at about 430 nm (2.4.7) is not more than 0.15.

**pH** (2.4.24). 8.0 to 10.0, determined 10 minutes after dissolution in a 10.0 per cent w/v solution.



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**Specific optical rotation** (2.4.22). +258° to +287°, determined in a 0.25 per cent w/v solution in a 0.4 per cent w/v solution of *potassium hydrogen phthalate*.

***N,N*-Dimethylaniline** (2.3.21). Not more than 20 ppm, determined by Method B.

**Dichloromethane**. Not more than 0.2 per cent w/w, determined in the following manner.

Determine by gas chromatography (2.4.13).

*Internal standard solution*. A 0.02 per cent v/v solution of *1,2-dichloroethane* in *water*.

*Test solution*. Dissolve 1.0 g of the substance under examination in sufficient *water* to produce 10 ml.

*Reference solution (a)*. Dissolve 1.0 g of the substance under examination in 10 ml of the internal standard solution.

*Reference solution (b)*. Mix equal volumes of the internal standard solution and a 0.02 per cent v/v solution of *dichloromethane* in *water*.

Chromatographic system
- a glass column 1.5 m x 5 mm, packed with acid-washed silanised diatomaceous support (100 to 120 mesh) coated with 10 per cent w/w of *polyethylene glycol 1000*,
- temperature:
  column. 60°,
- inlet port and detector.150°,
- flow rate. 40 ml per minute of the carrier gas.

Calculate the percentage w/w of dichloromethane, assuming its relative density (2.4.29) to be 1.325 g.

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

**Water** (2.3.43). Not more than 2.0 per cent, determined on 0.3 g.

**Assay**. Determine by liquid chromatography (2. 4.14).

*Solvent mixture*. Mix 10 ml of *1 M monobasic potassium phosphate* and 1 ml of *1 M acetic acid* and dilute to 1000 ml with *water*.

*Test solution*. Transfer an accurately weighed quantity containing about 100 mg of ampicillin to a 100-ml volumetric flask and dissolve in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound and dilute to 100.0 ml with the solvent mixture. Use this solution promptly after preparation.

*Reference solution (a)*. Weigh accurately a suitable quantity of *ampicillin RS*, dissolve in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound to obtain a solution having a known concentration of about 1 mg per ml. Use this solution promptly after preparation.

*Reference solution (b)*. Dissolve *caffeine* in reference solution (a) to obtain a solution containing about 0.12 mg per ml.

Chromatographic system
- a stainless steel column 30 cm x 4.0 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: a mixture of 900 volumes of *water*, 80 volumes of *acetonitrile*, 10 volumes of *1 M monobasic potassium phosphate*, and 1 ml of *1 M acetic acid*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (b). The resolution between the caffeine and ampicillin peaks is not less than 2.0. The relative retention times are about 0.5 for ampicillin and 1.0 for caffeine.

Inject reference solution (a). The test is not valid unless the capacity factor is not more than 2.5, the tailing factor is not more than 1.4 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and reference solution (a).

Calculate the percentage content of $C_{16}H_{19}N_3O_4S$.

*Ampicillin Sodium intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.*

**Bacterial Endotoxins** (2.2.3). Not more than 0.15 Endotoxin Unit per mg.

*Ampicillin Sodium intended for use in the manufacture of parenteral preparations without a further appropriate sterilization procedure complies with the following additional requirement.*

**Sterility** (2.2.11). Complies with the test for sterility.

**Storage**. Store protected from moisture at a temperature not exceeding 30°. If it is intended for use in the manufacture of parenteral preparations, the container should be sterile, tamper-evident and sealed so as to exclude micro-organisms.

**Labelling**. The label states whether or not the material is intended for use in the manufacture of parenteral preparations.

## Ampicillin Injection

Ampicillin Sodium Injection

Ampicillin Injection is a sterile material consisting of Ampicillin Sodium with or without buffering agents and other excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.



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*The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).*

**Storage.** The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Ampicillin Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of ampicillin, $C_{16}H_{19}N_3O_4S$.

**Category.** Antibacterial

**Usual strengths.** The equivalent of 100 mg, 250 mg, 500 mg and 1 g of ampicillin.

**Description.** A white or almost white powder; hygroscopic.

*The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injections) and with the following requirements.*

## Identification

A. Determine by infrared absorption spectophotometry (2.4.6). Compare the spectrum with that obtained with *ampicillin sodium RS* or with the reference spectrum of ampicillin sodium.

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

C. A 5 per cent w/v solution gives the reactions of sodium salts (2.3.1).

## Tests

**Appearance of solution.** A 10 per cent w/v solution is clear, when examined immediately after preparation (2.4.1), and the absorbance of the solution at about 430 nm is not more than 0.15.

**pH** (2.4.24). 8.0 to 10.0, determined 10 minutes after dissolution in a 10 per cent w/v solution.

**Specific optical rotation** (2.4.22). +258° to +287°, determined in a 0.25 per cent w/v solution in a 0.4 per cent w/v solution of *potassium hydrogen phthalate*.

***N,N*-Dimethylaniline** (2.3.21). Not more than 20 ppm, determined by Method B.

**Dichloromethane.** Not more than 0.2 per cent w/w, determined in the following manner.

Determine by gas chromatography (2.4.13).

*Internal standard solution.* A 0.02 per cent v/v solution of *1,2-dichloroethane* in *water*.

*Test solution.* Dissolve 1.0 g of the substance under examination in sufficient *water* to produce 10 ml.

*Reference solution (a).* Dissolve 1.0 g of the substance under examination in 10 ml of the internal standard solution.

*Reference solution (b).* Mix equal volumes of the internal standard solution and a 0.02 per cent v/v solution of *dichloromethane* in *water*.

Chromatographic system
- a glass column 1.5 m x 5 mm, packed with acid-washed silanised diatomaceous support (100 to 120 mesh) coated with 10 per cent w/w of *polyethylene glycol 1000*,
- temperature:
  column. 60°,
- inlet port and detector.150°,
- flow rate. 40 ml per minute of the carrier gas.

Calculate the percentage w/w of dichloromethane, assuming its relative density (2.4.29) to be 1.325 g.

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

**Bacterial Endotoxins** (2.2.3). Not more than 0.15 Endotoxin Unit per mg of ampicillin.

**Sterility** (2.2.11). Complies with the test for sterility.

**Water** (2.3.43). Not more than 2.0 per cent, determined on 0.3 g.

**Assay.** Determine by liquid chromatography (2. 4.14).

*Solvent mixture.* Mix 10 ml of *1 M monobasic potassium phosphate* and 1 ml of *1 M acetic acid* and dilute to 1000 ml with *water*.

*Test solution.* Determine the weight of the contents of 10 containers. Transfer an accurately weighed quantity of the mixed contents of the 10 containers containing about 100 mg of ampicillin to a 100-ml volumetric flask, add about 80 ml of the solvent mixture and dissolve by shaking and mixing if necessary, with the aid of ultrasound. Dilute to 100.0 ml with the solvent mixture and filter. Use this solution promptly after preparation.

*Reference solution (a).* Weigh accurately a suitable quantity of *ampicillin RS,* dissolve in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound to obtain a solution having a known concentration of about 1 mg per ml. Use this solution promptly after preparation.

*Reference solution (b).* Dissolve *caffeine* in reference solution (a) to obtain a solution containing about 0.12 mg per ml.

Chromatographic system
- a stainless steel column 30 cm x 4.0 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: a mixture of 900 volumes of *water,* 80 volumes of *acetonitrile,* 10 volumes of *1 M*



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*monobasic potassium phosphate*, and 1 ml of *1 M acetic acid*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (b). The resolution between the caffeine and ampicillin peaks is not less than 2.0. The relative retention times are about 0.5 for ampicillin and 1.0 for caffeine.

Inject reference solution (a). The test is not valid unless the capacity factor is not more than 2.5, the tailing factor is not more than 1.4 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and reference solution (a).

Calculate the content of $C_{16}H_{19}N_3O_4S$ in the injection.

**Storage**. Store protected from moisture, in a sterile, tamper-evident container sealed so as to exclude micro-organisms, at a temperature not exceeding 30°.

**Labelling**. The label states the quantity of Ampicillin Sodium contained in the sealed container in terms of the equivalent amount of anhydrous ampicillin.

# Ampicillin Oral Suspension

Ampicillin Oral Suspension is a mixture consisting of Ampicillin or Ampicillin Trihydrate with buffering agents and other excipients. It contains a suitable flavouring agent. It is filled in a sealed container.

The suspension is constituted by dispersing the contents of the sealed container in the specified volume of Water just before issue.

Ampicillin oral suspension contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of ampicillin, $C_{16}H_{19}N_3O_4S$.

The constituted suspension, when stored at the temperature and for the period stated on the label during which it may be expected to be satisfactory for use, contains not less than 80.0 per cent of the stated amount of ampicillin, $C_{16}H_{19}N_3O_4S$.

**Storage**. Store protected from moisture at a temperature not exceeding 30°.

**Usual strengths**. Ampicillin 125 mg per 5 ml; Ampicillin 250 mg per 5 ml.

## Identification

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

The constituted suspension complies with the tests stated under Oral liquids and with the following tests.

**pH** (2.4.24). 4.0 to 7.0.

**Assay**. Determine by liquid chromatography (2. 4.14).

*Solvent mixture*. Mix 10 ml of *1 M monobasic potassium phosphate* and 1 ml of *1 M acetic acid* and dilute to 1000 ml with *water*.

*Test solution*. Transfer an accurately weighed quantity containing about 100 mg of ampicillin to a 100-ml volumetric flask and dilute to 100.0 ml with the solvent mixture and filter. Use this solution promptly after preparation.

*Reference solution (a)*. Weigh accurately a suitable quantity of *ampicillin RS*, dissolve in the solvent mixture by shaking and mixing with the aid of ultrasound if necessary, to obtain a solution having a known concentration of about 1 mg per ml. Use this solution promptly after preparation.

*Reference solution (b)*. Dissolve *caffeine* in reference solution (a) to obtain a solution containing about 0.12 mg per ml.

Chromatographic system
- a stainless steel column 30 cm x 4.0 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: a mixture of 900 volumes of *water*, 80 volumes of *acetonitrile*, 10 volumes of *1 M monobasic potassium phosphate*, and 1 ml of *1 M acetic acid*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (b). The resolution between the caffeine and ampicillin peaks is not less than 2.0. The relative retention times are about 0.5 for ampicillin and 1.0 for caffeine.

Inject reference solution (a). The test is not valid unless the capacity factor is not more than 2.5, the tailing factor is not more than 1.4 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and reference solution (a).

Determine the weight per ml (2.4.29) of the suspension and calculate the content of $C_{16}H_{19}N_3O_4S$, weight in volume.

Repeat the procedure using a portion of the constituted suspension that has been stored at the temperature and for the period stated on the label.

**Labelling**. The label states (1) the quantity of active ingredient in terms of the equivalent amount of ampicillin when the active ingredient is Ampicillin Trihydrate; (2) the temperature of storage and the period during which the constituted suspension may be expected to be satisfactory for use.



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# Ampicillin Dispersible Tablets

Dispersible Ampicillin Tablets

Ampicillin Dispersible Tablets contain Ampicillin or Ampicillin Trihydrate in a suitable dispersible base.

Ampicillin Dispersible Tablets contain Ampicillin or Ampicillin Trihydrate equivalent to not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of ampicillin, $C_{16}H_{19}N_3O_4S$.

**Usual strengths**. The equivalent of 125 mg and 250 mg of ampicillin.

## Identification

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

## Tests

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

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets.

Determine by liquid chromatography (2. 4.14).

*Solvent mixture*. Mix 10 ml of *1 M monobasic potassium phosphate* and 1 ml of *1 M acetic acid* and dilute to 1000 ml with *water*.

*Test solution*. Transfer an accurately weighed quantity of the powdered tablets containing about 100 mg of ampicillin to a 100-ml volumetric flask, add about 80 ml of the solvent mixture, shake for 15 minutes and mix with the aid of ultrasound to achieve complete dissolution. Dilute to 100.0 ml with the solvent mixture and filter. Use this solution promptly after preparation.

*Reference solution (a)*. Weigh accurately a suitable quantity of *ampicillin RS*, dissolve in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound to obtain a solution having a known concentration of about 1 mg per ml. Use this solution promptly after preparation.

*Reference solution (b)*. Dissolve *caffeine* in reference solution (a) to obtain a solution containing about 0.12 mg per ml.

Chromatographic system
- a stainless steel column 30 cm x 4.0 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: a mixture of 900 volumes of *water*, 80 volumes of *acetonitrile*, 10 volumes of *1 M monobasic potassium phosphate*, and 1 ml of *1 M acetic acid*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (b). The resolution between the caffeine and ampicillin peaks is not less than 2.0. The relative retention times are about 0.5 for ampicillin and 1.0 for caffeine.

Inject reference solution (a). The test is not valid unless the capacity factor is not more than 2.5, the tailing factor is not more than 1.4 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

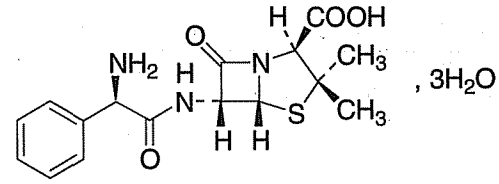
Inject alternately the test solution and reference solution (a).

Calculate the percentage content of $C_{16}H_{19}N_3O_4S$ in the tablets.

**Storage**. Store protected from moisture at a temperature not exceeding 30°.

**Labelling**. The label states (1) the strength in terms of the equivalent amount of ampicillin (when Ampicillin Trihydrate is used); (2) that the tablets should be dispersed in water immediately before use.

# Ampicillin Trihydrate




$C_{16}H_{19}N_3O_4S,3H_2O$ Mol. Wt. 403.5

Ampicillin Trihydrate is (6*R*)-6-(α-phenyl-D-glycylamino)penicillanic acid trihydrate.

Ampicillin Trihydrate contains not less than 96.0 per cent and not more than 100.5 per cent of $C_{16}H_{19}N_3O_4S$, calculated on the anhydrous basis.

**Category**. Antibacterial.

**Dose**. The equivalent of 2 to 6 g of ampicillin daily, in divided doses.

**Description**. A white, crystalline powder.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ampicillin trihydrate RS or* with the reference spectrum of ampicillin trihydrate.



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

### Tests

**Appearance of solution**. Dissolve 1.0 g in 10 ml of *1 M hydrochloric acid* and a further 1.0 g in a mixture of 3 ml of *dilute ammonia solution* and 7 ml of *water*. Both solutions when freshly prepared are not more opalescent than opalescence standard OS2 (2.4.1).

**pH** (2.4.24). 3.5 to 5.5, determined in a 0.25 per cent w/v solution.

**Specific optical rotation** (2.4.22). +280° to +305°, determined in a 0.25 per cent w/v solution.

***N,N*-Dimethylaniline** (2.3.21). Not more than 20 ppm, determined by Method B.

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

**Sulphated ash** (2.3.18). Not more than 0.5 per cent.

**Water** (2.3.43). 12.0 per cent to 15.0 per cent, determined on 0.1 g.

**Assay**. Determine by liquid chromatography (2. 4.14).

*Solvent mixture*. Mix 10 ml of *1 M monobasic potassium phosphate* and 1 ml of *1 M acetic acid* and dilute to 1000 ml with *water*.

*Test solution*. Transfer an accurately weighed quantity containing about 100 mg of ampicillin to a 100-ml volumetric flask and dissolve in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound and dilute to 100.0 ml with the solvent mixture. Use this solution promptly after preparation.

*Reference solution (a)*. Weigh accurately a suitable quantity of *ampicillin RS*, dissolve in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound to obtain a solution having a known concentration of about 1 mg per ml. Use this solution promptly after preparation.

*Reference solution (b)*. Dissolve *caffeine* in reference solution (a) to obtain a solution containing about 0.12 mg per ml.

Chromatographic system

- a stainless steel column 30 cm x 4.0 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: a mixture of 909 volumes of *water*, 80 volumes of *acetonitrile*, 10 volumes of *1 M monobasic potassium phosphate*, and 1 ml of *1 M acetic acid*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (b). The resolution between the caffeine and ampicillin peaks is not less than 2.0. The relative retention times are about 0.5 for ampicillin and 1.0 for caffeine.

Inject reference solution (a). The test is not valid unless the capacity factor is not more than 2.5, the tailing factor is not more than 1.4 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and reference solution (a).

Calculate the percentage content of $C_{16}H_{19}N_3O_4S$.

**Storage**. Store at a temperature not exceeding 30°.

## Alpha Amylase

Diastase

Alpha Amylase is an amylolytic enzyme or a mixture of enzymes obtained from fungi such as *Aspergillus oryzae* or from a non-pathogenic variant of bacteria such as *Bacillus subtilis* and with the specific activity for converting starch into dextrin and maltose. It may contain suitable harmless diluents such as Lactose or Dibasic Calcium Phosphate.

Alpha Amylase has amylase activity of not less than 800 Units which represents the number of grams of dry, soluble maize or corn starch digested by 1.0 g of Alpha Amylase under the conditions of the Assay.

**Category**. Digestive enzyme

**Dose**. 200 to 500 mg.

**Description**. A cream to light brown-coloured powder; almost odourless or with faint characteristic odour; hygroscopic.

### Tests

**Loss on drying** (2.4.19). Not more than 5.0 per cent, determined on 1.0 g by drying in an oven at 105° for 1 hour.

**Assay**. Weigh accurately a quantity containing 100 Units of amylase activity and triturate with 200 ml of *buffer solution pH 6.0* (*for bacterial amylase*) or of *acetate buffer pH 5.0* (*for fungal amylase*) and add sufficient *buffer solution pH 6.0* or *acetate buffer pH 5.0*, as appropriate, to produce 1000.0 ml. Dilute 10.0 ml to 100.0 ml with *buffer solution pH 6.0* or *acetate buffer pH 5.0*, as appropriate, to give the test solution; filter if necessary (1 ml of the test solution should be capable of digesting about 10 mg of dry soluble maize or corn starch). Into each of six stoppered test-tubes add 5.0 ml of *starch substrate* without touching the sides of the test-tube. Place the test-tubes in a water-bath maintained at 40° ± 0.1°. When the temperature of the solution in the tubes has reached 40°, add 0.35 ml, 0.4 ml, 0.45 ml, 0.5 ml, 0.55 ml and 0.6 ml of the test



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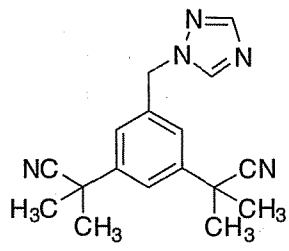
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solution to each of the test-tubes marked 1 to 6 respectively and record the time of addition. Mix thoroughly and replace the tubes in the water-bath. After exactly 60 minutes remove the tubes and cool rapidly in cold *water*. Add to each tube 0.05 ml of *0.02 M iodine* and mix well. Note the tube containing the lowest volume of test solution that does not show a bluish or violet tinge (if there is doubt, warm the solution slightly, when the colour distinction is prominent). From this volume calculate the number of grams of dry soluble maize or corn starch digested by 1.0 g of the substance under examination. This represents the number of Units of amylase activity per g.

**Storage**. Store protected from light and moisture.

# Anastrozole



$C_{17}H_{19}N_5$ Mol. Wt. 293.4

Anastrozole is a,a,a´,a´-tetramethyl-5-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-benzene diacetonitrile

Anastrozole contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{17}H_{19}N_5$, calculated on the anhydrous basis.

**Category**. Anticancer.

**Description**. A white to off white, crystalline powder.

*CAUTION — Anastrozole is cytotoxic; extra care required to prevent inhaling particles and exposing the skin to it.*

## Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *anastrozole RS* or with the reference spectrum of anastrozole.

## Tests

**Melting range** (2.4.21). 81° to 84°.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture.* 50 volumes of *acetonitrile* and 50 volumes of *water*.

*Test solution.* Dissolve 50 mg of the substance under examination in 100.0 ml of the solvent mixture.

*Reference solution.* Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with phenyl group ( 5 µm),
- mobile phase: a mixture of 65 volumes of *water*, 35 volumes of *acetonitrile* and 0.5 volume of *orthophosphoric acid*, adjust pH to 3.0 with *1 M sodium hydroxide*,
- flow rate. 1 ml per minute,
- spectrophometer set at 220 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and the reference solution. In the chromatogram obtained with the test solution, area of any secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent). The sum of areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the peak in the chromatogram obtained with the reference solution (0.1 per cent).

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

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 0.5 per cent, determined on 1.0 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve 50 mg of the substance under examination in 100.0 ml of the solvent mixture. Dilute 10.0 ml of this solution to 50.0 ml with the solvent mixture.

*Reference solution.* A 0.01 per cent w/v solution of *anastrozole RS* in the solvent mixture.

Use the chromatographic system as described under Related substances.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{17}H_{19}N_5$.

**Storage**. Store protected from light and moisture.



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# Anastrozole Tablets

Anastrozole Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of anastrozole, $C_{17}H_{19}N_5$.

**Usual strength**. 1 mg.

## Identification

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

## Tests

**Uniformity of content**. Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14).

*Test solution*. Disperse 1 tablet in 10.0 ml of the solvent mixture.

*Reference solution*. A 0.01 per cent w/v solution of *anastrozole RS* in the solvent mixture.

Use chromatographic system as described under Assay.

Inject the reference solution and the test solution.

Calculate the content of $C_{17}H_{19}N_5$ in the tablet.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. 50 volumes of *acetonitrile* and 50 volumes of *water*.

*Test solution*. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 10 mg of Anastrozole, shake with 70 ml of the solvent mixture, dilute to 100.0 ml with the solvent mixture and filter.

*Reference solution*. A 0.01 per cent w/v solution of *anastrozole RS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with phenyl group (5 µm),
- mobile phase: a mixture of 65 volumes of *water*, 35 volumes of *acetonitrile* and 0.5 volume of *orthophosphoric acid*, adjusted to pH 3.0 with *1 M sodium hydroxide*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the theoretical plates is not less than 2000 and tailing factor is not more than 2.0.

Inject the reference solution and the test solution.

Calculate the content of $C_{17}H_{19}N_5$ in the tablets.

**Storage**. Store protected from moisture, at a temperature not exceeding 30°.

# Anticoagulant Citrate Dextrose Solution

ACD Solution

Anticoagulant Citrate Dextrose Solution is a sterile solution of Sodium Citrate, Citric Acid and Dextrose in Water for Injections.

Anticoagulant Citrate Dextrose Solution contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amounts of Sodium Citrate, $C_6H_5Na_3O_7,2H_2O$, Citric Acid monohydrate, $C_6H_8O_7,H_2O$ (or Anhydrous Citric Acid, $C_6H_8O_7$), and Dextrose, $C_6H_{12}O_6,H_2O$. It contains no antimicrobial agent.

**Category**. Anticoagulant for storage of whole blood.

**Usual strengths**.

| | Solution A | Solution B |
|---|---|---|
| Sodium Citrate | 2.20 g | 1.32 g |
| Citric Acid (Anhydrous) | 0.73 g | 0.44 g |
| or Citric Acid (Monohydrate) | 0.80 g | 0.48 g |
| Dextrose (Monohydrate) | 2.45 g | 1.47 g |
| Water for Injection | 100 ml | 100 ml |

*NOTE — 15 ml of solution A or 25 ml of solution B are to be used for 100 ml of whole blood.*

**Description**. A clear, colourless or faintly straw-coloured liquid; odourless.

## Identification

A. To 1 ml add 0.05 ml of *potassium cupri-tartrate solution*; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.

B. Gives the reactions of sodium salts (2.3.1).

C. To 2 ml (for Solution A) add 3 ml of *water* or to 4 ml (for Solution B) add 1 ml of *water*. The resulting solution gives reaction A of citrates (2.3.1).

## Tests

**pH** (2.4.24). 4.5 to 5.5.

**Bacterial Endotoxins** (2.2.3). Not more than 5.56 Endotoxin Units per ml.

**Sterility** (2.2.11). Complies with the test for sterility.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).



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**Assay**. *For sodium citrate* — Pipette 50.0 ml into a beaker and titrate with *1.3 M hydrochloric acid* to a pH of 1.98 ± 0.02, determining the end-point potentiometrically (2.4.25). Carry out a blank titration with 50 ml of *water*.

1 ml of *1.3 M hydrochloric acid* is equivalent to 0.1274 g of $C_6H_5Na_3O_7,2H_2O$.

*For free citric acid* — Pipette 20.0 ml into a conical flask and titrate with *0.1 M sodium hydroxide* using *phenolphthalein solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.006404 g of $C_6H_8O_7$ or 0.007005 g of $C_6H_8O_7,H_2O$.

*For dextrose* — Determine the optical rotation in a 2-dm tube (2.4.22). The observed rotation multiplied by 1.0425, represents the weight of $C_6H_{12}O_6,H_2O$ in 100 ml of the solution.

**Storage**. Store protected from light in a single dose, tamper-evident container of colourless, transparent glass or of a suitable plastic material.

**Labelling**. The label states (1) whether the contents are Solution A or Solution B; (2) volume of the solution required per 100 ml of whole blood or the volume of the solution required per volume of whole blood to be collected; (3) where applicable, the maximum amount of blood to be collected in the container.

# Anticoagulant Citrate Phosphate Dextrose Solution

CPD Solution

Anticoagulant Citrate Phosphate Dextrose Solution is a sterile solution of Sodium Citrate, Citric Acid, Sodium Dihydrogen Phosphate Dihydrate and Dextrose in Water for Injection.

Anticoagulant Citrate Phosphate Dextrose Solution contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amounts of Sodium Citrate, $C_6H_5Na_3O_7,2H_2O$, Citric Acid, $C_6H_8O_7,H_2O$, Sodium Dihydrogen Phosphate Dihydrate, $NaH_2PO_4,2H_2O$ and Dextrose, $C_6H_{12}O_6,H_2O$. It contains no antimicrobial agent.

**Category**. Anticoagulant for storage of whole blood.

**Usual strengths**.

| | |
|---|---|
| Sodium Citrate | 2.630 g |
| Citric Acid (Monohydrate) | 0.327 g |
| Dextrose (Monohydrate) | 2.550 g |
| Sodium Dihydrogen Phosphate (Dihydrate) | 0.251 g |
| Water for Injection to | 100 ml |

*NOTE — 14 ml are to be used for 100 ml of whole blood.*

**Description**. A clear, colourless or faintly straw-coloured liquid; odourless.

## Identification

A. To 1 ml add 0.05 ml of *potassium cupri-tartrate solution*; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.

B. Gives the reactions of sodium salts and reaction B of phosphates (2.3.1).

C. To 2 ml add 3 ml of *water*. The resulting solution gives reaction A of citrates (2.3.1).

## Tests

**pH** (2.4.24). 5.0 to 6.0.

**Bacterial Endotoxins** (2.2.3). Not more than 5.56 Endotoxin Units per ml.

**Sterility** (2.2.11). Complies with the test for sterility.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. *For sodium citrate* — Dilute 25.0 ml to 100.0 ml with *water* and mix. Dilute 5.0 ml of the resulting solution to 100.0 ml with *water* and mix. Transfer 1.0 ml of this solution to a test-tube, add 1.3 ml of *pyridine*, swirl to mix, add 5.7 ml of *acetic anhydride*, mix and immediately place in a water-bath at 31° ± 0.5°. Allow the colour to develop for 35 minutes and measure the absorbance of the resulting solution at about 425 nm (2.4.7) using as the blank solution 1 ml of *water* treated in the same manner. Prepare a calibration curve by measuring the absorbance of solutions prepared by treating in the same manner 1 ml quantities of suitable dilutions of a solution in *water* containing 2.5 mg per ml of $C_6H_8O_7$, prepared by using *anhydrous citric acid*, previously dried for 3 hours at 90°. Calculate the total citrate content, as $C_6H_8O_7$, in mg per ml of the solution under examination from the expression 0.2 C, where C is the concentration in µg per ml of $C_6H_8O_7$, read from the curve.

Calculate the quantity, in mg, of $C_6H_5Na_3O_7,2H_2O$ in 1 ml of the solution under examination from the expression 1.53 $(A - B)$, where $A$ is the concentration in mg per ml of total citrate as $C_6H_8O_7$ and $B$ is the concentration in mg per ml of free citric acid in the solution.

*For free citric acid* — Pipette 20.0 ml into a conical flask and titrate with *0.1 M sodium hydroxide* using *phenolphthalein solution* as indicator.

From the volume of *0.1 M sodium hydroxide* required subtract a volume, in ml, equal to 1.28 times the number of mg of $NaH_2PO_4,2H_2O$ present, as determined in the Assay for sodium acid phosphate.



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1 ml of the remainder is equivalent to 0.007005 g of $C_6H_8O_7,H_2O$.

*For sodium dihydrogen phosphate dihydrate* — Dilute 5.0 ml to 100.0 ml with *water*. Transfer 5.0 ml to a 25-ml graduated flask and add 10.0 ml of a 2.8 per cent w/v solution of *sulphuric acid* followed by 2.0 ml of a 2.5 per cent w/v solution of *ammonium molybdate*, mixing after each addition. Add 1.0 ml of *aminohydroxynaphthalenesulphonic acid solution* and sufficient *water* to produce 25.0 ml, mix and keep aside at 25° for 10 minutes. Measure the absorbance ($A_1$) of the resulting solution at the maximum at about 660 nm (2.4.7) using as the blank 5 ml of *water* treated in the same manner. Calculate the content of $NaH_2PO_4,2H_2O$ in each ml of the solution under examination from the absorbance ($A_2$) obtained by simultaneously carrying out the operation using 5.0 ml of a solution of *potassium dihydrogen phosphate* containing 0.11 mg of $KH_2PO_4$ per ml (C) and from the expression

$$22.92\,C\,(A_1/A_2).$$

*For dextrose* — Weigh a clean, medium- porosity sintered-glass crucible containing a few glass beads. To 50 ml of *potassium cupri-tartrate solution* add the glass beads from the weighed crucible, 45 ml of *water* and 5.0 ml of the solution under examination. Heat the solution at such a rate that it begins to boil in 3.5 to 4 minutes, boil the solution for exactly 2 minutes and filter immediately through the weighed crucible, taking care to transfer all the glass beads to the crucible, along with the precipitate. Wash the precipitate with hot *water* and then with 10 ml of *ethanol (95 per cent)* and dry it to constant weight at 110°. Carry out a blank determination.

1 mg of the precipitate is equivalent to 0.000496 g of $C_6H_{12}O_6,H2O$.

**Storage**. Store in a single dose, tamper-evident container of colourless, transparent glass or of a suitable plastic material, protected from light.

**Labelling**. The label states (1) the composition and volume of the solution; (2) volume of the solution required per 100 ml of whole blood or the volume of the solution required per volume of whole blood to be collected; (3) where applicable, the maximum amount of blood to be collected in the container.

# Anticoagulant Citrate Phosphate Dextrose Adenine Solution

CPDA Solution

Anticoagulant Citrate Phosphate Dextrose Adenine Solution is a sterile solution of Citric Acid, Sodium Citrate, Sodium Dihydrogen Phosphate Dihydrate, Dextrose and Adenine in *Water for Injection*.

Anticoagulant Citrate Phosphate Dextrose Adenine Solution contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amounts of total Sodium, Na, total Citrate, $C_6H_5O_7$, Sodium Dihydrogen Phosphate Dihydrate, $NaH_2PO_4,2H_2O$, Adenine,$C_5H_5N_5$ and Dextrose Monohydrate, $C_6H_{12}O_6,H_2O$. It contains no antimicrobial agent.

**Category**. Anticoagulant for storage of whole blood.

**Usual strengths**.

| | |
|---|---|
| Citric Acid (Anhydrous) | 0.2990 g |
| Sodium Citrate (Dihydrate) | 2.6300 g |
| Sodium Dihydrogen Phosphate (Dihydrate) | 0.2510 g |
| Adenine | 0.0275 g |
| Dextrose (Monohydrate) | 3.1900 g |
| Water for Injection to | 100 ml |

*NOTE — 14 ml is to be used for 100 ml of whole blood.*

**Description**. A clear, colourless or faintly straw-coloured liquid; odourless.

## Identification

A. To 1 ml add 0.05 ml of *potassium cupri-tartrate solution*; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.

B. Gives the reaction B of phosphates and the reactions of sodium salts (2.3.1).

C. To 2 ml add 3 ml of *water*. The resulting solution gives reaction A of citrates (2.3.1).

D. In the test for adenine in the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (c).

## Tests

**pH** (2.4.24). 5.0 to 6.0.

**Bacterial Endotoxins** (2.2.3). Not more than 5.56 Endotoxin Units per ml.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. *For total sodium* — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.3), measuring at 589 nm and using *sodium solution FP* or *sodium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

*For total citrate* — Dilute 5.0 ml of the solution under examination to 1000.0 ml with *water* and mix. Transfer 1.0 ml of



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this solution to a test-tube, add 1.3 ml of *pyridine*, swirl to mix, add 5.7 ml of *acetic anhydride*, mix and immediately place in a water-bath at 31° ± 1°. Allow the colour to develop for 33 ± 1 minutes and measure the absorbance of the resulting solution at about 425 nm (2.4.7), using as the blank 1 ml of *water* treated in the same manner. Prepare a calibration curve by measuring the absorbance of the solutions prepared by treating in the same manner 1 ml quantities of suitable dilutions of a solution in *water* containing 1.0 mg per ml of $C_6H_8O_7$, prepared by using *anhydrous citric acid*, previously dried for 3 hours at 90°. Calculate the total citrate content, as $C_6H_8O_7$, in mg per ml of the solution under examination from the expression 0.2 C, where C is the concentration in µg per ml of $C_6H_8O_7$, read from the curve.

*For sodium dihydrogen phosphate dihydrate* — Dilute 5.0 ml to 100.0 ml with *water*. Transfer 5.0 ml of this solution to a 25-ml volumetric flask and add 10.0 ml of a 2.8 per cent w/v solution of *sulphuric acid* followed by 2.0 ml of a 2.5 per cent w/v solution of *ammonium molybdate*, mixing after each addition. Add 1.0 ml of *aminohydroxynaphthalenesulphonic acid solution* and sufficient *water* to produce 25.0 ml. Mix and keep aside at 25° for 10 minutes. Measure the absorbance (A1) of the resulting solution at about 660 nm (2.4.7), using as the blank 5 ml of *water* treated in the same manner. Calculate the content of $NaH_2PO_4,2H_2O$ in each ml of the solution under examination from the absorbance (A2) obtained by simultaneously carrying out the operation using 5.0 ml of a solution of *potassium dihydrogen phosphate* containing 0.11 mg of $KH_2PO_4$ per ml (C) using the expression

$$25\,C\,(A1/A2).$$

*For adenine* — Determine by liquid chromatography (2.4.14).

*Test solution*. Substance under examination.

Reference solutions (a), (b) and (c) are prepared by dissolving accurately weighed quantities of *adenine RS* in *dilute hydrochloric acid* in three separate volumetric flasks, diluting with the same solvent to volume and mixing to obtain reference solutions having known concentrations of about 0.25 mg, 0.275 mg and 0.30 mg of adenine per ml respectively.

*Reference solution (d)*. A solution containing 0.0275 per cent w/v each of *adenine RS* and *purine* in *dilute hydrochloric acid*.

Chromatographic system

- a stainless steel column 30 cm x 4 mm, packed with irregular or spherical, totally porous silica gel (10 µm) having a chemically bonded strongly acidic cation-exchange coating,
- mobile phase: dissolve 3.45 g of *ammonium dihydrogen phosphate* in 950 ml of *water* in a 1000-ml volumetric flask, add 10 ml of *glacial acetic acid*, dilute to volume with *water* and mix,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (d) at least four times and record the chromatograms. The test is not valid unless the relative standard deviation of the peak response of adenine is not more than 2.5 per cent, the relative standard deviation of the retention time of adenine is not more than 2.0 per cent and the resolution factor of adenine and purine is not less than 3.0.

Inject separately the test solution and reference solutions (a), (b) and (c). Record the chromatograms and measure the responses for the major peaks. Plot the responses against the concentrations in mg of adenine per ml of reference solutions (a), (b) and (c).

Calculate the quantity, in mg, of $C_5H_5N_5$ in each ml of the solution under examination as the value read directly from the standard curve corresponding to the response obtained with the test solution.

*For dextrose* — Weigh a clean, medium porosity sintered-glass crucible containing a few glass beads. To 50 ml of *potassium cupri-tartrate solution* add the glass beads from the weighed crucible, 45 ml of *water* and 5.0 ml of the solution under examination. Heat the solution at such a rate that it begins to boil in 3.5 to 4 minutes, boil the solution for exactly 2 minutes and filter immediately through the weighed crucible, taking care to transfer all the glass beads with the precipitate to the crucible. Wash the precipitate with hot *water* and then with 10 ml of *ethanol (95 per cent)* and dry it to constant weight at 110°. Carry out a blank determination.

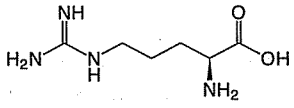
1 mg of the precipitate is equivalent to 0.000496 g of $C_6H_{12}O_6$.

**Storage**. Store protected from light, in single dose, tamper-proof containers made of a suitable plastic material in a cool place.

**Labelling**. The label states (1) the composition and volume of the solution; (2) volume of the solution required per 100 ml of whole blood or the volume of the solution required per volume of whole blood to be collected; (3) where applicable, the maximum amount of blood to be collected in the container.

# Arginine

L-Arginine




$C_6H_{14}N_4O_2$ Mol. Wt. 174.2



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Arginine contains not less than 98.5 per cent and not more than 101.5 per cent of $C_6H_{14}N_4O_2$, calculated on the dried basis.

**Category**. α- amino acid.

### Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *L-arginine RS* or with the reference spectrum of arginine.

### Tests

**Specific optical rotation** (2.4.22). +26.3° to +27.7°, determined in an 8.0 per cent w/v solution in *6 M hydrochloric acid.*

**Chlorides** (2.3.12). 0.5 g complies with the limit test for chlorides (500 ppm).

**Sulphates** (2.3.17). ). 0.5 g complies with the limit test for sulphates (300 ppm).

**Iron** (2.3.14). 0.75 g complies with the limit test for iron (30 ppm).

**Heavy metals** (2.3.13). 0.75 g complies with the limit test for heavy metals Method A (15 ppm).

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

*Mobile phase*. A mixture of 70 volumes of *isopropyl alcohol* and 30 volumes of *ammonia.*

*Test solution*. Weigh accurately about 1.0 g of the substance under examination and dissolve in sufficient *2 M hydrochloric acid* to produce 100.0 ml.

*Reference solution (a)*. A 0.005 per cent w/v solution of *L-arginine RS* in *0.1 M hydrochloric acid.*

*Reference solution (b)*. A solution containing 0.04 per cent w/v of each of *L-arginine RS* and *L-lysine hydrochloride RS* in *0.1 M hydrochloric acid.*

Apply to the plate 5 µl of each solution. After development, dry the plate at 100° until the ammonia disappears completely. Spray the plate with a 0.2 per cent w/v solution of *ninhydrin* in a mixture of 95 volumes of *butyl alcohol* and 5 volumes of *2 M acetic acid.* Heat the plate at 105° for 15 minutes. Cool and examine in daylight. Any secondary spot in the chromatogram obtained with the test solution is not larger or more intense than the principal spot in the chromatogram obtained with reference solution (a). The test is not valid unless in the chromatogram obtained with reference solution (b), two clearly separated spots are seen.

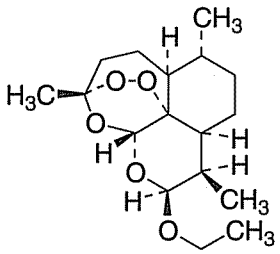
**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.08 g and dissolve in a mixture of 3 ml of *formic acid* and 50 ml of *glacial acetic acid.* Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.00871 g of $C_6H_{14}N_4O_2$.

## Arteether

α-β Arteether



$C_{17}H_{28}O_5$ Mol. Wt. 312.4

α-arteether is (3*R*,5a*S*,6*R*,10*R*,12*S*,12a*R*)-10-Ethoxydecahydro-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano[4,3-j]-1,2-benzodioxepin.

β-arteether is (3*R*,5a*S*,6*R*,10*S*,12*S*,12a*R*)-10-Ethoxydecahydro-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano[4,3-j]-1,2-benzodioxepin.

Arteether contains α-isomer not less than 25.0 per cent and not more than 35.0 per cent and β-isomer not less than 65.0 per cent and not more than 75.0 per cent and total arteether is not less than 95.0 per cent and not more than 105.0 per cent of $C_{17}H_{28}O_5$, calculated on the dried basis.

**Category**. Antimalarial.

**Description**. A light yellow coloured lipophilic semi-solid.

### Identification

*Test A may be omitted if test B is carried out. Test B may be omitted if test A is carried out.*

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

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

### Tests

**Appearance of solution**. A 5.0 per cent w/v solution in *hexane* is clear (2.4.1) and colourless (2.4.1).



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**Specific optical rotation** (2.4.22). +100.0° to +120.0°, at 20°, determined in a 1.0 per cent w/v solution in *methanol.*

**Related substances.** Determine by thin layer chromatography (2.4.17), coating the plate with *silica gel G.*

*Mobile phase.* A mixture of 90 volumes of *hexane* and 10 volume of *ethyl acetate.*

*Test solution.* Dissolve 0.5 g of the substance under examination in 10.0 ml of *chloroform*.

*Reference solution (a).* A 0.15 per cent w/v solution of *arteether RS* in *chloroform*.

*Reference solution (b).* A 0.10 per cent w/v solution of β-*arteether RS* in *chloroform*.

*Reference solution (c).* Dilute 5.0 ml of reference solution (b) to 10.0 ml with *chloroform.*

Apply to the plate 6 µl of each solution. After development, dry the plate at 60° for 15 minutes. Spray with a 4 per cent w/v solution of *vanillin* in *sulphuric acid* and examine in daylight. Any spot in the chromatogram obtained with the test solution other than the principal spots is not more intense than the spot in the chromatogram obtained with reference solution (b). Not more than one such spot is more intense than that in the chromatogram obtained with reference solution (c).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 2 per cent, determined on 1.0 g at 35° under vacuum for 3 hours.

**Assay.** Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve 0.15 g of the substance under examination in 100.0 ml of *acetonitrile.*

*Reference solution.* A 0. 15 per cent w/v solution of *arteether RS* in *acetonitrile.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 70 volumes of *acetonitrile* and 30 volumes of *water,*
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume. 20 µl.

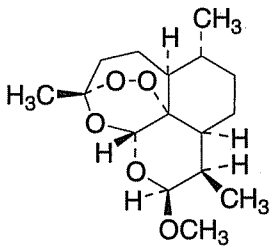
Inject the reference solution. The relative retention time with respect to β-arteether, for α-arteether is about 0.7. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of total arteether, $C_{17}H_{28}O_5$, and of the α- and β-isomers.

**Storage.** Store protected from light and moisture.

# Artemether



$C_{16}H_{26}O_5$ Mol. Wt. 298.4

Artemether is (3*R*,5a*S*,6*R*,8a*S*,9*R*,10*S*,12*R*,12a*R*)-Decahydro-10-methoxy-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano[4,3-*j*]-1,2-benzodioxepin.

Artemether contains not less than 97.0 per cent and not more than 102.0 per cent of $C_{16}H_{26}O_5$, calculated on the dried basis.

**Category.** Antimalarial.

**Description.** A white crystals or a white crystalline powder.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

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

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

C. Dissolve 30 mg in 1 ml of *anhydrous ethanol* and add 0.1 g of *potassium iodide.* Heat the mixture on a water-bath. A yellow colour is produced.

D. Dissolve 30 mg in 6 ml of *anhydrous ethanol.* Add a few drops on a white porcelain dish and add 1 drop of *vanillin sulphuric acid TS.* A pink colour is produced.

## Tests

**Specific optical rotation** (2.4.22). +166.0° to +173.0° at 20°, determined in a 1.0 per cent w/v solution in *anhydrous ethanol.*

**Related substances.** Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve 0.1 g of the substance under examination in 10.0 ml of the mobile phase.

*Reference solution.* A 0.005 per cent w/v solution of the substance under examination in the mobile phase.

Use chromatographic system as described in the Assay.

Inject the test solution and the reference solution. In the chromatogram obtained with the test solution, the area of any



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secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent). The area of not more than one such peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.25 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with the reference solution (1.0 per cent). Ignore any peak with an area less than 0.1 times that of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying over *phosphorous pentaoxide* under vacuum at 2.67 kPa.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve 100 mg of the substance under examination in 10.0 ml of the mobile phase.

*Reference solution.* A 1.0 per cent w/v solution of *artemether RS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 62 volumes of *acetonitrile,* 38 volumes of *water,*
- flow rate.1.5 ml per minute,
- spectrophotometer set at 216 nm,
- injection volume. 20 µl.

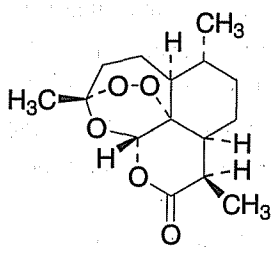
Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{16}H_{26}O_5$.

**Storage**. Store protected from light and moisture.

# Artemisinin



$C_{15}H_{22}O_5$ Mol.Wt. 282.3

Artemisinin is (3*R*,5a*S*,6*R*,8a*S*,12*S*,12a*R*)-octahydro-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano[4,3-*j*]-1,2-benzodioxepin-10(3*H*)-one.

Artemisinin contains not less than 97.0 per cent and not more than 102.0 per cent of artemisinin, $C_{15}H_{22}O_5$, calculated on the dried basis.

**Category**. Antimalarial.

**Description**. Colourless needles or a white crystalline powder.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

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

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

C. Dissolve 5 mg in 0.5 ml of *anhydrous ethanol* and add 0.5 ml of *hydroxylamine hydrochloride* and 0.25 ml of *2 M sodium hydroxide,* heat the mixture in a water-bath to boiling, cool, add 2 drops of *1 M hydrochloric acid* and 2 drops of *ferric chloride solution*. A deep violet colour is produced.

D. Dissolve 5 mg in 0.5 ml of *anhydrous ethanol*. Add 1 ml of *potassium iodide solution*, 2.5 ml of *dilute sulphuric acid solution* and 4 drops of *starch solution*, a violet colour is immediately produced.

## Tests

**Specific optical rotation** (2.4.22). +75.0° to +78.0°, at 20°, determined in a 1.0 per cent w/v solution in *anhydrous ethanol.*

**Related substances**. Determine by thin layer chromatography (2.4.17), coating the plate with *silica gel G.*

*Mobile phase*. A mixture of 50 volumes of *light petroleum* and 50 volumes of *ether.*

*Test solution*. Dissolve 0.1 g of the substance under examination in 10.0 ml of *toluene.*

*Reference solution (a)*. A 0.005 per cent w/v solution of *artemisinin RS* in *toluene.*

*Reference solution (b)*. Dilute 5.0 ml of reference solution (a) to 10.0 ml with *toluene.*

Apply to the plate 10 µl of each solution. Dry the plate in air, spray with *anisaldehyde sulphuric acid TS,* heat the plate at 105° for 7 minutes and examine under day light. Any secondary spot obtained with the test solution is not more intense than that obtained with reference solution (a) (0.5 per cent). Not more than one such spot is more intense than that obtained with reference solution (b) (0.25 per cent).



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**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 80°.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve 100 mg of the substance under examination in 100.0 ml of the mobile phase.

*Reference solution.* A 0.1 per cent w/v solution of *artemisinin RS* in the mobile phase.

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- mobile phase: a mixture of 60 volumes of *acetonitrile* and 40 volumes of *water*,
- flow rate.0.6 ml per minute,
- spectrophotometer set at 216 nm,
- injection volume. 20 µl.

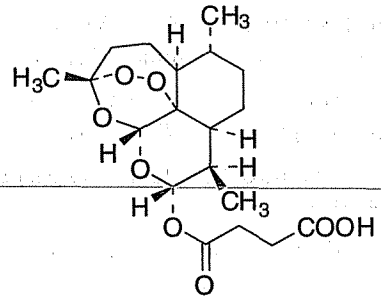
Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{15}H_{22}O_5$.

**Storage**. Store protected from light and moisture.

# Artesunate



$C_{19}H_{28}O_8$ Mol. Wt. 384.4

Artesunate is (3*R*,5a*S*,6*R*,8a*S*,9*R*,10*R*,12*R*,12a*R*)-decahydro-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano-[4,3-*j*]-1,2-benzodioxepin-10-ol hydrogen succinate.

Artesunate contains not less than 97.0 per cent and not more than 102.0 per cent of $C_{19}H_{28}O_8$, calculated on the dried basis.

**Category**. Antimalarial.

**Description**. A white crystalline powder.

## Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *artesunate RS* or with the reference spectrum of artesunate.

## Tests

**pH** (2.4.24). 3.5 to 4.5, determined on 1.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). + 4.5° to + 6.5°, determined in a 1.0 per cent w/v solution in *dichloromethane* at 20°.

**Related substances.** Determine by liquid chromatography (2.4.14).

*NOTE—Use freshly prepared solution, do not sonicate.*

*Test solution.* Dissolve 40 mg of the substance under examination in 10.0 ml of *water.*

*Reference solution.* Dilute 1.0 ml of the test solution to 100.0 ml with *water.*

Chromatographic system
- a stainless steel column 12.5 cm x 3.5 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 30°,
- mobile phase: a mixture of equal volumes of *acetonitrile* and buffer solution pH 3.0 prepared by dissolving 1.36 g of *potassium dihydrogen phosphate* in 1000 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid*,
- flow rate. 0.6 ml per minute,
- spectrophotometer set at 216 nm,
- injection volume. 20 µl.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent). The area of one such peak is not more than 0.25 times the area of the principal peak in the chromatogram obtained with the reference solution (0.25 per cent). The sum of areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent).

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

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in oven at 105° for 3 hours.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture.* 50 volumes of mobile phase and 50 volumes of *methanol.*

*Test solution.* Dissolve about 400 mg of the substance under examination in 70 ml of the solvent mixture, sonicate for 15 minutes and dilute to 100.0 ml with the solvent mixture.



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*Reference solution*. A 0.4 per cent w/v solution of *artesunate RS* in the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil ODS),
- mobile phase: a mixture of 30 volumes of a solution containing 3.85 g of *ammonium acetate* and 1 ml of *triethylamine* in 1000 ml of *water*, adjusted to pH 5.5 with *acetic acid* and 70 volumes of *methanol*,
- flow rate. 0.6 ml per minute,
- spectrophotometer set at 216 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

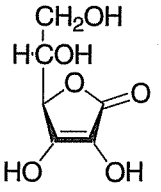
Inject the reference solution and the test solution.

Calculate the content of $C_{19}H_{28}O_8$.

**Storage**. Store protected from light and moisture.

# Ascorbic Acid

Vitamin C; L-Ascorbic Acid



$C_6H_8O_6$ Mol. Wt. 176.1

Ascorbic Acid is (*R*)-5-[(*S*)-1,2-dihydroxyethyl)-3,4-dihydroxy-5(*H*)-furan-2-one.

Ascorbic Acid contains not less than 99.0 per cent and not more than 100.5 per cent of $C_6H_8O_6$.

**Category**. Vitamin (antiscorbutic) and pharmaceutical aid (antioxidant).

**Dose**. Prophylactic, 25 to 75 mg daily; therapeutic, not less than 250 mg daily, in divided doses.

**Description**. A white to very pale yellow crystalline powder or colourless crystals; odourless. On exposure to light it gradually darkens.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests C and D may be omitted if tests A and B are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ascorbic acid RS* or with the reference spectrum of ascorbic acid.

B. Add 2 ml of a 2 per cent w/v solution to a few ml of *2,6-dichlorophenolindophenol solution*; the solution is decolourised.

C. Dilute 1 ml of a 2 per cent w/v solution with 5 ml of *water* and add 1 drop of a freshly prepared 5 per cent w/v solution of *sodium nitroprusside* and 2 ml of *dilute sodium hydroxide solution*. Add 0.6 ml of *hydrochloric acid* dropwise and stir; the yellow colour turns blue.

D. To 2 ml of a 2 per cent w/v solution add 2 ml of *water*, 0.1 g of *sodium bicarbonate* and about 20 mg of *ferrous sulphate*, shake and allow to stand; a deep violet colour is produced. Add 5 ml of *1 M sulphuric acid*; the colour disappears.

## Tests

**Appearance of solution**. A 5.0 per cent w/v solution in *water* is clear (2.4.1), and not more intensely coloured than reference solution BYS7 (2.4.1).

**pH** (2.4.24). 2.2 to 2.5, determined in a 5.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). +20.5° to +21.5°, determined in a 10.0 per cent w/v solution.

**Light absorption**. Absorbance of a 0.001 per cent w/v solution in *0.01 M hydrochloric acid* at the maximum at about 244 nm (2.4.7) is about 0.56.

**Oxalic acid**. Dissolve 0.25 g in 5 ml of *water* and neutralise to *litmus paper* with *2 M sodium hydroxide*. Add 1 ml of *2 M acetic acid* and 0.5 ml of *0.5 M calcium chloride*. Any opalescence, after 60 minutes, is not more intense than that produced by treating 5 ml of a solution prepared by dissolving 70 mg of *oxalic acid* in 500 ml of *water* in a similar manner (0.3 per cent).

**Heavy metals** (2.3.13). 1.0 g dissolved in 25 ml of *water* complies with the limit test for heavy metals, Method A (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Assay**. Weigh accurately about 0.1 g and dissolve in a mixture of 100 ml of freshly boiled and cooled *water* and 25 ml of *1 M sulphuric acid*. Immediately titrate with *0.05 M iodine*, using *starch solution* as indicator until a persistent blue-violet colour is obtained.

1 ml of *0.05 M iodine* is equivalent to 0.008806 g of $C_6H_8O_6$.

**Storage**. Store protected from light and moisture avoiding contact with metals. It undergoes rapid decomposition in solutions in contact with air.



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# Ascorbic Acid Injection

Vitamin C Injection; L-Ascorbic Acid Injection

Ascorbic Acid Injection is a sterile solution of Sodium Ascorbate or of Ascorbic Acid prepared with the aid of Sodium Hydroxide or Sodium Carbonate or Sodium Bicarbonate in Water for Injections.

Ascorbic Acid Injection contains not less than 95.0 per cent and not more than 115.0 per cent of the stated amount of ascorbic acid, $C_6H_8O_6$.

**Usual strength**. 500 mg in 2 ml.

**Description**. A clear, colourless liquid.

## Identification

A. To a volume containing 5 mg of Ascorbic Acid add 0.5 ml of *0.1 M hydrochloric acid* and 3 drops of *sodium nitroprusside solution* followed immediately by 1 ml of *0.1 M sodium hydroxide*; a transient blue colour is produced.

B. To a volume containing 40 mg of Ascorbic Acid add 4 ml of *0.1 M hydrochloric acid* and 4 drops of *methylene blue solution* and warm to 40°; the deep blue colour becomes appreciably lighter or is completely discharged within 3 minutes.

C. The solution responds to the flame test for sodium salts (2.3.1).

## Tests

**pH** (2.4.24). 5.5 to 7.0.

**Oxalic acid**. Dilute a volume containing 0.25 g of Ascorbic Acid in 5 ml of *water* and neutralise to *litmus paper* with *2 M sodium hydroxide*. Add 1 ml of *2 M acetic acid* and 0.5 ml of *0.5 M calcium chloride*. Any opalescence, after 60 minutes, is not more intense than that produced by treating 5 ml of a solution prepared by dissolving 70 mg of *oxalic acid* in 500 ml of *water* in a similar manner (0.3 per cent).

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. Measure accurately a volume containing about 50 mg of Ascorbic Acid and transfer to a 250-ml volumetric flask. Add 20 ml of *metaphosphoric-acetic acids solution*, dilute with *water* to 250.0 ml and mix. Pipette 10.0 ml into a 50-ml Erlenmeyer flask, add 5 ml of *metaphosphoric-acetic acids solution* and titrate with *standard 2,6-dichlorophenolindophenol solution*, until the pink colour persists for at least 10 seconds, the titration occupying not more than 2 minutes. Repeat the operation with a mixture of 5.5 ml of *metaphosphoric-acetic acid solution* and 15 ml of *water* omitting the preparation being examined. From the difference calculate the ascorbic acid in each ml of the injection from the ascorbic acid equivalent of the standard 2,6-dichlorophenolindophenol solution.

**Storage**. Store protected from light, in a single dose container.

# Ascorbic Acid Tablets

Vitamin C Tablets; L-Ascorbic Acid Tablets

Ascorbic Acid Tablets contain not less than 95.0 per cent and not more than 115.0 per cent of the stated amount of ascorbic acid, $C_6H_8O_6$. The tablets may contain permitted flavouring agents.

**Usual strengths**. 50 mg; 100 mg; 500 mg.

## Identification

A. Shake a quantity of the powdered tablets with sufficient *water* to make approximately the equivalent of a 2 per cent w/v solution of Ascorbic Acid and filter. The filtrate (solution A) is acid to *litmus solution*.

B. To solution A add a few ml of *2,6-dichlorophenolindophenol solution*; the solution is decolourised.

C. To 1 ml of solution A, add about 0.1 ml of *2 M nitric acid* and 0.05 ml of *silver nitrate solution*; a grey precipitate is produced.

## Tests

**Disintegration**. The test does not apply to Ascorbic Acid Tablets containing 500 mg or more of Ascorbic Acid.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.15 g of Ascorbic Acid and dissolve as completely as possible in a mixture of 30 ml of *water* and 20 ml of *1 M sulphuric acid*. Titrate with *0.1 M ceric ammonium sulphate* using *ferroin sulphate solution* as indicator.

1 ml of *0.1 M ceric ammonium sulphate* is equivalent to 0.008806 g of $C_6H_8O_6$.

**Storage**. Store protected from light and moisture avoiding contact with metals.

**Labelling**. For tablets containing 500 mg or more of Ascorbic Acid the label states, where applicable, that the tablets should be chewed before swallowing.

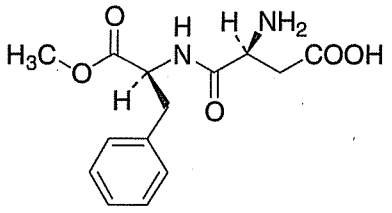


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# Aspartame



$C_{14}H_{18}N_2O_5$ Mol. Wt. 294.3

Aspartame is *N*-L-α-aspartyl-L-phenylalanine-1-methyl ester.

Aspartame contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{14}H_{18}N_2O_5$, calculated on the dried basis.

**Category**. Pharmaceutical aid (sweetening agent).

**Description**. A white, crystalline powder; odourless.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *aspartame RS*.

B. When examined in the range 230 nm to 300 nm (2.4.7), a 0.1 per cent w/v solution in *ethanol (95 per cent)* shows absorption maxima at about 247 nm, 252 nm, 258 nm and 264 nm.

## Tests

**pH** (2.4.24). About 5.0, determined in a 0.8 per cent w/v solution.

**Specific optical rotation** (2.4.22). +14.5° to +16.5°, determined at 20° in a 4.0 per cent w/v solution in *15 M formic acid* within 30 minutes of preparing the solution.

**Light absorption** (2.4.7). Absorbance of a 1.0 per cent w/v solution in *2 M hydrochloric acid*, prepared with the aid of ultrasound, at the maximum at about 430 nm, not more than 0.022.

**5-Benzyl-3,6-dioxo-2-piperazineacetic acid**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 0.5 g of the substance under examination in 100 ml of a mixture of 10 volumes of *methanol* and 90 volumes of *water*.

*Reference solution*. A 0.0075 per cent w/v solution of *5-benzyl-3,6-dioxo-2-piperazine-acetic acid RS* in a mixture of 10 volumes of *methanol* and 90 volumes of *water*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (3 to 10 µm),
- mobile phase: dissolve 5.6 g of *potassium dihydrogen phosphate* in 820 ml of *water*, adjust to pH 4.3 with *phosphoric acid* and dilute to 1000 ml with *methanol*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

Inject the test solution and reference solution. Record the chromatograms. The test is not valid if the relative standard deviations for replicate injections is more than 4.0 per cent and the symmetry factor of the principle peak in the chromatogram obtained with the reference solution is more than 2.0.

In the chromatogram obtained with the test solution the response obtained for any peak at a retention time corresponding to that of *5-benzyl-3,6-dioxo-2-piperazineacetic acid RS* is not greater than the response obtained for the peak in the chromatogram of the reference solution corresponding to not more than 1.5 per cent of 5-benzyl-3,6-dioxo-2-piperazineacetic acid.

**Other Related substances**. Carry out the test for 5-Benzyl-3,6-dioxo-2-piperazineacetic acid, using reference solution (b) prepared by diluting 2.0 ml of the test solution to 100 ml with a mixture of 10 volumes of *methanol* and 90 volumes of *water*. Inject 20 µl of the test solution and reference solution (b), record the chromatograms and measure the peak responses. Continue elution of the test solution for twice the retention time of the aspartame peak. The sum of the areas of any peaks observed in the chromatogram obtained with the test solution, other than the peaks for aspartame and 5-benzyl-3,6-dioxo-2-piperazineacetic acid, is not more than the area of the aspartame peak obtained with reference solution (b)(2.0 per cent).

**Arsenic** (2.3.10). Mix 3.3 g with 3 g of *anhydrous sodium carbonate*, add 10 ml of *bromine solution* and mix thoroughly. Evaporate to dryness on a water-bath, gently ignite, dissolve the cooled residue in 16 ml of *brominated hydrochloric acid AsT* and add 45 ml of *water*. Remove the excess of *bromine* with 2 ml of *stannous chloride AsT*. The resulting solution complies with the limit test for arsenic (3 ppm).

**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

**Sulphated ash** (2.3.18). Not more than 0.2 per cent, determined on 2.0 g.

**Loss on drying** (2.4.19). Not more than 4.5 per cent, determined on 1.0 g by drying in an oven at 105° for 4 hours.

**Assay**. Weigh accurately about 0.3 g, dissolve in 1.5 ml of *anhydrous formic acid*, add 60 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration. A blank titration of more than 0.1 ml may be indicative of excessive



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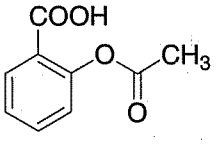
water content. In such a case, repeat the test after taking precautions to maintain anhydrous conditions throughout.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02943 g of $C_{14}H_{18}N_2O_5$.

**Storage**. Store protected from light and moisture.

## Aspirin

Acetylsalicylic Acid




$C_9H_8O_4$ Mol. Wt. 180.2

Aspirin is 2-acetoxybenzoic acid.

Aspirin contains not less than 99.5 per cent and not more than 100.5 per cent of $C_9H_8O_4$, calculated on the dried basis.

**Category**. Non-steroidal antiinflammatory; antirheumatic; antithrombotic.

**Dose**. As analgesic and antipyretic, 300 to 600 mg four to six times a day; as antirheumatic, 1 to 2 g four to six times a day, upto 10 g daily; as antithrombotic, 75 mg daily.

**Description**. Colourless crystals or a white, crystalline powder; odourless or almost odourless.

### Identification

*Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.*

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

B. Boil about 0.5 g with 10 ml of *sodium hydroxide solution* for 3 minutes, cool and add 10 ml of *dilute sulphuric acid*; a white, crystalline precipitate is produced and the odour of acetic acid is perceptible. Filter, dissolve the precipitate in about 2 ml of *water* and add *ferric chloride test solution*; a deep violet colour is produced.

C. To the filtrate obtained in test B add 3 ml of *ethanol (95 per cent)* and 3 ml of *sulphuric acid* and warm; the odour of ethyl acetate is perceptible.

### Tests

**Appearance of solution**. A 1.0 per cent w/v solution in *ethanol (95 per cent)* is clear (2.4.1) and not more intensely coloured than reference solution BS8 (2.4.1).

**Clarity of solution in alkali**. A 5.0 per cent w/v solution in a warm 5 per cent w/v solution of *sodium carbonate* is clear (2.4.1).

**Arsenic** (2.3.10). Mix 5.0 g with 3 g of *anhydrous sodium carbonate*, add 10 ml of *bromine solution* and mix thoroughly. Evaporate to dryness on a water-bath, gently ignite, and dissolve the cooled residue in 16 ml of *brominated hydrochloric acid* and 45 ml of *water*. Remove the excess of bromine with 2 ml of *stannous chloride AsT*. The resulting solution complies with the limit test for arsenic (2 ppm).

**Heavy metals**. Not more than 10 ppm, determined by the following method. Dissolve 2.0 g in 25 ml of *acetone*, add 1 ml of *water* and 10 ml of *hydrogen sulphide solution*; any colour produced is not more intense than that produced by mixing 25 ml of *acetone*, 1.0 ml of *lead standard solution (20 ppm Pb)* and 10 ml of *hydrogen sulphide solution*.

**Chlorides** (2.3.12). Boil 1.75 g with 75 ml of *water* for 5 minutes, cool, add sufficient *water* to restore the original volume and filter. 25 ml of the filtrate complies with the limit test for chlorides (430 ppm).

**Sulphates** (2.3.17). 10 ml of the filtrate obtained in the test for Chlorides complies with the limit test for sulphates (600 ppm).

**Readily carbonisable substances**. Dissolve 0.5 g in 5 ml of *sulphuric acid* (containing 94.5 per cent to 95.5 per cent w/w of $H_2SO_4$); any colour produced is not more intense than that of reference solution BYS4 (2.4.1).

**Salicylic acid**. Dissolve 2.5 g in sufficient *ethanol (95 per cent)* to produce 25.0 ml (test solution). To each of two matched Nessler cylinders add 48 ml of *water* and 1 ml of a freshly prepared *acid ferric ammonium sulphate solution*. Into one cylinder add 1.0 ml of a freshly prepared 0.01 per cent w/v solution of *salicylic acid* and into the other pipette 1.0 ml of the test solution. Mix the contents of the cylinders; after 30 seconds, the colour in the cylinder containing the test solution is not more intense than that in the cylinder containing the standard solution (0.1 per cent).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying over *phosphorus pentoxide* at a pressure of 1.5 to 2.5 kPa.

**Assay**. Weigh accurately about 1.5 g, dissolve in 15 ml of *ethanol (95 per cent)*, add 50.0 ml of *0.5 M sodium hydroxide*, boil gently for 10 minutes, cool and titrate the excess of alkali with *0.5 M hydrochloric acid* using *phenol red solution* as indicator. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of sodium hydroxide required.



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1 ml of *0.5 M sodium hydroxide* is equivalent to 0.04504 g of $C_9H_8O_4$.

**Storage**. Store protected from moisture at a temperature not exceeding 30°.

## Aspirin Tablets

Acetylsalicylic Acid Tablets

Aspirin Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of aspirin, $C_9H_8O_4$.

**Usual strengths**. 75 mg; 150 mg; 300 mg; 600 mg.

### Identification

Boil a quantity of the powdered tablets containing 0.5 g of Aspirin with 10 ml of *sodium hydroxide solution* for 3 minutes, cool and add 10 ml of *dilute sulphuric acid*; a white, crystalline precipitate is produced and the odour of acetic acid is perceptible. Filter, dissolve the precipitate in about 2 ml of *water* and add *ferric chloride test solution*; a deep violet colour is produced.

### Tests

**Salicylic acid**. Shake a quantity of the powdered tablets containing 0.2 g of Aspirin with 4 ml of *ethanol (95 per cent)*, dilute to 100.0 ml with *water*, filter immediately, transfer 50 ml of the *filtrate* to a Nessler cylinder, add 1.0 ml of freshly prepared acid *ferric ammonium sulphate solution*, mix and allow to stand for 1 minute; the violet colour produced is not more intense than that produced by adding 1 ml of freshly prepared *acid ferric ammonium sulphate solution* to a mixture of 3.0 ml of a freshly prepared 0.01 per cent w/v solution of *salicylic acid*, 2 ml of *ethanol (95 per cent)* and sufficient *water* to produce 50 ml contained in a second Nessler cylinder (0.3 per cent).

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.5 g of Aspirin, add 30.0 ml of *0.5 M sodium hydroxide*, boil gently for 10 minutes, cool and titrate the excess of alkali with *0.5 M hydrochloric acid* using *phenol red solution* as indicator. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of sodium hydroxide required.

1 ml of *0.5 M sodium hydroxide* is equivalent to 0.04504 g of $C_9H_8O_4$.

**Storage**. Store protected from moisture at a temperature not exceeding 30°.

## Soluble Aspirin Tablets

Dispersible Aspirin Tablets; Calcium Aspirin Tablets

Soluble Aspirin Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of aspirin, $C_9H_8O_4$.

**Usual strength**. 300 mg.

### Identification

A. The tablets effervesce on the addition of *water*.

B. Boil 0.1 g of the powdered tablets with 10 ml of *water* and 0.5 ml of *ferric chloride test solution*; a violet-red colour is produced.

### Tests

**Salicylic acid**. To a quantity of the powdered tablets containing 0.5 g of Aspirin add 25.0 ml of *chloroform*, shake vigorously for 2 minutes and filter through a dry filter paper. Evaporate 5.0 ml of the filtrate rapidly to dryness in a dish in a current of dry air at room temperature. Dissolve the residue in 2 ml of *ethanol (95 per cent)*, transfer to a Nessler cylinder, using a further 1 ml of *ethanol (95 per cent)* to rinse the dish, dilute to 50 ml with *water*, add 1 ml of *acid ferric ammonium sulphate solution*, mix, and allow to stand for 1 minute; the violet colour produced is not more intense than that produced by adding 1 ml of *acid ferric ammonium sulphate solution* to a mixture of 2.0 ml of a freshly prepared 0.15 per cent w/v solution of *salicylic acid*, 3 ml of *ethanol (95 per cent)* and sufficient *water* to produce 50 ml contained in a second Nessler cylinder (3 per cent).

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.3 g of Aspirin, dissolve in 10 ml of *0.5 M sulphuric acid* and boil under a reflux condenser for 1 hour. Cool, transfer to a separating funnel with the aid of small quantities of *water*, and extract the liberated salicylic acid with four quantities, each of 20 ml, of *ether*. Wash the combined ether extracts with two quantities, each of 5 ml of *water*, remove the ether in a current of air at a temperature not exceeding 30°, dissolve the residue in 20 ml of *0.5 M sodium hydroxide*, and dilute to 200.0 ml with *water*. Transfer 50.0 ml to a stoppered flask, add 50.0 ml of *0.05 M bromine* and 5 ml of *hydrochloric acid*, protect the mixture from light and shake repeatedly during 25 minutes. Add 20 ml of *potassium iodide solution*, shake thoroughly and titrate with *0.1 M sodium thiosulphate* using *starch solution*, added towards the end of the titration, as indicator.

1 ml of *0.05 bromine* is equivalent to 0.003003 g of $C_9H_8O_4$.

**Storage**. Store protected from moisture at a temperature not exceeding 30°.

**Labelling**. The label states that the tablets should be dispersed in water immediately before use.



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# Aspirin and Caffeine Tablets

Acetylsalicylic Acid and Caffeine Tablets

Aspirin and Caffeine Tablets contain not less than 330 mg and not more than 370 mg of aspirin, $C_9H_8O_4$, and not less than 27.5 mg and not more 32.5 mg of caffeine, $C_8H_{10}N_4O_2$.

**Usual strength**. Aspirin 0.4 g and caffeine 30 mg.

## Identification

A. Boil 1 g of the powdered tablets with 10 ml of *1 M sodium hydroxide*, cool and filter. Acidify the filtrate with *1 M sulphuric acid*; a white precipitate is produced. Dissolve the precipitate in about 2 ml of *water* and add *ferric chloride test solution*; a deep violet colour is produced.

B. Shake 0.5 g of the powdered tablets with 10 ml of *water* for 5 minutes, filter and add 10 ml of *1 M sodium hydroxide*. Extract with three quantities, each of 30 ml of *chloroform*, washing each extract with the same 10 ml of *water*. Filter the combined extracts through absorbent cotton and evaporate the filtrate to dryness. Reserve a quantity of the residue for test C. Dissolve 10 mg of the residue in 1 ml of *hydrochloric acid*, add 0.1 g of *potassium chlorate* and evaporate to dryness in a porcelain dish; a reddish residue remains which becomes purple on exposure to ammonia vapour.

C. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001per cent w/v solution of the residue reserved in Test B shows an absorption maximum at about 273 nm.

## Tests

**Salicylic acid**. Shake a quantity of the powdered tablets containing 0.5 g of Aspirin with 50.0 ml of *chloroform* and 10 ml of *water* and allow to separate. Filter the chloroform layer through a dry filter paper and evaporate 10 ml of the filtrate to dryness at room temperature using a rotary evaporator. To the residue add 4 ml of *ethanol (95 per cent)*, stir well, dilute to 100 ml with *water* at a temperature not exceeding 10°, filter immediately, rapidly transfer 50 ml to a Nessler cylinder, add 1 ml of freshly prepared *acid ferric ammonium sulphate solution*, mix and allow to stand for 1 minute; the violet colour produced is not more intense than that produced by adding 1 ml of acid *S ammonium sulphate solution* to a mixture of 3.0 ml of a freshly prepared 0.01 per cent w/v solution of *salicylic acid*, 2 ml of *ethanol (95 per cent)* and sufficient *water* to produce 50 ml contained in a second Nessler cylinder (0.6 per cent).

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets.

*For aspirin* — Weigh accurately a quantity of the powder containing about 0.7 g of Aspirin, add 20 ml of *water* and 2 g of *sodium citrate* and heat under a reflux condenser for 30 minutes. Cool, wash the condenser with 30 ml of warm *water* and titrate with *0.5 M sodium hydroxide* using *phenolphthalein solution* as indicator.

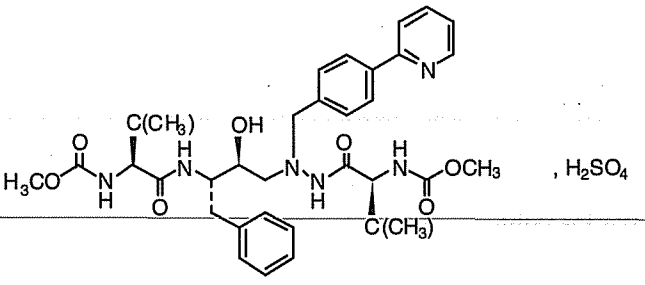
1 ml of *0.5 M sodium hydroxide* is equivalent to 0.04504 g of $C_9H_8O_4$.

*For caffeine* — Weigh accurately a quantity of the powder containing about 30 mg of Caffeine add 200 ml of *water* and shake for 30 minutes. Add sufficient *water* to produce 250.0 ml and filter. To 10.0 ml of the filtrate add 10 ml of *1 M sodium hydroxide* and extract immediately with five quantities, each of 30 ml, of *chloroform*, washing each extract with the same 10 ml of *water*. Filter the combined chloroform extracts, if necessary, through absorbent cotton previously moistened with *chloroform*. Evaporate the solution to dryness and dissolve the residue as completely as possible in *water*, warming gently if necessary. Cool, add sufficient *water* to produce 100.0 ml, mix and filter if necessary. Measure the absorbance of the resulting solution at the maximum at about 273 nm (2.4.7).

Calculate the content of $C_8H_{10}N_4O_2$ taking 504 as the specific absorbance at 273 nm.

**Storage**. Store protected from moisture.

# Atazanavir Sulphate




$C_{38}H_{52}N_6O_7.H_2SO_4$ Mol. Wt. 802.9

Atazanavir Sulphate is salt with sulphuric acid of (3*S*,8*S*,9S,12*S*)-3,12-bis(1,1-dimethylethyl)-8-hydroxy-4,11-dioxo-9-(phenylmethyl)-6-[[4-(2-pyridinyl)phenyl]methyl]-2,5,6,10,13-pentaazatetradecanedioic acid dimethyl ester.

Atazanavir Sulphate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{38}H_{52}N_6O_7.H_2SO_4$, calculated on the dried basis.

**Category**. Antiretroviral.

**Description**. A white to pale yellow crystalline powder.



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### Identification

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

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

### Tests

**Specific optical rotation** (2.4.22). -44° to -48°, determined on 1.0 per cent w/v solution in a mixture of equal volumes of *methanol* and *water* at 22°.

**Sulphate**. Not less than 11.6 per cent w/w and not more than 12.8 per cent w/w.

Weigh accurately about 0.2 g and sonicate with 30 ml of *methanol*, add 30 ml of *water*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.0049 g of sulphate.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. Equal volumes of mobile phase A and mobile phase B.

*Test solution*. Dissolve 100 mg of the substance under examination in 100.0 ml of the solvent mixture.

*Reference solution*. A 0.0005 per cent w/v solution of *atazanavir sulphate RS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Inertsil ODS-3),
- column temperature. 45°,
- mobile phase: A. dissolve 6.0 g of *sodium dihydrogen orthophosphate monohydrate* in *water*, add 2.0 ml of *orthophosphoric acid* and dilute to 1000 ml with *water*, adjust the pH to 2.5 with *triethylamine*,
  B. *acetonitrile*,
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

| Time (in min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0-15 | 60 | 40 |
| 32-44 | 30 | 70 |
| 45-55 | 60 | 40 |

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent) and sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent).

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

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 10 mg of the substance under examination in 100.0 ml of the mobile phase.

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

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (such as Inertsil ODS-3),
- column temperature. 45°,
- mobile phase. a mixture of 45 volumes of buffer solution prepared by dissolving 6.0 g of *sodium dihydrogen orthophosphate monohydrate* in *water*, add 2.0 ml of *orthophosphoric acid* and dilute to 1000 ml with *water*, adjusted to pH 2.5 with *triethylamine* and 55 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the theoretical plates are not less than 2500, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{38}H_{52}N_6O_7.H_2SO_4$.

**Storage**. Store protected from light and moisture, at a temperature not exceeding 30°.

## Atazanavir Capsules

### Atazanavir Sulphate Capsules

Atazanavir Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of atazanavir, $C_{38}H_{52}N_6O_7$.



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**Usual strengths**. 150 mg; 200 mg; 300 mg; 400 mg.

## Identification

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

B. When examined in the range from 200 nm to 400 nm (2.4.7), a 0.012 per cent w/v solution in 0.2 per cent v/v solution of *hydrochloric acid* shows an absorption maximum as obtained with *atazanavir sulphate RS* of the same concentration.

## Tests

**Dissolution** (2.5.2).

Apparatus No. 1 (Use sinkers, if required),

Medium. 1000 ml of *0.025 M hydrochloric acid,*

Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Dilute the filtrate, if necessary, with the dissolution medium. Measure the absorbance at the maximum at about 300 nm (2.4.7). Calculate the content of $C_{38}H_{52}N_6O_7$ in the medium from the absorbance obtained from a solution of known concentration of *atazanavir sulphate RS*.

D. Not less than 75 per cent of the stated amount of $C_{38}H_{52}N_6O_7$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture.* 10 volumes of mobile phase A and 10 volumes of mobile phase B.

*Test solution.* Mix the contents of 20 capsules. Disperse the content of capsules containing about 50 mg of Atazanavir with 30 ml of the solvent mixture, sonicate for 15 minutes and dilute to 50.0 ml with the solvent mixture and filter.

*Reference solution.* A 0.001 per cent w/v solution of *atazanavir sulphate RS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Inertsil ODS-3),
- column temperature. 45°,
- mobile phase: A. dissolve 6.0 g of *sodium dihydrogen orthophosphate monohydrate* and 2 ml of *orthophosporic acid* in 1000 ml of *water*, adjusted to pH 2.5 with *triethylamine or orthophosphoric acid*,
  B. *acetonitrile,*
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume.20 µl.

| Time (in min) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0 – 15 | 60 | 40 |
| 15 – 32 | 60 → 30 | 40 → 70 |
| 32- 44 | 30 | 70 |
| 44 -45 | 30 → 60 | 70 → 40 |
| 45 – 55 | 60 | 40 |

Inject the reference solution. The test is not valid unless the theoretical plates of the principal peak is not less than 2000 and the tailing factor is not more than 2.0. The relative standard deviation for replicate injections is not more than 2.0 per cent.

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

**Uniformity of content**. Comply with the test stated under Capsules.

Determine by liquid chromatography (2.4.14), as described under Assay using the following as the test solution.

*Test solution.* Transfer the content of 1 capsule into a 200-ml volumetric flask. Add about 150 ml of mobile phase, sonicate for 15 minutes and dilute to volume with the mobile phase, filter. Further dilute 5.0 ml of this solution to 10.0 ml with the mobile phase.

Inject the reference solution and the test solution.

Calculate the content of $C_{38}H_{52}N_6O_7$ in the capsule.

**Other tests**. Comply with the tests stated under Capsules.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh and mix the contents of 20 capsules. Disperse a quantity of the mixed content containing about 20 mg of Atazanavir with 150 ml of the mobile phase, sonicate for 15 minutes and dilute to 250.0 ml with the mobile phase, filter.

*Reference solution.* A 0.009 per cent w/v solution of *atazanavir sulphate RS* in the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Inertsil ODS),
- column temperature. 40°,
- mobile phase: a mixture of 60 volumes of *acetonitrile* and 40 volumes of *water*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 250 nm,
- injection volume. 5 µl.



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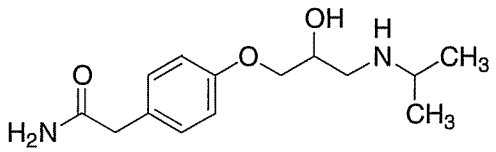
Inject the reference solution. The test is not valid unless the theoretical plates is not less than 2000 and the tailing factor is not more than 2.0. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{38}H_{52}N_6O_7$ in the capsules.

**Storage**. Store at a temperature not exceeding 30°.

# Atenolol




$C_{14}H_{22}N_2O_3$ Mol. Wt. 266.3

Atenolol is (*RS*)-4-(2-hydroxy-3-isopropylaminopropoxy)phenylacetamide.

Atenolol contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{14}H_{22}N_2O_3$, calculated on the dried basis.

**Category**. Antihypertensive.

**Dose**. 50 to 100 mg, daily, in 1 or 2 doses.

**Description**. A white or almost white powder.

## Identification

*Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.*

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

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.01 per cent w/v solution in *methanol* shows absorption maxima at about 275 nm and 282 nm. The ratio of the absorbance at the maximum at about 275 nm to that at the maximum at about 282 nm is 1.15 to 1.20.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 99 volumes of *methanol* and 1 volume of *strong ammonia solution*.

*Test solution*. Dissolve 1.0 g of the substance under examination in sufficient *methanol* to produce 100 ml.

*Reference solution*. A 1.0 per cent w/v solution of *atenolol RS* in *methanol*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in a current of warm air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

## Tests

**Appearance of solution**. A 1.0 per cent w/v solution is clear (2.4.1), and not more intensely coloured than degree 6 of the appropriate range of reference solutions.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution (a)*. Dissolve 50 mg of the substance under examination in 20 ml of the mobile phase and dilute to 25 ml with the mobile phase.

*Test solution (b)*. Dissolve 50 mg of the substance under examination in 0.1 ml of *dimethyl sulphoxide*, if necessary heating the mixture by placing the container in a water-bath for a few seconds and dilute to 25 ml with the mobile phase.

*Reference solution (a)*. Dilute 0.5 ml of the test solution (a) to 100 ml with the mobile phase.

*Reference solution (b)*. Dissolve 50 mg of *atenolol impurity standard RS* in 0.1 ml of *dimethyl sulphoxide*, if necessary heating the mixture by placing the container in a water-bath for a few seconds and dilute to 25 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: dissolve 1.0 g of *sodium octanesulphonate* and 0.4 gm of *tetrabutyl ammonium hydrogen sulphate* in 1000 ml of a mixture of 20 volumes of *tetrahydrofuran*, 180 volumes of *methanol* and 800 volumes of a 0.34 per cent w/v solution of *potassium dihydrogen phosphate* and adjust the pH to 3.0 with *phosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 226 nm,
- injection volume. 20 µl.

Equilibrate the column with the mobile phase for about 30 minutes and adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (a) is at least 50 per cent of the full scale of the recorder.

Inject reference solution (b). The resulting chromatogram is similar to that of the specimen chromatogram provided with *atenolol impurity standard RS* in that the peak due to bis-ether precedes and is separated from that due to tertiary amine, which normally appears as a doublet. If necessary, adjust the



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concentration of sodium octanesulphonate; if its concentration is increased, the retention time of the tertiary amine is prolonged.

Inject separately test solution (a) and reference solution (a). Continue the chromatography for four times the retention time of the principal peak. The area of any secondary peak in the chromatogram obtained with test solution (a) is not greater than half the area of the principal peak obtained with reference solution (a) (0.25 per cent); the sum of the areas of any secondary peaks is not greater than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 10 per cent of that of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

If the substance under examination is found to contain more than 0.15 per cent of bis-ether, its compliance is confirmed by repeating the chromatography using 10 µl of test solution (b).

**Chlorides** (2.3.12). Dissolve 0.25 g in a mixture of 1 ml of *2 M nitric acid* and 15 ml of *water*. The solution complies with the limit test for chlorides without further addition of *2 M nitric acid* (0.1 per cent).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.2 g and dissolve in 80 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02663 g of $C_{14}H_{22}N_2O_3$.

# Atenolol Tablets

Atenolol Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of atenolol, $C_{14}H_{22}N_2O_3$.

**Usual strengths**. 50 mg; 100 mg.

## Identification

A. Heat a quantity of the powdered tablets containing about 0.1 g of Atenolol with 15 ml of *methanol* to 50°, shake for 5 minutes, filter (Whatman No. 42 paper is suitable) and evaporate the filtrate to dryness on a water-bath. Warm the residue with 10 ml of *0.1 M hydrochloric acid*, shake and filter. Add to the filtrate sufficient *1 M sodium hydroxide* to make it alkaline, extract with 10 ml of *chloroform*, dry by shaking with *anhydrous sodium sulphate*, filter, evaporate the filtrate to dryness on a water-bath and dry the residue at 105° for 1hour. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *atenolol RS* or with the reference spectrum of atenolol.

B. When examined in the range 230 nm to 360 nm (2.4.7), the solution obtained in the Assay shows absorption maxima at about 275 nm and 282 nm.

## Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Shake a quantity of the powdered tablets containing 25 mg of Atenolol with 25 ml of the mobile phase and mix with the aid of ultrasound for 20 minutes, filter (such as Whatman GF/C filter) and use the filtrate.

*Reference solution (a)*. Dilute 1 volume of the test solution to 200 volumes with the mobile phase.

*Reference solution (b)*. Dissolve 10 mg of *atenolol impurity standard RS* in 0.1 ml of *dimethyl sulphoxide* with the aid of gentle heat, dilute to 10 ml with the mobile phase and mix.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: dissolve 0.8 g of *sodium octanesulphonate* and 0.4 gm of *tetrabutyl- ammonium hydrogen sulphate* in 1000 ml of a mixture of 20 volumes of *tetrahydrofuran*, 180 volumes of *methanol* and 800 volumes of a 0.34 per cent w/v solution of *potassium dihydrogen phosphate* and adjust the pH to 3.0 with *phosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 226 nm,
- injection volume. 20 µl.

Inject each solution. The test is not valid unless the chromatogram obtained with reference solution (b) resembles the reference chromatogram supplied with the atenolol impurity standard RS in that the peak due to bis-ether precedes and is separated from that due to tertiary amine, which is normally a doublet. If necessary, adjust the concentration of sodium octanesulphonate in the mobile phase; if its concentration is increased, the retention time of the tertiary amine is prolonged.

In the chromatogram obtained with the test solution, the area of any peak corresponding to 4-(2-hydroxy-3-isopropylamino-propoxy)phenylacetic acid (blocker acid) is not greater than the area of the peak in the chromatogram obtained with reference solution (a) (0.5 per cent) and the area of any peak corresponding to either tertiary amine or bis-ether is not greater than half the area of the peak in the chromatogram obtained with reference solution (a) (0.25 per cent).

**Other tests**. Comply with the tests stated under Tablets.



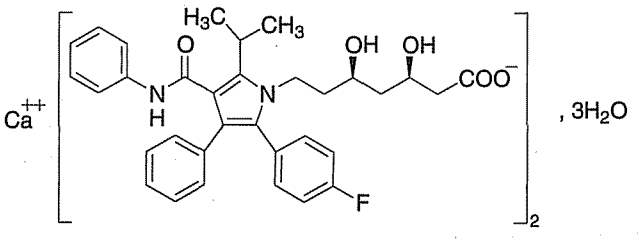
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**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.2 g of Atenolol, transfer to a 500-ml volumetric flask using 300 ml of *methanol*, heat the resulting suspension to 60° and shake for 15 minutes. Cool, dilute to 500.0 ml with *methanol*, filter through a sintered-glass funnel (Porosil G3) and dilute a suitable volume of the filtrate with sufficient *methanol* to produce a solution containing 0.01 per cent w/v of Atenolol. Measure the absorbance of the resulting solution at the maximum at about 275 nm (2.4.7). Calculate the content of $C_{14}H_{22}N_2O_3$ taking 53.7 as the value of the specific absorbance at 275 nm.

# Atorvastatin Calcium



$C_{66}H_{68}CaF_2N_4O_{10}$ Mol. Wt. 1155.4

Atorvastatin Calcium is calcium salt of (β*R*,8*R*)-2-(4-fluorophenyl)-α,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1*H*-pyrrole-1-heptanoic acid trihydrate.

Atorvastatin Calcium contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{66}H_{68}CaF_2N_4O_{10}$, calculated on the anhydrous basis.

**Category**. Antihyperlipidaemic.

**Description**. A white to off-white, crystalline powder.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *atorvastatin calcium RS* or with the reference spectrum of atorvastatin calcium.

B. It gives reactions of calcium salts (2.3.1).

## Tests

**Specific optical rotation** (2.4.22). - 6.0° to -12.0°, determined in a 1.0 per cent w/v solution in *dimethylsulphoxide*.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. A mixture of 40 volumes of *acetonitrile* and 60 volumes of *water*.

*Test solution*. Dissolve 50 mg of the substance under examination in 10 ml of *methanol* and dilute to 100 ml with the solvent mixture.

*Reference solution (a)*. A 0.5 per cent w/v solution of *atorvastatin calcium RS* in *methanol*. Dilute 5 ml of the solution to 50 ml with the solvent mixture.

*Reference solution (b)*. Dilute 1 ml of reference solution (a) to 100 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 92.5 volumes of *acetonitrile* and 7.5 volumes *tetrahydrofuran*,
  B. a mixture of 58 volumes of a buffer solution prepared by dissolving 5.75 g of *ammonium dihydrogen orthophosphate* in 1000 ml of *water* and 42 volumes of mobile phase A,
  C. a mixture of 20 volumes of the buffer solution, 20 volumes of mobile phase A and 60 volumes of *methanol*,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 246 nm,
- injection volume. 20 µl,
- injection delay 10 minutes.

| Time (in min.) | Flow rate (ml per minute) | Mobile phase B (per cent v/v) | Mobile phase C (per cent v/v) |
|---|---|---|---|
| 0 | 1.8 | 100 | 0 |
| 20 | 1.8 | 100 | 0 |
| 35 | 1.5 | 25 | 75 |
| 40 | 1.5 | 25 | 75 |
| 55 | 1.5 | 0 | 100 |
| 60 | 1.8 | 100 | 0 |

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 5000 theoretical plates and the tailing factor is not more than 1.5.

Inject alternatively the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any individual secondary peak is not more than half the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than 2 times the area of the peak in the chromatogram obtained with reference solution (b) (2.0 per cent). Ignore any peak with an area less than 0.05 times the area of the peak obtained in the chromatogram obtained in the chromatogram obtained with reference solution (b) (0.05 per cent)

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



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**Water** (2.3.43). 3.0 per cent to 7.0 per cent, determined on 0.3 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. A mixture of 40 volumes of *acetonitrile* and 60 volumes of *water*.

*Test solution*. Dissolve 80 mg of the substance under examination in 20 ml of *methanol* and dilute to 200 ml with the solvent mixture. Dilute this solution with the solvent mixture to produce a solution containing 0.008 per cent w/v of Atorvastatin Calcium.

*Reference solution*. Dissolve 20 mg of *atorvastatin calcium RS* in 5 ml of *methanol* and dilute to 50 ml with the solvent mixture. Dilute the solution with the solvent mixture to produce a solution containing 0.008 per cent w/v of Atorvastatin Calcium.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 58 volumes of a buffer solution prepared by dissolving 5.75 g of *ammonium dihydrogen orthophosphate* in 1000 ml of *water* and 42 volumes of a mixture of 92.5 volumes of *acetonitrile* and 7.5 volumes of *tetrahydrofuran.*,
- flow rate. 1.8 ml per minute,
- spectrophotometer set at 246 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent,

Inject alternatively the test solution and the reference solution.

Calculate the content of $C_{66}H_{68}CaF_2N_4O_{10}$.

**Storage**. Store protected from light at a temperature not exceeding 30°.

# Atorvastatin Tablets

Atorvastatin Calcium Tablets

Atorvastatin Tablets contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of atorvastatin, $C_{66}H_{68}F_2N_4O_{10}$.

**Usual strengths**. 10 mg; 20 mg; 40 mg; 80 mg.

## Identification

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

## Tests

**Dissolution** (2.5.2).

Apparatus No. 1,

Medium. 900 ml of *phosphate buffer pH 6.8,*

Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Determine by liquid chromatography (2.4.14).

*Test solution*. Use the filtrate, diluted if necessary, with the dissolution medium.

*Reference solution*. Dissolve an accurately weighed quantity of *atorvastatin calcium RS* in *methanol,* and dilute quantitatively with the dissolution medium to obtain a solution of about the same concentration as the test solution.

Use the chromatographic system as described under Assay.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Calculate the content of $C_{66}H_{68}F_2N_4O_{10}$.

D. Not less than 70 per cent of the stated amount of $C_{66}H_{68}F_2N_4O_{10}$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. A mixture of 40 volumes of *acetonitrile* and 60 volumes of the buffer solution (see below).

*Test solution*. Weigh accurately a quantity of the powdered tablets containing 50 mg of atorvastatin, disperse in 10 ml of *methanol,* add 20 ml of the solvent mixture, disperse with the aid of ultrasound, if required, and dilute to 100 ml with the solvent mixture and filter.

*Reference solution (a)*. Weigh accurately a suitable quantity of *atorvastatin calcium RS,* dissolve in 5 ml of *methanol* and dilute to 50 ml with the solvent mixture, to produce 0.05 per cent of atorvastatin.

*Reference solution (b)*. Dilute 1 ml of *reference solution (a)* to 100 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 92.5 volumes of *acetonitrile* and 7.5 volumes *tetrahydrofuran*,
  B. a mixture of 58 volumes of a buffer solution prepared by dissolving 5.75 g of *ammonium dihydrogen orthophosphate* in 1000 ml of *water* and 42 volumes of mobile phase A,



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C. a mixture of 20 volumes of the buffer solution, 20 volumes of mobile phase A and 60 volumes of *methanol*,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 246 nm,
- injection volume. 20 µl,
- injection delay 10 minutes.

| Time (in min.) | Flow rate (ml per minute) | Mobile phase B (per cent v/v) | Mobile phase C (per cent v/v) |
|---|---|---|---|
| 0 | 1.8 | 100 | 0 |
| 20 | 1.8 | 100 | 0 |
| 35 | 1.5 | 25 | 75 |
| 40 | 1.5 | 25 | 75 |
| 55 | 1.5 | 0 | 100 |
| 60 | 1.8 | 100 | 0 |

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 5000 theoretical plates and the tailing factor is not more than 1.5.

Inject alternatively the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of the areas of all the secondary peaks is not more than 4 times the area of the peak in the chromatogram obtained with reference solution (b) (4.0 per cent). Ignore any peak with an area less than 0.05 times the area of the peak obtained with reference solution (b) (0.05 per cent).

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. A solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* and 0.9 g of *sodium hydroxide* in 1000 ml of *water* and adjusting the pH to 6.8 with *phosphoric acid* or *sodium hydroxide*.

*Test solution*. Weigh and powder 20 tablets. Weigh accurately a quantity of the powdered tablets containing about 80 mg of atorvastatin and disperse in sufficient *methanol* to produce a solution containing 0.016 per cent w/v of atorvastatin. Disperse with the aid of ultrasound, if required, and filter. Dilute the filtrate with sufficient of the solvent mixture to produce a solution containing 0.008 per cent w/v of atorvastatin.

*Reference solution*. Weigh accurately a suitable quantity of *atorvastatin calcium RS* and dissolve in sufficient *methanol* to produce a solution containing 0.08 per cent of atorvastatin. To 5 ml of this solution, add 20 ml of *methanol* and dilute to 50 ml with the solvent mixture to produce a solution containing 0.008 per cent w/v of atorvastatin.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of a buffer solution prepared by dissolving 1.54 g of *ammonium acetate* in 1000 ml of *water* and adjusting the pH to 4.0 with *glacial acetic acid*, and 50 volumes of a mixture of 92.5 volumes of *acetonitrile* and 7.5 volumes of *tetrahydrofuran*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 246 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject alternatively the test solution and the reference solution.

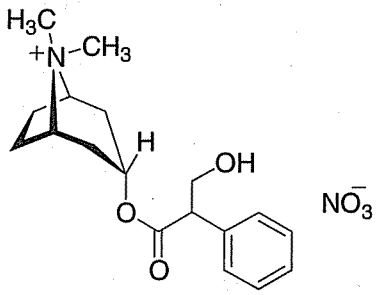
Calculate the content of $C_{66}H_{68}F_2N_4O_{10}$ in the tablets.

**Storage**. Store protected from moisture at a temperature not exceeding 30°.

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

## Atropine Methonitrate

Methylatropine Nitrate




$C_{18}H_{26}N_2O_6$ Mol. Wt. 366.4

Atropine Methonitrate is (*RS*)-(1*R*,3*r*,5*S*)-8-methyl-3-tropoyloxytropanium nitrate.

Atropine Methonitrate contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{18}H_{26}N_2O_6$, calculated on the dried basis.

**Category**. Anticholinergic.

**Dose**. In the treatment of congenital hypertrophic pyloric stenosis in infants, 200 to 600 µg, half an hour before feeds.

**Description**. Colourless crystals or a white, crystalline powder.



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## Identification

*Test A may be omitted if tests B, C, and D are carried out. Tests B and C may be omitted if tests A and D are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *atropine methonitrate RS*.

B. To 0.05 ml of a 1 per cent w/v solution add 0.05 ml of a 0.1 per cent w/v solution of *diphenylamine* in *nitrogen-free sulphuric acid*; an intense blue colour is produced.

C. To 2.5 ml of a 10 per cent w/v solution add 2.5 ml of *water* and 2 ml of *dilute sodium hydroxide solution*; no precipitate is produced.

D. Add about 1 mg to 4 drops of *fuming nitric acid* and evaporate to dryness on a water-bath; a yellow residue is obtained. To the cooled residue add 2 ml of *acetone* and 4 drops of a 3 per cent w/v solution of *potassium hydroxide* in *methanol*; a violet colour is produced.

## Tests

**Appearance of solution.** A 5.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1) and not more intensely coloured than reference solution BS8 (2.4.1).

**pH** (2.4.24). 6.0 to 7.5, determined in a 5.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). –0.25° to +0.05°, determined in a 10.0 per cent w/v solution, using a 2-dm tube (distinction from hyoscyamine).

**Silver.** To 10 ml of a 10.0 per cent w/v solution add 0.1 ml of *sodium sulphide solution*. The solution is not more intensely coloured than reference solution BS8 (2.4.1).

**Halides** (2.3.12). 15 ml of a 5.0 per cent w/v solution in *carbon dioxide-free water* complies with the limit test for chlorides, using 0.3 ml of *chloride standard solution (25 ppm Cl )* for preparing the standard.

**Apomethylatropine.** A 0.1 per cent w/v solution in *0.01 M hydrochloric acid* shows absorption maxima at about 252 nm and 257 nm (2.4.7). The ratio of the absorbance at about 257 nm to that at about 252 nm is not less than 1.17.

**Related substances.** Determine by thin-layer chromatography (2.4.17) coating the plate with *silica gel G*.

*Mobile phase.* A mixture of 60 volumes of *ethyl acetate*, 15 volumes of *anhydrous formic acid*, 15 volumes of *water* and 10 volumes of *methanol*.

*Test solution.* A 4.0 per cent w/v solution of the substance under examination in *methanol (90 per cent)*.

*Reference solution.* Dilute 5 ml of the test solution to 100 ml with *methanol (90 per cent)*, mix and dilute 10 ml of the resulting solution to 100 ml with *methanol (90 per cent)*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate at 105° until the odour of the solvent is not detectable. Allow it to cool to room temperature and spray with *dilute potassium iodobismuthate solution* until spots appear. Any secondary spot in the chromatogram obtained with test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

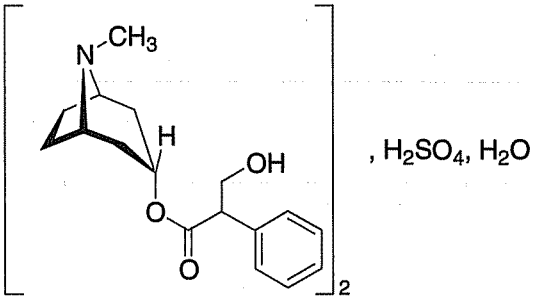
**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay.** Weigh accurately about 0.3 g and dissolve in 50 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03664 g of $C_{18}H_{26}N_2O_6$.

**Storage.** Store protected from light.

# Atropine Sulphate




$(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$ Mol. Wt. 694.8

Atropine Sulphate is (*RS*)-(1*R*,3*r*,5*S*)-3-tropoyloxytropanium sulphate monohydrate.

Atropine Sulphate contains not less than 99.0 per cent and not more than 101.0 per cent of atropine sulphate, $(C_{17}H_{23}NO_3)_2$, $H_2SO_4$, calculated on the anhydrous basis.

**Category.** Anticholinergic; antidote to cholinesterase inhibitors.

**Dose.** As anticholinergic, orally, 250 µg to 2 mg daily in single or divided doses; by subcutaneous, intramuscular, or by intravenous injection, 400 µg to 600 µg four to six times a day; as antidote to cholinesterase inhibitors, by intravenous injection, 2 to 4 mg initially, followed by intramuscular injection, 2 mg repeated every 5 to 10 minutes.

**Description.** Colourless crystals or a white, crystalline powder; odourless.



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### Identification

*Test A may be omitted if tests B and C are carried out. Test B may be omitted if tests A and C are carried out.*

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

B. To a 2 per cent w/v solution add *sodium hydroxide solution*, filter and transfer the precipitate with *water*. Dry the precipitate at 60°. To 5 mg of the residue add 5 drops of *fuming nitric acid* and evaporate to dryness on a water-bath. Cool the faintly yellow coloured residue and add 2 ml of *acetone* and 4 drops of a 3 per cent w/v solution of *potassium hydroxide* in *methanol*; a violet colour is produced.

C. A 5 per cent w/v solution gives the reactions of sulphates (2.3.1).

### Tests

**pH** (2.4.24). 4.5 to 6.2, determined in a 2.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). –0.50° to +0.05°, determined in a 10.0 per cent w/v solution, using a 2-dm tube (distinction from hyoscyamine).

**Apoatropine**. Absorbance of a 0.1 per cent w/v solution in *0.01 M hydrochloric acid* at about 245 nm, not more than 0.4 (2.4.7).

**Foreign alkaloids and decomposition products**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 90 volumes of *acetone*, 7 volumes of *water* and 3 volumes of *strong ammonia solution*.

*Test solution*. A 2.0 per cent w/v solution of the substance under examination in *methanol*.

*Reference solution (a)*. Dilute 1 ml of the test solution to 100 ml with *methanol*.

*Reference solution (b)*. Dilute 25 ml of reference solution (a) to 50 ml with *methanol*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate at 105° for 15 minutes. Allow it to cool to room temperature and spray with *dilute potassium iodobismuthate solution*. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Water** (2.3.43). Not more than 4.0 per cent, determined on 0.5 g.

**Assay**. Weigh accurately about 0.5 g, dissolve in 30 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.06768 g of $(C_{17}H_{23}NO_3)_2, H_2SO_4$.

**Storage**. Store protected from light.

## Atropine Injection

### Atropine Sulphate Injection

Atropine Injection is a sterile solution of Atropine Sulphate in Water for Injections.

Atropine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of atropine sulphate, $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$.

**Usual strengths**. 500 µg per ml; 600 µg per ml; 1 mg per ml.

### Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 50 volumes of *chloroform*, 40 volumes of *acetone* and 10 volumes of *diethylamine*.

*Test solution*. Evaporate a volume of the injection containing 5 mg of Atropine Sulphate to dryness on a water-bath, triturate the residue with 1 ml of *ethanol (95 per cent)*, allow to stand and use the supernatant liquid.

*Reference solution*. A 0.5 per cent w/v solution of *atropine sulphate RS* in *ethanol (95 per cent)*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate at 105° for 20 minutes, allow it to cool to room temperature and spray with *potassium iodobismuthate solution*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with reference solution (a) corresponds to the peak in the chromatogram obtained with reference solution (b).

### Tests

**pH** (2.4.24). 3.0 to 5.5.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. Determine by gas chromatography (2.4.13).



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*Test solution.* Add 1.0 ml of a 0.25 per cent w/v solution of *homatropine hydrobromide RS* (internal standard) in *methanol* (solution A), 1 ml of *5 M ammonia* to a volume of the injection containing 2.5 mg of Atropine Sulphate, diluted if necessary to 5 ml with *water* and extract with two quantities, each of 10 ml, of *0.1 M hydrochloric acid.* Wash the combined extracts with 5 ml of *chloroform,* shake the combined extracts with 1 g of *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness. Dissolve the residue in 5.0 ml of *dichloromethane*. To 1.0 ml of this solution add 0.2 ml of a mixture of 4 volumes of *N,O-bis(trimethylsilyl)acetamide* and 1 volume of *trimethylchlorosilane*, mix and allow to stand for 30 minutes.

*Reference solution (a).* Prepare in the same manner as the test solution but omitting the addition of solution A.

*Reference solution (b).* Add 1 ml of solution A and 1 ml of *5 M ammonia* to 5.0 ml of a 0.05 per cent w/v solution of *atropine sulphate RS.*

Chromatographic system

- a glass column 1.5 m x 4 mm, packed with acid-washed, silanised diatomaceous support (80 to 100 mesh) coated with 3 per cent w/w of phenyl methyl silicone fluid (50 per cent phenyl) ( such as OV-17),
- temperature:
  column. 220°,
  inlet port and detector. 260°,
- flow rate. 30 ml per minute of the carrier gas.

Calculate the content of $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$ in the injection.

**Storage.** Store protected from light.

# Atropine Eye Ointment

Atropine Sulphate Eye Ointment

Atropine Eye Ointment is a sterile preparation of Atropine Sulphate in an eye ointment base.

Atropine Eye Ointment contains not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of atropine sulphate, $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$.

**Usual strength.** 1.0 per cent w/w.

## Identification

Determine by thin- layer chromatography (2.4.17), coating the plate with *silica gel G.*

*Mobile phase.* A mixture of 50 volumes of *chloroform*, 40 volumes of *acetone* and 10 volumes of *diethylamine.*

*Test solution.* Dissolve a quantity of the ointment containing 10 mg of Atropine Sulphate as completely as possible in 10 ml of *light petroleum (40° to 60°)* and extract with two quantities, each of 10 ml, of *0.05 M sulphuric acid*, washing each acid solution with the same 5 ml of *light petroleum (40° to 60°).* Mix the acid solutions, make alkaline with *dilute ammonia solution*, and extract with two quantities, each of 15 ml, of *chloroform.* Remove the *chloroform* and dissolve the residue in 2 ml of *ethanol (95 per cent).*

*Reference solution.* A 0.5 per cent w/v solution of *atropine sulphate RS* in *ethanol (95 per cent).*

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate at 105° for 20 minutes, allow it to cool to room temperature and spray with *potassium iodobismuthate solution.* The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

## Tests

**Other tests.** Complies with the tests stated under Eye Ointments.

**Assay.** Determine by gas chromatography (2.4.13).

*Test solution* (a). Dissolve a quantity of the ointment containing about 10 mg of Atropine Sulphate in 15 ml of *chloroform*, add 2 ml of 0.5 per cent w/v solution of *homatropine hydrobromide RS* (internal standard) in *methanol* (solution A) and extract with two quantities, each of 10.0 ml, of *0.1 M hydrochloric acid.* Wash the combined extracts with 10 ml of *chloroform*, add 2 ml of *5 M ammonia.* Extract with two quantities, each of 10 ml, of *chloroform*, shake the combined extracts with 2 g of *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness. Dissolve the residue in 5.0 ml of *dichloromethane*. To 1.0 ml of this solution add 0.2 ml of a mixture of 4 volumes of *N,O-bis (trimethylsilyl) acetamide* and 1 volume of *trimethylchlorosilane,* mix and allow to stand for 30 minutes.

*Reference solution (a).* Prepare in the same manner as the test solution but omitting the addition of solution A.

*Reference solution (b).* Add 2.0 ml of solution A and 2.0 ml of *5 M ammonia* to 20.0 ml of a 0.05 per cent w/v solution of *atropine sulphate RS* and complete the procedure described under the test solution beginning at the words "Extract with two quantities, each of 10.0 ml, of *chloroform*,.....".

Chromatographic system

- a glass column 1.5 m x 4mm, packed with acid-washed, silanised diatomaceous support (80 to 100 mesh) coated with 3 per cent w/w of phenyl methyl silicone fluid (50 per cent phenyl) (such as OV-17),
- temperature:
  column. 220°,
  inlet port and detector. 260°,
- flow rate. 30 ml per minute of the carrier gas.

Calculate the content of $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$ in the ointment.

**Storage.** Store at a temperature not exceeding 30°.



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# Atropine Tablets

Atropine Sulphate Tablets

Atropine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of atropine sulphate, $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$.

## Identification

A. Determine by thin- layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 50 volumes of *chloroform*, 40 volumes of *acetone* and 10 volumes of *diethylamine*.

*Test solution*. Shake a quantity of the powdered tablets containing 5 mg of Atropine Sulphate with 1 ml of *ethanol (95 per cent)*, centrifuge and use the supernatant liquid.

*Reference solution*. A 0.5 per cent w/v solution of *atropine sulphate RS* in *ethanol (95 per cent)*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate at 105° for 20 minutes, allow it to cool to room temperature and spray with *potassium iodobismuthate solution*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. The powdered tablets give the reactions of sulphates (2.3.1).

## Tests

**Uniformity of content**. Comply with the test stated under Tablets.

Determine by gas chromatography (2.4.13).

*Test solution*. Powder one tablet and shake in a centrifuge tube with 5 ml of *0.1 M hydrochloric acid*. Add 1 ml of a 0.06 per cent w/v solution of *homatropine hydrobromide RS* (internal standard) in *methanol* (solution A), extract with two quantities, each of 5 ml, of *chloroform* and discard the chloroform extracts. Add 1 ml of *5 M ammonia*. Extract with two quantities, each of 5 ml, of *chloroform*, shake the combined extracts with 1 g of *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness. Dissolve the residue in 0.5 ml of a mixture of 20 volumes of *dichloromethane* 4 volumes of *N, O-bis (trimethylsilyl)- acetamide* and 1 volume of *trimethylchlorosilane*, mix and allow to stand for 30 minutes.

*Reference solution (a)*. Add 1 ml of solution A and 1 ml of *5 M ammonia* to 5.0 ml of a 0.012 per cent w/v solution of *atropine sulphate RS*. Extract with two quantities, each of 5 ml, of *chloroform* and complete the procedure described under solution (1) beginning at the words "shake the combined extracts with 1 g of *anhydrous sodium sulphate*,.....".

*Reference solution (b)*. Prepare in the same manner as reference solution (a) but omitting the addition of solution A.

Carry out the chromatographic procedure described under Assay.

Calculate the content of $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$ in the tablet.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by gas chromatography (2.4.13).

*Test solution*. Weigh and powder 20 tablets. Shake a quantity of the powdered tablets containing about 5 mg of Atropine Sulphate with 10 ml of *0.1 M hydrochloric acid*, add 1 ml of a 0.5 per cent w/v solution of *homatropine hydrobromide RS* (internal standard) in *methanol* (solution A), extract with two quantities, each of 10 ml, of *chloroform* and discard the chloroform extracts. Add 1 ml of *5 M ammonia*. Extract with two quantities, each of 10 ml, of *chloroform*, shake the combined extracts with 2 g of *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness. Dissolve the residue in 2.0 ml of *dichloromethane*. To 1.0 ml of this solution, add 0.2 ml of a mixture of 4 volumes of *N,O-bis(trimethylsilyl) acetamide* and 1 volume of *trimethylchlorosilane*, mix and allow to stand for 30 minutes.

*Reference solution (a)*. Add 1 ml of solution A and 1 ml of *5 M ammonia* to 10 ml of a 0.05 per cent w/v solution of *atropine sulphate RS*. Extract with two quantities, each of 10 ml, of *chloroform* and complete the procedure described under the test solution beginning at the words "shake the combined extracts with 2 g of....".

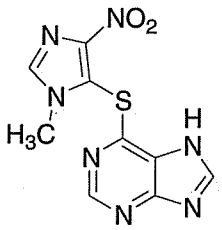
*Reference solution (b)*. Prepare in the same manner as reference solution (a) but omitting the addition of solution A.

Chromatographic system

- a glass column 1.5 m x 4 mm, packed with acid-washed, silanised diatomaceous support (80 to 100 mesh) coated with 3 per cent w/w of phenyl methyl silicone fluid (50 per cent phenyl) ( such as OV-17),
- temperature:
  column. 220°,
  inlet port and detector. 260°,
- flow rate. 30 ml per minute of the carrier gas.

Calculate the content of $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$ in the tablets.

# Azathioprine



$C_9H_7N_7O_2S$ Mol. Wt. 277.3



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Azathioprine is 6-[(1-methyl-4-nitro-1*H*-imidazol-5yl) sulphanyl]-7*H*-purine.

Azathioprine contains not less than 98.5 per cent and not more than 101.0 per cent of $C_9H_7N_7O_2S$, calculated on the dried basis.

**Category**. Immunosuppressant.

**Description**. A pale-yellow powder.

### Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *azathioprine RS*.

B. Dissolve 0.15 g in 30 ml of *dimethyl sulphoxide* and dilute to 500 ml with *0.1 M hydrochloric acid*. Dilute 25 ml of this solution to 1000 ml with *0.1 M hydrochloric acid*.

When examined in the range 230 nm to 350 nm (2.4.7), the resulting solution shows an absorption maximum at about 280 nm; absorbance at 280 nm, about 0.600 to 0.660.

C. To about 20 mg add 100 ml of *water*, heat and filter. To 5 ml of the filtrate add 1 ml of *hydrochloric acid* and about 10 mg of *zinc powder*, stand for 5 minutes. The solution becomes yellow. Filter, cool in iced water, add 0.1 ml of *sodium nitrite solution* and 0.1 g of *sulphamic acid* and shake until the bubbles disappear. Add 1 ml of *α-naphthol solution*. A pale-pink precipitate is formed.

### Tests

**Acidity or alkalinity**. To 0.5 g add 25 ml of *carbon dioxide free water*, shake for 15 minutes and filter. To 20 ml of the filtrate add 0.1 ml of *methyl red solution*. Not more than 0.2 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

**5-Chloro-1-methyl-4-nitroimidazole and 6-mercaptopurine**. Determine by thin-layer chromatography (2.4.17), coating the plate with *cellulose GF254*.

*Mobile phase*. *Butanol* saturated with *dilute ammonia solution*.

*NOTE – Prepare the following solutions immediately before use.*

*Test solution*. Dissolve 0.2 g of the substance under examination in *dilute ammonia solution* and add sufficient *dilute ammonia solution* to produce 10 ml.

*Reference solution (a)*. A 0.02 per cent w/v solution of *chloromethylnitroimidazole RS* in *dilute ammonia solution*.

*Reference solution (b)*. A 0.02 per cent w/v solution of *mercaptopurine* in *dilute ammonia solution*.

Apply to the plate 5 µl of each solution. After development, dry the plate at 50° and examine in ultraviolet light at 254 nm. In the chromatogram obtained with the test solution, any spots corresponding to chloromethylnitroimidazole and mercaptopurine are not more intense than the spots in the chromatograms obtained with reference solution (a) (1.0 per cent) and reference solution (b) (1.0 per cent).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 0.5 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.25 g and dissolve in 25 ml of *dimethylformamide*. Titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.02773 g of $C_9H_7N_7O_2S$.

**Storage**. Store protected from light.

## Azathioprine Tablets

Azathioprine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of azathioprine, $C_9H_{77}O_2S$.

**Usual strength**. 50 mg.

### Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *cellulose F 254*.

*Mobile phase*. A mixture of *butan-1-ol* saturated with *6 M ammonia*.

*Test solution*. Shake a quantity of the powdered tablets containing 0.2 g of Azathioprine with 50 ml of *6 M ammonia*, filter through a glass micro fibre paper (such as Whatman GF/C) and use the filtrate.

*Reference solution*. A 0.4 per cent w/v solution of *azathioprine RS* in *6 M ammonia*.

Apply to the plate 5 µl of each solution. After removal of the plate, dry the plate at 50° and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. Heat a quantity of the powdered tablets containing 20 mg of Azathioprine with 100 ml of *water* and filter. To 5 ml of the filtrate add 1 ml of *hydrochloric acid* and 10 mg of *zinc powder*



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and allow to stand for 5 minutes; a yellow colour is produced. Filter, cool in ice, add 0.1 ml of a 10 per cent w/v solution of *sodium nitrite* and 0.1 g of *sulphamic acid* and shake until the bubbles disappear. Add 1 ml of *2-naphthol solution*; a pale pink precipitate is produced.

### Tests

**5-Chloro-1-methyl-4-nitroimidazole and 6-mercaptopurine.** Determine by thin-layer chromatography (2.4.17), coating the plate with *cellulose F 254*.

*Mobile phase*. A mixture of *butan-1-ol* saturated with *6 M ammonia*.

*Test solution*. Shake a quantity of the powdered tablets containing 0.2 g of Azathioprine with 10 ml of *6 M ammonia* and filter through a glass micro fibre filter paper (such as Whatman GF/C).

*Reference solution (a)*. A solution containing 2.0 per cent w/v of *azathioprine RS* and 0.02 per cent w/v of *6-mercaptopurine* in *6 M ammonia*.

*Reference solution (b)*. A 0.02 per cent w/v solution of *6-mercaptopurine* in *6 M ammonia*.

*Reference solution (c)*. A 0.02 per cent w/v solution of *chloromethylnitroimidazole RS* in *6 M ammonia*.

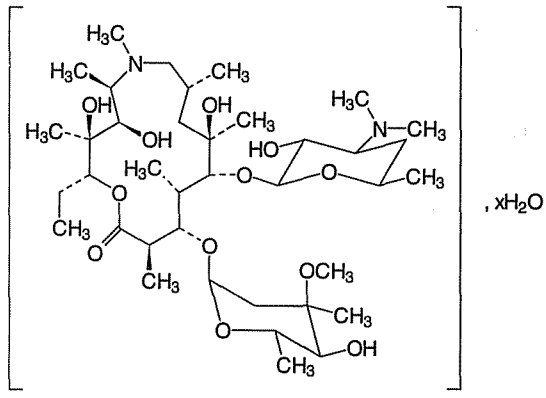
Apply to the plate 5 µl of each solution. After removal of the plate, dry the plate at 50° and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution corresponding to 6-mercaptopurine in the chromatogram obtained with reference solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b). Any spot corresponding to 5-chloro-1-methyl-4-nitroimidazole in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (c).

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Shake a quantity of the powder containing about 0.15 g of Azathioprine with 20 ml of *dimethyl sulphoxide* for 15 minutes and dilute to 500.0 ml with *0.1 M hydrochloric acid*, filter. Dilute 25.0 ml of the filtrate to 1000.0 ml with *0.1 M hydrochloric acid*. Measure the absorbance of the resulting solution at the maximum at about 280 nm (2.4.7) using *0.1 M hydrochloric acid* as the blank. Calculate the content of $C_9H_7N_7O_2S$ using a solution of *azathioprine RS* of the same concentration in *0.1 M hydrochloric acid*.

**Storage**. Store protected from light.

## Azithromycin




$C_{38}H_{72}N_2O_{12}.xH_2O$ with x = 1 or 2 Mol. Wt. 749.0 (anhydrous)

Azithromycin is (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*R*,14*R*)-13-[2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one monohydrate or dihydrate.

Azithromycin contains not less than 96.0 per cent and not more than 102.0 per cent of $C_{38}H_{72}N_2O_{12}$, calculated on the anhydrous basis.

**Category**. Antibacterial.

**Description**. A white or almost white powder.

### Identification

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

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

### Tests

**Appearance of solution.** Dissolve 0.5 g in *anhydrous ethanol* and dilute to 50.0 ml with the same solvent (solution A). Solution A is clear (2.4.1) and colourless (2.4.1).

**pH** (2.4.24). 9.0 to 11.0 determined in a solution prepared by dissolving 0.1 g in 25.0 ml of *methanol* and further diluting to 50.0 ml with *carbon dioxide-free water*.

**Specific optical rotation** (2.4.22). −45.0° to −49.0°, determined in solution A.

**Related substances.** Determine by liquid chromatography (2.4.14).



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*NOTE — Prepare the solutions immediately before use.*

*Solvent mixture.* Prepare a 0.173 per cent w/v solution of *ammonium dihydrogen phosphate* with the pH adjusted to 10.0 with *strong ammonia solution.* To 350 ml of this solution add 300 ml of *acetonitrile* and 350 ml of *methanol.* Mix well.

*Test solution.* Dissolve 0.2 g of the substance under examination in the solvent mixture and dilute to 25 ml with the solvent mixture.

*Reference solution (a).* Dilute 1 ml of the test solution to 100 ml with the solvent mixture.

*Reference solution (b).* A solution containing 0.01 per cent w/v of *azithromycin RS* and *6-demethyl-azithromycin RS (azithromycin impurity A)* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with end-cappped octadecylsilyl amorphous organosilica polymer for mass spectrometry (5µm) (such as Waters Xterra),
- column temperature. 60°,
- mobile phase: A. a 0.18 per cent w/v solution of *anhydrous disodium hydrogen phosphate* with the pH adjusted to 8.9 with *dilute phosphoric acid* or with *dilute sodium hydroxide solution*,
  B. a mixture of 25 volumes of *methanol* and 75 volumes of *acetonitrile*,
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 50 µl.

| Time (in min.) | Mobile phase A (per cent v/v) | Mobile Phase B (per cent v/v) |
|---|---|---|
| 0-25 | 50→45 | 50→55 |
| 25-30 | 45→40 | 55→60 |
| 30-80 | 40→25 | 60→75 |
| 80-81 | 25→50 | 75→50 |
| 81-93 | 50 | 50 |

Inject reference solution (b). The chromatogram obtained shows peaks corresponding to azithromycin and azithromycin impurity A. The test is not valid unless the resolution between these two peaks is at least 7.0.

Inject the test solution and reference solution (a). In the chromatogram obtained with the test solution, the area of any secondary peak eluting with relative retention time of about 1.3 due to 3-deoxyazithromycin (azithromycin impurity B) is not more than twice the area of principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent). The sum of the areas of all the other secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (3.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

**Heavy metals** (2.3.13). 0.8 g complies with the limit test for heavy metals, Method B (25 ppm).

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Water** (2.3.43). 1.8 per cent to 6.5 per cent, determined on 0.2 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture.* 40 volumes of *acetonitrile* and 60 volumes of *water.*

*Test solution.* Dissolve 0.1g of the substance under examination in the solvent mixture and dilute to 100 ml with the solvent mixture.

*Reference solution (a)* . A 0.1 per cent w/v solution of *azithromycin RS* in the solvent mixture.

*Reference solution (b).* A solution containing 0.01 per cent w/v of *azithromycin RS* and *6-demethyl-azithromycin RS (azithromycin impurity A RS)* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with end capped polar embedded octadecylsilyl amorphous organosilica polymer (5 µm) (such as Waters Xterra),
- column temperature. 70°,
- mobile phase: a mixture of 10 volumes of 3.484 per cent w/v solution of *dipotassium hydrogen phosphate* previously adjusted to pH 6.5 with *orthophosphoric acid*, 35 volumes of *acetonitrile* and 55 volumes of *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume. 100 µl.

Inject reference solution (b). The chromatogram obtained shows peaks corresponding to azithromycin and azithromycin impurity A. The test is not valid unless the resolution between these two peaks is at least 7.0.

Inject the test solution and reference solution (a).

Calculate the content of $C_{38}H_{72}N_2O_{12}$.

**Storage**. Store protected from moisture.

## Azithromycin Capsules

Azithromycin Capsules contain not less than 90.0 percent and not more than 110.0 percent of the stated amount of azithromycin, $C_{38}H_{72}N_2O_{12}$.

**Usual strength**. 500 mg.



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## Identification

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

## Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*NOTE — Prepare the solutions immediately before use.*

*Solvent mixture*. Prepare a 0.173 per cent w/v solution of *ammonium dihydrogen phosphate* with the pH adjusted to 10.0 with *strong ammonia solution*. Transfer 350 ml of this solution, add 300 ml of *acetonitrile* and 350 ml of *methanol*. Mix well.

*Test solution*. Dissolve a suitable weighed quantity of the mixed contents of 20 capsules containing about 0.2 g of Azithromycin in the solvent mixture by shaking mechanically, dilute to 25.0 ml with the solvent mixture and filter.

*Reference solution (a)*. Dilute 1ml of the test solution to 100 ml with the solvent mixture.

*Reference solution (b)*. A solution containing 0.01 per cent w/v of *azithromycin RS* and *6-demethyl-azithromycin RS (azithromycin impurity A RS)* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with end-cappped octadecylsilyl amorphous organosilica polymer for mass spectrometry (5µm) (such as Waters Xterra),
- column temperature 60°,
- mobile phase: A. a solution of 0.18 per cent w/v solution of *anhydrous disodium hydrogen phosphate* with the pH adjusted to 8.9 with *dilute phosphoric acid* or with *dilute sodium hydroxide solution*,
  B. a mixture of 25 volumes of *methanol* and 75 volumes of *acetonitrile*,
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 50 µl.

| Time (in min.) | mobile phase A (per cent v/v) | Mobile Phase B (per cent v/v) |
|---|---|---|
| 0-25 | 50→45 | 50→55 |
| 25-30 | 45→40 | 55→60 |
| 30-80 | 40→25 | 60→75 |
| 80-81 | 25→50 | 75→50 |
| 81-93 | 50 | 50 |

Inject reference solution (b). The chromatogram obtained shows peaks corresponding to azithromycin and azithromycin impurity A. The test is not valid unless the resolution between these two peaks is at least 7.0.

Inject the test solution and reference solution (a).

Inject the test solution and reference solution (a). In the chromatogram obtained with the test solution, the area of any secondary peak eluting with an relative retention time of about 1.3 due to 3-deoxyazithromycin (azithromycin impurity B) is not more than twice the area of principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent). The sum of the areas of all the other secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (3.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

**Dissolution** (2.5.2).

Apparatus No. 1

Medium. 900 ml of a buffer solution prepared by adding to 6 litres of *0.1 M dibasic sodium phosphate* about 40 ml of *hydrochloric acid* to adjust the pH to 6.0, adding 600 mg of *trypsin* and mixing.

Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

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

*Test solution*. The filtrate from the dissolution medium suitably diluted with the mobile phase, if necessary.

*Reference solution*. A solution of *azithromycin RS* in the dissolution medium suitably diluted with the mobile phase to obtain a solution having the same concentration as that of the test solution.

Calculate the content of $C_{38}H_{72}N_2O_{12}$ in the medium.

D. Not less than 75 per cent of the stated amount of $C_{38}H_{72}N_2O_{12}$.

**Water** (2.3.43). Not more than 5.0 per cent determined on 0.2 g of the contents of the capsules.

**Other tests**. Comply with the tests stated under Capsules.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. 40 volumes of *acetonitrile* and 60 volumes of *water*.

*Test solution*. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 0.1 g of Azithromycin, dissolve in about 75 ml of the solvent mixture by shaking mechanically, dilute to 100 ml with the solvent mixture and filter.



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*Reference solution(a).* A 0.1 per cent w/v solution of *azithromycin RS* in the solvent mixture.

*Reference solution (b).* A solution containing 0.01 per cent w/v of *azithromycin RS* and *6-demethyl-azithromycin RS (azithromycin impurity A RS)* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with end capped polar embedded octadecylsilyl amorphous organosilica polymer (5 µm) (such as Waters Xterra),
- column temperature 70°,
- mobile phase: a mixture of 10 volumes of a 3.484 per cent w/v solution of *dipotassium hydrogen phosphate* with the pH previously adjusted to 6.5 with *orthophosphoric acid*, 35 volumes of *acetonitrile* and 55 volumes of *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume. 100 µl.

Inject reference solution (b). The chromatogram obtained shows peaks corresponding to azithromcyin and azithromycin impurity A. The test is not valid unless the resolution between these two peaks is at least 7.0.

Inject the test solution and reference solution (a).

Calculate the content of $C_{38}H_{72}N_2O_{12}$ in the capsules.

**Storage.** Store protected from moisture. Where packaged in unit-of-use containers, each container contains six 250-mg capsules and the label indicates the intended sequential day of use for each capsule.

# Azithromycin Oral Suspension

Azithromycin Oral Suspension is a dry mixture of Azithromycin with buffering agents and other excipients, or is a homogeneous suspension in a suitable vehicle.

The suspension is constituted by dispersing the contents of the sealed container in the specified volume of Water just before use.

Azithromycin Oral Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of azithromycin, $C_{38}H_{72}N_2O_{12}$.

**Usual strength.** 40 mg per ml.

When stored at the temperature and for the period stated on the label during which the constituted suspension may be expected to be satisfactory for use, it contains not less than 80.0 per cent of the stated amount of azithromycin, $C_{38}H_{72}N_2O_{12}$.

*The contents of the sealed container comply with the following test.*

**Water** (2.3.43). Not more than 1.5 per cent, determined on 0.5 g.

**Storage.** Store protected from moisture.

*The constituted suspension or the suspension complies with the tests stated under Oral liquids and with the following tests.*

## Identification

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

## Tests

**pH** (2.4.24). 7.5 to 11.0.

**Related substances.** Determine by liquid chromatography (2.4.14).

*NOTE — Prepare the solutions immediately before use.*

*Solvent mixture.* Prepare a 0.173 per cent w/v solution of *ammonium dihydrogen phosphate* with the pH adjusted to 10.0 with *strong ammonia solution*. Transfer 350 ml of this solution add 300 ml of *acetonitrile* and 350 ml of *methanol*. Mix well.

*Test solution.* Weigh accurately a quantity of the oral suspension containing about 0.2 g of Azithromycin, dissolve in the solvent mixture, dilute to 25.0 ml with the solvent mixture and filter.

*Reference solution (a).* Dilute 1ml of the test solution to 100 ml with the solvent mixture.

*Reference solution (b).* A solution containing 0.01 per cent w/v of *azithromycin RS* and *6-demethyl-azithromycin RS (azithromycin impurity A RS)* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with end-cappped octadecylsilyl amorphous organosilica polymer for mass spectrometry (5µm) (such as Waters Xterra),
- column temperature. 60°,
- mobile phase: A. a 0.18 per cent w/v solution of *anhydrous disodium hydrogen phosphate* with the pH adjusted to 8.9 with *dilute orthophosphoric acid* or with *dilute sodium hydroxide solution*,

  B. a mixture of 25 volumes of *methanol* and 75 volumes of *acetonitrile*,
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 50 µl.



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| Time (in min.) | Mobile phase A (per cent v/v) | Mobile Phase B (per cent v/v) |
|---|---|---|
| 0-25 | 50→45 | 50→55 |
| 25-30 | 45→40 | 55→60 |
| 30-80 | 40→25 | 60→75 |
| 80-81 | 25→50 | 75→50 |
| 81-93 | 50 | 50 |

Inject reference solution (b). The chromatogram obtained shows peaks corresponding to azithromycin and azithromycin impurity A. The test is not valid unless the resolution between these two peaks is at least 7.0.

Inject the test solution and reference solution (a). In the chromatogram obtained with the test solution, the area of any secondary peak eluting with an relative retention time of about 1.3 due to 3-deoxyazithromycin (azithromycin impurity B) is not more than twice the area of principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent). The sum of the areas of all the other secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (3.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. 40 volumes of *acetonitrile* and 60 volumes of 3.484 per cent w/v solution of *dipotassium hydrogen phosphate* with the pH previously adjusted to 6.5 with *orthophosphoric acid*.

*Test solution*. Weigh accurately a quantity of the oral suspension containing about 0.1 g of Azithromycin , dissolve in the solvent mixture, dilute to 100.0 ml with the solvent mixture and filter.

*Reference solution (a)*. A 0.1 per cent w/v solution of *azithromycin RS* in the solvent mixture.

*Reference solution (b)*. A solution containing 0.01 per cent w/v of *azithromycin RS* and *6-demethyl-azithromycin RS (azithromycin impurity A RS)* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with end capped polar embedded octadecylsilyl amorphous organosilica polymer (5µm) (such as Waters Xterra),
- column temperature 70°,
- mobile phase: a mixture of 10 volumes of a 3.484 per cent w/v solution of *dipotassium hydrogen phosphate* with the pH previously adjusted to 6.5 with *orthophosphoric acid*, 35 volumes of *acetonitrile* and 55 volumes of *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume. 100 µl.

Inject reference solution (b). The chromatogram obtained shows peaks corresponding to azithromcyin and azithromycin impurity A. The test is not valid unless the resolution between these two peaks is at least 7.0.

Inject the test solution and reference solution (a).

Determine the weight per ml (2.4.29) of the suspension and calculate the content of $C_{38}H_{72}N_2O_{12}$, weight in volume.

## Azithromycin Tablets

Azithromycin Tablets contain not less than 90.0 percent and not more than 110.0 percent of the stated amount of azithromycin, $C_{38}H_{72}N_2O_{12}$.

**Usual strengths**. 250 mg; 500 mg.

### Identification

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

### Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*NOTE — Prepare the solutions immediately before use.*

*Solvent mixture*. Prepare a 0.173 per cent w/v solution of *ammonium dihydrogen phosphate* with the pH adjusted to 10.0 with *strong ammonia solution*. Transfer 350 ml of this solution add 300 ml of *acetonitrile* and 350 ml of *methanol*. Mix well.

*Test solution*. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.2 g of Azithromycin, dissolve in the solvent mixture by shaking mechanically, dilute to 25.0 ml with the solvent mixture and filter.

*Reference solution (a)*. Dilute 1 ml of the test solution to 100 ml with the solvent mixture.

*Reference solution (b)*. A solution containing 0.01 per cent w/v of *azithromycin RS* and *6-demethyl-azithromycin RS (azithromycin impurity A RS)* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with end-cappped octadecylsilyl amorphous organosilica polymer for mass spectrometry (5 µm) (such as Waters Xterra),
- column temperature 60°,



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- mobile phase: A. a 0.18 per cent w/v solution of *anhydrous disodium hydrogen phosphate* with the pH adjusted to 8.9 with *dilute orthophosphoric acid* or with *dilute sodium hydroxide solution*,
  B. a mixture of 25 volumes of *methanol* and 75 volumes of *acetonitrile*,
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 50 µl.

| Time (min.) | Mobile phase A (per cent v/v) | Mobile Phase B (per cent v/v) |
|---|---|---|
| 0-25 | 50→45 | 50→55 |
| 25-30 | 45→40 | 55→60 |
| 30-80 | 40→25 | 60→75 |
| 80-81 | 25→50 | 75→50 |
| 81-93 | 50 | 50 |

Inject reference solution (b). The chromatogram obtained shows peaks corresponding to azithromycin and azithromycin impurity A. The test is not valid unless the resolution between these two peaks is at least 7.0

Inject the test solution and reference solution (a). In the chromatogram obtained with the test solution, the area of any secondary peak eluting with an relative retention time of about 1.3 due to 3-deoxyazithromycin (azithromycin impurity B) is not more than twice the area of principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent). The sum of the areas of all the other secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (3.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

**Dissolution** (2.5.2).

Apparatus No. 1

Medium. 900 ml of a buffer solution prepared by adding to 6 litres of *0.1 M dibasic sodium phosphate* about 40 ml of *hydrochloric acid*, adjusting the pH to 6.0, adding 600 mg of *trypsin*, and mixing.

Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter through a filter.

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

*Test solution.* The filtrate from the dissolution medium suitably diluted with the mobile phase, if necessary.

*Reference solution.* A solution of *azithromycin RS* in the dissolution medium suitably diluted with the mobile phase to obtain a solution having the same concentration as that of the test solution.

Calculate the content of $C_{38}H_{72}N_2O_{12}$ in the medium.

D. Not less than 75 per cent of the stated amount of $C_{38}H_{72}N_2O_{12}$.

**Water** (2.3.43). Not more than 6.0 per cent determined on 0.2 g of the powdered tablets.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture.* 40 volumes of *acetonitrile* and 60 volumes of *water*.

*Test solution.* Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.1 g of Azithromycin, dissolve in the solvent mixture by shaking mechanically, dilute to 100 ml with the solvent mixture and filter.

*Reference solution (a)* . A 0.1 per cent w/v solution of *azithromycin RS* in the solvent mixture.

*Reference solution (b).* A solution containing 0.01 per cent w/v of *azithromycin RS* and *6-demethyl-azithromycin RS (azithromycin impurity A RS)* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with end capped polar embedded octadecylsilyl amorphous organosilica polymer (5 µm) (such as Waters Xterra),
- column temperature. 70°,
- mobile phase: a mixture of 10 volumes of a 3.484 per cent w/v solution of *dipotassium hydrogen phosphate* with the pH previously adjusted to 6.5 with *orthophosphoric acid*, 35 volumes of *acetonitrile* and 55 volumes of *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume. 100 µl.

Inject reference solution (b). The chromatogram obtained shows peaks corresponding to azithromcyin and azithromycin impurity A. The test is not valid unless the resolution between these two peaks is at least 7.0.

Inject the test solution and reference solution (a).

Calculate the content of $C_{38}H_{72}N_2O_{12}$ in the tablets.



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# Bacitracin

Bacitracin is a mixture of polypeptides produced by the growth of certain strains of *Bacillus licheniformis* and *B. subtilis* (Fam. Bacillaceae). Its main components are Bacitracin A1, B1, B2 and B3.

Bacitracin has a potency of not less than 60 Units of bacitracin activity per mg, calculated on the dried basis.

**Category**. Antibacterial (for topical use).

**Description**. A white or almost white powder; odourless or with a faint odour; hygroscopic.

## Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 75 parts of *phenol* and 25 parts of *water*.

*Test solution*. Dissolve 5 mg of the substance under examination in a mixture of 0.5 ml of *hydrochloric acid* and 0.5 ml of *water*, heat in a sealed tube at 135° for 5 hours, evaporate to dryness on a water-bath, continue to heat until the odour of *hydrogen chloride* is no longer detectable and dissolve the residue in 0.5 ml of *water*.

*Reference solution*. Prepare in the same manner as the test solution but using *bacitracin zinc RS* in place of the substance under examination.

Apply to the plate 5 µl of each solution as bands 10 mm wide. Place the plate in the tank so that it is not in contact with the mobile phase and allow to stand for at least 12 hours before development. Allow the mobile phase to rise 10 cm. Dry the plate at 105°, spray with *ethanolic ninhydrin solution* and heat at 110° for 5 minutes. The bands in the chromatogram obtained with the test solution correspond to those in the chromatogram obtained with the reference solution.

B. Shake 5 mg with 1 ml of *water*, add 1 ml of a 0.2 per cent w/v solution of *ninhydrin* in *1-butanol* and 0.5 ml of *pyridine* and heat at 100° for 5 minutes; a deep purple colour is produced.

## Tests

**Appearance of solution**. A 1.0 per cent w/v solution in *carbon dioxide-free water* is clear, (2.4.1), and not more intensely coloured than reference solution BYS6 (2.4.1).

**pH** (2.4.24). 5.5 to 7.5, determined in a 1.0 per cent w/v solution.

**Composition**. Determine by liquid chromatography (2.4.14).

*NOTE — prepare the solution immediately before use.*

*Test solution*. Dissolve 0.1 g of the substance under examination in 50.0 ml of the mobile phase.

*Reference solution (a)*. Dissolve 20 mg of *bacitracin zinc RS* in *water*, add 0.2 ml of *dilute hydrochloric acid* and dilute to 10.0 ml with *water*.

*Reference solution (b)*. Dilute 5.0 ml of the test solution to 100.0 ml with the mobile phase.

*Reference solution (c)*. Dilute 1.0 ml of reference solution (b) to 10.0 ml with the mobile phase.

*Reference solution (d)*. Dissolve 50 mg of substance under examination in 25 ml of a 4.0 per cent w/v solution of *sodium edetate*, adjust the pH to 7.0 with *dilute sodium hydroxide*. Heat on boiling water bath for 30 minutes.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: add a mixture of 52 volumes of *methanol*, 4 volumes of *acetonitrile* and 30 volumes of *water* to 100 volumes of a 3.48 per cent w/v solution of *dipotassium hydrogen phosphate*, adjust the pH to 6.0 with a 2.72 per cent w/v solution of *potassium dihydrogen orthophosphate*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 100 µl.

Inject reference solution (a). The relative retention time with reference to bacitracin A for bacitracin B1 is about 0.6, for bacitracin B3 is about 0.8, for bacitracin impurity E is about 2.5. The test is not valid unless the peak- to- valley ratio between the peaks due to bacitracin B1 and bacitracin B2 is not less than 1.2.

Inject the test solution, reference solution (a) and (c). Run the chromatogram three times the retention time of the peak due to Bacitracin A. In the chromatogram obtained with the test solution, the area of the any peak corresponding to Bacitracin A is not less than 40.0 per cent. The sum of area of the peaks corresponding to bacitracin A, bacitracin B1, bacitracin B2 and bacitracin B3 is not less than 70 per cent. Ignore any peak with an area less than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent).

**Related peptides**. Determine by liquid chromatography (2.4.14) as described under Composition.

Inject the test solution. The sum of areas of all the peaks eluting before the peak due to Bacitracin B1 is not more than 20.0 per cent.

**Impurity E**. Determine by liquid chromatography (2.4.14) as described under Composition.

Spectrophotometer set at 254 nm and at 300 nm for reference solution (d).



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Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of peak due to bacitracin impurity E is not more than 1.2 times the area of the peak in the chromatogram obtained with reference solution (b) (6.0 per cent).

**Sulphated ash** (2.3.18). Not more than 3.0 per cent.

**Loss on drying** (2.4.19). Not more than 5.0 per cent, determined on 0.5 g by drying in an oven over *phosphorus pentoxide* at 60° at a pressure not exceeding 0.1 kPa for 3 hours.

**Assay**. Determine by the microbiological assay of antibiotics, Method A (2.2.10), and express the results in Units per mg.

**Bacterial endotoxins** (2.2.3). Not more than 0.8 Endotoxin Unit per mg of bacitracin, if intended for use in the manufacture of ophthalmic dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

**Sterility** (2.2.11). Complies with the test for sterility, if intended for the preparation of ophthalmic dosage forms without a further appropriate sterilization procedure.

**Storage**. Store protected from moisture, at a temperature not exceeding 8°.

**Labelling**. The label states (1) the number of Units per mg; (2) whether or not the contents are intended for use in the manufacture of ophthalmic preparations.

# Bacitracin Zinc

Bacitracin Zinc is the zinc complex of Bacitracin.

Bacitracin Zinc has a potency of not less than 60 Units of bacitracin activity per mg, calculated on the dried basis.

**Category**. Antibacterial (for topical use).

**Description**. A white or light yellowish-grey powder; odourless or with a faint odour; hygroscopic.

## Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 75 volumes of *phenol* and 25 volumes of *water*.

*Test solution*. Dissolve 5 mg of the substance under examination in a mixture of 0.5 ml of *hydrochloric acid* and 0.5 ml of *water*, heat in a sealed tube at 135° for 5 hours, evaporate to dryness on a water-bath, continue to heat until the odour of *hydrogen chloride* is no longer detectable and dissolve the residue in 0.5 ml of water.

*Reference solution*. Prepare in the same manner as the test solution but using *bacitracin zinc RS* in place of the substance under examination.

Apply to the plate 5 µl of each solution as bands 10 mm wide. Place the plate in the tank so that it is not in contact with the mobile phase and allow to stand for at least 12 hours before development. Allow the mobile phase to rise 10 cm. Dry the plate at 105°, spray with *ethanolic ninhydrin solution* and heat at 110° for 5 minutes. The bands in the chromatogram obtained with the test solution correspond to those in the chromatogram obtained with the reference solution.

B. Shake 5 mg with 1 ml of *water*, add 1 ml of a 0.2 per cent w/v solution of *ninhydrin* in *1-butanol* and 0.5 ml of *pyridine* and heat at 100° for 5 minutes; a deep purple colour is produced.

C. Ignite 0.15 g and allow to cool. The residue on dissolving in 1 ml of *2 M hydrochloric acid* and diluting with 4 ml of *water* gives the reactions of zinc salts (2.3.1).

## Tests

**pH** (2.4.24). 6.0 to 7.5, determined on the filtrate obtained by shaking 1.0 g with 10 ml of *carbon dioxide-free water*.

**Composition**. Determine by liquid chromatography (2.4.14).

*NOTE — prepare the solution immediately before use.*

*Test solution*. Dissolve 0.1 g of the substance under examination in 50.0 ml of the mobile phase.

*Reference solution (a)*. Dissolve 20 mg of *bacitracin zinc RS* in *water*, add 0.2 ml of *dilute hydrochloric acid* and dilute to 10.0 ml with *water*.

*Reference solution (b)*. Dilute 5.0 ml of the test solution to 100.0 ml with the mobile phase.

*Reference solution (c)*. Dilute 1.0 ml of reference solution (b) to 10.0 ml with the mobile phase.

*Reference solution (d)*. Dissolve 50 mg of substance under examination in 25 ml of a 4.0 per cent w/v solution of *sodium edetate*, adjust the pH to 7.0 with *dilute sodium hydroxide*. Heat on boiling water bath for 30 minutes.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: add a mixture of 52 volumes of *methanol*, 4 volumes of *acetonitrile* and 30 volumes of *water* to 100 volumes of a 3.48 per cent w/v solution of *dipotassium hydrogen phosphate*, adjust the pH to 6.0 with a 2.72 per cent w/v solution of *potassium dihydrogen orthophosphate*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 100 µl.

Inject reference solution (a). The relative retention time with reference to bacitracin A for bacitracin B1 is about 0.6, for



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bacitracin B3 is about 0.8, for bacitracin impurity E is about 2.5. The test is not valid unless the peak- to- valley ratio between the peaks due to bacitracin B1 and bacitracin B2 is not less than 1.2.

Inject the test solution, reference solution (a) and (c). Run the chromatogram three times the retention time of the peak due to Bacitracin A. In the chromatogram obtained with the test solution, the area of the any peak corresponding to Bacitracin A is not less than 40.0 per cent. The sum of area of the peaks corresponding to bacitracin A, bacitracin B1, bacitracin B2 and bacitracin B3 is not less than 70 per cent. Ignore any peak with an area less than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent).

**Related peptides**. Determine by liquid chromatography (2.4.14) as described under Composition.

Inject the test solution. The sum of areas of all the peaks eluting before the peak due to Bacitracin B1 is not more than 20.0 per cent.

**Impurity E**. Determine by liquid chromatography (2.4.14) as described under Composition.

Spectrophotometer set at 254 nm and at 300 nm for reference solution (d).

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of peak due to bacitracin impurity E is not more than 1.2 times the area of the peak in the chromatogram obtained with reference solution (b) (6.0 per cent).

**Zinc content**. 4.0 per cent to 8.0 per cent, calculated on the dried basis, determined by the following method. Weigh accurately about 0.2 g and dissolve in 20 ml of *water* and 3 ml of *strong ammonia-ammonium chloride solution* and titrate with *0.01 M disodium edetate* using *mordant black 11 mixture* as indicator.

1 ml of *0.01 M disodium edetate* is equivalent to 0.000654 g of Zn.

**Loss on drying** (2.4.19). Not more than 5.0 per cent, determined on 0.5 g by drying in an oven over *phosphorus pentoxide* at 60° at a pressure not exceeding 0.1 kPa for 3 hours.

**Assay**. Weigh accurately about 0.1 g, suspend in 10 ml of *water* and 0.5 ml of *2 M hydrochloric acid* and add sufficient *water* to produce 200.0 ml. Allow to stand at room temperature for 30 minutes. Determine by the microbiological assay of antibiotics, Method A (2.2.10), and express the results in Units per mg.

**Pyrogens** (2.2.8). If intended for administration by spraying into internal body cavities without a further appropriate procedure for the removal of pyrogens, it complies with the test for pyrogens. Inject per kilogram of the rabbit's mass 1 ml of the supernatant liquid obtained by centrifuging a suspension containing 11mg per milliliter in a 0.9 per cent solution of *sodium chloride*.

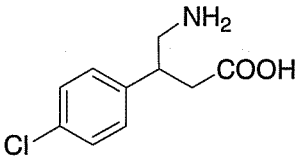
*Bacitracin Zinc intended for administration as a spray in internal body cavities without a further appropriate sterilization procedure complies with the following additional requirement.*

**Sterility** (2.2.11). Complies with the test for sterility.

**Storage**. Store protected from moisture at a temperature not exceeding 30°. If it is intended for administration as a spray in internal body cavities, the container should be sterile and sealed so as to exclude micro-organisms.

**Labelling**. The label states (1) the number of Units per mg; (2) whether or not the contents are intended for administration as a spray in internal body cavities.

# Baclofen




$C_{10}H_{12}ClNO_2$ Mol. Wt. 213.7

Baclofen is (*RS*)-4-amino-3-(4-chlorophenyl)butyric acid.

Baclofen contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{10}H_{12}ClNO_2$, calculated on the anhydrous basis.

**Category**. Muscle relaxant.

**Description**. A white or almost white powder.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *baclofen RS or* with the refernce spectrum of baclofen.

B. When examined in the range 220 nm to 320 nm (2.4.7), a 0.07 per cent w/v solution shows three absorption maxima, at 259 nm, 266 nm and 275 nm. The specific absorbances at these maxima are 9.8 to 10.8, 11.5 to 12.7 and 8.4 to 9.3, respectively.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*.

*Mobile phase*. A mixture of 5 volumes of *anhydrous formic acid*, 5 volumes of *water*, 20 volumes of *methanol*, 30 volumes of *chloroform* and 40 volumes of *ethyl acetate*.



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

*Reference solution.* A 0.1 per cent w/v solution of *baclofen RS* in the mobile phase.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate and spray with *ninhydrin solution* until the plate is slightly wet. Place the plate in an oven maintained at 100° for 10 minutes. Examine in daylight. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

### Tests

**Appearance of solution**. Dissolve 0.5 g in *1 M sodium hydroxide* and dilute to 25 ml with the same solvent. The freshly prepared solution is not more intensely coloured than reference solution BY5 (2.4.1).

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve 25 mg of the substance under examination in the mobile phase and dilute to 10 ml with the mobile phase.

*Reference solution (a).* A 0.25 per cent w/v solution of *(4RS)-4-(4-chlorophenyl)pyrrolidin-2-one RS* (baclofen impurity A) in the mobile phase.

*Reference solution (b).* Dilute 1 ml of reference solution (a) to 100 ml with the mobile phase.

*Reference solution (c).* Dilute 2 ml of the test solution to 100 ml with the mobile phase.

*Reference solution (d).* Dilute 2 ml of the test solution and 2 ml of reference solution (a) to 100 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a solution of 1.822 g of *sodium hexanesulphonate* in 1000 ml of a mixture of 560 volumes of *water*, 440 volumes of *methanol* and 5 volumes of *glacial acetic acid,*
- flow rate. 2 ml per minute,
- spectrophotometer set at 266 nm,
- injection volume. 20 µl.

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (c) is at least 50 per cent of the full scale of the recorder. Inject reference solution (d). The test is not valid unless the resolution between the peaks corresponding to baclofen and impurity A is at least 2.0.

Inject the test solution, reference solution (b) and reference solution (c). Continue the chromatography for 5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of the peak corresponding to baclofen impurity A is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of the areas of all such peaks is not greater than the area of the principal peak in the chromatogram obtained with reference solution (c) (2.0 per cent).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 1.0 per cent, determined on 1.0 g.

**Assay**. Weigh accurately about 0.15 g and dissolve in 50 ml of *anhydrous acetic acid.* Titrate with *0.1 M perchloric acid,* determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02137 g of $C_{10}H_{12}ClNO_2$.

**Storage**. Store protected from moisture.

## Baclofen Oral Solution

Baclofen Oral Solution is a solution of Baclofen in a suitable aqueous vehicle.

Baclofen Oral Solution contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of baclofen, $C_{10}H_{12}ClNO_2$.

**Usual strength**. 1 mg per ml.

### Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

*Solvent mixture.* 35 volumes of *acetonitrile* and 65 volumes of *water.*

*Mobile phase.* A mixture of 20 volumes of *glacial acetic acid,* 20 volumes of *water* and 80 volumes of *butan-1-ol.*

*Test solution.* Dilute a volume of the oral solution containing 5 mg of Baclofen to 100 ml with the solvent mixture.

*Reference solution.* A 0.005 per cent w/v solution of *baclofen RS* in the solvent mixture.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in air. Place an evaporating dish containing a mixture of 4 ml of *water,* 1 ml of *7 M hydrochloric acid* and 0.5 g of *potassium permanganate* in a



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chromatography tank, close the tank and allow to stand for 2 minutes. Place the plate in the tank, close the tank and leave the plate in contact with the vapour for 1 minute. After removal of the plate, place it in a current of cold air until an area of coating below the line of application shows only a faint blue colour on the addition of 0.05 ml of *potassium iodide and starch solution*. Spray the plate with *potassium iodide and starch solution* and examine in daylight. The chromatogram obtained with the test solution exhibits a spot that corresponds to the spot in the chromatogram obtained with the reference solution.

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

### Tests

**Lactam**. Determine by liquid chromatography (2.4.14) as described under Assay using the following solutions.

*Test solution*. Use the test solution prepared for the Assay.

*Reference solution (a)*. A solution containing 0.0002 per cent w/v of *(4RS)-4-(4-chlorophenyl)pyrrolidin-2-one RS (baclofen impurity A)* in the mobile phase.

*Reference solution (b)*. A solution containing 0.01 per cent w/v of *baclofen RS*, 0.0003 per cent w/v of *propyl 4-hydroxybenzoate*, 0.0003 per cent w/v of *methyl 4-hydroxybenzoate* and 0.0002 per cent w/v of *baclofen impurity A* in the mobile phase.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to methyl-4-hydroxybenzoate and baclofen impurity A and between the peaks due to baclofen impurity A and propyl-4-hydroxybenzoate is at least 5.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution the area of any peak corresponding to baclofen impurity A (lactam) is not greater than the area of the peak in the chromatogram obtained with reference solution (a) (2.0 per cent).

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

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dilute a weighed quantity of the oral solution containing about 5 mg of Baclofen to 50.0 ml with the mobile phase.

*Reference solution (a)*. A 0.01 per cent w/v solution of *baclofen RS* in the mobile phase.

*Reference solution (b)*. A solution containing 0.01 per cent w/v of *baclofen RS*, 0.0003 per cent w/v of *propyl 4-hydroxybenzoate* and 0.0002 per cent w/v of *baclofen impurity A RS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (10 µm) (Such as Nucleosil C18),
- mobile phase: a solution prepared by dissolving 5 g of *sodium dodecyl sulphate* in a mixture of 5 ml of *orthophosphoric acid* and 650 ml of *water* and diluting to 1000 ml with *acetonitrile*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 218 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to baclofen impurity A and propyl-4-hydroxybenzoate is at least 5.0.

Inject alternately the test solution and reference solution (a).

Determine the weight per ml (2.4.29) of the oral solution and calculate the content of $C_{10}H_{12}ClNO_2$, weight in volume.

**Storage**. Store protected from light in a refrigerator (2° and 8°). Do not freeze.

## Baclofen Tablets

Baclofen tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of baclofen, $C_{10}H_{12}ClNO_2$.

**Usual strengths**. 10 mg; 20 mg.

### Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Solvent mixture*. 4 volumes of *absolute ethanol* and 1 volume of *glacial acetic acid*.

*Mobile phase*. A mixture of 80 volumes of *butan-1-ol*, 20 volumes of *glacial acetic acid* and 20 volumes of *water*.

*Test solution*. Shake a quantity of the powdered tablets containing 20 mg of Baclofen with 20 ml of the solvent mixture for 30 minutes and filter.

*Reference solution*. A 0.1 per cent w/v solution of *baclofen RS* in the solvent mixture.

Apply to the plate 5 µl of each solution. After development, dry the plate in air, spray with *ninhydrin solution* and heat at 100° for 10 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

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

### Tests

**Lactam**. Determine by liquid chromatography (2.4.14).



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*Test solution.* Mix with the aid of ultrasound a quantity of the powdered tablets containing 0.1 g of Baclofen with 50 ml of the mobile phase for 30 minutes, shaking occasionally to disperse the sample, and filter through a glass-fibre filter (such as Whatman GF/C).

*Reference solution (a).* A solution containing 0.004 per cent w/v of *(4RS)-4-(4-chlorophenyl)pyrrolidin-2-one RS (baclofen impurity A)* in the mobile phase.

*Reference solution (b).* A solution containing 0.2 per cent w/v of *baclofen RS* and 0.004 per cent w/v of *(4RS)-4-(4-chlorophenyl)pyrrolidin-2-one RS* (baclofen impurity A) in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (10 µm) (Such as Spherisorb ODS 1),
- mobile phase: a mixture of 5 volumes of *glacial acetic acid*, 440 volumes of *methanol* and 560 volumes of water containing 1.822 g per litre of *sodium hexanesulphonate*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 266 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to baclofen and baclofen impurity A is at least 2.0.

Inject the test solution and reference solution (a). In the chromatogram obtained with the test solution the area of any peak corresponding to baclofen impurity A (lactam) is not greater than the area of the peak in the chromatogram obtained with the reference solution (2.0 per cent).

**Dissolution** (2.5.2)

Apparatus No 1
Medium. 900 ml of *0.1 M hydrochloric acid*
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

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

*Test solution.* Use the filtrate as given above.

*Reference solution.* A 0.001 per cent w/v solution of *baclofen RS* in the dissolution medium.

Calculate the content of $C_{10}H_{12}ClNO_2$ in the medium.

D. Not less than70 per cent of the stated amount of $C_{10}H_{12}ClNO_2$.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Determine by liquid chromatography (2.4.14).

*Test solution.* Add a quantity of whole tablets containing 0.1 g of Baclofen to 25 ml of a mixture of 100 volumes of *water* and 1 volume of *glacial acetic acid* and disperse with the aid of ultrasound. Dilute to 50.0 ml with *methanol,* filter and use the filtrate.

*Reference solution.* A solution containing 0.2 per cent w/v of *baclofen RS* in a mixture of 100 volumes of *methanol,* 100 volumes of *water* and 1 volume of *glacial acetic acid.*

Chromatographic system

- a stainless steel column 20 cm × 4.6 mm, packed with octadecylsilyl silica gel (10 µm) (such as Nucleosil C18),
- mobile phase: *0.01 M sodium hexanesulphonate* in a mixture of 100 volumes of *methanol,* 100 volumes of *water* and 1 volume of *glacial acetic acid,*
- flow rate. 2 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume. 20 µl.

Inject alternately the test solution and the reference solution. Calculate the content of $C_{10}H_{12}ClNO_2$ in the tablets.

# Barium Sulphate

$BaSO_4$ Mol. Wt. 233.4

**Category.** Diagnostic aid (radio-opaque medium for gastrointestinal tract).

**Description.** A fine, heavy, white powder, free from gritty particles; odourless.

## Identification

A. Boil 0.2 g with 5 ml of a 50 per cent w/v solution of *sodium carbonate* for 5 minutes, add 10 ml of *water* and filter. Reserve the residue for test B. Acidify the filtrate with *dilute hydrochloric acid*; the solution gives the reactions of sulphates (2.3.1).

B. Wash the residue obtained in test A three times with successive small quantities of *water*. To the residue add 5 ml of *dilute hydrochloric acid*, filter and add to the filtrate 0.3 ml of *dilute sulphuric acid*; a white precipitate is formed which is insoluble in *dilute sodium hydroxide solution.*

## Tests

**Acidity or alkalinity.** Heat 5.0 g with 20 ml of *carbon dioxide-free water* on a water-bath for 5 minutes and filter. To 10 ml of the filtrate add 1 drop of *bromothymol blue solution.* Not more than 0.5 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the solution.

**Arsenic** (2.3.10). Disperse 5.0 g in 50 ml of *water* and add 10 ml of *stannated hydrochloric acid AsT.* The resulting solution complies with the limit test for arsenic (2 ppm).



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**Heavy metals** (2.3.13). Boil 4.0 g with a mixture of 2 ml of *glacial acetic acid* and 48 ml of *water* for 10 minutes. Add *water* to make upto 50 ml, filter and reject the first 5 ml of the filtrate. 25 ml of the filtrate complies with the limit test for heavy metals, Method A (10 ppm).

**Phosphate**. Boil 1 g with a mixture of 3 ml of *nitric acid* and 5 ml of *water* for 5 minutes and add *water* to restore the original volume. Filter through a filter paper previously washed with *dilute nitric acid*. Add to the warm filtrate an equal volume of *ammonium molybdate solution*; no yellow precipitate is formed.

**Sulphide**. Boil 10 g with a mixture of 10 ml of *dilute hydrochloric acid* and 90 ml of *water* for 10 minutes. Expose a *lead acetate paper* to the vapours; the paper does not darken.

**Acid-soluble substances**. Cool the mixture obtained in the test for Sulphide, add *water* to restore the original volume and filter through a filter paper previously washed with a mixture of 10 ml of *dilute hydrochloric acid* and 90 ml of *water*, returning the first portions, if necessary, to obtain a clear filtrate. Evaporate 50 ml of the filtrate to dryness on a water-bath and add 2 drops of *hydrochloric acid* and 10 ml of hot *water*. Filter again through acid-washed paper, prepared as directed above, wash the filter paper with 10 ml of hot *water* and evaporate the combined filtrate and washings. Dry the residue at 105°, cool and weigh (0.3 per cent).

**Soluble barium salts**. Digest the residue obtained in the test for Acid-soluble substances with 10 ml of *water* and filter through a filter paper previously washed with a mixture of 10 ml of *dilute hydrochloric acid* and 90 ml of *water*. Add 0.5 ml of *dilute sulphuric acid* to the clear filtrate and set aside for 30 minutes; no turbidity is produced.

**Bulkiness**. Place 5.0 g in a glass-stoppered 50-ml graduated cylinder having the 50-ml graduation mark 14 cm from the base. Add *water* to 50 ml, shake the mixture for 5 minutes and allow to stand for 15 minutes; it does not settle below the 15 ml mark.

**Loss on ignition** (2.4.20). Not more than 2.5 per cent, determined on 1.0 g at 600°.

## Barium Sulphate Suspension

Barium Meal

Barium Sulphate Suspension is a dry mixture of Barium Sulphate with suitable flavours, colours, preservatives and suspending/dispersing agents.

Barium Sulphate Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of barium sulphate, $BaSO_4$.

**Description**. A white or coloured, fine powder or granules.

### Identification

A. Ignite 1 g to constant weight. Cool, boil 0.2 g of the residue with 5 ml of a 50 per cent w/v solution of *sodium carbonate* for 5 minutes, add 10 ml of *water* and filter. Reserve the residue for test B. Acidify the filtrate with *dilute hydrochloric acid*; the solution gives the reactions of sulphates (2.3.1).

B. Wash the residue obtained in test A three times with successive small quantities of *water*. To the residue add 5 ml of *dilute hydrochloric acid*, filter and add to the filtrate 0.3 ml of *dilute sulphuric acid*; a white precipitate is formed which is insoluble in *dilute sodium hydroxide solution*.

### Tests

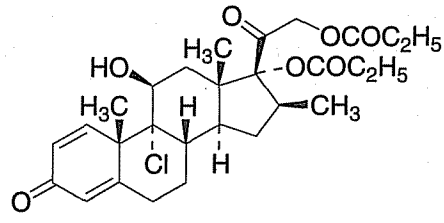
**pH** (2.4.24). 4.0 to 8.0, determined in a 75.0 per cent w/v suspension in *water*.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for 4 hours.

**Assay**. Weigh accurately about 0.6 g in a platinum crucible, add 5 g of *sodium carbonate* and 5 g of *potassium carbonate* and mix. Heat to 1000° and maintain at this temperature for 15 minutes. Allow to cool and suspend the residue in 150 ml of *water*. Wash the crucible with 2 ml of *acetic acid* and add to the suspension. Cool in ice and filter by decantation, transferring as little of the solid matter as possible to the filter. Wash the residue with successive quantities of a 2 per cent w/v solution of *sodium carbonate* until the washings are free from sulphate and discard the washings. Add 5 ml of *dilute hydrochloric acid* to the filter and wash through into the vessel containing the bulk of the solid matter with *water*. Add 5 ml of *hydrochloric acid* and dilute to 100 ml with *water*. Add 10 ml of a 40 per cent w/v solution of *ammonium acetate*, 25 ml of a 10 per cent w/v solution of *potassium dichromate* and 10 g of *urea*. Cover, digest in an oven at 80° to 85° for 16 hours and filter while still hot through a sintered-glass filter (porosity No. 4), washing the precipitate initially with a 0.5 per cent w/v solution of *potassium dichromate* and finally with 2 ml of *water*. Dry to constant weight at 105°.

1 g of the residue is equivalent to 0.9213 g of $BaSO_4$.

## Beclomethasone Dipropionate




$C_{28}H_{37}ClO_7$ Mol. Wt. 521.1



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Beclomethasone Dipropionate is 9α-chloro-11β-hydroxy-16β-methyl-3,20-dioxopregna-1,4-diene-17,21-diyldipropionate.

Beclomethasone Dipropionate contains not less than 96.0 per cent and not more than 103.0 per cent of $C_{28}H_{37}ClO_7$, calculated on the dried basis.

**Category**. Adrenocortical steroid.

**Dose**. By inhalation, for an adult, 2 inhalations, each of 50 µg, three or four times a day up to a maximum of 20 inhalations in 24 hours; for a child, 1 or 2 inhalations, each of 50 µg, three or four times a day up to a maximum of 10 inhalations in 24 hours.

**Description**. A white to creamy-white, crystalline powder; odourless.

### Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *beclomethasone dipropionate RS* or with the reference spectrum of beclomethasone dipropionate.

B. Determine by the oxygen flask method (2.3.34), on 25 mg and use a mixture of 20 ml of *water* and 1 ml of *1 M sodium hydroxide* as the absorbing liquid. The liquid gives reaction A of chlorides (2.3.1).

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

### Tests

**Specific optical rotation** (2.4.22). +88.0° to +94.0°, determined in a 1.0 per cent w/v solution in *dioxan*.

**Light absorption**. Dissolve 50.0 mg in sufficient *ethanol (95 per cent)* to produce 100.0 ml and dilute 2.0 ml of this solution to 50.0 ml with the same solvent. Absorbance of the resulting solution at the maximum at about 238 nm, 0.57 to 0.60 (2.4.7).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh accurately about 70 mg of the substance under examination, dissolve in *methanol* and dilute to 50.0 ml with same solvent. To 4.0 ml of this solution add 4.0 ml of a 0.12 per cent w/v solution of *testosterone propionate RS* (internal standard).

*Reference solution*. Dissolve an accurately weighed quantity of *beclomethasone dipropionate RS* in *methanol* and dilute to obtain a solution having a known concentration of about 1.4 mg per ml. To 4.0 ml of this solution add 4.0 ml of a 0.12 per cent w/v solution of *testosterone propionate RS* (internal standard).

Chromatographic system
- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a mixture of 3 volumes of *acetonitrile* and 2 volumes of *water*, or such that the retention time of beclomethasone dipropionate is approximately 6 minutes and that of testsosterone propionate is approximately 10 minutes,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 3.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the percentage content of $C_{28}H_{37}ClO_7$.

**Storage**. Store protected from light.

## Beclomethasone Inhalation

Beclomethasone Dipropionate Inhalation;
Beclomethasone Inhalation Aerosol

Beclomethasone Inhalation is a suspension of Beclomethasone Dipropionate in a suitable liquid in a suitable pressurised container.

Beclomethasone Inhalation delivers not less than 80.0 per cent and not more than 120.0 per cent of the stated amount per inhalation of beclomethasone dipropionate, $C_{28}H_{37}ClO_7$, by actuation of the valve.

**Usual strength**. 50 µg per metered dose.

### Identification

A. Discharge the container a sufficient number of times at low relative humidity into a mortar to obtain about 2 mg of anhydrous Beclomethasone Dipropionate. Heat at 110° for 2 hours at a pressure of 2kPa, cool, grind the residue thoroughly with 0.1 g of potassium bromide, add a further 0.2 g of potassium bromide and mix thoroughly.

On the resultant dispersion determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *beclomethasone dipropionate RS* or with the reference spectrum of beclomethasone dipropionate.

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



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## Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 3 volumes of *methanol* and 97 volumes of *dichloroethane*.

*Test solution*. Discharge from the container into a small, dry flask a sufficient number of times to obtain 50 mg of Beclomethasone Dipropionate and dissolve the residue in 2 ml of *acetone*. Evaporate the solution to a volume such that the whole solution can be applied to the plate.

*Reference solution (a)*. A 0.1 per cent w/v solution of *beclomethasone dipropionate RS* in *acetone*.

*Reference solution (b)*. Dilute 5 ml of reference solution (a) to 10 ml with *acetone*.

*Reference solution (c)*. Dilute 5 ml of reference solution (a) to 20 ml with *acetone*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with *alkaline tetrazolium blue solution* and heat at 50° for 5 minutes. Cool and spray again with *alkaline tetrazolium blue solution*. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a), not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b) (1 per cent) and any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.5 per cent). Ignore any spot with an $R_f$ value of more than 0.85.

**Other tests**. Complies with the tests stated under Inhalation Preparations (Pressurised metered-dose Preparations).

Follow the procedure described under Assay wherever the amount of active substance is to be determined in any test.

**Assay**. Carry out the test for Content of active ingredient delivered per actuation stated under Inhalation Preparations (Pressurised metered-dose Preparations).

Use 40 ml of *dehydrated methanol* as the solvent. Discharge the number of deliveries that constitute the minimum recommended dose, keep the solution on a water-bath for 5 minutes to expel the propellants. Transfer the solution and washings to a flask containing sufficient *testosterone propionate RS* (internal standard) in *methanol* that, on dilution to a suitable volume with appropriate amounts of *water* and *methanol*, the final solution contains 0.00015 per cent w/v each of testosterone propionate and beclomethasone dipropionate in the methanol-water mixture in the proportions 70:30 by volume.

Determine by liquid chromatography (2.4.14).

*Test solution*. The diluted solution obtained as given above.

*Reference solution*. A solution containing 0.00015 per cent w/v each of the internal standard and *beclomethasone dipropionate RS* in the mobile phase.

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 50°
- mobile phase: a mixture of 70 volumes of *methanol* and 30 volumes of *water*, adjusted if necessary so that the resolution between the peaks due to beclomethasone dipropionate and the internal standard is not less than 2.0,
- flow rate. 2 ml per minute,
- spectrophotometer set at 239 nm,
- injection volume. 20 µl.

Inject the test solution and the reference solution. The test is not valid unless the resolution between the two principal peaks in the chromatogram obtained with the reference solution is at least 2.0.

Calculate the amount of $C_{28}H_{37}ClO_7$ delivered per actuation of the valve.

Determine the content of active ingredient a second and third time by repeating the procedure on the middle ten and on the last ten successive combined actuations of the valve. For each of the three determinations the average content of $C_{28}H_{37}ClO_7$ delivered per actuation of the valve meets the requirements.

**Storage**. Store protected from moisture at a temperature not exceeding 30°.

**Labelling**. The label states the amount of active ingredient delivered per inhalation.

# White Beeswax

White Beeswax is obtained by bleaching Yellow Beeswax.

**Category**. Pharmaceutical aid.

**Description**. Yellowish-white pieces or plates, translucent when thin, with a fine-grained, matt, non-crystalline fracture; becomes soft and pliable when warmed by hand. Odour, faint and characteristic and similar to that of yellow beeswax.

## Tests

**Melting range** (2.4.21). 61° to 65°, determined by Method IV.

**Acid value** (2.3.23). 5 to 15, determined by the following method. Weigh accurately about 5.0 g in a 250-ml conical flask fitted



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with a reflux condenser, add 40 ml of *xylene* and a few glass beads, heat until dissolved, add 20 ml of *ethanol (95 per cent)* and 0.5 ml of *phenolphthalein solution* and titrate the hot solution with *0.5 M ethanolic potassium hydroxide* until a red colour persists for at least 10 seconds ($n_1$ ml). Repeat the procedure omitting the substance under examination ($n_2$ ml). Calculate the Acid value from the expression $28.05(n_1 - n_2)/w$, where $w$ is the weight, in g, of the substance taken.

**Ester value** (2.3.26). 75 to 95, determined by subtracting the Acid value from the Saponification value.

**Ratio number**. The Ester value divided by the Acid value is between 5 and 19.

**Saponification value** (2.3.37). 87 to 104, determined by the following method. Weigh accurately about 2.0 g, add 30 ml of a mixture of equal volumes of *xylene* and *ethanol (95 per cent)* and a few glass beads, heat until dissolved, add 25.0 ml of *0.5 M ethanolic potassium hydroxide* and heat under a reflux condenser for 3 hours. Titrate the hot solution immediately with *0.5 M hydrochloric acid* using 1 ml of *phenolphthalein solution* as indicator, bringing the solution back to boil several times during the titration ($n_1$ ml). Repeat the procedure omitting the substance under examination ($n_2$ ml). Calculate the Saponification value from the expression $28.05(n_2 - n_1)/w$, where $w$ is the weight, in g, of the substance taken.

**Fats, fatty acids, Japan wax and resin**. Boil 5.0 g for 10 minutes with 80 ml of a 10 per cent w/v solution of *sodium hydroxide*, replace the water lost by evaporation, cool, filter the solution through a plug of glass wool and acidify with *hydrochloric acid*; no precipitate is produced.

**Ceresin, paraffin and other waxes**. To 3.0 g in a 100-ml round-bottomed flask add 30 ml of a 4 per cent w/v solution of *potassium hydroxide* in *aldehyde-free ethanol (95 per cent)* and boil gently under a reflux condenser for 2 hours. Remove the condenser and immediately insert a thermometer, place the flask in a water-bath at 80° and allow to cool with continuous swirling. The solution may be opalescent, but no precipitate is formed before the temperature reaches 65°.

**Glycerin and other polyhydric alcohols**. To 0.2 g add 10 ml of *ethanolic potassium hydroxide solution*, heat under a reflux condenser in a water-bath for 30 minutes, add 50 ml of *1 M sulphuric acid*, cool and filter. Rinse the flask and filter with *1 M sulphuric acid*, combine the filtrate and washings and dilute to 100 ml with *1 M sulphuric acid* (solution A). Into two matched test-tubes introduce, respectively, 1 ml of solution A and 1 ml of a 0.001 per cent w/v solution of *glycerin* in *1 M sulphuric acid* (solution B). Add 0.5 ml of a 1.07 per cent w/v solution of *sodium periodate* to each tube, mix, allow to stand for 5 minutes, add to each tube 1 ml of *decolorised fuchsin solution* and mix; any precipitate disappears. Place the tubes in a beaker containing water at 40° and observe for 10 to 15 minutes during cooling. Any bluish violet colour in the tube containing solution A is not more intense than that in the tube containing solution B (0.5 per cent w/w, calculated as glycerin).

## Yellow Beeswax

Yellow beeswax is the wax obtained by melting the walls of the honeycomb of the bee, *Apis mellifera* Linn. with hot water and removing the foreign matter.

**Category**. Pharmaceutical aid.

**Description**. Yellow or light brown pieces or plates, with a fine-grained, matt, non-crystalline fracture; becomes soft and pliable when warmed by hand. Odour, faint and characteristic. It is tasteless and does not stick to the teeth.

### Tests

**Melting range** (2.4.21). 61° to 65°, determined by Method IV.

**Acid value** (2.3.23). 5 to 15, determined by the following method. Weigh accurately about 5.0 g in a 250-ml conical flask fitted with a reflux condenser, add 40 ml of *xylene* and a few glass beads, heat until dissolved, add 20 ml of *ethanol (95 per cent)* and 0.5 ml of *phenolphthalein solution* and titrate the hot solution with *0.5 M ethanolic potassium hydroxide* until a red colour persists for at least 10 seconds ($n_1$ ml). Repeat the procedure omitting the substance under examination ($n_2$ ml). Calculate the Acid value from the expression $28.05(n_1 - n_2)/w$, where $w$ is the weight, in g, of the substance taken.

**Ester value** (2.3.26). 75 to 95, determined by subtracting the Acid value from the Saponification value.

**Ratio number**. The Ester value divided by the Acid value is between 5 and 19.

**Saponification value** (2.3.37). 87 to 104, determined by the following method. Weigh accurately about 2.0 g, add 30 ml of a mixture of equal volumes of *xylene* and *ethanol (95 per cent)* and a few glass beads, heat until dissolved, add 25.0 ml of *0.5 M ethanolic potassium hydroxide* and heat under a reflux condenser for 3 hours. Titrate the hot solution immediately with *0.5 M hydrochloric acid* using 1 ml of *phenolphthalein solution* as indicator, bringing the solution back to boil several times during the titration ($n_1$ ml). Repeat the procedure omitting the substance under examination ($n_2$ ml). Calculate the Saponification value from the expression $28.05(n_2 - n_1)/w$, where $w$ is the weight, in g, of the substance taken.

**Fats, fatty acids, Japan wax and resin**. Boil 5 g for 10 minutes with 80 ml of a 10 per cent w/v solution of *sodium hydroxide*, replace the water lost by evaporation, cool, filter the solution through a plug of glass wool and acidify with *hydrochloric acid*; no precipitate is produced.



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**Ceresin, paraffin and other waxes.** To 3.0 g in a 100-ml round-bottomed flask add 30 ml of a 4 per cent w/v solution of *potassium hydroxide* in *aldehyde-free ethanol (95 per cent)* and boil gently under a reflux condenser for 2 hours. Remove the condenser and immediately insert a thermometer, place the flask in a water-bath at 80° and allow to cool with continuous swirling. The solution may be opalescent, but no precipitate is formed before the temperature reaches 65°.

**Glycerin and other polyhydric alcohols.** To 0.2 g add 10 ml of *ethanolic potassium hydroxide solution*, heat under a reflux condenser in a water-bath for 30 minutes, add 50 ml of *1 M sulphuric acid*, cool and filter. Rinse the flask and filter with *1 M sulphuric acid*, combine the filtrate and washings and dilute to 100 ml with *1 M sulphuric acid* (solution A). Into two matched test-tubes introduce, respectively, 1 ml of solution A and 1 ml of a 0.001 per cent w/v solution of *glycerin* in *1 M sulphuric acid* (solution B). Add 0.5 ml of a 1.07 per cent w/v solution of *sodium periodate* to each tube, mix, allow to stand for 5 minutes, add to each tube 1 ml of *decolorised fuchsin solution* and mix; any precipitate disappears. Place the tubes in a beaker containing water at 40° and observe for 10 to 15 minutes during cooling. Any bluish violet colour in the tube containing solution A is not more intense than that in the tube containing solution B (0.5 per cent w/w, calculated as glycerin).

**Storage.** Store in well-closed containers.

## Bentonite

Bentonite is a natural, colloidal, hydrated aluminium silicate that has been processed to remove grit and non-swelling components of the ore.

**Category.** Pharmaceutical aid (suspending agent).

**Description.** A very fine, pale buff or cream-coloured to greyish-white powder, free or almost free from gritty particles.

### Identification

Fuse 1 g with 2 g of *anhydrous sodium carbonate*, warm the residue with 10 ml of *water*, filter, wash the filter with 5 ml of *water* and reserve the combined filtrate and washings. Dissolve the residue in 10 ml of *dilute hydrochloric acid*; the solution gives the reactions of aluminium salts, (2.3.1). Add to the reserved filtrate and washings 3 ml of *hydrochloric acid*; a gelatinous precipitate is produced.

### Tests

**pH** (2.4.24). 9.0 to 10.5, determined in a 2.0 per cent w/v suspension in *water*.

**Heavy metals** (2.3.13). To 5.0 g add 7.5 ml of *2 M hydrochloric acid* and 27.5 ml of *water*, boil for 5 minutes, centrifuge and filter the supernatant liquid. Wash the residue with *water*, filter, combine the filtrates and dilute to 50 ml with *water*. To 5 ml of the solution add 5 ml of *water*, 10 ml of *hydrochloric acid* and 25 ml of *4-methyl-2-pentanone*, shake for 2 minutes, allow the layers to separate and evaporate the aqueous layer to dryness on a water-bath. Dissolve the residue in 1 ml of *5 M acetic acid*, dilute to 25 ml and filter. The resulting solution complies with the limit test for heavy metals, Method D (50 ppm). Prepare the standard using *lead standard solution (1 ppm Pb)*.

**Sedimentation volume.** In a mortar, mix 6.0 g with 0.3 g of *light magnesium oxide*, freshly calcined. Mix the powder progressively with 200 ml of *water*. Shake for 1 hour and place 100 ml of the suspension in a 100-ml graduated cylinder. After 24 hours the volume of the clear supernatant liquid is not greater than 2 ml.

**Swelling power.** Add 2.0 g in twenty portions at intervals of 2 minutes to 100 ml of a 1 per cent w/v solution of *sodium lauryl sulphate* in a 100-ml graduated cylinder about 3 cm in diameter. Allow each portion to settle before adding the next and let it stand for 2 hours. The apparent volume of the sediment at the bottom of the cylinder is not less than 24 ml.

**Coarse particles.** To 20 g add 1000 ml of *water* and mix for 15 minutes at not less than 5000 rpm. Transfer to a wet sieve of nominal aperture of 75 mm, previously dried at 100° to 105° and weighed, and wash with three quantities, each of 500 ml, of *water*, ensuring that any agglomerates are dispersed. Dry at 100° to 105° and weigh. The weight of the matter on the sieve is not more than 0.1 g (0.5 per cent).

**Microbial contamination** (2.2.9). 1g is free from Escherichia coli.

**Loss on drying** (2.4.19). Not more than 15.0 per cent, determined on 1.0 g by drying in an oven at 105°.

## Benzalkonium Chloride

Benzalkonium Chloride is a mixture of alkylbenzyldimethylammonium chlorides, the alkyl groups having chain lengths of $C_8$ to $C_{18}$.

Benzalkonium Chloride contains not less than 95.0 per cent and not more than 104.0 per cent of alkylbenzyldimethylammonium chlorides, calculated as $C_{22}H_{40}ClN$ on the dried basis.

**Category.** Antiseptic.

**Description.** A white or yellowish-white powder or gelatinous, yellowish-white fragments, hygroscopic, soapy to the touch.

### Identification

A. Dilute 0.1 g with 10 ml of *water*. To 5 ml add 1.5 ml of *dilute nitric acid*; a white precipitate is produced which is soluble in



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*ethanol (95 per cent)*. To the remainder add 1.5 ml of *mercuric chloride solution*; a white precipitate is produced which is soluble in *ethanol (95 per cent)*.

B. Dissolve 0.25 g in 1 ml of *sulphuric acid*, add 0.1 g of *potassium nitrate*, heat on a water-bath for 5 minutes, cool, dilute with *water* to 10 ml, add 0.5 g of *zinc powder*, and heat on a water-bath for 5 minutes. To 2 ml of the clear supernatant liquid add 0.5 ml of *sodium nitrite solution*, cool in ice and add to 3 ml of *2-naphthol solution*; an orange red colour is produced.

C. To 25 mg add 1 ml of *2 M nitric acid*; a white precipitate is produced which dissolves on addition of 5 ml of *ethanol (95 per cent)*. The resulting solution gives reaction A of chlorides (2.3.1).

### Tests

**Acidity or alkalinity**. Dissolve 0.5 g in 50 ml of *carbon dioxide-free water*, add 0.1 ml of *bromocresol purple solution* and titrate with *0.1 M hydrochloric acid* or with *0.1 M sodium hydroxide*. Not more than 0.1 ml is required to change the colour of the solution.

**Ammonia compounds**. Boil 0.1 g with 3 ml of *sodium hydroxide solution*; no odour of ammonia is produced.

**Foreign amines**. Dissolve 0.1 g in 5 ml of *water* and add 3 ml of *1 M sodium hydroxide*; no precipitate is formed. Heat to boiling; the odour of amines is not perceptible.

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Water** (2.3.43). Not more than 10 per cent, determined on 0.3 g.

**Assay**. Weigh accurately about 2.0 g, dissolve in sufficient *water* to produce 100.0 ml. Transfer 25.0 ml to a separating funnel, add 25 ml of *chloroform*, 10 ml of *0.1 M sodium hydroxide* and 10.0 ml of a freshly prepared 5 per cent w/v solution of *potassium iodide*. Shake well, allow to separate and discard the chloroform layer. Shake the aqueous solution with three further quantities, each of 10 ml, of *chloroform* and discard the chloroform layer. Add 40 ml of *hydrochloric acid*, cool and titrate with *0.05 M potassium iodate* until the solution becomes pale brown in colour. Add 2 ml of *chloroform* and continue the titration until the chloroform becomes colourless. Titrate a mixture of 20 ml of *water*, 10.0 ml of a freshly prepared 5 per cent w/v solution of potassium iodide and 40 ml of *hydrochloric acid* with *0.05 M potassium iodate* in a similar manner; the difference between the titrations represents the amount of *0.05 M potassium iodate* required.

1 ml of *0.05 M potassium iodate* is equivalent to 0.0354 g of $C_{22}H_{40}ClN$.

**Storage**. Avoid contact with metals.

## Benzalkonium Chloride Solution

Benzalkonium Chloride Solution is a solution of a mixture of alkylbenzyldimethylammonium chlorides, the alkyl groups having chain lengths of $C_8$ to $C_{18}$. It may contain *ethanol (95 per cent)*. In making Benzalkonium Chloride Solution, the *ethanol (95 per cent)* may be replaced by Industrial Methylated Spirit, diluted so as to be of equivalent strength.

Benzalkonium Chloride Solution contains not less than 49.0 per cent w/v and not more than 51.0 per cent w/v of alkylbenzyldimethylammonium chlorides, calculated as $C_{22}H_{40}ClN$. It may contain not more than 16.0 per cent v/v of ethanol, $C_2H_6O$.

**Category**. Antiseptic detergent.

**Description**. A clear, colourless or slightly yellow, syrupy liquid; odour, aromatic.

### Identification

A. Dilute 0.2 ml with 10 ml of *water*. To 5 ml add 1.5 ml of *dilute nitric acid*; a white precipitate is produced which is soluble in *ethanol (95 per cent)*. To the remainder add 1.5 ml of *mercuric chloride solution*; a white precipitate is produced which is soluble in *ethanol (95 per cent)*.

B. Evaporate 0.5 ml to dryness on a water-bath, dissolve the residue in 1 ml of *sulphuric acid*, add 0.1 g of *potassium nitrate*, heat on a water-bath for 5 minutes, cool, dilute with *water* to 10 ml, add 0.5 g of *zinc powder*, and heat on a water-bath for 5 minutes. To 2 ml of the clear supernatant liquid add 0.5 ml of *sodium nitrite solution*, cool in ice and add to 3 ml of *2-naphthol solution*; an orange red colour is produced.

C. To 0.05 ml add 1 ml of *2 M nitric acid*; a white precipitate is produced which dissolves on addition of 5 ml of *ethanol (95 per cent)*. The resulting solution gives reaction A of chlorides (2.3.1).

### Tests

**Acidity or alkalinity**. Dissolve 1.0 g in 50 ml of *carbon dioxide-free water*, add 0.1 ml of *bromocresol purple solution* and titrate with *0.1 M hydrochloric acid* or with *0.1 M sodium hydroxide*. Not more than 0.1 ml is required to change the colour of the solution.

**Ammonia compounds**. Boil 0.2 ml with 3 ml of *sodium hydroxide solution*; no odour of ammonia is produced.

**Foreign amines**. To a volume containing 0.1 g of benzalkonium chloride add sufficient *water* to produce 5 ml and add 3 ml of *1 M sodium hydroxide*; no precipitate is formed. Heat to boiling; the odour of amines is not perceptible.

**Ethanol** *(if present)* (2.3.45). Not more than 16.0 per cent v/v, determined by Method I or II, as applicable.



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**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Assay**. Weigh accurately about 4.0 g, dissolve in sufficient *water* to produce 100.0 ml. Transfer 25.0 ml to a separating funnel, add 25 ml of *chloroform,* 10 ml of *0.1 M sodium hydroxide* and 10.0 ml of a freshly prepared 5 per cent w/v solution of *potassium iodide*. Shake well, allow to separate and discard the chloroform layer. Shake the aqueous solution with three further quantities, each of 10 ml, of *chloroform* and discard the chloroform layer. Add 40 ml of *hydrochloric acid,* cool and titrate with *0.05 M potassium iodate* until the solution becomes pale brown in colour. Add 2 ml of *chloroform* and continue the titration until the chloroform becomes colourless. Titrate a mixture of 20 ml of *water*, 10.0 ml of a freshly prepared 5 per cent w/v solution of potassium iodide and 40 ml of *hydrochloric acid* with *0.05 M potassium iodate* in a similar manner; the difference between the titrations represents the amount of *0.05 M potassium iodate* required.

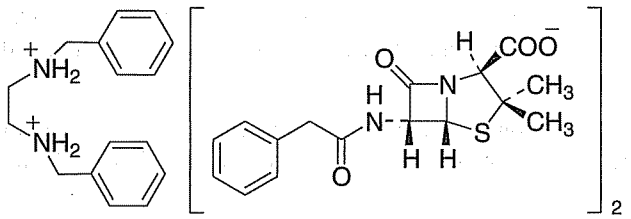
1 ml of *0.05 M potassium iodate* is equivalent to 0.0354 g of $C_{22}H_{40}ClN$. Determine the relative density (2.4.29), and calculate the amount of $C_{22}H_{40}ClN$, weight in volume.

**Storage**. Avoid contact with metals.

**Labelling**. The label states, where appropriate, the content of *ethanol (95 per cent)* or Industrial Methylated Spirit.

## Benzathine Penicillin

Benzathine Benzylpenicillin; Benzathine Penicillin G



$C_{16}H_{20}N_2,(C_{16}H_{18}N_2O_4S)_2$ Mol. Wt. 909.0

Benzathine Penicillin is *N,N'*-dibenzylethylenediammonium bis[(6*R*)-6-(2 phenylacetamido)penicillanate] containing a variable amount of water.

Benzathine Penicillin contains not less than 96.0 per cent and not more than 100.5 per cent of $C_{16}H_{20}N_2,(C_{16}H_{18}N_2O_4S)_2$ and not less than 24.0 per cent and not more than 27.0 per cent of $C_{16}H_{20}N_2$, both calculated on the anhydrous basis.

**Category**. Antibacterial.

**Dose**. Orally, 225 mg (300,000 Units) to 450 mg (600,000 Units) every 6 hours; by intramuscular injection, 225 mg (300,000 Units) to 750 mg (1,000,000 Units). Prophylactic, by intramuscular injection, 900 mg (1,200,000 Units) every 2 or 3 weeks.

[900 mg of Benzathine Penicillin is approximately equivalent to 720 mg of benzylpenicillin (1,200,000 Units of penicillin)].

**Description**. A white, crystalline powder; almost odourless.

### Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *benzathine penicillin RS*.

B. Shake 0.1 g with 1 ml of *1 M sodium hydroxide* for 2 minutes, add 2 ml of *ether*, shake for 1 minute and allow to separate. Evaporate 1 ml of the ether layer to dryness, dissolve the residue in 2 ml of *glacial acetic acid* and add 1 ml of *potassium dichromate solution*; a golden yellow precipitate is formed.

C. Shake 0.1 g with 2 ml of *1 M sodium hydroxide* for 2 minutes, extract the mixture with two quantities, each of 3 ml, of *ether;* evaporate the combined extracts and dissolve the residue in 1 ml of *ethanol (50 per cent)*. Add 5 ml of *picric acid solution*, heat at 90° for 5 minutes and allow to cool slowly; the precipitate, after recrystallisation from *ethanol (25 per cent)* containing a small quantity of *picric acid*, melts at about 214° (2.4.21).

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

### Tests

**pH** (2.4.24). 5.0 to 7.5, determined in a saturated solution.

**Related substances**. Determine by liquid chromatography (2.4.14).

Prepare the solutions immediately before use. Avoid any overheating during the preparation of the solutions.

*Test solution*. Dissolve an accurately weighed quantity of about 70 mg of the substance under examination in 25 ml of *methanol* with the aid of ultrasound (for about 2 minutes). Dilute to 50.0 ml with a solution containing 6.8 g per litre of *potassium dihydrogen phosphate* and 1.02 g per litre of *disodium hydrogen phosphate*.

*Reference solution (a)*. Dissolve an accurately weighed quantity of about 70 mg of *benzathine penicillin RS* in 25 ml of *methanol* with the aid of ultrasound (for about 2 minutes). Dilute to 50.0 ml with a solution containing 6.8 g per litre of *potassium dihydrogen phosphate* and 1.02 g per litre of *disodium hydrogen phosphate*.



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*Reference solution (b).* Dilute 1 ml of reference solution (a) to 100 ml with mobile phase A.

Chromatographic system

- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 40°,
- mobile phase: A. a mixture of 10 volumes of a 34 g per litre solution of *potassium dihydrogen phosphate* adjusted to pH 3.5 with *phosphoric acid*, 30 volumes of *methanol* and 60 volumes of *water*,

  B. a mixture of 10 volumes of a 34 g per litre solution of *potassium dihydrogen phosphate* adjusted to pH 3.5 with *phosphoric acid*, 30 volumes of *water* and 60 volumes of *methanol*,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 220 nm,
- injection volume. 20 µl.

| Time (in min) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0 – 10 | 75 | 25 |
| 10 – 20 | 75 → 0 | 25 →100 |
| 20 – 55 | 0 | 100 |
| 55 – 70 | 75 | 25 |

Inject reference solution (a). The relative retention time with reference to benzylpenicillin: benzathine = 0.3 to 0.4; benzylpenicilloic acids benzathide = about 2.4. If necessary, adjust the concentration of *methanol* in the mobile phase.

Inject the test solution and reference solution (b). The area of any secondary peak obtained with the test solution corresponding to benzylpenicilloic acid benzathide is not more than twice the sum of the areas of the two principal peaks in the chromatogram obtained with reference solution (b) (2 per cent).The area any other secondary peak obtained with the test solution is not more than the sum of the areas of the two principal peaks in the chromatogram obtained with reference solution (b) (1 per cent). Disregard any peak with an area 0.05 times the sum of the areas of the two principal peaks in the chromatogram obtained with reference solution (b) (0.05 per cent).

**Water** (2.3.43). 5.0 to 8.0 per cent, determined on 0.3 g.

**Assay**. Determine by liquid chromatography, (2.4.14) as given under the test for Related substances using the following mobile phase.

*Mobile phase.* a mixture of 10 volumes of *phosphate buffer solution pH 3.5,* 35 volumes of *methanol,* and 55 volumes of *water.*

Inject alternately the test solution and reference solution (a). Calculate the percentage contents of $C_{16}H_{20}N_2$ and of $C_{16}H_{20}N_2,(C_{16}H_{18}N_2O_4S)_2$. Calculate the content of $C_{16}H_{20}N_2,(C_{16}H_{18}N_2O_4S)_2$ by multiplying the percentage content of benzylpenicillin by 1.36.

*Benzathine Penicillin intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.*

**Bacterial endotoxins** (2.2.3). Not more than 0.13 Endotoxin Unit per ml of a solution prepared in the following manner. Suspend 20 mg of the substance under examination in 20 ml of *0.1 M sodium hydroxide*, dilute 1 ml to 100 ml and use the supernatant liquid.

**Sterility** (2.2.11). Complies with the test for sterility.

**Storage**. Store protected from moisture at a temperature not exceeding 30°. If the material is intended for use in the manufacture of parenteral preparations the container should be sterile and sealed so as to exclude micro-organisms.

**Labelling** The label states whether or not the contents are intended for use in the manufacture of parenteral preparations.

## Benzathine Penicillin Injection

Benzathine Benzylpenicillin Injection; Benzathine Penicillin G Injection

Benzathine Penicillin Injection is a sterile material consisting of Benzathine Penicillin with or without suspending agents, buffering agents and other excipients. It is filled in a sealed container.

The injection is constituted by suspending the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

**Usual strength**. 450 mg (600,000 Units). (Each mg of Benzathine Penicillin is approximately equivalent to 1,330 Units of penicillin).

**Storage**. The constituted suspension should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Benzathine Penicillin Injection contains not less than 95.0 per cent and not more than 110.0 per cent of the stated amount of benzathine penicillin, $C_{16}H_{20}N_2$, $(C_{16}H_{18}N_2O_4S)_2$.

**Description**. A white crystalline powder, almost odourless.

*The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.*



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## Identification

A. Shake 0.1 g with 1 ml of *1 M sodium hydroxide* for 2 minutes, add 2 ml of *ether*, shake for 1 minute and allow to separate. Evaporate 1 ml of the ether layer to dryness, dissolve the residue in 2 ml of *glacial acetic acid* and add 1 ml of *potassium dichromate solution*; a golden yellow precipitate is formed.

B. Shake 0.1 g with 2 ml of *1 M sodium hydroxide* for 2 minutes, extract the mixture with two quantities, each of 3 ml, of *ether*, evaporate the combined extracts and dissolve the residue in 1 ml of *ethanol (50 per cent)*. Add 5 ml of *picric acid solution*, heat at 90° for 5 minutes and allow to cool slowly; the precipitate, after recrystallisation from *ethanol (25 per cent)* containing a small quantity of *picric acid*, melts at about 214° (2.4.21).

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

## Tests

**pH** (2.4.24). 5.0 to 7.5, determined in a suspension obtained by reconstituting as directed on the label.

**Consistency**. To a quantity containing 60,000 Units add 2 ml of *water* and shake thoroughly. The resulting suspension passes through a 23G hypodermic needle.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Prepare the solutions immediately before use. Avoid any overheating during the preparation of the solutions.*

*Test solution*. Dissolve an accurately weighed quantity containing about 70 mg of Benzathine Penicillin in 25 ml of *methanol* with the aid of ultrasound (for about 2 minutes). Dilute to 50.0 ml with a solution containing 6.8 g per litre of *potassium dihydrogen phosphate* and 1.02 g per litre of *disodium hydrogen phosphate*.

*Reference solution (a)*. Dissolve an accurately weighed quantity of about 70 mg of *benzathine penicillin RS* in 25 ml of *methanol* with the aid of ultrasound (for about 2 minutes). Dilute to 50.0 ml with a solution containing 6.8 g per litre of *potassium dihydrogen phosphate* and 1.02 g per litre of *disodium hydrogen phosphate*.

*Reference solution (b)*.Dilute 1 ml of reference solution (a) to 100 ml with mobile phase A.

Chromatographic system

- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 40°,
- mobile phase: A. a mixture of 10 volumes of a 34 g per litre solution of *potassium dihydrogen phosphate* adjusted to pH 3.5 with *phosphoric acid*, 30 volumes of *methanol* and 60 volumes of *water*,

  B. a mixture of 10 volumes of a 34 g per litre solution of *potassium dihydrogen phosphate* adjusted to pH 3.5 with *phosphoric acid*, 30 volumes of *water* and 60 volumes of *methanol*,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 220 nm,
- injection volume. 20 µl.

| Time (in min) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0 – 10 | 75 | 25 |
| 10 – 20 | 75 → 0 | 25 →100 |
| 20 – 55 | 0 | 100 |
| 55 – 70 | 75 | 25 |

Inject reference solution (a). The relative retention time with reference to benzylpenicillin for benzathine is about 0.3 to 0.4; for benzylpenicilloic acids benzathide is about 2.4.

Inject the test solution and reference solution (b). The area of any secondary peak obtained with the test solution corresponding to benzylpenicilloic acids benzathide is not more than twice the sum of the areas of the two principal peaks in the chromatogram obtained with reference solution (b) (2.0 per cent).The area of any other secondary peak obtained with the test solution is not more than the sum of the areas of the two principal peaks in the chromatogram obtained with reference solution (b) (1 per cent). Disregard any peak with an area 0.05 times the sum of the areas of the two principal peaks in the chromatogram obtained with reference solution (b) (0.05 per cent).

**Bacterial endotoxins** (2.2.3) Not more than 0.13 Endotoxin Unit per ml of a solution prepared by suspending 20 mg of the substance under examination in 20 ml of *0.1 M sodium hydroxide*, diluting 1 ml to 100 ml and using the supernatant.

**Water** (2.3.43). 5.0 to 8.0 per cent, determined on 0.3 g.

**Assay**. Determine by liquid chromatography, (2.4.14) as given under the test for Related substances using the following mobile phase.

*Mobile phase.* a mixture of 10 volumes of *phosphate buffer solution pH 3.5*, 35 volumes of *methanol*, and 55 volumes of *water*.

Inject alternately the test solution and reference solution (a).

Calculate the content of $C_{16}H_{20}N_2$,$(C_{16}H_{18}N_2O_4S)_2$ by multiplying the percentage content of benzylpenicillin by 1.36.

**Labelling**. The label states (1) the directions for constituting the suspension; (2) the names of any added buffering agents or other pharmaceutical aids; (3) that the preparation is meant for intramuscular injection only.



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# Fortified Benzathine Penicillin Injection

Fortified Benzathine Benzylpenicillin Injection;
Fortified Benzathine Penicillin G Injection

Benzathine Penicillin Injection is a sterile material consisting of Benzathine Penicillin and Procaine Penicillin with or without suspending agents, buffering agents and other excipients. It is filled in a sealed container.

The injection is constituted by suspending the contents of the sealed container in the requisite amount of sterile Water for Injections containing Benzylpenicillin Sodium immediately before use.

**Storage**. The constituted suspension should be used immediately after preparation but, in any case, within the period and under the conditions recommended by the manufacturer.

Fortified Benzathine Penicillin Injection contains not less than 90.0 per cent and not more than 125.0 per cent of the stated amount of benzathine penicillin, not less than 95.0 per cent and not more than 125.0 per cent of the stated amount of procaine penicillin, not less than 90.0 per cent and not more than 130.0 per cent of the stated amount of benzylpenicillin sodium, all in terms of Units of penicillin.

**Category**. Antibacterial.

**Usual strength**. Benzathine Penicillin, 450 mg (600,000 Units), Procaine Penicillin, 300 mg (300,000 Units) and Benzylpenicillin, 180 mg (300,000 Units).

*The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.*

## Identification

A. Give the reaction for penicillins (2.3.1).

B. Give reaction B of penicillins and cephalosporins (2.3.1).

C. Shake 0.1 g with 1 ml of *1 M sodium hydroxide* for 2 minutes, add 2 ml of *ether*, shake for 1 minute and allow to separate. Evaporate 1 ml of the ether layer to dryness, dissolve the residue in 2 ml of *glacial acetic acid* and add 1 ml of *potassium dichromate solution*; a golden yellow precipitate is formed.

D. Give the reactions of sodium salts (2.3.1).

## Tests

**Stability**. Using an aseptic technique prepare the suspension as directed on the label in an individual unopened container and determine the concentration of benzylpenicillin sodium by the method described below using an accurately measured quantity of the suspension, withdrawn aseptically from the container. Store the remainder of the suspension in the closed container at 4° for 7 days and then repeat the determination of benzylpenicillin sodium.

The concentration of benzylpenicillin sodium in the stored injection is not less than 80 per cent of the concentration found in the freshly prepared suspension.

**Consistency**. To a quantity containing 600,000 Units of Benzathine Penicillin, 300,000 Units each of Procaine Penicillin and Benzylpenicillin add 2 ml of *water* and shake thoroughly. The resulting suspension passes readily through a 22G hypodermic needle.

**Bacterial endotoxins** (2.2.3). Not more than 0.13 Endotoxin Unit per ml of a solution prepared by suspending 20 mg of the substance under examination in 20 ml of *0.1 M sodium hydroxide*, diluting 1 ml to 100 ml and using the supernatant.

**Water** (2.3.43). Not more than 7.5 per cent, determined on 0.3 g.

**Assay**. *For benzathine penicillin* — Shake a quantity of the mixed contents of 10 containers containing 1 g of Benzathine Penicillin with 30 ml of a saturated solution of *sodium chloride* and 10 ml of *5 M sodium hydroxide* and extract with four successive quantities, each of 50 ml, of *ether*. Wash the combined ether extracts with three successive quantities, each of 5 ml, of *water*, extracting each aqueous washing with the same 25 ml of *ether*. Combine the ether extracts, evaporate to a low bulk, add 2 ml of *ethanol* and evaporate to dryness. Dissolve the residue in 50 ml of *glacial acetic acid* and titrate with *0.1 M perchloric acid* using 1 ml of *1-naphtholbenzein solution* as indicator.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04545 g of $C_{18}H_{50}N_6O_8S_2$. Calculate the apparent content of Benzathine Penicillin.

Calculate the content of procaine penicillin, as determined by the method given below in the weight of the sample used in this assay, multiply this content by a factor of 1.544 and deduct the figure from the apparent content of benzathine penicillin; the result is the content of benzathine penicillin. (1 mg of benzathine penicillin is approximately equivalent to 1330 Units of penicillin).

*For procaine penicillin* — To a quantity of the mixed contents of 10 containers containing 0.25 g of Procaine Penicillin add 100 ml of *water*, shake well, dilute to 200.0 ml with *water*, mix and filter. Dilute 5.0 ml of the filtrate to 250.0 ml with *buffer solution pH 7.0* and measure the absorbance of the resulting solution at the maximum at about 290 nm, using *buffer solution pH 7.0* as the blank (2.4.7). Calculate the content of procaine penicillin taking 310 as the specific absorbance at 290 nm. (1 mg of procaine penicillin is equivalent to 1009 Units of penicillin).



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*For benzylpenicillin sodium* — Shake a quantity of the mixed contents of 10 containers containing 0.15 g of Benzylpenicillin Sodium with water until dissolved and dilute to 500.0 ml with *water*. Dilute 25.0 ml of the resulting solution to 100.0 ml with *phosphate buffer pH 6.8*. Place two quantities, each of 2.0 ml, of the resulting solution in separate stoppered tubes. To one tube add 10.0 ml of *imidazole-mercury reagent*, mix, stopper the tube and immerse in a water-bath at 60° for 35 minutes, swirling occasionally. Remove from the water-bath and cool rapidly to 20° (solution A). Add 10.0 ml of *imidazole solution* to the second tube, mix, stopper the tube and allow to stand at 20° for 35 minutes, swirling occasionally (solution B). Without delay measure the absorbance of solutions A and B at about 325 nm (2.4.7), using as the blank a mixture of 2.0 ml of *water* and 10.0 ml of *imidazole-mercury reagent* for solution A and a mixture of 2.0 ml of *water* and 10.0 ml of *imidazole solution* for solution B. Calculate the content of total penicillins as $C_{16}H_{17}N_2NaO_4S$ from the difference between the absorbances of solutions A and B, from the difference obtained by repeating the procedure using 0.15 g of *benzylpenicillin sodium RS* in place of the contents of the sealed containers. Calculate the content of benzylpenicillin sodium by subtracting the contents of benzathine penicillin and procaine penicillin, both expressed as benzylpenicillin sodium, $C_{16}H_{17}N_2NaO_4S$.

**Labelling**. The label on the sealed container states (1) the quantity of Benzathine Penicillin, Benzylpenicillin Sodium and Procaine Penicillin contained in it; (2) the directions for reconstituting the suspension; (3) the names of the added suspending agent, buffering agent and any other pharmaceutical aid; (4) that the preparation is intended for intramuscular injection only.

# Benzathine Penicillin Tablets

Benzathine Benzylpenicillin Tablets; Benzathine Penicillin G Tablets

Benzathine Penicillin Tablets contain Benzathine Penicillin equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the stated number of Units of penicillin.

**Usual strength**. 200,000 Units of penicillin.

## Identification

A. Shake 0.1 g with 1 ml of *1 M sodium hydroxide* for 2 minutes, add 2 ml of *ether*, shake for 1 minute and allow to separate. Evaporate 1 ml of the ether layer to dryness, dissolve the residue in 2 ml of *glacial acetic acid* and add 1 ml of *potassium dichromate solution*; a golden yellow precipitate is formed.

B. Shake 0.1 g with 2 ml of *1 M sodium hydroxide* for 2 minutes, extract the mixture with two quantities, each of 3 ml, of *ether*, evaporate the combined extracts and dissolve the residue in 1 ml of *ethanol (50 per cent)*. Add 5 ml of *picric acid solution*, heat at 90° for 5 minutes and allow to cool slowly; the precipitate, after recrystallisation from *ethanol (25 per cent)* containing a small quantity of *picric acid*, melts at about 214° (2.4.21).

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

## Tests

**Water** (2.3.43). Not more than 8.0 per cent, determined on the powdered tablets.

**Other tests**. Comply with the tests stated under Tablets.

**Related substances**. Determine by liquid chromatography (2.4.14).

Prepare the solutions immediately before use. Avoid any overheating during the preparation of the solutions.

*Test solution*. Weigh and powder 20 tablets. Dissolve an accurately weighed quantity containing about 70 mg of Benzathine Penicillin in 25 ml of *methanol* with the aid of ultrasound for 2 minutes and allow to stand for 15 minutes. Dilute to 50.0 ml with a solution containing 6.8 g per litre of *potassium dihydrogen phosphate* and 1.02 g per litre of *disodium hydrogen phosphate* mix and filter.

*Reference solution (a)*. Dissolve an accurately weighed quantity of about 70 mg of *benzathine penicillin RS* in 25 ml of *methanol* with the aid of ultrasound (for about 2 minutes). Dilute to 50.0 ml with a solution containing 6.8 g per litre of *potassium dihydrogen phosphate* and 1.02 g per litre of *disodium hydrogen phosphate*.

*Reference solution (b)*.Dilute 1 ml of reference solution (a) to 100 ml with mobile phase A.

Chromatographic system

- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded ot porous silica (5 µm),
- column temperature. 40°,
- mobile phase: A. a mixture of 10 volumes of a 34 g per litre solution of *potassium dihydrogen phosphate* adjusted to pH 3.5 with *phosphoric acid*, 30 volumes of methanol and 60 volumes of *water*,
  B. a mixture of 10 volumes of a 34 g per litre solution of *potassium dihydrogen phosphate* adjusted to pH 3.5 with *phosphoric acid*, 30 volumes of *water* and 60 volumes of *methanol*,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 220 nm,
- injection volume. 20 µl.



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| Time (min) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0 – 10 | 75 | 25 |
| 10 – 20 | 75 → 0 | 25 → 100 |
| 20 – 55 | 0 | 100 |
| 55 – 70 | 75 | 25 |

Inject reference solution (a). Relative retention time with reference to benzylpenicillin: benzathine = 0.3 to 0.4; benzylpenicilloic acids benzathide = about 2.4. If necessary, adjust the concentration of *methanol* in the mobile phase.

Inject the test solution and reference solution (b). The area of any secondary peak obtained with the test solution corresponding to benzylpenicilloic acids benzathide is not more than twice the sum of the areas of the two principal peaks in the chromatogram obtained with reference solution (b) (2 per cent). The area any other secondary peak obtained with the test solution is not more than the sum of the areas of the two principal peaks in the chromatogram obtained with reference solution (b) (1 per cent). Disregard any peak with an area 0.05 times the sum of the areas of the two principal peaks in the chromatogram obtained with reference solution (b) (0.05 per cent).

**Assay**. Determine by liquid chromatography, (2.4.14) as given under the test for Related substances using the following mobile phase.

*Mobile phase:* a mixture of 10 volumes of *phosphate buffer pH 3.5,* 35 volumes of *methanol,* and 55 volumes of *water.*

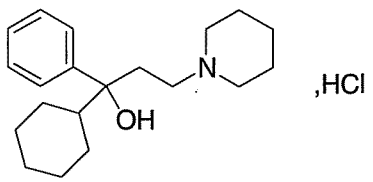
Inject alternately the test solution and reference solution (a).

Calculate the percentage content of $C_{16}H_{20}N_2,(C_{16}H_{18}N_2O_4S)_2$ by multiplying the percentage content of benzylpenicillin by 1.36.

**Storage**. Store at a temperature not exceeding 30°.

# Benzhexol Hydrochloride

Trihexyphenidyl Hydrochloride




$C_{20}H_{31}NO,HCl$ Mol. Wt. 337.9

Benzhexol Hydrochloride is (*RS*)-1-cyclohexyl-1-phenyl-3-piperidinopropan-1-ol hydrochloride.

Benzhexol Hydrochloride contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{20}H_{31}NO,HCl$, calculated on the dried basis.

**Category**. Antiparkinsonian.

**Dose**. 1 mg, gradually increased to a usual maintenance dose of 5 to 15 mg daily in 3 to 4 divided doses.

**Description**. A white or creamy-white, crystalline powder; odourless or almost odourless.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *benzhexol hydrochloride RS* or with the reference spectrum of benzhexol hydrochloride.

B. Dissolve 0.5 g in 5 ml of warm *methanol* and make just alkaline to *litmus paper* with *5 M sodium hydroxide*; a precipitate is produced, which, after recrystallisation from *methanol* melts at about 114° (2.4.21).

C. Gives the reactions of chlorides (2.3.1).

## Tests

**pH** (2.4.24). 5.2 to 6.2, determined in a solution prepared by dissolving 1.0 g in 50 ml of *carbon dioxide-free water* with the aid of heat, cooling and diluting to 100.0 ml with the same solvent.

**Optical rotation** (2.4.22). -0.10° to +0.10°, determine in a 5.0 per cent w/v solution in a mixture of 20 volumes of *methanol* and 80 volumes of *dichloromethane.*

**Related substances.** Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve 20 mg of the substance under examination in 10.0 ml of the mobile phase.

*Reference solution (a).* Dilute 1.0 ml of the test solution to 200.0 ml with the mobile phase. Dilute 10.0 ml of the solution to 50.0 ml with the mobile phase.

*Reference solution (b).* Dilute 10 mg of *1-phenyl-3-(piperidin-1-yl)propan-1-one RS (trihexyphenidyl impurity A RS)* in 10.0 ml of the mobile phase.

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

*Reference solution (d).* Add 1.0 ml of the test solution to 1.0 ml of the reference solution (b) and dilute to 100.0 ml with the mobile phase.

Chromatographic system

– a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),



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- mobile phase: a mixture of 20 volumes of *water* add 0.2 ml of *triethylamine,* adjust the pH to 4.0 with *orthophosphoric acid* and 80 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

Inject reference solution (d). The test is not valid unless the resolution between the peaks due to trihexyphenidyl and trihexyphenidyl impurity A not less than 4.0.

Inject the test solution and reference solution (a) and (c). Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of secondary peak corresponding to trihexyphenidyl impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (a) (0.1 per cent) and the sum of the areas of all secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.7 g and dissolve in 50 ml of *anhydrous glacial acetic acid* previously neutralised using *1-naphtholbenzein solution* as indicator, warming and cooling, if necessary. Add 15 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid* to the full colour change of the indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03379 g of $C_{20}H_{31}NO,HCl$.

# Benzhexol Tablets

Benzhexol HydrochlorideTablets; Trihexyphenidyl Hydrochloride Tablets

Benzhexol Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of benzhexol hydrochloride, $C_{20}H_{31}NO,HCl$.

**Usual strengths**. 2 mg; 5 mg.

## Identification

A. Shake a quantity of the powdered tablets with 20 ml of *water* and filter. The filtrate yields a yellow precipitate with *trinitrophenol solution* and a white precipitate with *5 M sodium hydroxide*.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 90 volumes of *chloroform* and 10 volumes of *methanol*.

*Test solution*. Shake a quantity of the powdered tablets with sufficient *chloroform* to produce a solution containing 0.2 per cent w/v of Benzhexol Hydrochloride and filter.

*Reference solution*. A 0.2 per cent w/v solution of *benzhexol hydrochloride RS* in *chloroform*.

Apply to the plate 10 µl of each solution. After development remove the plate, allow it to dry in air and spray with *dilute potassium iodobismuthate solution*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

**Uniformity of content**. Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14).

*Test solution*. Disperse well one tablet in 5.0 ml of *water* in an ultrasonic bath, add 10 ml of *methanol,* shake for 15 minutes, dilute to 25.0 ml with *methanol*, mix and filter through a filter with a maximum pore size of 0.2 µm.

*Reference solution*. A solution containing 0.008 per cent w/v of *benzhexol hydrochloride RS* and 0.004 per cent w/v of *3-piperidylpropiophenone hydrochloride RS* in the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- mobile phase: 800 volumes of *acetonitrile*, 200 volumes of *water* and 0.2 volume of *triethylamine*, the pH of the mixture being adjusted to 4.0 with *phosphoric acid*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the resolution factor between the two principal peaks in the chromatogram obtained with the reference solution is greater than 4.0.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_{20}H_{31}NO,HCl$ in the tablet.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Determine by liquid chromatography (2.4.14), using the following solutions.

*Test Solution*. Disperse well a quantity of the powdered tablets containing about 5 mg of Benzhexol Hydrochloride in 5.0 ml of



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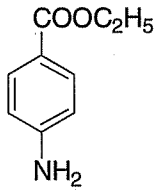
*water* in an ultrasonic bath, add 10 ml of *methanol,* shake for 30 minutes, dilute to 25.0 ml with *methanol* mix and filter through a filter with a maximum pore size of 0.2 µm.

*Reference solution.* A solution containing 0.02 per cent w/v of *benzhexol hydrochloride RS* and 0.01 per cent w/v of *3-piperidylpropiophenone hydrochloride RS* in the mobile phase.

Carry out the chromatographic procedure described under Uniformity of content.

Calculate the content of $C_{20}H_{31}NO,HCl$ in the tablets.

# Benzocaine




$C_9H_{11}NO_2$ Mol. Wt. 165.2

Benzocaine is ethyl 4-aminobenzoate.

Benzocaine contains not less than 99.0 per cent and not more than 101.0 per cent of $C_9H_{11}NO_2$, calculated on the dried basis.

**Category.** Local anaesthetic.

**Description.** Colourless crystals or a white, crystalline powder; odourless.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

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

B. Dissolve 10 mg in 1 ml of *water* with the aid of one drop of *dilute hydrochloric acid* and add 2 drops of a 10 per cent w/v solution of *sodium nitrite* and 2 drops of a solution of 10 mg of *2-naphthol* in 5 ml of *sodium hydroxide solution*; a deep red colour is produced. On setting aside the solution for some time, a scarlet precipitate is produced.

C. Dissolve 0.2 g in 10 ml of *water* with the aid of *dilute hydrochloric acid* (solution A) and divide into 2 parts. To one part of solution A add *iodine solution*; a precipitate is obtained (distinction from orthocaine).

D. To the other part of solution A add *potassium mercuri-iodide solution*; no precipitate is obtained (distinction from procaine).

## Tests

**Appearance of solution.** A 5.0 per cent w/v solution in *ethanol (95 per cent)* is clear (2.4.1), and colourless (2.4.1).

**Acidity or alkalinity.** Dissolve 0.5 g in 5 ml of *ethanol (95 per cent),* add 10 ml of *water* and one drop of *phenolphthalein solution*; no pink colour is produced. Add 0.5 ml of *0.01 M sodium hydroxide*; the solution develops a pink colour.

**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

**Chlorides.** Dissolve 0.2 g in 5 ml of *ethanol (95 per cent)* previously acidified with a few drops of *dilute nitric acid* and add few drops of *silver nitrate solution*; no turbidity is produced immediately.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

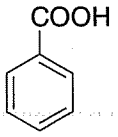
**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying over *phosphorus pentoxide* at a pressure of 1.5 to 2.5 kPa.

**Assay.** Weigh accurately about 0.4 g and dissolve in a mixture of 25 ml of *hydrochloric acid* and 50 ml of *water*. Cool to 10°. Determine by the nitrite titration (2.3.31).

1 ml of *0.1 M sodium nitrite* is equivalent to 0.01652 g of $C_9H_{11}NO_2$.

**Storage.** Store protected from light.

# Benzoic Acid




$C_7H_6O_2$ Mol. Wt. 122.1

Benzoic Acid contains not less than 99.5 per cent and not more than 100.5 per cent of $C_7H_6O_2$, calculated on the anhydrous basis.

**Category.** Antifungal agent; pharmaceutical aid (anti- microbial preservative).

**Description.** Colourless, light crystals, scales or needles; odour, slight and characteristic.

## Identification

A. Warm gently 0.2 g with 20 ml of *water*, add 1 ml of *1 M sodium hydroxide* and filter. To the filtrate add *ferric chloride test solution*; a buff coloured precipitate is produced.

B. When examined in the range 220 nm to 360 nm, a 0.001 per cent w/v solution in *methanol* shows an absorption maximum



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only at about 225 nm; absorbance at about 225 nm, about 0.8 (2.4.7).

C. A 1 per cent w/v solution is acidic to *methyl red solution*.

### Tests

**Appearance of solution**. A 5.0 per cent w/v solution in *ethanol (95 per cent)* is clear (2.4.1), and colourless (2.4.1).

**Arsenic** (2.3.10). Mix 5.0 g with 3 g of *anhydrous sodium carbonate*, add 10 ml of *bromine solution* and mix thoroughly. Evaporate to dryness on a water-bath, gently ignite and dissolve the cooled residue in 16 ml of *brominated hydrochloric acid* and 45 ml of *water*. Remove the excess of bromine with 2 ml of *stannous chloride AsT*. The resulting solution complies with the limit test for arsenic (2 ppm).

**Heavy metals**. Not more than 10 ppm, determined by the following method. Dissolve 2.0 g in 25 ml of *acetone* and add 2 ml of *water* and 10 ml of *hydrogen sulphide solution*; any colour produced is not more intense than that of a solution prepared with 25 ml of *acetone*, 2.0 ml of *lead standard solution (10 ppm Pb)* and 10 ml of *hydrogen sulphide solution*.

**Readily oxidisable substances**. Add 1 ml of *sulphuric acid* to 100 ml of *water*, heat to boiling and add dropwise *0.1 M potassium permanganate* until the pink colour persists for 30 seconds. Dissolve exactly 1 g in the hot solution and titrate with *0.1 M potassium permanganate* to a pink colour that persists for 15 seconds; not more than 0.5 ml of *0.1 M potassium permanganate* is required.

**Readily carbonisable substances**. Dissolve 0.5 g in 5 ml of *sulphuric acid* and allow to stand for 5 minutes. The colour of the solution is not more intense than that of reference solution YS5 (2.4.1).

**Cinnamic acid**. Warm 0.1 g with 0.1 g of *potassium permanganate* and 5 ml of *dilute sulphuric acid*; no odour of benzaldehyde is developed.

**Chlorinated compounds**. Dissolve 0.33 g in 5 ml of *0.5 M sodium carbonate*, evaporate to dryness and heat the residue until completely charred, keeping the temperature below 400°. Extract the residue with a mixture of 10 ml of *water* and 12 ml of *dilute nitric acid* and filter; the filtrate complies with the limit test for chlorides (2.3.12).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 0.7 per cent, determined on 0.25 g and using a mixture of 1 volume of *methanol* and 2 volumes of *pyridine* as the solvent.

**Assay**. Weigh accurately about 1.0 g and dissolve in 15 ml of warm *ethanol (95 per cent)* previously neutralised to *phenolphthalein solution*. Add 20 ml of *water* and titrate with *0.5 M sodium hydroxide* using *phenolphthalein solution* as indicator.

1 ml of *0.5 M sodium hydroxide* is equivalent to 0.06106 g of $C_7H_6O_2$.

## Compound Benzoic Acid Ointment

Benzoic and Salicylic Acids Ointment; Whitfield's Ointment

Compound Benzoic Acid Ointment is an ointment containing 6.0 per cent w/w of Benzoic Acid and 3.0 per cent w/w of Salicylic Acid in a suitable ointment base. Other strengths may also be prepared with Benzoic Acid and Salicylic Acid being in the ratio of about 2 to 1.

Compound Benzoic Acid Ointment contains not less than 5.7 per cent and not more than 6.3 per cent w/w of benzoic acid, $C_7H_6O_2$, and not less than 2.85 per cent and not more than 3.15 per cent w/w of salicylic acid, $C_7H_6O_3$.

**Category**. Antifungal (topical).

### Identification

Carry out the method for thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 80 volumes of *toluene* and 20 volumes of *glacial acetic acid*.

*Test solution*. Warm 1 g of the ointment with 10 ml of *chloroform*, cool and filter.

*Reference solution*. A solution containing 0.6 per cent w/v of *benzoic acid* and 0.3 per cent w/v of *salicylic acid* in *chloroform*.

Apply to the plate 2 µl of each solution. After development, dry the plate in a current of air and examine in ultraviolet light at 254 nm. The two principal spots in the chromatogram obtained with the test solution correspond to those in the chromatogram obtained with the reference solution. Examine the plate in ultraviolet light at 365 nm. A blue fluorescent spot in the chromatogram obtained with the test solution corresponds in colour and position to the one in the chromatogram obtained with the reference solution. Spray the plate with *ferric chloride test-solution*. The chromatogram obtained with the test solution shows a purple spot corresponding in position to the blue fluorescent spot observed in ultraviolet light at 365 nm and corresponding in colour and position to the spot in the chromatogram obtained with the reference solution.

### Tests

**Assay**. *For benzoic acid* — Weigh accurately about 2.5 g, dissolve with the aid of gentle heat, as completely as possible,



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in 50 ml of a mixture of equal volumes of *ethanol (95 per cent)* and *ether*, previously neutralised to *phenolphthalein solution* and titrate with *0.1 M sodium hydroxide* using *phenolphthalein solution* as indicator.

1 ml of *0.1 M sodium hydroxide*, after deducting 1 ml for each 0.01381 g of $C_7H_6O_3$ in the weight of the ointment taken (calculated from the result of the Assay for *salicylic acid*) is equivalent to 0.01221 g of $C_7H_6O_2$.

*For salicylic acid* — Weigh accurately about 2.5 g, dissolve with the aid of gentle heat, as completely as possible, in 50 ml of *ether*, and extract with 5 quantities, each of 10 ml, of a saturated solution of *sodium bicarbonate*, washing each extract with the same 50 ml of *ether*. Combine the aqueous extracts, cautiously add *hydrochloric acid* until the solution is distinctly acid to litmus paper and extract with 4 quantities, each of 25 ml, of *ether*; combine the extracts and evaporate the *ether* at a temperature below 40°. Dissolve the residue in 5 ml of *0.5 M sodium hydroxide*, add 50.0 ml of *0.1 M bromine* and 5 ml of *hydrochloric acid*, shake repeatedly during 15 minutes and allow to stand for 15 minutes. Add 10 ml of *potassium iodide solution* and titrate with *0.1 M sodium thiosulphate* using *starch solution*, added towards the end of the titration, as indicator. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of bromine required.

1 ml of *0.1 M bromine* is equivalent to 0.002302 g of $C_7H_6O_3$.

**Storage**. Store at a temperature not exceeding 30°.

## Benzoic Acid Solution

Benzoic Acid Solution contains 50 g of benzoic acid, 750 ml of propylene glycol, diluted to 1000 ml with purified water.

Benzoic Acid Solution contains not less than 4.75 per cent w/v and not more than 5.25 per cent w/v of benzoic acid, $C_7H_6O_2$.

**Category**. Antifungal.

### Identification

To 5 ml, add 30 ml of *1 M sulphuric acid* and extract the precipitated acid with three 25 ml quantities of *light petroleum ether (boiling range, 40° to 60°)*. Wash the combined extracts with three 25 ml quantities of *water*, filter through absorbent cotton and evaporate to dryness. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *benzoic acid RS* or with the reference spectrum of benzoic acid.

B. Melting point (2.4.21). About 121°.

### Tests

**Weight per ml** (2.4.29). 1.045 to 1.055 g.

**Assay**. To 10 ml, add 20 ml of *ethanol (95 per cent)* previously neutralised to *phenolphthalein solution* and titrate with *0.1 M sodium hydroxide* using *phenolphthalein solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.01221g of $C_7H_6O_2$.

**Labelling**. The label states (1) the date after which the solution is not intended to be used; (2) the conditions under which it should be stored.

## Benzoin

Benzoin is the balsamic resin obtained from *Styrax benzoin* Dryander or *Styrax paralleloneurus* Perkins, known in commerce as Sumatra Benzoin or from *Styrax tonkinensis* (Pierre) Craib ex Hartwich, or other species of the Section *Anthostyrax* of the genus *Styrax*, known in commerce as Siam Benzoin (Fam. Styraceae).

Benzoin contains not less than 25.0 per cent of total balsamic acids, calculated as cinnamic acid, $C_9H_8O_2$, in Sumatra Benzoin and as benzoic acid, $C_7H_6O_2$, in Siam Benzoin, calculated on the dried basis.

**Category**. Topical protectant; expectorant and comforting by steam inhalation in acute laryngitis.

**Description**. *Unground Sumatra Benzoin* — Blocks or lumps of varying size, made up of tears compacted together, with a reddish-brown, reddish-grey or greyish-brown resinous mass, known in commerce as block benzoin. It also occurs in the form of tears with cream-coloured to yellowish surfaces; when fractured they exhibit milky-white surfaces; odour, balsamic which accentuates on digestion with boiling *water*.

*Unground Siam Benzoin* — Pebble-like tears of variable size and shape, compressed, yellowish-brown to rusty-brown externally, milky white on fracture, hard and brittle at ordinary temperatures but softened by heat; odour, balsamic.

### Identification

A. To a solution in *ethanol (95 per cent)* add *water*; the solution becomes milky, and the mixture is acid to *litmus paper*.

B. Heat 0.5 g in a dry test-tube; it melts and evolves white fumes, which form a white needle-shaped crystalline sublimate.



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C. Heat 0.5 g in a test-tube with 5 ml of *potassium permanganate solution*; a strong odour of benzaldehyde is obtained with Sumatra Benzoin.

D. Determine by thin-layer chromatography (2.4.17), coating the plate with *kieselguhr*.

*Mobile phase*. A mixture of 93 volumes of *toluene* and 7 volumes of *ethyl acetate*.

*Test solution*. Dissolve 2.0 g of the substance under examination in 100 ml of *ethanol (95 per cent)*.

*Reference solution (a)*. A 0.05 per cent w/v solution of *benzoic acid RS* in *chloroform*.

*Reference solution (b)*. A 0.05 per cent w/v solution of *cinnamic acid RS* in *chloroform*.

*Reference solution (c)*. A 0.05 per cent w/v solution of *coniferyl benzoate RS* in *chloroform*.

*Reference solution (d)*. A 0.05 per cent w/v solution of *cinnamoyl cinnamate RS* in *chloroform*.

*Reference solution (e)*. A 0.05 per cent w/v solution of *propyl cinnamate RS* in *chloroform*.

*Reference solution (f)*. A 0.05 per cent w/v solution of *cinnamoyl benzoate RS* in *chloroform*.

After development, dry the plate in air until the odour of the solvent is no longer detectable and spray with *anisaldehyde-sulphuric acid reagent*. Heat the plate at 110° for 5 minutes and examine in ultraviolet light at 254 nm. In the case of Sumatra Benzoin, the chromatogram obtained with the test solution exhibits four intense spots corresponding to spots in the chromatograms obtained with reference solutions (b), (c), (d) and (e). In the case of Siam Benzoin, it exhibits intense spots corresponding to spots in the chromatograms obtained with reference solutions (a), (c), (d) and (f).

## Tests

**Dammar gum**. Determine by thin-layer chromatography (2.4.17), coating the plate with *aluminium oxide G*.

*Mobile phase*. A mixture of 60 volumes of *ether* and 40 volumes of *light petroleum (80° to 100°)*.

*Test solution*. Dissolve by warming 0.2 g of the substance under examination in 10 ml of *ethanol (90 per cent)* and centrifuge.

Apply to the plate 5 µl of the test solution. Allow the mobile phase to rise 10 cm. Dry the plate in air, spray with *anisaldehyde-sulphuric acid reagent* and heat at 100° to 105° for 5 minutes. The chromatogram does not show any prominent spot with an Rf value between 0.4 and 1.0.

**Foreign organic matter** (2.6.1). Not more than 1.0 per cent.

**Ethanol-soluble extractive**. Not less than 75.0 per cent in Sumatra Benzoin and not less than 90.0 per cent in Siam Benzoin, determined by the following method. Weigh accurately about 2 g, in coarse powder, in a tared extraction thimble and insert the thimble in a Soxhlet or other suitable continuous extraction apparatus. Place 0.1 g of *sodium hydroxide* in the receiving flask of the apparatus, extract with *ethanol (95 per cent)* until extraction is complete (about 5 hours), dry the thimble to constant weight at 105° and calculate the ethanol-soluble extractive from the increase in weight of the thimble.

**Acid-insoluble ash** (2.3.19). Not more than 1.0 per cent in Sumatra Benzoin and not more than 0.5 per cent in Siam Benzoin, determined on 2.0 g.

**Loss on drying** (2.4.19). Not more than 10.0 per cent, determined on 2.0 g, in coarse powder, by drying over *phosphorus pentoxide* at a pressure not exceeding 2.7 kPa for 4 hours.

**Assay**. Weigh accurately about 1.25 g and boil with 25 ml of *dilute ethanolic potassium hydroxide solution* under a reflux condenser for 1 hour. Remove the ethanol and digest the residue with 50 ml of hot *water* until diffused. Cool the liquid, add 150 ml of *water* and 1.5 g of *magnesium sulphate* dissolved in 50 ml of *water*. Mix thoroughly and set aside for 10 minutes. Filter, wash the residue on the filter with 20 ml of water, acidify the combined filtrate and washings with *hydrochloric acid* and extract with successive quantities of 50, 40, 30, 30 and 30 ml of *ether*. Combine the ether extracts and discard the aqueous portion. Extract with successive quantities of 20, 20, 10, 10 and 10 ml of *sodium bicarbonate solution*, washing each aqueous extract with the same 20 ml of *ether*. Discard the ether layers, acidify the combined aqueous extracts with *hydrochloric acid* and extract with successive quantities of 30, 20, 20 and 10 ml of *chloroform*, filtering each chloroform extract through a plug of cotton wool on which a layer of *anhydrous sodium sulphate* is placed. Evaporate the chloroform on a water-bath until about 10 ml remains and remove the remainder in a current of air stopping immediately when the last trace of solvent is removed. Dissolve the residue by warming with 10 ml of *ethanol (95 per cent)*, previously neutralised to *phenol red solution*, cool and titrate with *0.1 M sodium hydroxide* using *phenol red solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.01482 g of total balsamic acids, calculated as cinnamic acid, $C_9H_8O_2$, in Sumatra Benzoin and 0.01221 g of total balsamic acids, calculated as benzoic acid, $C_7H_6O_2$, in Siam Benzoin.

**Storage**. Store protected from light at a temperature not exceeding 30°.



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**Labelling**. The label states whether the material is Sumatra Benzoin or Siam Benzoin.

## Compound Benzoin Tincture

Friars' Balsam

| | |
|---|---|
| Benzoin, in *moderately coarse powder* | 100 g |
| Prepared Storax | 75 g |
| Tolu Balsam | 25 g |
| Aloes, in *moderately coarse powder* | 20 g |
| Ethanol (90 per cent) sufficient to produce | 1000 ml |

Macerate the Benzoin, Prepared Storax, Tolu Balsam and Aloes with 800 ml of Ethanol (90 per cent) in a closed vessel for not less than 2 days with occasional shaking. Filter and pass sufficient Ethanol (90 per cent) through the filter to produce the required volume.

Compound Benzoin Tincture contains not less than 4.5 per cent w/v of total balsamic acids, calculated as cinnamic acid, $C_9H_8O_2$.

**Category**. Topical protectant; expectorant and comforting by steam inhalation in acute laryngitis.

### Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *kieselguhr*.

*Mobile phase*. A mixture of 93 volumes of *toluene* and 7 volumes of *ethyl acetate*.

*Test solution*. Dilute 1 ml of the tincture with 4 ml of *ethanol (95 per cent)*. (The chromatographic profile may vary depending on the variety of Benzoin used).

*Reference solution (a)*. A 0.05 per cent w/v solution of *benzoic acid RS* in *chloroform*.

*Reference solution (b)*. A 0.05 per cent w/v solution of *cinnamic acid RS* in *chloroform*.

*Reference solution (c)*. A 0.05 per cent w/v solution of *coniferyl benzoate RS* in *chloroform*.

*Reference solution (d)*. A 0.05 per cent w/v solution of *cinnamoyl cinnamate RS* in *chloroform*.

*Reference solution (e)*. A 0.05 per cent w/v solution of *propyl cinnamate RS* in *chloroform*.

*Reference solution (f)*. A 0.05 per cent w/v solution of *cinnamoyl benzoate RS* in *chloroform*.

Apply to the plate 20 µl of each solution. After development, dry the plate in air until the odour of the solvent is no longer detectable and spray with *anisaldehyde-sulphuric acid reagent*. Heat the plate at 110° for 5 minutes and examine in ultraviolet light at 254 nm. In the case of Sumatra Benzoin, the chromatogram obtained with the test solution exhibits four intense spots corresponding to spots in the chromatograms obtained with reference solutions (b), (c), (d) and (e). In the case of Siam Benzoin, it exhibits intense spots corresponding to spots in the chromatograms obtained with reference solutions (a), (c), (d) and (f).

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *kieselguhr*.

*Mobile phase*. A mixture of 93 volumes of *toluene* and 7 volumes of *ethyl acetate*.

*Test solution*. Dilute 1 ml of the *tincture* with 4 ml of *ethanol (95 per cent)*.

*Reference solution*. A 0.05 per cent w/v solution of *styrene RS* in *chloroform*.

Apply to the plate 20 µl of each solution. After development, dry the plate in air until the odour of the solvent is no longer detectable and spray with *anisaldehyde-sulphuric acid reagent*. Heat the plate at 110° for 5 minutes and examine in ultraviolet light at 254 nm. The chromatogram obtained with the test solution exhibits an intense spot corresponding to the spot in the chromatogram obtained with the reference solution (Prepared Storax).

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *kieselguhr*.

*Mobile phase*. A mixture of 93 volumes of *toluene* and 7 volumes of *ethyl acetate*.

*Test solution*. Dilute 1 ml of the *tincture* with 4 ml of *ethanol (95 per cent)*.

*Reference solution (a)*. A 0.05 per cent w/v solution of *benzoyl benzoate RS* in *chloroform*.

*Reference solution (b)*. A 0.05 per cent w/v solution of *benzoyl cinnamate RS* in *chloroform*.

*Reference solution (c)*. A 0.05 per cent w/v solution of *eugenol RS* in *chloroform*.

*Reference solution (d)*. A 0.05 per cent w/v solution of *vanillin RS* in *chloroform*.

Apply to the plate 20 µl of each solution. After development, dry the plate in air until the odour of the solvent is no longer detectable and spray with *anisaldehyde-sulphuric acid reagent*. Heat the plate at 110° for 5 minutes and examine in ultraviolet light at 254 nm. The chromatogram obtained with the test solution exhibits spots corresponding to the spots in the chromatogram obtained with reference solutions, (a), (b), (c) and (d) (Tolu Balsam).



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D. Carry out the method for thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 100 volumes of *ethyl acetate*, 13.5 volumes of *methanol* and 10 volumes of *water*.

*Test solution*. Dilute 1 ml of the *tincture* with 4 ml of *ethanol (95 per cent)*.

*Reference solution*. A 0.5 per cent w/v solution of *barbaloin RS* in *methanol*.

Apply to the plate 50 µl of each solution as bands 20 mm long and not more than 3 mm wide. Allow the mobile phase to rise 10 cm. Dry the plate in air until the odour of the solvent is no longer detectable, spray with a 10 per cent w/v solution of *potassium hydroxide* in *methanol* and examine in ultraviolet light at 365 nm. The chromatogram obtained with the test solution exhibits a yellow fluorescent band corresponding to the band obtained in the chromatogram obtained with the reference solution and a light blue fluorescent band with a lower Rf value due to aloesine. Heat the plate at 110° for 5 minutes; a violet fluorescent band just below the band corresponding to barbaloin may also be seen in the chromatogram obtained with the test solution (Aloes).

### Tests

**Weight per ml** (2.4.29). 0.870 g to 0.885 g.

**Ethanol content**. 70.0 to 77.0 per cent v/v, determined by Method II (2.3.45).

**Total solids**. Not less than 13.5 per cent w/v, determined on 1 ml by drying in an oven at 105° for 4 hours.

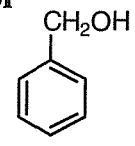
**Assay**. Evaporate 10 ml to a thick consistency on a water-bath. Boil the residue with 25 ml of *ethanolic potassium hydroxide solution* under a reflux condenser for 1 hour. Remove the ethanol and digest the residue with 50 ml of hot *water* until diffused. Cool the liquid, add 150 ml of *water* and 1.5 g of *magnesium sulphate* dissolved in 50 ml of *water*. Mix thoroughly and set aside for 10 minutes. Filter, wash the residue on the filter with 20 ml of water, acidify the combined filtrate and washings with *hydrochloric acid* and extract with successive quantities of 50, 40, 30, 30 and 30 ml of *ether*. Combine the ether extracts and discard the aqueous portion. Extract with successive quantities of 20, 20, 10, 10 and 10 ml of *sodium bicarbonate solution*, washing each aqueous extract with the same 20 ml of *ether*. Discard the ether layers, acidify the combined aqueous extracts with *hydrochloric acid* and extract with successive quantities of 30, 20, 20 and 10 ml of *chloroform*, filtering each chloroform extract through a plug of cotton wool on which a layer of *anhydrous sodium sulphate* is placed. Evaporate the chloroform on a water-bath until about 10 ml remains and remove the remainder in a current of air stopping immediately when the last trace of solvent is removed. Dissolve the residue by warming with 10 ml of *ethanol (95 per cent)*, previously neutralised to *phenol red solution*, cool and titrate with *0.1 M sodium hydroxide* using *phenol red solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.01482 g of total balsamic acids, calculated as cinnamic acid, $C_9H_8O_2$.

**Storage**. Store protected from light in tightly-closed containers and avoid exposure to direct sunlight and to excessive heat.

**Labelling**. The label states that it is flammable.

## Benzyl Alcohol




$C_7H_8O$ Mol. Wt. 108.1

Benzyl Alcohol contains not less than 97.0 per cent of $C_7H_8O$.

**Category**. Local anaesthetic; disinfectant.

**Description**. A colourless liquid; almost odourless; taste, sharp and burning.

### Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *benzyl alcohol RS* or with the reference spectrum of benzyl alcohol.

### Tests

**Appearance of solution**. A 1.0 per cent v/v solution is clear (2.4.1), colourless and oily liquid (2.4.1).

**Peroxide value** (2.3.35). Not more than 5.0.

**Wt. per ml** (2.4.29). 1.04 g to 1.05 g.

**Distillation range** (2.4.8). None distils below 200° and not less than 94 per cent distils between 202° and 208°.

**Refractive index** (2.4.27). 1.536 to 1.542.

**Acid Value** (2.3.23). Not more than 0.5.

**Related substances**. Determine by gas chromatography (2.4.13).

*Test solution*. The substance under examination.

*Reference solution (a)*. Dissolve 0.1 g of *ethylbenzene* in 10.0 ml of the test solution. Dilute 2.0 ml of this solution to 20.0 ml with the test solution.

*Reference solution (b)*. Dissolve 2.0 g of *dicyclohexyl* in 10.0 ml of the test solution. Dilute 2.0 ml of this solution to 20.0 ml with the test solution.

*Reference solution (c)*. Dissolve 0.75 g of *benzaldehyde* and 0.5 g of *cyclohexylmethanol* in 25.0 ml of the test solution. Add 1.0 ml of this solution to a mixture of 2.0 ml of reference



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solution (a) and 3.0 ml of reference solution (b) and dilute to 20.0 ml with the test solution.

*Reference solution (d).* Dissolve 0.25 g of *benzaldehyde* and 0.5 g of *cyclohexylmethanol* in 25.0 ml of the test solution. Add 1.0 ml of this solution to a mixture of 2.0 ml of reference solution (a) and 2.0 ml of reference solution (b) and dilute to 20.0 ml with the test solution.

Chromatographic system

- a glass column 30 m x 0.32 mm, packed with fused silica coated with macrogol 20000 (film thickness 0.5 µm),
- temperature:

| column | time (min.) | temperature (°) |
|---|---|---|
| | 0-34 | 50-220 |
| | 34-69 | 220 |

- inlet port. 200° and detector. 310°,
- flame ionization detector,
- linear velocity. 25 cm/second using nitrogen as carrier gas,

Benzyl alcohol not intended for parenteral use

Inject 0.1 µl reference solution (c). The relative retention time with reference to benzyl alcohol for ethyl benzene is about 0.28, for dicyclohexyl is about 0.59, for benzyl alcohol impurity A is about 0.68 and for benzyl alcohol impurity B is about 0.71. The test is not valid unless the resolution between the peaks due to benzyl alcohol impurity A and benzyl alcohol impurity B is not less than 3.0.

Inject the test solution and reference solution (c). In the chromatogram obtained with the test solution, the area of secondary peak corresponding to benzyl alcohol impurity A is not more than the area of the peak due to benzyl alcohol impurity A in the chromatogram obtained with reference solution (c) (0.15 per cent). The area of secondary peak corresponding to benzyl alcohol impurity B is not more than the area of the peak due to benzyl alcohol impurity B in the chromatogram obtained with reference solution (c) (0.1 per cent). The sum of the areas of all other secondary peaks with relative retention time less than benzyl alcohol is not more than 4 times the area of the peak due to ethylbenzene in the chromatogram obtained with reference solution (c) (0.04 per cent). The sum of the areas of all other secondary peaks with relative retention time more than benzyl alcohol is not more than the area of the peak due to dicyclohexyl in the chromatogram obtained with reference solution (c) (0.3 per cent). Ignore any peak with an area less than 0.01 times the area of the peak due to ethylbenzene in the chromatogram obtained with reference solution (c) (0.0001 per cent).

Benzyl alcohol intended for parenteral use

Inject 0.1 µl reference solution (d). The relative retention time with reference to benzyl alcohol for ethyl benzene is about 0.28, for dicyclohexyl is about 0.59, for benzyl alcohol impurity A is about 0.68 and for benzyl alcohol impurity B is about 0.71. The test is not valid unless the resolution between the peaks due to benzyl alcohol impurity A and benzyl alcohol impurity B is not less than 3.0.

Inject the test solution and reference solution (d). In the chromatogram obtained with the test solution, the area of secondary peak corresponding to benzyl alcohol impurity A is not more than the area of the peak due to benzyl alcohol impurity A in the chromatogram obtained with reference solution (d) (0.05 per cent). The area of secondary peak corresponding to benzyl alcohol impurity B is not more than the area of the peak due to benzyl alcohol impurity B in the chromatogram obtained with reference solution (d) (0.1 per cent). The sum of the areas of all other secondary peaks with relative retention time less than benzyl alcohol is not more than twice the area of the peak due to ethylbenzene in the chromatogram obtained with reference solution (d) (0.02 per cent). The sum of the areas of all other secondary peaks with relative retention time more than benzyl alcohol is not more than the area of the peak due to dicyclohexyl in the chromatogram obtained with reference solution (d) (0.2 per cent). Ignore any peak with an area less than 0.01 times the area of the peak due to ethylbenzene in the chromatogram obtained with reference solution (c) (0.0001 per cent).

**Loss on drying** (2.4.19). Not more than 0.05 per cent.

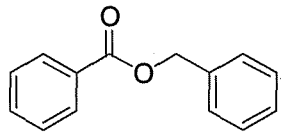
**Assay.** To 1.5 g add *25* ml *of* a mixture of 1 volume of *acetic anhydride* and 7 volumes of *pyridine* and heat on a water-bath for thirty minutes. Cool, add 25 ml of *water,* and titrate with *1 M sodium hydroxide,* using *phenolphthalein solution* as indicator. Repeat the operation without the substance under examination; the difference between the titrations represents the amount of alkali required by the benzyl alcohol.

1 ml of *1 M sodium hydroxide* is equivalent to 0.1081 g of $C_7H_8O$.

**Storage.** Store protected from moisture and at a temperature not exceeding 8°.

**Labelling.** The label states, where appropriate, the contents are intended for use in the manufacture of parenteral preparations.

## Benzyl Benzoate



$C_{14}H_{12}O_2$ Mol. Wt. 212.2

Benzyl Benzoate is the benzyl ester of benzoic acid.



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Benzyl Benzoate contains not less than 99.0 per cent and not more than 100.5 per cent w/w of $C_{14}H_{12}O_2$.

**Category**. Anti-parasitic (for topical treatment of scabies).

**Description**. Colourless crystals or a clear, colourless, oily liquid; odour, faintly aromatic.

### Identification

A. Boil 2 g with 25 ml of *ethanolic potassium hydroxide solution* for 2 hours in a flask fitted with a reflux condenser. Remove the ethanol on a water-bath, add 50 ml of *water* to the liquid remaining in the flask and distill until the liquid distilling is no longer turbid. Preserve the distillate for test B. To the liquid remaining in the flask add *dilute hydrochloric acid* till it is neutral and divide the mixture into two parts. To one part add *ferric chloride test solution*; a buff-coloured precipitate is produced. To the other part add *hydrochloric acid*; a white crystalline precipitate of benzoic acid is produced.

B. To the distillate obtained in test A, add 2.5 g of *potassium permanganate* and 2 ml of *sodium hydroxide solution*, boil for 15 minutes in a flask fitted with a reflux condenser, cool and filter. To the filtrate add *dilute hydrochloric acid* till it is neutral and divide the mixture into two parts. To one part add *ferric chloride test solution*; a buff-coloured precipitate is produced. To the other part add *hydrochloric acid*; a white crystalline precipitate of benzoic acid is produced.

### Tests

**Congealing temperature** (2.4.10). Not below 17.0°.

**Relative density** (2.4.29). 1.113 to 1.118.

**Refractive index** (2.4.27). 1.567 to 1.569.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Assay**. Boil a convenient quantity of *ethanol (95 per cent)* thoroughly to expel carbon dioxide and neutralise to *phenolphthalein solution*. Weigh accurately about 2 g of the substance under examination, dissolve in 5 ml of the neutralised ethanol contained in a hard-glass flask and neutralise the free acid in the solution with *0.5 M ethanolic potassium hydroxide* using 0.2 ml of *phenolphthalein solution* as indicator. Add 40 ml of *0.5 M ethanolic potassium hydroxide* and boil under a reflux condenser on a water-bath for 1 hour. Add 20 ml of *water* and titrate the excess of alkali with *0.5 M hydrochloric acid* using a further 0.2 ml of *phenolphthalein solution* as indicator. Repeat the operation without the substance under examination. The difference between the titrations represents the alkali required to saponify the benzyl benzoate.

1 ml of *0.5 M ethanolic potassium hydroxide* is equivalent to 0.1061 g of $C_{14}H_{12}O_2$.

**Storage**. Store protected from light and air in well-filled containers.

## Benzyl Benzoate Application

Benzyl Benzoate Application contains 25 per cent w/w of Benzyl Benzoate in a suitable oil-in-water emulsified basis.

Benzyl Benzoate Application contains not less than 22.5 per cent and not more than 27.5 per cent w/w of benzyl benzoate, $C_{14}H_{12}O_2$.

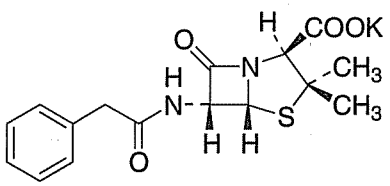
**Assay**. Weigh accurately about 8.0 g and dissolve in 10 ml of *ethanol (95 per cent)* previously neutralised with *0.1 M sodium hydroxide* contained in a hard-glass flask and neutralise the free acid in the solution with *0.5 M ethanolic potassium hydroxide* using 0.2 ml of *phenolphthalein solution* as indicator. Add 40 ml of *0.5 M ethanolic potassium hydroxide* and boil under a reflux condenser on a water-bath for 1 hour. Add 20 ml of *water* and titrate the excess of alkali with *0.5 M hydrochloric acid* using a further 0.2 ml of *phenolphthalein solution* as indicator. Repeat the operation without the substance under examination. The difference between the titrations represents the alkali required to saponify the benzyl benzoate.

1 ml of *0.5 M ethanolic potassium hydroxide* is equivalent to 0.1061 g of $C_{14}H_{12}O_2$.

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

## Benzylpenicillin Potassium

Penicillin G Potassium




$C_{16}H_{17}KN_2O_4S$ Mol. Wt. 372.5

Benzylpenicillin Potassium is potassium (6*R*)-6-(2-phenylacetamido)penicillanate, produced by the growth of certain strains of *Penicillium notatum* or related organisms, or obtained by any other means.

Benzylpenicillin Potassium contains not less than 96.0 per cent and not more than 100.5 per cent of penicillins, calculated as $C_{16}H_{17}KN_2O_4S$ on the dried basis.

**Category**. Antibacterial.

**Dose**. By intramuscular or by slow intravenous injection or by infusion, the equivalent of 1.2 to 2.4 g of benzylpenicillin daily, in 4 divided doses.

**Description**. A white or almost white, crystalline powder.



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## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *benzylpenicillin potassium RS*.

B. Gives reaction A of potassium salts (2.3.1).

## Tests

**pH** (2.4.24). 5.5 to 7.5, determined in a 10.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). +270° to +300°, determined in a 2.0 per cent w/v solution in *carbon dioxide-free water*.

**Light absorption** (2.4.7). Dissolve 94 mg in sufficient *water* to produce 50.0 ml. Measure the absorbance of the solution at about 325 nm, at about 280 nm and at the maximum at about 264 nm, diluting the solution, if necessary, for the measurement at the maximum at about 264 nm. Absorbances at about 325 nm and 280 nm, not more than 0.10 and that at the maximum at about 264 nm, calculated on the basis of the undiluted solution (0.188 per cent w/v), 0.80 to 0.88.

**Related substances**. Determine by liquid chromatography (2.4.14) as given under Assay. Inject reference solution (d) and elute isocratically using the chosen mobile phase. Inject test solution (b) and start the elution isocratically. Immediately after elution of the benzylpenicillin peak start the following linear gradient:

| Time (min) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) | Comment |
|---|---|---|---|
| 0 – 20 | 70 → 0 | 30 → 100 | linear gradient |
| 20 – 35 | 0 | 100 | Isocratic |
| 35 – 50 | 70 | 30 | re-equilibration |

Inject *water* and use the same elution pattern to obtain a blank. In the chromatogram obtained with test solution (b) the area of any peak, other than the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with reference solution (d) (1.0 per cent).

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Determine by liquid chromatography (2.4.14).

*NOTE—Prepare the solutions immediately before use.*

*Test solution (a)*. Dissolve 50.0 mg of the substance under examination in *water* and dilute to 50.0 ml with the same solvent.

*Test solution (b)*. Dissolve 80.0 mg of the substance under examination in *water* and dilute to 20.0 ml with the same solvent.

*Reference solution (a)*. Dissolve 50.0 mg of *benzylpenicillin sodium RS* in *water* and dilute to 50.0 ml with the same solvent.

*Reference solution (b)*. Dissolve 10 mg of *benzylpenicillin sodium RS* and 10 mg of *phenylacetic acid RS* in *water* and dilute to 50.0 ml with the same solvent.

*Reference solution (c)*. Dilute 1.0 ml of reference solution (a) to 20.0 ml with *water*. Dilute 1.0 ml of the solution to 50.0 ml with the same solvent.

*Reference solution (d)*. Dilute 4.0 ml of reference solution (a) to 100.0 ml with *water*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 10 volumes of a 68 g per litre solution of *potassium dihydrogen phosphate* adjusted to pH 3.5 with a 500 g per litre solution of *dilute phosphoric acid*, 30 volumes of *methanol* and 60 volumes of *water*,

  B. a mixture of 10 volumes of a 68 g per litre solution of *potassium dihydrogen phosphate* adjusted to pH 3.5 with a 500 g per litre solution of *dilute phosphoric acid*, 40 volumes of *water* and 50 volumes of *methanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume. 20 µl.

Equilibrate the column with a mobile phase ratio A:B of 70:30.

Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is at least 6.0 (if necessary, adjust the ratio A:B of the mobile phase) and the capacity factor for the second peak (benzylpenicillin) is 4.0 to 6.0.

Inject reference solution (c). Adjust the system to obtain a peak with a signal-to-noise ratio of at least 3.

Inject alternately test solution (a) and reference solution (a).

Calculate the percentage content of benzylpenicillin potassium by multiplying the percentage content of benzylpenicillin sodium by 1.045.

*Benzylpenicillin Potassium intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.*

**Bacterial endotoxins** (2.2.3). Not more than 0.16 Endotoxin Unit per mg.

*Benzylpenicillin Potassium intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.*

**Sterility** (2.2.11). Complies with the test for sterility.



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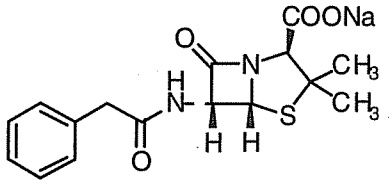


**Storage**. Store protected from moisture at a temperature not exceeding 30°. If it is intended for use in the manufacture of parenteral preparations, the container should be sterile and sealed so as to exclude micro-organisms.

**Labelling**. The label states whether or not the contents are intended for use in the manufacture of parenteral preparations.

## Benzylpenicillin Sodium

Penicillin G Sodium




$C_{16}H_{17}N_2NaO_4S$ Mol. Wt. 356.4

Benzylpenicillin Sodium is sodium (6*R*)-6-(2-phenylacetamido) penicillanate, produced by the growth of certain strains of *Penicillium notatum* or related organisms, or obtained by any other means.

Benzylpenicillin Sodium contains not less than 96.0 per cent and not more than 100.5 per cent of penicillins, calculated as $C_{16}H_{17}N_2NaO_4S$ on the dried basis.

**Category**. Antibacterial.

**Dose**. By intramuscular or by slow intravenous injection or by infusion, the equivalent of 1.2 to 2.4 g of benzylpenicillin daily, in 4 divided doses.

**Description**. A white or almost white, crystalline powder.

### Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *benzylpenicillin sodium RS*.

B. Gives reaction A of sodium salts (2.3.1).

### Tests

**pH** (2.4.24). 5.5 to 7.5, determined in a 10.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). +285° to +310°, determined in a 2.0 per cent w/v solution in *carbon dioxide-free water*.

**Light absorption** (2.4.7). Dissolve 90 mg in sufficient *water* to produce 50.0 ml. Measure the absorbance of the solution at about 325 nm, at about 280 nm and at the maximum at about 264 nm, diluting the solution, if necessary, for the measurement at about 264 nm. Absorbances at about 325 nm and 280 nm, not more than 0.10 and that at the maximum at about 264 nm, calculated on the basis of the undiluted solution (0.18 per cent w/v), 0.80 to 0.88.

**Related substances**. Determine by liquid chromatography (2.4.14) as given under Assay. Inject reference solution (d) and elute isocratically using the chosen mobile phase. Inject test solution (b) and start the elution isocratically. Immediately after elution of the benzylpenicillin peak start the following linear gradient:

| Time (min) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) | Comment |
|---|---|---|---|
| 0 – 20 | 70 → 0 | 30 → 100 | linear gradient |
| 20 – 35 | 0 | 100 | Isocratic |
| 35 – 50 | 70 | 30 | re-equilibration |

Inject *water* and use the same elution pattern to obtain a blank. In the chromatogram obtained with test solution (b) the area of any peak, other than the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with reference solution (d) (1.0 per cent).

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Determine by liquid chromatography, (2.4.14).

Prepare the solutions immediately before use.

*Test solution (a)*. Dissolve 50.0 mg of the substance under examination in *water* and dilute to 50.0 ml with the same solvent.

*Teat solution (b)*. Dissolve 80.0 mg of the substance under examination in *water* and dilute to 20.0 ml with the same solvent.

*Reference solution (a)*. Dissolve 50.0 mg of *benzylpenicillin sodium RS* in *water* and dilute to 50.0 ml with the same solvent.

*Reference solution (b)*. Dissolve 10 mg of *benzylpenicillin sodium RS* and 10 mg of *phenylacetic acid RS* in *water* and dilute to 50.0 ml with the same solvent.

*Reference solution (c)*. Dilute 1.0 ml of reference solution (a) to 20.0 ml with *water*. Dilute 1.0 ml of the solution to 50.0 ml with the same solvent.

*Reference solution (d)*. Dilute 4.0 ml of reference solution (a) to 100.0 ml with *water*.

Chromatographic system

- a stainless steel a column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 10 volumes of a 68 g per litre solution of *potassium dihydrogen phosphate* adjusted to pH 3.5 with a 500 g per litre solution of *dilute phosphoric acid*, 30 volumes of *methanol* and 60 volumes of *water*,

  B. a mixture of 10 volumes of a 68 g per litre solution of *potassium dihydrogen* phosphate adjusted to pH 3.5 with a 500 g per litre solution of *dilute phosphoric acid*, 40 volumes of *water* and 50 volumes of *methanol*,
- flow rate. 1 ml per minute,



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- spectrophotometer set at 225 nm,
- injection volume. 20 µl.

Equilibrate the column with a mobile phase ratio A:B of 70:30.

Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is at least 6.0 (if necessary, adjust the ratio A:B of the mobile phase) and the capacity factor for the second peak (benzylpenicillin) is 4.0 to 6.0.

Inject reference solution (c). Adjust the system to obtain a peak with a signal-to-noise ratio of at least 3.

Inject alternately test solution (a) and reference solution (a).

Calculate the percentage content of $C_{16}H_{17}N_2NaO_4S$.

*Benzylpenicillin Sodium intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.*

**Bacterial endotoxins** (2.2.3). Not more than 0.16 Endotoxin Unit per mg.

*Benzylpenicillin Sodium intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.*

**Sterility** (2.2.11). Complies with the test for sterility.

**Storage**. Store protected from moisture at a temperature not exceeding 30°. If it is intended for use in the manufacture of parenteral preparations, the container should be sterile and sealed so as to exclude micro-organisms.

**Labelling**. The label states whether or not the contents are intended for use in the manufacture of parenteral preparations.

**Usual strengths**. The equivalent of 150 mg (250,000 Units), 300 mg (500,000 Units) and 600 mg (1,000,000 Units) of Benzylpenicillin.

**Storage**. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Benzylpenicillin Injection contains not less than 95.0 per cent and not more than 110.0 per cent of the stated amount of penicillins, calculated as $C_{16}H_{18}N_2O_4S$.

**Description**. A white or almost white crystalline powder.

*The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.*

### Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *benzylpenicillin potassium RS* .

B. Gives reaction A of potassium or sodium salts (2.3.1).

### Tests

**pH** (2.4.24). 5.5 to 7.5, determined in a 10.0 per cent w/v solution.

**Related substances**. Determine by liquid chromatography (2.4.14) as given under Assay. Inject reference solution (d) and elute isocratically using the chosen mobile phase. Inject test solution (b) and start the elution isocratically. Immediately after elution of the benzylpenicillin peak start the following linear gradient:

| Time (min) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) | Comment |
|---|---|---|---|
| 0 – 20 | 70 → 0 | 30 → 100 | linear gradient |
| 20 – 35 | 0 | 100 | Isocratic |
| 35 – 50 | 70 | 30 | re-equilibration |

Inject *water* and use the same elution pattern to obtain a blank. In the chromatogram obtained with test solution (b) the area of any peak, other than the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with reference solution (d) (1.0 per cent).

**Bacterial endotoxins** (2.2.3). Not more than 0.16 Endotoxin Unit per mg.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Determine by liquid chromatography, (2.4.14).

*NOTE—Prepare the solutions immediately before use.*

Determine the weight of the contents of 10 containers.

## Benzylpenicillin Injection

Penicillin G Injection

Benzylpenicillin Injection is a sterile material consisting of Benzylpenicillin Potassium or Benzylpenicillin Sodium with or without buffering agents and other excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

*The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).*



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*Test solution (a)*. Dissolve 50.0 mg of the mixed contents of the 10 containers in *water* and dilute to 50.0 ml with the same solvent.

*Teat solution (b)*. Dissolve 80.0 mg of the substance under examination in *water* and dilute to 20.0 ml with the same solvent.

*Reference solution (a)*. Dissolve 50.0 mg of *benzylpenicillin sodium RS* in *water* and dilute to 50.0 ml with the same solvent.

*Reference solution (b)*. Dissolve 10 mg of *benzylpenicillin sodium RS* and 10 mg of *phenylacetic acid RS* in *water* and dilute to 50.0 ml with the same solvent.

*Reference solution (c)*. Dilute 1.0 ml of reference solution (a) to 20.0 ml with *water*. Dilute 1.0 ml of the solution to 50.0 ml with *water*.

*Reference solution (d)*. Dilute 4.0 ml of reference solution (a) to 100.0 ml with *water*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 10 volumes of a 68 g per litre solution of *potassium dihydrogen phosphate* adjusted to pH 3.5 with a 500 g per litre solution of *dilute phosphoric acid*, 30 volumes of *methanol* and 60 volumes of *water*,

  B. a mixture of 10 volumes of a 68 g per litre solution of *potassium dihydrogen phosphate* adjusted to pH 3.5 with a 500 g per litre solution of *dilute phosphoric acid*, 40 volumes of *water* and 50 volumes of *methanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume. 20 µl.

Equilibrate the column with a mobile phase ratio A:B of 70:30.

Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is at least 6.0 (if necessary, adjust the ratio A:B of the mobile phase) and the capacity factor for the second peak (benzylpenicillin) is 4.0 to 6.0.

Inject reference solution (c). Adjust the system to obtain a peak with a signal-to-noise ratio of at least 3.

Inject alternately test solution (a) and reference solution (a).

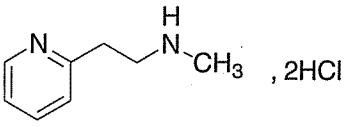
Calculate the content of benzylpenicillin sodium in the injection.

1 mg of $C_{16}H_{17}N_2NaO_4S$ is equivalent to 0.9383 mg of $C_{16}H_{18}N_2O_4S$.

**Storage**. Store protected from moisture at a temperature not exceeding 30°.

**Labelling**. The label states (1) whether the contents are Benzylpenicillin Potassium or Benzylpenicillin Sodium; (2) the name of any added buffering agents.

# Betahistine Hydrochloride




$C_8H_{12}N_2$, 2HCl Mol. Wt. 209.1

Betahistine Hydrochloride is *N*-methyl-2-(2-pyridyl) ethylamine dihydrochloride.

Betahistine Hydrochloride contains not less than 98.5 per cent and not more than 102.0 per cent of $C_8H_{12}N_2$, 2HCl, calculated on the dried basis.

**Category**. Antihistaminic.

**Description**. A white to off-white, crystalline powder; sometimes clumped, odourless or almost odourless, very hygroscopic.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *betahistine hydrochloride RS* or with the reference spectrum of betahistine hydrochloride.

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

C. Gives the reaction A of chlorides (2.3.12).

## Tests

**Appearance of solution**. A 10 per cent w/v solution in *water* is clear (2.4.1) and not more intensely coloured than reference solution B8 (2.4.1).

**pH** (2.4.24). 2.0 to 3.0, determined in a 10 per cent w/v solution in *water*.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 40 mg of the substance under examination in 100 ml of the mobile phase.

*Reference solution (a)*. A 0.04 per cent w/v solution of *betahistine dihydrochloride RS* in the mobile phase.

*Reference solution (b)*. Dilute 1 ml of reference solution (a) to 100 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 3.0 mm packed with octadecylsilane chemically bonded to porous silica (5 µm),
- mobile phase: dissolve 0.45 g *ammonium acetate* and 0.4 ml *glacial acetic acid* in 650 ml of *water*, add 350 ml of *acetonitrile* and add 2.88 g of *sodium laurylsulphate* and mix,



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- flow rate. 0.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Inject reference solution (a). The test is not valid unless the tailing factor is not more than 2.0 and the column efficiency in not less than 5000 theoretical plates.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with the reference solution (b) (2.0 per cent).

**Heavy metals** (2.3.13). 1 g complies with the limit test for heavy metals, Method C ( 20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 2.0 per cent, determined on 1 g by drying in an oven at 105°.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 40 mg of the substance under examination in 100.0 ml of the mobile phase.

*Reference solution*. A 0.04 per cent w/v solution of *betahistine dihydrochloride RS* in the mobile phase.

Use chromatographic system as described under Related substances.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_8H_{12}N_2$, 2HCl.

**Storage**. Store protected from light.

# Betahistine Tablets

## Betahistine Hydrochloride Tablets

Betahistine Tablets contain Betahistine Dihydrochloride.

Betahistine Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of betahistine dihydrochloride, $C_8H_{12}N_2$, 2HCl.

**Usual strengths**. 8 mg; 16 mg.

### Identification

Dissolve the powdered tablet containing about 0.1 g of Betahistine Hydrochloride in 5 ml of *water*, add 0.5 ml of *5 M sodium hydroxide*, extract with 5 ml of *dichloromethane*, filter the dichloromethane layer through *anhydrous sodium sulphate* with 2 ml of *dichloromethane* and evaporate the solution to dryness. The residue complies with the following test.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *betahistine hydrochloride RS*.

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

### Tests

**Dissolution** (2.5.2).

Apparatus. No 2,

Medium. 900 ml of *Phosphate citrate buffer pH 6.8*,

Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 256 nm (2.4.7). Calculate the content of $C_8H_{12}N_2$, 2HCl in the medium from the absorbance obtained from a solution of known concentration of *betahistine hydrochloride RS* in the same medium.

D. Not less than 80 per cent of the stated amount of $C_8H_{12}N_2$, 2HCl.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh and powder 20 tablets. Weigh accurately a quantity of the powdered tablet containing 32 mg of Betahistine Dihydrochloride, disperse in 50 ml of mobile phase and dilute to 100 ml with mobile phase and filter.

*Reference solution (a)*. A 0.032 per cent w/v solution of *betahistine hydrochloride RS* in mobile phase.

*Reference solution (b)*. Dilute 1 ml of reference solution (a) to 100 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 50°,
- mobile phase: dissolve 2.76 g of *sodium dihydrogen phosphate monohydrate* and 1.60 g of *sodium dodecylsulphate* in 600 ml of *water*, add 1.2 g of *hexylamine* and 400 ml of *acetonitrile*, and mix, adjusted pH to 3.5 with *orthophosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Inject reference solution (a). The test is not valid unless the tailing factor is not more than 2.0 and the column efficiency in not less than 2000 theoretical plates.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b)



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(0.5 per cent) and the sum of areas of all the secondary peaks is not more than 1.5 times the area of the peak in the chromatogram obtained with the reference solution (b) (1.5 per cent).

**Uniformity of content**. Comply with the tests stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay.

*Test solution.* Crush one tablet in 100-ml volumetric flask. Add about 50 ml of mobile phase and swirl for 10 minutes, make up to volume with mobile phase and filter.

Calculate the content of $C_8H_{12}N_2$, 2HCl in the tablet.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh and powder 20 tablets. Weigh accurately a quantity of the powdered tablet containing 32 mg of Betahistine Dihydrochloride, disperse in 50 ml of mobile phase and dilute to 100.0 ml with mobile phase and filter.

*Reference solution.* A 0.032 per cent w/v solution of *betahistine hydrochloride RS* in mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 50°,
- mobile phase: dissolve 2.76 g of *sodium dihydrogen phosphate monohydrate* and 1.6 g of *sodium dodecylsulphate* in 600 ml of *water.* add 0.4 g of *hexylamine* and 400 ml of *acetonitrile*, adjust the pH to 3.5 with *orthophosphoric acid*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

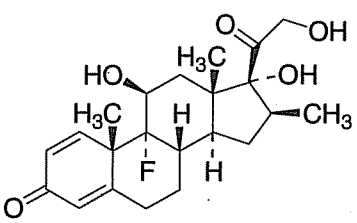
Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, the column efficiency in not less than 2000 theoretical plates. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_8H_{12}N_2$, 2HCl in the tablet.

**Storage**. Store protected from light.

# Betamethasone




$C_{22}H_{29}FO_5$ Mol. Wt. 392.5

Betamethasone is 9α-fluoro-11β,17α,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione.

Betamethasone contains not less than 96.0 per cent and not more than 104.0 per cent of $C_{22}H_{29}FO_5$, calculated on the dried basis.

**Category**. Adrenocortical steroid.

**Dose**. 0.5 to 5 mg daily, in divided doses.

**Description**. A white to creamy-white powder; odourless.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests C and D may be omitted if tests A and B are carried out.*

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

B. Determine by thin-layer chromatography (2.4.17), coating the plate with a suitable silica gel containing a fluorescent indicator with an optimal intensity at 254 nm (such as Merck silica gel 60 F254).

*Mobile phase.* A mixture of 85 volumes of *ether*, 10 volumes of *toluene* and 5 volumes of *1-butanol* saturated with *water.*

*Test solution.* Dissolve 25 mg of the substance under examination in 10 ml of a mixture of 90 volumes of *chloroform* and 10 volume of *methanol.*

*Reference solution (a).* A 0.25 per cent w/v solution of *betamethasone RS* in a mixture of 90 volumes of *chloroform* and 10 volumes of *methanol.*

*Reference solution (b).* A 0.125 per cent w/v solution of each of the substance under examination and *betamethasone RS* in the same solvent mixture.

*Reference solution (c).* A 0.125 per cent w/v solution of each of the substance under examination and *dexamethasone RS* in the same solvent mixture.

Apply to the plate 2 µl of each solution. After development, dry the plate in air and spray with *ethanolic sulphuric acid (20 per cent).* Heat at 120° for 10 minutes or until spots are produced, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution is similar in colour in daylight, in fluorescence in ultraviolet light at 365 nm, position and size to the principal spot in the chromatogram obtained with reference solution (a) and the chromatogram obtained with reference solution (b) shows only one spot. The test is not valid unless the chromatogram obtained with reference solution (c) shows two principal spots that are close to one another but separated.

C. Heat 0.5 ml of *chromic-sulphuric acid* in a test-tube (5 cm × about 6 mm) in a naked flame until white fumes are evolved;



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the solution wets the sides of the tube readily and there is no greasiness. Add 2 or 3 mg of the substance under examination and again heat in a naked flame until white fumes appear; the solution does not wet the sides of the tube and does not pour easily from the tube.

D. Place 2 ml of a 0.01 per cent w/v solution in *ethanol* in a stoppered tube, add 10 ml of *phenylhydrazine solution*, mix, warm in a water-bath at 60° for 20 minutes and cool immediately; absorbance of the resulting solution at about 450 nm (2.4.7), not more than 0.25.

## Tests

**Specific optical rotation** (2.4.22). +114.0° to +122.0°, determined in a 0.5 per cent w/v solution in *dioxan*.

**Light absorption** (2.4.7). Absorbance of a 0.001 per cent w/v solution in *ethanol (95 per cent)* at the maximum at about 240 nm, 0.37 to 0.40.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 25.0 mg of the substance under examination in a mixture of equal volumes of *acetonitrile* and *methanol* and dilute to 10.0 ml with the same solvent.

*Reference solution (a)*. Dissolve 2 mg of *betamethasone RS* and 2 mg of *methylprednisolone RS* in mobile phase A and dilute to 100.0 ml with the same mobile phase.

*Reference solution (b)*. Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 45°,
- mobile phase: A. a mixture of 250 ml of *acetonitrile* and 700 ml of *water*, allowed to equilibrate, sufficient water added to produce 1000 ml and mixed,
  B. *acetonitrile*,
- flow rate. 2.5 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

| Time (min) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) | Comment |
|---|---|---|---|
| 0 – 15 | 100 → 0 | 0 | isocratic |
| 15 – 40 | 0 → 100 | 100 | linear gradient |
| 41 – 46 | 100 → 0 | 0 | equilibration |

Equilibrate the column with mobile phase B for at least 30 minutes and then with mobile phase A for 5 minutes. For subsequent chromatograms, use the conditions described from 40 minutes to 46 minutes.

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (b) is not less than 50 per cent of the full scale of the recorder.

Inject reference solution (a). When the chromatograms are recorded in the conditions described above, the retention times are: methylprednisolone, about 11.5 minutes and betamethasone, about 12.5 minutes. The test is not valid unless the resolution between the peaks corresponding to methylprednisolone and betamethasone is at least 1.5; if necessary, adjust the concentration of acetonitrile in mobile phase A.

Inject separately a mixture of equal volumes of *acetonitrile* and *methanol* as blank, the test solution and reference solution (b). In the chromatogram obtained with the test solution: the area of any peak, other than the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and not more than one such peak has an area greater than half the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent); the sum of the areas of all the peaks, other than the principal peak, is not greater than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent). Disregard any peak due to the blank and any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Dissolve 0.1 g in *alcohol* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of the solution to 100.0 ml with *alcohol*. Measure the absorbance of the resulting solution at the maximum at about 238.5 nm (2.4.7).

Calculate the content of $C_{22}H_{29}FO_5$ taking 395 as the specific absorbance at 238.5 nm.

**Storage**. Store protected from light.

# Betamethasone Tablets

Betamethasone Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of betamethasone, $C_{22}H_{29}FO_5$.

**Usual strengths**. 0.5 mg; 1.0 mg.



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## Identification

Powder a few tablets and extract with *chloroform*. Evaporate the extract to dryness. The residue complies with the following tests.

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

B. Place 2 ml of a 0.01 per cent w/v solution in *ethanol* in a stoppered tube, add 10 ml of *phenylhydrazine solution*, mix, warm in a water-bath at 60° for 20 minutes and cool immediately; absorbance of the resulting solution at about 450 nm (2.4.7), not more than 0.25.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Solvent mixture*. A mixture of 90 volumes of *acetone* and 10 volumes of *formamide*.

*Mobile phase*. *Chloroform*.

*Test solution*. Dissolve 25 mg of the residue in 10 ml of the solvent mixture.

*Reference solution (a)*. Dissolve 25 mg of *betamethasone RS* in 10 ml of the solvent mixture.

*Reference solution (b)*. Mix equal volumes of the test solution and reference solution (a).

*Reference solution (c)*. Mix equal volumes of the test solution and a 0.25 per cent w/v solution of *dexamethasone RS* in the solvent mixture.

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v)*. Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot. The chromatogram obtained with reference solution (c) shows two closely running spots.

## Tests

**Related substances**. Transfer a quantity of the powdered tablets containing about 2 mg of Betamethasone to a glass-stoppered 50-ml centrifuge tube. Pipette 20 ml of *ethanol (95 per cent)* into the tube, shake for 2 minutes and allow to stand for 20 minutes with occasional shaking. Centrifuge the mixture for 5 minutes. Pipette 10 ml of the clear supernatant liquid into a glass-stoppered tube and evaporate the ethanol on a water-bath with the aid of a current of air to about 0.5 ml, then evaporate without heat to dryness. Pipette 1 ml of a mixture of 9 volumes of *chloroform* and 1 volume of *methanol*, insert the stopper and mix. Centrifuge, if necessary, to remove any insoluble material. Use this solution as the test solution.

Determine by thin-layer chromatography (2.4.17), coating the plate with a suitable silica gel containing a fluorescent indicator with an optimal intensity at 254 nm (such as Merck silica gel 60 F254).

*Mobile phase*. A mixture of 77 volumes of *dichloromethane*, 15 volumes of *ether*, 8 volumes of *methanol* and 1.2 volumes of *water*.

*Reference solution (a)*. A 0.02 per cent w/v solution of the substance under examination in a mixture of 90 volumes of *chloroform* and 10 volumes of *methanol*.

*Reference solution (b)*. A 0.01 per cent w/v solution of the substance under examination in the same solvent mixture.

*Reference solution (c)*. A 0.1 per cent w/v solution of each of the substance under examination and *prednisone RS* in the same solvent mixture.

Apply to the plate 5 µl of each solution. After development, dry the plate in air until the odour of solvents is no longer detectable and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated principal spots.

**Dissolution** (2.5.2).

Apparatus. No 1,
Medium. 900 ml of *water* and 1 ml of 0.05 per cent w/v solution of *testosterone RS* (internal standard) in *methanol*,
Speed and time. 50 rpm and 45 minutes.

Use one tablet in the vessel for each test.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

*Test solution*. The filtrate obtained as given above.

*Reference solution*. Dilute a mixture of 1.0 ml each of a 0.05 per cent w/v solution of *betamethasone RS* in *methanol* and 1 ml



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of a 0.05 per cent w/v solution of *testosterone RS* in *methanol* to 900 ml with *water*.

Chromatographic system
- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of *methanol* and 40 volumes of *water*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

D. Not less than 75 per cent of the stated amount of $C_{22}H_{29}FO_5$.

**Uniformity of content**. Comply with the test stated under Tablets. Determine by liquid chromatography (2.4.14).

*NOTE — Protect the solutions from light.*

*Test solution*. Finely crush one tablet, add 20.0 ml of a 0.002 per cent w/v solution of *hydrocortisone* (internal standard) in *methanol (50 per cent)*, shake for 10 minutes and filter through a glass-fibre filter paper.

*Reference solution*. A solution containing 0.0025 per cent w/v of *betamethasone RS* and 0.002 per cent w/v of *hydrocortisone*.

Chromatographic system
- a stainless steel column 20 cm x 5 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 53 volumes of *water* and 47 volumes of *methanol*.
- flow rate. 1.4 ml per minute,
- spectrophotometer set at 238 nm,
- injection volume. 20 µl.

Calculate the content of $C_{22}H_{29}FO_5$ in the tablet.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*NOTE — Protect the solutions from light.*

*Test solution*. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 2.5 mg of Betamethasone, add 20.0 ml of *methanol (50 per cent)*, shake for 10 minutes and filter through a glass-fibre paper.

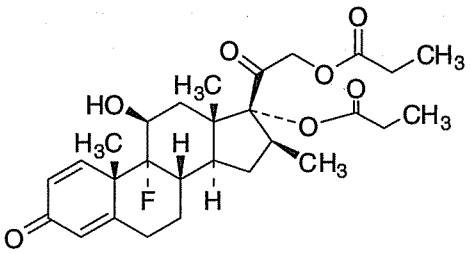
*Reference solution (a)*. A solution containing 0.0125 per cent w/v of *betamethasone RS* and 0.01 per cent w/v of *hydrocortisone RS* (internal standard).

*Reference solution (b)*. Prepare in the same manner as the test solution but use 20.0 ml of a 0.01 per cent w/v solution of *hydrocortisone* in *methanol (50 per cent)* in place of 20.0 ml of *methanol (50 per cent)*.

Carry out the chromatographic procedure described under Uniformity of content. Calculate the content of $C_{22}H_{29}FO_5$ in the tablets.

**Storage**. Store protected from light.

# Betamethasone Dipropionate



$C_{28}H_{37}FO_7$ Mol. Wt. 504.6

Betamethasone Dipropionate is 9α-fluoro-11β,17α,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17α,21-dipropionate.

Betamethasone Dipropionate contains not less than 97.0 per cent and not more than 103.0 per cent of $C_{28}H_{37}FO_7$, calculated on the dried basis.

**Description**. A white or almost white, crystalline powder.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *betamethasone dipropionate RS* or with the reference spectrum of betamethasone dipropionate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Solvent mixture*. 10 volumes of *methanol* and 90 volumes of *dichloromethane*.

*Mobile phase*. Add 1.2 volumes of *water* and 8 volumes of *methanol* in a mixture of 15 volumes of *ether* and 77 volumes of *dichloromethane*.

*Test solution*. Dissolve 10 mg of the substance under examination in 10.0 ml of the solvent mixture.

*Reference solution (a)*. A 0.1 per cent w/v solution of *betamethasone dipropionate RS* in the solvent mixture.

*Reference solution (b)*. A 0.1 per cent w/v solution of *betamethasone acetate RS* in the solvent mixture. Dilute 5.0 ml of this solution to 10 ml with reference solution (a).

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with reference solution (a). Spray with *ethanolic sulphuric acid*. Heat at 120° for



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10 minutes or until the spots appear, allow to cool. Examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with reference solution (a). Reference solution (b) gives two clearly separated spots.

### Tests

**Specific optical rotation** (2.4.22). +63 ° to +70°, determined on a 1.0 per cent w/v solution of *dioxan*.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve 62.5 mg of the substance under examination in 25.0 ml of the mobile phase.

*Reference solution (a).* Dissolve 2.5 mg of *betamethasone dipropionate RS* and 2.5 mg of *anhydrous beclometasone dipropionate RS* in 50.0 ml of the mobile phase.

*Reference solution (b).* Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 40 volumes of *water* and 60 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to betamethasone dipropionate and beclometasone dipropionate is not less than 2.5.

Inject reference solution (b) and the test solution. Run the chromatogram 2.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.75 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent) and not more than one such peak has an area more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). The sum of all the secondary peaks is not more than 1.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.5 per cent). Ignore any peak with an area less than 0.025 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 0.5 g by drying in an oven at 105°.

**Assay**. Dissolve 50 mg in 100.0 ml of *ethanol (95 per cent).* Dilute 2.0 ml of this solution to 50.0 ml with *ethanol (95 per cent)* and measure the absorbance of the resulting solution at the maximum at about 240 nm (2.4.7). Calculate the content of $C_{28}H_{37}FO_7$ taking 305 as the specific absorbance at 240 nm.

**Storage**. Store protected from light.

## Betamethasone Cream

Betamethasone Dipropionate Cream

Betamethasone Cream contains an amount of betamethasone dipropionate, $C_{28}H_{37}FO_7$ equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of betamethasone, $C_{22}H_{29}FO_5$ in a suitable cream base.

**Usual strength**. 0.05 per cent w/w.

### Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 70 volumes of *chloroform* and 10 volumes of *acetone*.

*Test solution.* Transfer about 1.5 g of Cream to a glass-stoppered, 50-ml centrifuge tube. Add 15 ml of a *methanolic hydrochloric acid solution* prepared by mixing 1 volume of *dilute hydrochloric acid* (1 in 120) with 4 volumes of *methanol.* Shake to obtain a homogeneous mixture. Add 30 ml of *hexane*, mix for 10 minutes, and centrifuge. Using a suitable syringe, transfer the lower aqueous phase to a second centrifuge tube, add about 20 ml of *water* and mix. Extract this aqueous mixture with chloroform by shaking, centrifuging, and removing the lower, chloroform phase with a syringe. Evaporate the chloroform on a steam bath with the aid of a stream of nitrogen to dryness, cool, and dissolve the residue in chloroform to obtain a solution containing about 150 µg of betamethasone dipropionate per ml.

*Reference solution.* A 0.015 per cent w/v solution of *betamethasone dipropionate RS* in *chloroform.*

Apply to the plate 40 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with the reference solution.

### Tests

**Other tests**. Complies with the tests stated under Cream.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture.* A 0.1 per cent v/v solution of *acetic acid* in *methanol.*

*Internal standard solution.* A 0.045 per cent w/v solution of *beclomethasone dipropionate RS* in the solvent mixture.



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*Test solution.* Shake a quantity of Cream containing about 2 mg of Betamethasone Dipropionate with 5.0 ml of internal standard solution and 10.0 ml of the solvent mixture. Heat in a water-bath at 60°, shaking intermittently, until the cream melts. Remove from the bath, and shake vigorously until the specimen has resolidified. Repeat the heating and shaking. Freeze in an ice-methanol bath for about 15 minutes, and centrifuge at 2500 rpm for about 5 minutes. Transfer a portion of the supernatant to a suitable vial.

*Reference solution.* A 0.02 per cent w/v solution of *betamethasone dipropionate RS* in the solvent mixture.

Transfer 10.0 ml of this solution to a suitable vial, and add 5.0 ml of internal standard solution, to obtain a solution having known concentrations of about 0.133 mg of Betamethasone Dipropionate and about 0.15 mg of Beclomethasone Dipropionate per ml.

Chromatographic system

- a stainless steel column 30 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of *acetonitrile* and 50 volumes of *water,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 25 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0.

Inject the reference solution and the test solution.

Calculate the content of betamethasone, $C_{22}H_{29}FO_5$ in the Cream.

**Storage.** Preserve in collapsible tubes or tight containers and at a temperature of 30°, excursions permitted between 15° and 30°. Protect from freezing.

# Betamethasone Lotion

## Betamethasone Dipropionate Lotion

Betamethasone Lotion contains an amount of betamethasone dipropionate, $C_{28}H_{37}FO_7$ equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of betamethasone, $C_{22}H_{29}FO_5$ in a suitable lotion base.

**Usual strength.** 0.05 per cent w/w.

### Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

*Mobile phase.* A mixture of 70 volumes of *chloroform* and 10 volumes of *acetone.*

*Test solution.* Shake a quantity of Lotion containing about 0.6 mg of Betamethasone Dipropionate with 10 ml of *0.1 M hydrochloric acid* and 4 ml of *chloroform* for 10 minutes. Centrifuge at 2000 rpm for about 5 minutes. Transfer the chloroform layer to a suitable vial.

*Reference solution.* A 0.015 per cent w/v solution of *betamethasone dipropionate RS* in *chloroform.*

Apply to the plate 40 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with the reference solution.

### Tests

**Other tests.** Complies with the tests stated under Lotion.

**Assay.** Determine by liquid chromatography (2.4.14).

*Solvent mixture.* A 0.1 per cent v/v solution of *acetic acid* in *methanol.*

*Internal standard solution.* A 0.09 per cent w/v solution of *beclomethasone dipropionate RS* in *chloroform.*

*Test solution.* Disperse a quantity of Lotion containing about 1.2 mg of Betamethasone Dipropionate with 10.0 ml of *0.1 M hydrochloric acid* in a capped 50-ml centrifuge tube. Add 2.0 ml of internal standard solution and 2.0 ml of *chloroform.* Cap, and shake vigorously for about 2 minutes, or disperse on a vortex mixer for about 1 minute. Centrifuge at 2500 rpm for about 3 minutes. Transfer the chloroform phase to a suitable vial. Evaporate the chloroform under a stream of nitrogen at a slightly elevated temperature to dryness. Cool the vial to room temperature, add 4.0 ml of *methanol*, and swirl to dissolve the residue.

*Reference solution.* A 0.06 per cent w/v solution of *betamethasone dipropionate RS* in *chloroform.* Transfer 5.0 ml of this solution to a suitable vial, and add 5.0 ml of internal standard solution to obtain a solution having known concentrations of about 0.3 mg of Betamethasone Dipropionate and about 0.45 mg of Beclomethasone Dipropionate per ml. To 10.0 ml of *0.1 M hydrochloric acid* in a capped 5-ml centrifuge tube add 4.0 ml of the prepared solution. Cap, and shake vigorously for about 2 minutes, or disperse on a vortex mixer for about 1 minute. Centrifuge at 2500 rpm for about 3 minutes. Transfer the chloroform phase to a suitable vial. Evaporate the chloroform under a stream of nitrogen at a slightly elevated temperature to dryness. Cool the vial to room temperature, add 4.0 ml of *methanol*, and swirl to dissolve the residue.

Chromatographic system

- a stainless steel column 30 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),



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- mobile phase: a mixture of 50 volumes of *acetonitrile* and 50 volumes of *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 25 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0.

Inject the reference solution the test solution.

Calculate the content of betamethasone, $C_{22}H_{29}FO_5$ in the Lotion.

**Storage**. Store protected from light and moisture, at a temperature not exceeding 30°.

# Betamethasone Ointment

Betamethasone Dipropionate Ointment

Betamethasone Ointment contains an amount of betamethasone dipropionate, $C_{28}H_{37}FO_7$ equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of betamethasone, $C_{22}H_{29}FO_5$, in a suitable ointment base.

**Usual strength**. 0.05 per cent w/w.

## Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 70 volumes of *chloroform* and 10 volumes of *acetone*.

*Test solution*. Shake about 1.5 g of Ointment with 15 ml of *methanolic hydrochloric acid solution* prepared by mixing 1 volume of *dilute hydrochloric acid* (1 in 120) with 4 volumes of *methanol*. Add 30 ml of *hexane*, mix for 10 minutes, and centrifuge. Using a suitable syringe, transfer the lower aqueous phase to a second centrifuge tube, add about 20 ml of *water*, and mix. Extract this aqueous mixture with *chloroform* by shaking, centrifuge and removing the lower, chloroform phase with a syringe. Evaporate the chloroform on a steam bath with the aid of a stream of nitrogen to dryness, cool, and dissolve the residue in chloroform to obtain a solution containing about 150 µg of betamethasone dipropionate per ml.

*Reference solution*. A 0.015 per cent w/v solution of *betamethasone dipropionate RS* in *chloroform*.

Apply to the plate 40 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with the reference solution.

## Tests

**Other tests**. Complies with the tests stated under Ointment.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. A 0.1 per cent v/v solution of *acetic acid* in *methanol*.

*Internal standard solution*. A 0.09 per cent w/v solution of *beclomethasone dipropionate RS* in *chloroform*.

*Test solution*. Disperse a quantity of Ointment containing about 2 mg of Betamethasone Dipropionate with 5.0 ml of internal standard solution and 10.0 ml of the solvent mixture. Heat in a water-bath at 70°, shaking intermittently until the ointment melts. Remove from the bath, and shake vigorously until the ointment has solidified. Repeat the heating and shaking operation. Freeze in an ice-methanol bath for about 15 minutes, and centrifuge at 2500 rpm for about 5 minutes. Transfer a portion of the supernatant to a suitable vial.

*Reference solution*. A 0.06 per cent w/v solution of *betamethasone dipropionate RS* in *chloroform*. Transfer 5.0 ml of this solution to a suitable vial, and add 5.0 ml of internal standard solution to obtain a solution having known concentrations of about 0.3 mg of Betamethasone Dipropionate and about 0.45 mg of Beclomethasone Dipropionate per ml. To 10.0 ml of *0.1 M hydrochloric acid* in a capped 5-ml centrifuge tube add 4.0 ml of the prepared solution. Cap, and shake vigorously for about 2 minutes, or disperse on a vortex mixer for about 1 minute. Centrifuge at 2500 rpm for about 3 minutes. Transfer the chloroform phase to a suitable vial. Evaporate the chloroform under a stream of nitrogen at a slightly elevated temperature to dryness. Cool the vial to room temperature, add 4.0 ml of *methanol*, and swirl to dissolve the residue.

Chromatographic system

- a stainless steel column 30 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of *acetonitrile* and 50 volumes of *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 25 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0.

Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{29}FO_5$ in the Ointment.

**Storage**. Store protected from light and moisture, at a temperature not exceeding 30°.

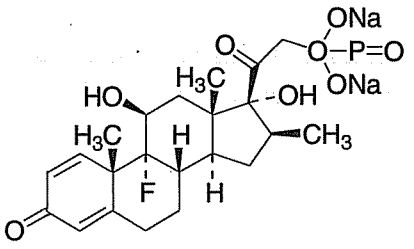


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# Betamethasone Sodium Phosphate




$C_{22}H_{28}FNa_2O_8P$ Mol. Wt. 516.4

Betamethasone Sodium Phosphate is 9α-fluoro-11β,17α,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione disodium phosphate.

Betamethasone Sodium Phosphate contains not less than 96.0 per cent and not more than 103.0 per cent of $C_{22}H_{28}FNa_2O_8P$, calculated on the anhydrous basis.

**Category**. Adrenocortical steroid.

**Dose**. The equivalent of 0.5 to 5 mg of betamethasone daily, in divided doses. In the treatment of acute adrenal insufficiency, by intravenous or intramuscular injection, the equivalent of 10 to 80 mg of betamethasone daily, in divided doses.

(6.5 mg of Betamethasone Sodium Phosphate is approximately equivalent to 5 mg of betamethasone).

**Description**. A white or almost white powder; odourless; very hygroscopic.

## Identification

A. To 2 ml of a 0.013 per cent w/v solution in *ethanol (95 per cent)* in a stoppered tube add 10 ml of *phenylhydrazine-sulphuric acid solution*, mix, warm in a water-bath at 60° for 20 minutes and cool immediately. Absorbance of the resulting solution at the maximum at about 450 nm, not more than 0.13 (2.4.7).

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A freshly prepared mixture of 30 volumes of *isopropyl alcohol*, 10 volumes of *acetic acid* and 10 volumes of *water*.

*Test solution*. Dissolve 0.25 g of the substance under examination in 100 ml of *water*.

*Reference solution (a)*. A 0.25 per cent w/v solution of *betamethasone sodium phosphate RS* in *water*.

*Reference solution (b)*. A mixture of equal volumes of the test solution and reference solution (a).

*Reference solution* (c). A mixture of equal volumes of the test solution and a 0.25 per cent w/v solution of *prednisolone sodium phosphate RS*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air until the odour of solvents is no longer detectable, spray with *ethanolic sulphuric acid (20 per cent)*, heat at 120° for 10 minutes, allow to cool, and examine in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot and the chromatogram obtained with reference solution (c) shows two closely running spots.

C. Heat 0.5 ml of *chromic-sulphuric acid* in a test-tube (5 cm x 6 mm) in a naked flame until white fumes are evolved; the solution wets the sides of the tube readily and there is no greasiness. Add 2 or 3 mg of the substance under examination and again heat in a naked flame until white fumes appear; the solution does not wet the sides of the tube and does not pour easily from the tube.

D. Dissolve 2 mg in 2 ml of *sulphuric acid* and allow to stand for 5 minutes; no red colour or yellowish-green fluorescence is produced (distinction from prednisolone sodium phosphate and hydrocortisone sodium phosphate).

E. Heat gently 40 mg with 2 ml of *sulphuric acid* until white fumes are evolved, add *nitric acid* dropwise until oxidation is complete and cool. Add 2 ml of *water*, heat until white fumes are again evolved, cool, add 10 ml of *water* and neutralise to *litmus paper* with *dilute ammonia solution*. The solution gives the reactions of sodium salts and of phosphates (2.3.1).

## Tests

**Appearance of solution**. A 2.0 per cent w/v solution is clear (2.4.1) and colourless (2.4.1).

**pH** (2.4.24). 7.5 to 9.0, determined in a 0.5 per cent w/v solution.

**Specific optical rotation** (2.4.22). +98.0° to +104.0°, determined in a 1.0 per cent w/v solution.

**Light absorption** (2.4.7). Ratio of the absorbance of the solution prepared as directed under Assay at the maximum at about 241 nm to that at about 263 nm, 1.70 to 1.90.

**Inorganic phosphate**. Not more than 0.5 per cent, calculated as $PO_4$, determined by the following method. Weigh accurately about 25 mg, dissolve in 10 ml of *water*, add 4 ml of *dilute sulphuric acid*, 1 ml of *ammonium molybdate solution* and 2 ml of *methylaminophenol with sulphite solution* and allow to stand for 15 minutes. Add sufficient *water* to produce 25.0 ml, allow to stand for further 15 minutes and measure the absorbance of the resulting solution at the maximum at about 730 nm (2.4.7). Calculate the content of phosphate from a



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calibration curve prepared by treating suitable aliquots of a 0.00143 per cent w/v solution of *potassium dihydrogen phosphate* in a similar manner.

**Free betamethasone and other derivatives.** Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

*Mobile phase. Methanol.*

*Test solution.* Dissolve 1.0 g of the substance under examination in 100 ml of *methanol.*

*Reference solution (a).* A 1.0 per cent w/v solution of *betamethasone sodium phosphate RS* in *methanol.*

*Reference solution (b).* A 0.02 per cent w/v solution of *betamethasone RS* in *methanol.*

Apply to the plate 2 µl of each solution. After development, dry the plate in air for 5 minutes and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution other than that corresponding to *betamethasone sodium phosphate RS* is not more intense than the spot in the chromatogram obtained with reference solution (b).

**Water** (2.3.43). Not more than 8.0 per cent, determined on 0.5 g.

**Assay.** Weigh accurately about 0.2 g and dissolve in sufficient *water* to produce 200.0 ml. Dilute 5.0 ml to 250.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 241 nm (2.4.7). Calculate the content of $C_{22}H_{28}FNa_2O_8P$, taking 297 as the specific absorbance at 241 nm.

**Storage.** Store protected from light and moisture.

# Betamethasone Eye Drops

Betamethasone Eye Drops are a sterile solution of Betamethasone Sodium Phosphate in Purified Water.

Betamethasone Eye Drops contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of betamethasone sodium phosphate, $C_{22}H_{28}FNa_2O_8P$.

**Usual strength.** 0.1 per cent w/v.

## Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

*Mobile phase.* A mixture of 60 volumes of *butanol,* 20 volumes of *acetic anhydride* and 20 volumes of *water.*

*Test solution.* Dilute the eye drops suitably with *water* to get a solution containing 0.1 per cent w/v of Betamethasone Sodium Phosphate.

*Reference solution (a).* A 0.1 per cent w/v solution of *betamethasone sodium phosphate RS* in *water.*

*Reference solution (b).* A mixture of equal volumes of the test solution and reference solution (a).

*Reference solution (c).* A mixture of equal volumes of reference solution (a) and 0.1 per cent w/v of *prednisolone sodium phosphate RS* in *water.*

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in air, heat at 110° for 10 minutes and examine in ultraviolet light at 254 nm. The chromatograms obtained with the test solution, reference solution (a) and reference solution (b) show single principal spots with similar $R_f$ values. The chromatogram obtained with reference solution (c) shows two principal spots with almost identical $R_f$ values.

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

C. To a volume containing 0.2 mg of Betamethasone Sodium Phosphate, add slowly 1 ml of *sulphuric acid* and allow to stand for 2 minutes. A brownish yellow colour but no red colour or yellowish green fluorescence is produced.

## Tests

**pH** (2.4.24).7.0 to 8.5.

**Related substances.** Determine by liquid chromatography (2.4.14).

*Test solution.* Dilute the eye drops if necessary to obtain a solution containing 0.1 per cent w/v of Betamethasone Sodium Phosphate.

*Reference solution (a).* Dilute 1 volume of the test solution to 50 volumes with *water.*

*Reference solution (b).* A solution containing 0.006 per cent w/v each of *betamethasone sodium phosphate RS* and *betamethasone RS.*

Chromatographic system

- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (10 µm) (Such as Spherisorb ODS 1),
- column temperature. 60°,
- mobile phase: a mixture of 60 volumes of *citro-phosphate buffer pH 5.0* and 40 volumes of *methanol,*
- flow rate. 2 ml per minute,
- spectrophotometer set at 241 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to betamethasone sodium phosphate and betamethasone is at least 3.5.



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Inject the test solution and reference solution (a) and record the chromatogram for three times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any peak corresponding to betamethasone is not more than 1.3 times the area of the principal peak in the chromatogram obtained with reference solution (a). The area of any other secondary peak is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a). The sum of the areas of all the secondary peaks is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a). Ignore any peak the area of which is less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a).

**Other tests**. Comply with the tests stated under Eye Drops.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution (a).* Mix a quantity of the eye drops containing 5 mg of Betamethasone Sodium Phosphate with 10 ml of *methanol* and dilute to 25.0 ml with *water.*

*Test solution (b).* Mix a quantity of the eye drops containing 5 mg of Betamethasone Sodium Phosphate with 10 ml of a 0.06 per cent w/v solution of *hydrocortisone* (internal standard) in *methanol* and dilute to 25.0 ml with *water.*.

*Reference solution.* Mix 5.0 ml of a 0.1 per cent w/v solution of *betamethasone sodium phosphate RS* in *water* (solution A) and 10 ml of the internal standard solution and dilute to 25.0 ml with *water.*

Chromatographic system

- a stainless steel column 20 cm x 5 mm, packed with octadecylsilane bonded to porous silica (10 µm) (Such as Spherisorb ODS 1),
- mobile phase: a mixture of 55 volumes of *citro-phosphate buffer pH 5.0* and 45 volumes of *methanol*,
- flow rate. 2 ml per minute.
- spectrophotometer set at 241 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject test solutions (a), (b) and reference solution.

Calculate the content of $C_{22}H_{28}FNa_2O_8P$ in the eye drops.

**Storage**. Store protected from light.

# Betamethasone Injection

## Betamethasone Sodium Phosphate Injection

Betamethasone Injection is a sterile solution of Betamethasone Sodium Phosphate in Water for Injections.

Betamethasone Injection contains not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of betamethasone, $C_{22}H_{29}FO_5$.

**Usual strength**. The equivalent of 4 mg of betamethasone per ml. (5.2 mg of Betamethasone Sodium Phosphate is approximately equivalent to 4 mg of betamethasone).

**Description**. A clear, colourless solution.

### Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

*Mobile phase.* A freshly prepared mixture of 30 volumes of *1-butanol,* 10 volumes of *acetic anhydride* and 10 volumes of *water.*

*Test solution.* Dilute the injection, if necessary, with *water* so that it contains the equivalent of 2 mg of betamethasone per ml.

*Reference solution (a).* A 0.25 per cent w/v solution of *betamethasone sodium phosphate RS* in *water.*

*Reference solution (b).* A mixture of equal volumes of the test solution and reference solution (a).

*Reference solution* (c). A mixture of equal volumes of the test solution and a 0.25 per cent w/v solution of *prednisolone sodium phosphate RS* in *water.*

Apply to the plate 5 µl of each solution. After development, dry the plate in air until the odour of solvents is no longer detectable, spray with *ethanolic sulphuric acid (20 per cent),* heat at 120° for 10 minutes, allow to cool, and examine in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot and the chromatogram obtained with reference solution (c) shows two closely running spots. Secondary spots due to excipients may also be seen in the chromatograms obtained with the test solution and reference solutions (b) and (c).

B. To a volume containing 4 mg of betamethasone, add 1 ml of *water* and sufficient *ethanol* to produce 40 ml. To 2 ml of this solution in a stoppered tube add 10 ml of *phenylhydrazine solution*, mix, warm in a water-bath at 60° for 20 minutes and cool immediately; absorbance of the resulting solution at the maximum at about 450 nm, not more than 0.1 (2.4.7).

### Tests

**pH** (2.4.24). 7.5 to 9.0.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).



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**Assay**. Measure accurately a volume containing about 20 mg of betamethasone and add sufficient *water* to produce 50.0 ml. To 5.0 ml add 20 ml of *water* and 2 ml of *0.1 M hydrochloric acid* and shake with two quantities, each of 25 ml, of *ether*. Wash the ethereal solutions separately with 2, 1 and 1 ml of *water*, add the washings to the acid solution and discard the ether solutions. To the combined acid solution and the washings add 2 ml of *0.1 M sodium hydroxide* and sufficient *water* to produce 200.0 ml. Measure the absorbance of the resulting solution at the maximum at about 241 nm (2.4.7), using as the blank a solution prepared in a similar manner but omitting the substance under examination. Calculate the content of $C_{22}H_{29}FO_5$ taking 391 as the specific absorbance at 241 nm.

**Storage**. Store protected from light at a temperature not exceeding 30°.

**Labelling**. The label states the strength in terms of the equivalent amount of betamethasone in a suitable dose-volume.

# Betamethasone Sodium Phosphate Tablets

Betamethasone Sodium Phosphate Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of betamethasone, $C_{22}H_{29}FO_5$.

**Usual strength**. The equivalent of 0.5 mg of betamethasone (0.65 mg of Betamethasone Sodium Phosphate is approximately equivalent to 0.5 mg of betamethasone).

## Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase.* A freshly prepared mixture of 30 volumes of *1-butanol*, 10 volumes of *acetic anhydride* and 10 volumes of *water*.

*Test solution.* Dissolve a quantity of the powdered tablets containing 2 mg of betamethasone in 25 ml of *water*, add 2.5 g of *sodium chloride* and 1 ml of *hydrochloric acid*, extract with 25 ml of *chloroform* and discard the chloroform layer. Extract with 2.5 ml of *tributyl phosphate* and discard the aqueous layer.

*Reference solution (a).* Prepare in the same manner as the test solution but using 2.5 mg of *betamethasone sodium phosphate RS* instead of the substance under examination.

*Reference solution (b).* A mixture of equal volumes of the test solution and reference solution (a).

*Reference solution (c).* A mixture of equal volumes of the test solution and a solution prepared in the same manner as the test solution but using 2.5 mg of *prednisolone sodium phosphate RS* instead of the substance under examination.

Apply to the plate 2 µl of each solution. After development, dry the plate in air until the odour of solvents is no longer detectable, spray with *ethanolic sulphuric acid (20 per cent)*, heat at 120° for 10 minutes, allow to cool, and examine in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot and the chromatogram obtained with reference solution (c) shows two closely running spots. Secondary spots due to excipients may also be seen in the chromatograms obtained with the test solution and reference solutions (b) and (c).

B. Mix a quantity of the powdered tablets containing 0.4 mg of betamethasone with 1 ml of *sulphuric acid* and allow to stand for 5 minutes; a pale yellow colour is produced.

## Tests

**Disintegration** (2.5.1). Not more than 5 minutes.

**Uniformity of content**. Comply with the test stated under Tablets. Determine by liquid chromatography (2.4.14).

*Test solution.* Powder one tablet and dissolve as completely as possible in 5 ml of *water* and add 5 ml of a 0.006 per cent w/v solution of *hydrocortisone* (internal standard) in *methanol*.

*Reference solution.* A mixture of equal volumes of a 0.0065 per cent w/v solution of *betamethasone sodium phosphate RS* in *water* and a 0.006 per cent w/v solution of *hydrocortisone* in *methanol*.

Chromatographic system

- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Spherisorb ODS),
- column temperature. 60°,
- mobile phase: a mixture of 55 volumes of *citro-phosphate buffer pH 5.0* and 45 volumes of *methanol*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 241 nm,
- injection volume. 20 µl.

Calculate the content of $C_{22}H_{29}FO_5$ in the tablet.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14)

*Test solution.* Weigh and powder 20 tablets. To a quantity of the powder containing 1.25 mg of betamethasone add 25.0 ml of a 0.006 per cent w/v solution of *hydrocortisone* (internal standard) in *methanol* and dilute to 50.0 ml with *water*.



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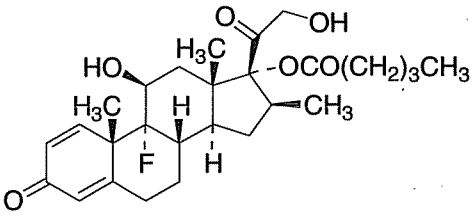
*Reference solution.* A mixture of equal volumes of a 0.0065 per cent w/v solution of *betamethasone sodium phosphate RS* in *water* and a 0.006 per cent w/v solution of *hydrocortisone* in *methanol.*

Carry out the chromatographic procedure described under Uniformity of content. Calculate the content of $C_{22}H_{29}FO_5$ in the tablets.

**Storage.** Store protected from light.

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

# Betamethasone Valerate



$C_{27}H_{37}FO_6$ Mol. Wt. 476.6

Betamethasone Valerate is a 9α–fluoro-11β,17α,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione-17-valerate.

Betamethasone Valerate contains not less than 96.0 per cent and not more than 102.0 per cent of $C_{27}H_{37}FO_6$, calculated on the dried basis.

**Category.** Adrenocortical steroid.

**Description.** A white to creamy-white powder.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *betamethasone valerate RS* or with the reference spectrum of betamethasone valerate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

*Solvent mixture.* A mixture of 90 volumes of *chloroform* and 10 volumes of *methanol.*

*Mobile phase.* A mixture of 95 volumes of *1,2-dichloroethane,* 5 volumes of *methanol* and 0.2 volume of *water.*

*Test solution.* Dissolve 25 mg of the substance under examination in 10 ml of the solvent mixture.

*Reference solution (a).* Dissolve 25 mg of *betamethasone valerate RS* in 10 ml of the solvent mixture.

*Reference solution (b).* Mix equal volumes of the test solution and reference solution (a).

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v).* Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

C. In the Assay, the retention time of the principal peak in the chromatogram obtained with the test solution corresponds to that of the peak due to *betamethasone valerate RS* in the chromatogram obtained with the reference solution.

D. Heat 50 mg with 2 ml of *0.5 M ethanolic potassium hydroxide* in a water-bath for 5 minutes. Cool, add 2 ml of *sulphuric acid* (50 per cent v/v) and boil gently for 1 minute; the odour of ethyl valerate is perceptible.

## Tests

**Specific optical rotation** (2.4.22). +75.0° to +82.0°, determined in a 1.0 per cent w/v solution in *dioxan.*

**Light absorption** (2.4.7). Absorbance of a 0.002 per cent w/v solution in *ethanol* at the maximum at about 240 nm, 0.63 to 0.67.

**Related substances.** Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh accurately 4 mg of the substance under examination add 10 ml of the mobile phase and shake well to dissolve.

Chromatographic system

- a stainless steel column 15 cm × 4.6 mm packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a mixture of 55 volumes of *acetonitrile,* 45 volumes of *water* and 0.1 volume of *glacial acetic acid,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Inject the test solution. The resolution between betamethasone valerate and any impurity is not less than 1.5 and the column efficiency is not less than 9000 theoretical plates.

Inject the test solution. Measure all the peak responses. Calculate the content of each impurity as a percentage of the sum of all the peak responses (1.0 per cent). Not more than 2.0 per cent of total impurities is found.



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**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh accurately about 60 mg of the substance under examination, dissolve in a 0.1 percent v/v solution of *glacial acetic acid* in *methanol* and dilute to 100.0 ml with the same solvent. To 5.0 ml of this solution add 10.0 ml of reference solution (b) and mix.

*Reference solution (a)*. Weigh accurately a suitable quantity of *betamethasone valerate RS* and dissolve in a 0.1 per cent v/v solution of *glacial acetic acid* in *methanol* to obtain a solution containing a known concentration of about 0.6 mg per ml. To 5.0 ml of this solution add 10.0 ml of reference solution (b) and mix.

*Reference solution (b)*. A 0.04 per cent w/v solution of *beclomethasone dipropionate RS* in a 0.1 per cent v/v solution of *glacial acetic acid* in *methanol*.

Chromatographic system
- a stainless steel column 30 cm × 4.0 mm, packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a mixture of 30 volumes of *acetonitrile* and 20 volumes of *water*,
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Inject reference solution (a). The relative retention times are about 1.7 for beclomethasone dipropionate and 1.0 for betamethasone valerate. The resolution between betamethasone valerate and beclomethasone dipropionate is not less than 4.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_{27}H_{37}FO_6$.

**Storage**. Store protected from light.

# Betamethasone Valerate Ointment

Betamethasone Valerate Ointment contains Betamethasone Valerate in a suitable ointment base.

Betamethasone Valerate Ointment contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of betamethasone, $C_{22}H_{29}FO_5$.

**Usual strengths**. The equivalent of 0.025 per cent w/w and 0.1 per cent w/w of betamethasone (120 mg of Betamethasone Valerate is approximately equivalent to 100 mg of betamethasone).

## Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 20 volumes of *chloroform*, 2 volumes of *acetone* and 1 volume of *ethanol*.

*Test solution*. Heat a quantity of the ointment containing 1 mg of betamethasone with 10 ml of *methanol* on a water-bath until it boils, shake vigorously, cool in ice for 30 minutes, filter, evaporate the filtrate to dryness in a current of nitrogen with gentle heating and dissolve the residue in 0.5 ml of *chloroform*.

*Reference solution*. A 0.24 per cent w/v solution of *betamethasone valerate RS* in *chloroform*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, heat at 105° for 5 minutes and spray while hot with *alkaline tetrazolium blue solution*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to *betamethasone valerate RS* in the chromatogram obtained with the reference solution.

## Tests

**Microbial contamination** (2.2.9). 1.0 g is free from *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**Other tests**. Complies with the tests stated under Ointments.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Heat a quantity of the accurately weighed ointment containing 2.5 mg of betamethasone with 10.0 ml of 0.04 per cent w/v solution of *beclomethasone dipropionate RS* (internal standard) in *methanol* containing 0.1 per cent v/v of *glacial acetic acid* and 5.0 ml of *methanol* containing 0.1 per cent v/v of *glacial acetic acid* on a water-bath until it boils, shake vigorously, cool in ice for 30 minutes, centrifuge and decant the supernatant solution into a stoppered flask.

*Reference solution*. Mix 5 ml of a 0.06 per cent w/v solution of *betamethasone valerate RS* in *methanol* containing 0.1 per cent v/v of *glacial acetic acid* and 10.0 ml of a 0.04 per cent w/v solution of *beclomethasone dipropionate RS* in *methanol* containing 0.1 per cent v/v of *glacial acetic acid*.

Chromatographic system
- a stainless steel column 30 cm × 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of *acetonitrile* and 40 volumes of *water*,
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume. 20 µl.



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Inject the reference solution. The relative retention times is about 1.7 for beclomethasone dipropionate and 1.0 for betamethasone valerate.

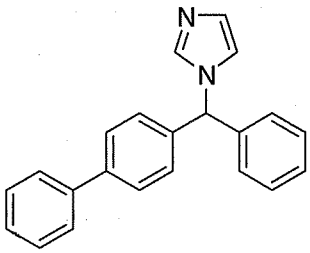
Inject alternately the test solution and the reference solution.

Calculate the content of $C_{22}H_{29}FO_5$ in the ointment.

**Storage**. Store protected from light. Avoid exposure to excessive heat.

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

## Bifonazole



$C_{22}H_{18}N_2$ Mol. Wt. 310.4

Bifonazole is 1-[(*RS*)-(biphenyl-4-yl)phenylmethyl]-1*H*-imidazole

Bifonazole contains not less than 98.0 per cent and not more than 100.5 per cent of $C_{22}H_{18}N_2$, calculated on the dried basis.

**Category**. Antifungal.

**Description.** A white or almost white, crystalline powder.

### Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *bifonazole RS* or with the reference spectrum of bifonazole.

### Tests

**Optical rotation** (2.4.22). -0.1° to +0.1°, determined in a 1.0 per cent w/v solution in *methanol*.

**Related substances**. Determined by liquid chromatography (2.4.14).

*Buffer solution pH 3.2*. Mix 2.0 ml of *orthophosphoric acid* with *water* and dilute to 1000.0 ml with the same solvent. Adjust to pH 3.2 with *triethylamine*.

*Test solution*. Dissolve 50 mg of the substance under examination in 25 ml of *acetonitrile* and dilute to 50.0 ml with buffer solution pH 3.2.

*Reference solution*. Dilute 0.25 ml of the test solution to 50.0 ml with buffer solution pH 3.2.

Chromatographic system

- a stainless steel column 12.5 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature 40°,
- mobile phase: A. a mixture of 20 volumes of *acetonitrile* and 80 volumes of *buffer solution pH 3.2*,
  B. a mixture of 20 volumes of *buffer solution pH 3.2* and 80 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 210 nm,
- injection volume. 50 µl.

| Time (min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0 - 8 | 60 | 40 |
| 8 - 12 | 60 → 10 | 40 → 90 |
| 12 - 30 | 10 | 90 |

Inject the reference solution and the test solution. Run the chromatogram 1.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 3 times the area of the principal peak in the chromatogram obtained with the reference solution (1.5 per cent). Sum of all the secondary peaks is not more than 4 times the area of the principal peak in the chromatogram obtained with the reference solution (2.0 per cent). Ignore the peaks having area less than 0.1 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Dissolve 0.25 g in 80 ml of *anhydrous acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03104 g of $C_{22}H_{18}N_2$.

## Bifonazole Cream

Bifonazole Cream contains Bifonazole in a suitable base.

Bifonazole Cream contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of bifonazole, $C_{22}H_{18}N_2$.

**Usual strength**. 1.0 per cent w/w.



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### Identification

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

### Tests

**Other tests**. Complies with the tests stated under Creams.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Shake a quantity of the cream containing about100 mg of Bifonazole with 40 ml of *methanol* in a 50 ml volumetric flask for 30 minutes and heat on a water-bath until the sample dissolves, allow to cool and dilute to volume with *methanol*. Freeze out the fatty phase under swirling 2 minutes in an ice-bath and filter through a membrane filter.

*Reference solution*. A 0.2 per cent w/v solution of *bifonazole RS* in *methanol*.

Chromatographic system

- a stainless steel column 12.5 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5µm) (such as LiChrospher 60 RP Select – B),
- column temperature. 40°,
- mobile phase: a mixture of 47 volumes of *acetonitrile*, 53 volumes of *0.02 M orthophosphoric acid*, adjusted to pH 5.0 with *ammonia solution*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 258 nm,
- injection volume. 10 µl.

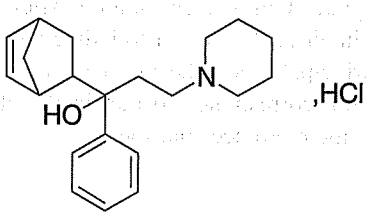
Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{18}N_2$ in the cream.

**Storage**. Store at a temperature not exceeding 30°.

## Biperiden Hydrochloride




$C_{21}H_{29}NO,HCl$ Mol. wt. 347.9

Biperiden Hydrochloride is (*RS*)-1-[(*RS*,2*RS*,4*RS*)-bicyclo-[2.2.1]hept-5-en-2-yl]-1-phenyl-3-(piperidin-1-yl)propan-1-ol hydrochloride.

Biperiden Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{21}H_{29}NO,HCl$, calculated on the dried basis.

**Category**. Anticholinergic.

**Dose**. Initially, 1 mg twice daily; maintenance dose, 2 mg to 6 mg daily, in divided doses.

**Description**. A white, crystalline powder.

### Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *biperiden hydrochloride RS*.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 100 volumes of *toluene*, 5 volumes of *diethylamine* and 5 volumes of *methanol*.

*Test solution*. Dissolve 0.5 g of the substance under examination in 100 ml of *methanol*.

*Reference solution (a)*. A 0.5 per cent w/v solution of *biperiden hydrochloride RS* in *methanol*.

*Reference solution (b)*. Dissolve 5 mg of (SR)-1-[(1RS, 2RS, 4RS)-bicyclo [2.2.1] hept-5-en-2-yl]-1-phenyl-3-(piperidin-1-yl]-propan-1-ol (*endo* form) in reference solution (a) and dilute to 2 ml with the same solution.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). Spray with *dilute iodobismuthate solution* and examine in daylight. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

C. To about 20 mg add 5 ml of *phosphoric acid*; a green colour develops.

D. Gives reaction A of chlorides (2.3.1).

### Tests

**Appearance of solution**. A 0.2 per cent solution in *carbon dioxide-free water* is not more opalescent than opalescence standard OS2 (2.4.1), and is colourless (2.4.1).

**pH** (2.4.24). 5.0 to 6.5, determined in a 0.2 per cent w/v solution.

**Related substances**. Determine by gas chromatography (2.4.13).



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*Test solution*. Dissolve 1.0 g of the substance under examination in 100 ml of *methanol*.

*Reference solution* (a). Dilute 1.0 ml of the test solution to 100 ml with *methanol* and mix. Dilute 10 ml of the resulting solution to 100 ml with *methanol*.

*Reference solution (b)*. To 1.0 ml of the test solution add 10 ml of *methanol* and 10 mg of (*SR*)-1-[(1*RS*, 2*RS*, 4*RS*)-bicyclo[2.2.1]hept-5-en-2-yl]-1-phenyl-3-(piperidin-1-yl) propan-l-ol (*endo* form) and sufficient *methanol* to produce 100 ml.

Chromatographic system

- a fused-silica capillary column, 50 m × 0.25 mm coated with poly (vinyl-phenylmethyl siloxane with thickness of 0.25 µm,
- flame ionisation detector,
- temperature:
- column. 200° for 5 minutes, then raised at the rate of 2° per minute to 270°,
- inlet port at 250° and detector at 300°,
- flow rate. 0.4 ml per minute of nitrogen (the carrier gas) and a split ratio of 1:250.

Inject 2 µl of each solution. When using a recorder, adjust the sensitivity of the system so that the heights of the two principal peaks in the chromatogram obtained with reference solution (b) are not less than 50 per cent of the full scale of the recorder. The test is not valid unless, in the chromatogram obtained with reference solution (b), the resolution between the first peak due to biperiden and the second peak due to (*SR*)-1-[(1*RS*, 2*RS*,4*RS*)]-bicyclo [2.2.1] hept-5-en-2-yl]-phenyl-3-(piperidin-1-yl)propa-1-ol (*endo* form) is at least 2.5; the principal peak in the chromatogram obtained with reference solution (a) has a signal-to-noise ratio of at least 6. For peaks with a retention time of 0.95 to 1.05 relative to biperiden, the area of any peak, other than the principal peak, is not greater than 0.5 per cent of the area of the principal peak and the sum of the areas of any such peaks is not greater than 1.0 per cent of the area of the principal peak. For peaks with relative retention times outside the above-mentioned range, the area of any peak is not greater than 0.1 per cent of the area of the principal peak and the sum of the areas of such peaks is not greater than 0.5 per cent of the area of the principal peak. Disregard any peak with an area less than 0.05 per cent of the area of the principal peak in the chromatogram obtained with the test solution.

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

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 2 hours.

**Assay**. Weigh accurately about 0.5 g, dissolve in 80 ml of *anhydrous glacial acetic acid*, warming slightly, if necessary to effect solution and cool. Add 10 ml of *mercuric acetate solution* and titrate with *0.1 M perchloric acid*, using 0.1ml of *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03479 g of $C_{21}H_{29}NO$, HCl.

**Storage**. Store protected from light.

# Biperiden Tablets

Biperiden Hydrochloride Tablets

Biperiden Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of biperiden hydrochloride, $C_{21}H_{29}NO$, HCl.

**Usual strength**. 2 mg.

## Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 100 volumes of *methanol* and 1.5 volumes of *strong ammonia solution*.

*Test solution*. Shake a quantity of the powdered tablets containing about 10 mg of Biperiden Hydrochloride with 5 ml of *water* and disperse the powder with the aid of ultra sound for a few minutes. Add 5 ml of *methanol* and mix again for 15 minutes. Filter the solution into a separator, add 2 ml of *1 M sodium hydroxide* and 10 ml of *chloroform* and shake for 3 minutes. Filter the chloroform layer into a stoppered flask and use the filtrate.

*Reference solution*. Prepare in a similar manner using 10 mg of *biperiden hydrochloride RS* in place of the substance under examination.

Apply to the plate 20 µl of each solution. After development, dry the plate in air and expose it to iodine vapours till spots appear. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

## Tests

**Dissolution** (2.5.2).

Apparatus. No. 1,
Medium. 500 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm and 45 minutes.



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Withdraw 75 ml of the solution and filter through a membrane filter disc with an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate. Transfer 50.0 ml of the clear filtrate into a suitable container, adjust the pH to 5.3 with *0.1 M sodium hydroxide*. Transfer this solution to a 100-ml volumetric flask and dilute with *water* to volume and mix.

Prepare a reference solution by weighing accurately about 80 mg of *biperiden hydrochloride RS* in sufficient *methanol* to produce 100.0 ml. Dilute 5.0 ml of this solution to 500.0 ml with *0.1 M hydrochloric acid* and mix. Transfer 25.0 ml of the resulting solution into a suitable container and adjust the pH to 5.3 with *0.1 M sodium hydroxide* and dilute to 100.0 ml with *water* (2 µg per ml).

Prepare a blank solution by treating 50 ml of *water* in place of the clear filtrate in the same manner as described for the test solution beginning at the words "adjust the pH to 5.3.....

Transfer 20.0 ml of the solutions into individual separators, each containing 10.0 ml of *phosphate-buffered bromocresol purple solution*. Add 40.0 ml of *chloroform* to each and shake for 10 minutes. After the layers have separated, filter each chloroform extract through a filter paper into separate, glass-stoppered flasks, discarding the first 10 ml of each filtrate.

Measure the absorbances of the solutions at the maximum at about 408 nm (2.4.7) against the blank solution. Calculate the content of $C_{21}H_{29}NO$,HCl in the medium from the absorbance obtained from the reference solution.

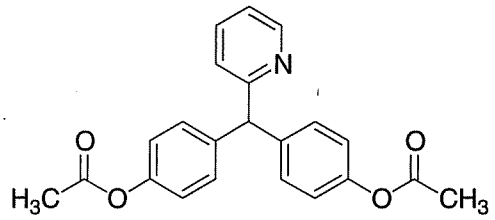
D. Not less than 75 per cent of the stated amount of $C_{21}H_{29}NO$, HCl.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 2 mg of Biperiden Hydrochloride and transfer to a 50-ml volumetric flask, add 12.5 ml of *water* and heat on a steam-bath for 15 minutes. Cool, dilute with *methanol* to volume and mix. Transfer 5.0 ml of the resulting solution to a separator, add 10.0 ml of *phosphate-buffered bromocresol purple solution*, extract with two quantities, each of 20 ml, of *chloroform* and allow to separate. Filter the chloroform extracts into a 50-ml volumetric flask through filter paper and make to volume. Measure the absorbance of the resulting solution at the maximum at about 408 nm (2.4.7), using a reagent blank of a mixture of 3 volumes of *methanol* and 1 volume of *water* and preparing the solution in a similar manner as that of the test solution omitting the substance under examination. Calculate the content of $C_{21}H_{29}NO$, HCl from the absorbance obtained by repeating the operation using a solution prepared by adding 5.0 ml of a 0.08 per cent w/v solution of *biperiden hydrochloride RS* in *methanol* to 25 ml of *water*, diluting to 100.0 ml with *methanol* and treating in the same manner as the test solution.

**Storage**. Store protected from light.

# Bisacodyl




$C_{22}H_{19}NO_4$ Mol. Wt. 361.4

Bisacodyl is bis(4-acetoxyphenyl)-2-pyridylmethane.

Bisacodyl contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{22}H_{19}NO_4$, calculated on the dried basis.

**Category**. Laxative.

**Dose**. 5 to 10 mg daily.

**Description**. A white or almost white, crystalline powder; odourless.

## Identification

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

B. When examined in the range 230 nm to 360 nm, a 0.001 per cent w/v solution in *0.1 M potassium hydroxide* in *methanol* shows an absorption maximum only at about 248 nm; absorbance at about 248 nm, about 0.65 (2.4.7).

## Tests

**Acidity or alkalinity**. Shake 1.0 g with 20 ml of *carbon dioxide-free water*, boil, cool and filter. Add 0.2 ml of *0.01 M sodium hydroxide* and 0.1 ml of *methyl red solution*. The resulting solution is yellow and not more than 0.4 ml of *0.01 M hydrochloric acid* is required to change the colour of the solution to red.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. A mixture of 4 volumes of *glacial acetic acid*, 30 volumes of *acetonitrile* and 66 volumes of *water*.

*Test solution*. Dissolve 0.5 g of substance under examination in 25 ml of *acetonitrile* and dilute to 50.0 ml with the solvent mixture.



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*Reference solution (a).* Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

*Reference solution (b).* Dissolve 2 mg of *bisacodyl for system suitability RS* (containing bisacodyl impurity A, B, C, D and E) in 1.0 ml of *acetonitrile* and dilute to 2.0 ml with the solvent mixture.

*Reference solution (c).* Dissolve 5 mg of *bisacodyl for peak identification RS* (containing bisacodyl impurity F) in 2.5 ml of *acetonitrile* and dilute to 5.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 45 volumes of *acetonitrile* and 55 volumes of 0.16 per cent w/v solution of *ammonium formate,* adjust the pH to 5.0 with *anhydrous formic acid,*
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume. 20 µl.

Inject reference solution (a). The relative retention time with reference to bisacodyl for 4,4'- (pyridine-2-ylmethylene)diphenol (bisacodyl impurity A) is about 0.2, for 2-((RS)-(4-hydroxyphenyl)(pyridine-2-yl)methyl)phenol (bisacodyl impurity B) is about 0.4, for 4-((RS)-(4-hydroxyphenyl)(pyridine-2-yl)methyl)phenyl acetate (bisacodyl impurity C) is about 0.45, for bisacodyl impurity D is about 0.8, for 2-((RS)-(4-acetyloxy)phenyl)(pyridine-2-yl)methyl)phenyl acetate (bisacodyl impurity E) is about 0.9, and for bisacodyl impurity F is about 2.6.

Inject reference solution (b). The test is not valid unless the peak-to -valley ratio between the peaks due to bisacodyl impurity E and bisacodyl is not less than 1.5.

Inject the test solution and reference solution (a). In the chromatogram obtained with the test solution, the area of secondary peaks corresponding to bisacodyl impurity A and B is not more than the area of corresponding peak in the chromatogram obtained with reference solution (a) (0.1 per cent), the area of secondary peak corresponding to bisascodyl impurity C and E is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent), the area of secondary peak corresponding to bisacodyl impurity D is not more than twice the area of corresponding peak in the chromatogram obtained with reference solution (a) (0.2 per cent), the area of secondary peak corresponding to bisacodyl impurity F is not more than 3 times the area of corresponding peak in the chromatogram obtained with reference solution (a) (0.3 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and sum of areas of all secondary peaks is not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 0.5 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.3 g and dissolve in 50 ml of *anhydrous glacial acetic acid.* Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03614 g of $C_{22}H_{19}NO_4$.

**Storage**. Store protected from light.

## Bisacodyl Suppositories

Bisacodyl Suppositories contain Bisacodyl in a suitable suppository basis.

Bisacodyl Suppositories contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of bisacodyl, $C_{22}H_{19}NO_4$.

**Usual strengths.** 5 mg; 10 mg.

### Identification

A. Dissolve a quantity of the suppositories containing 0.15 g of Bisacodyl as completely as possible in 150 ml of *light petroleum (40° to 60°),* filter, wash the residue with *light petroleum (40° to 60°)* until free from fatty material and dry at about 100°. Wash with a very small quantity of warm *chloroform* and dissolve the residue in 10 ml of a 1 per cent w/v solution of *sulphuric acid* (solution A). To 2 ml of the solution add one drop of *potassium mercuri-iodide solution*; a white precipitate is produced.

B. To 2 ml of the solution A add *sulphuric acid*; a reddish violet colour is produced.

C. Boil 2 ml of the solution A with a few drops of *nitric acid*; a yellow colour is produced. Cool and add *5 M sodium hydroxide*; the colour becomes yellowish brown.

### Tests

**Other tests**. Comply with the tests stated under Suppositories.

**Assay**. Weigh accurately a quantity of the suppositories containing about 50 mg of Bisacodyl, add 80 ml of *anhydrous*



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*glacial acetic acid* previously neutralised with *0.02 M perchloric acid* to *1-naphtholbenzein solution* and warm gently until solution is complete. Immediately titrate with *0.02 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.02 M perchloric acid* is equivalent to 0.007228 g of $C_{22}H_{19}NO_4$.

**Storage**. Store protected from light at a temperature not exceeding 30°.

## Bisacodyl Tablets

Bisacodyl Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of bisacodyl, $C_{22}H_{19}NO_4$. The tablets are rendered gastro-resistant by enteric coating or by other means.

**Usual strength**. 5 mg.

### Identification

A. Extract a quantity of the powdered tablets containing 50 mg of Bisacodyl with *chloroform*, filter, evaporate the filtrate to dryness and dissolve the residue in 10 ml of a 1 per cent w/v solution of *sulphuric acid* (solution A). To 2 ml of the solution add one drop of *potassium mercuri-iodide solution*; a white precipitate is produced.

B. To 2 ml of solution A add *sulphuric acid*; a reddish-violet colour is produced.

C. Boil 2 ml of solution A with a few drops of *nitric acid*; a yellow colour is produced. Cool and add *5 M sodium hydroxide*; the colour becomes yellowish-brown.

### Tests

**Uniformity of content**. Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14). As described under Assay using following test solution.

*Test solution.* Crush one tablet and disperse in 50 ml of the solvent mixture. Dilute 25.0 ml of this solution to 100.0 ml with the solvent mixture.

**Other tests**. Comply with the tests stated under Tablets.

In the test for Disintegration, use a 1.5 per cent w/v solution of *sodium bicarbonate* in place of mixed *phosphate buffer pH 6.8*.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture.* A mixture of 4 volumes of *glacial acetic acid,* 30 volumes of *acetonitrile* and 66 volumes of *water.*

*Test solution.* Weigh and powder 20 tablets. Disperse a quantity of powder tablets containing about 10 mg of Bisacodyl with 50 ml of the solvent mixture. Dilute 25.0 ml of this solution to 100.0 ml with the solvent mixture.

*Reference solution.* A 0.005 per cent w/v solution of *bisacodyl RS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with base deactivated octadecylsilane bonded to porous silica (5 µm) ( such as Symmetry C18),
- mobile phase: a mixture of 45 volumes of *acetonitrile* and 55 volumes of *0.025 M ammonium formate*, adjusted to pH 5.0 with *anhydrous formic acid,*
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume. 50 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not less than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{22}H_{19}NO_4$ in the tablets.

## Bismuth Subcarbonate

Bismuth Carbonate

$[Bi_2(CO_3)O_2]$

$CBi_2O_5$ Mol. Wt. 510.0

Bismuth Subcarbonate contains not less than 80.0 per cent and not more than 82.5 per cent of Bi, calculated on the dried basis.

**Category**. Antacid.

**Dose**. 1 to 4 g.

**Description**. A white or almost white powder; odourless.

### Identification

A. Gives the reactions of bismuth salts (2.3.1).

B. Gives reaction A of carbonates (2.3.1).

### Tests

**Appearance of solution**. Shake 5.0 g with 10 ml of *water,* add 20 ml of *nitric acid.* Heat to dissolve, cool and dilute to 100 ml with *water* (solution A). Solution A is not more opalescent than opalescence standard OS2 (2.4.1), and is colourless (2.4.1).

**Alkalis and alkaline-earth metals**. Not more than 1.0 per cent, determined by the following method. To 1.0 g add 10 ml of *water* and 10 ml of *5 M acetic acid*, boil for 2 minutes, cool, filter and wash the residue with 20 ml of *water*. To the combined filtrate and washings add 2 ml of *2 M hydrochloric acid* and



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20 ml of *water*. Boil, pass hydrogen sulphide through the boiling solution until no further precipitate is produced, filter and wash the residue with *water*. Evaporate the combined filtrate and washings to dryness on a water-bath and add 0.5 ml of *sulphuric acid*, ignite gently and allow to cool.

**Arsenic** (2.3.10). To 0.5 g in a distillation flask add 5 ml of *water* and 7 ml of *sulphuric acid*, cool and add 5 g of *hydrazine reducing mixture* and 10 ml of *hydrochloric acid*. Connect the flask to an air-condenser, heat gradually to boiling during 15 to 30 minutes and continue heating at such a rate that the distillation proceeds steadily and until the volume in the flask is reduced by half, or until 5 minutes after the condenser has become full of steam. Discontinue distillation before fumes of sulphur trioxide are evolved. Collect the distillate in a tube containing 15 ml of *water* cooled in ice. Wash the condenser with *water* and dilute the combined distillate and washings to 25 ml with *water*. The resulting solution complies with the limit test for arsenic (5 ppm). Use 2.5 ml of *arsenic standard solution (1 ppm As)* diluted to 25 ml with *water* to prepare the standard.

**Copper**. To 5 ml of solution A add 2 ml of *10 M ammonia*, dilute to 50 ml with *water* and filter. To 10 ml of the filtrate add 1 ml of a 0.1 per cent w/v solution of *sodium diethyldithiocarbamate*. Any colour produced is not more intense than that produced by treating at the same time and in the same manner a solution containing 0.25 ml of *copper standard solution (10 ppm Cu)* diluted to 10 ml with *water* (50 ppm).

**Lead**. To 10 ml of solution A add 10 ml of *1 M sulphuric acid*; the solution does not become cloudy.

**Silver**. To 2.0 g add 1 ml of *water* and 4 ml of *nitric acid*. Heat gently to dissolve and dilute to 11 ml with *water*. Cool, add 2 ml of *1 M hydrochloric acid* and allow to stand for 5 minutes protected from light. Any opalescence produced is not more intense than that obtained by treating at the same time and in the same manner a mixture of 10 ml of *silver standard solution (5 ppm Ag)*, 2 ml of *1 M hydrochloric acid* and 1 ml of *nitric acid* (25 ppm).

**Chlorides** (2.3.12). To 10 ml of solution A add 4 ml of *nitric acid* and 20 ml of *water*; the resulting solution complies with the limit test for chlorides (500 ppm).

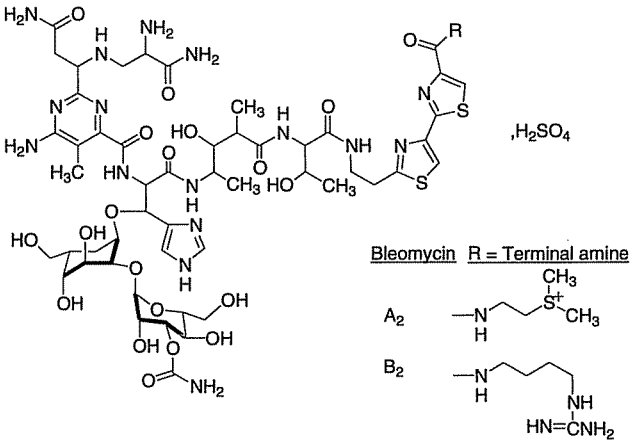
**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.5 g, dissolve in 3 ml *of nitric acid* and dilute to 250 ml with *water*. Add *strong ammonia solution* until cloudiness is first observed, add 0.5 ml of *nitric acid* and heat to 70°, maintaining the solution at this temperature until the solution becomes completely clear. Add about 50 mg of *xylenol orange mixture* and titrate with *0.1 M disodium edetate* until the colour changes from pinkish-violet to lemon yellow.

1 ml of *0.1 M disodium edetate* is equivalent to 0.02090 g of Bi.

**Storage**. Store protected from light.

## Bleomycin Sulphate




$C_{55}H_{84}N_{17}O_{21}S_3,H_2SO_4$ Mol. Wt. 1513.6
(Bleomycin $A_2$ Sulphate)

$C_{55}H_{84}N_{20}O_{21}S_2,H_2SO_4$ Mol. Wt. 1523.6
(Bleomycin $B_2$ Sulphate)

Bleomycin Sulphate is the sulphate salt of bleomycin, a mixture of basic cytotoxic glycopeptides produced by the growth of *Streptomyces verticillus* or produced by other means. Its main components are bleomycin $A_2$ and bleomycin $B_2$. Bleomycin $A_2$ sulphate is $N^1$-[3 (dimethylsulphonio)propyl]bleomycinamide hydrogen sulphate and Bleomycin $B_2$ is $N^1$-(guanidinobutyl)bleomycinamide sulphate.

Bleomycin Sulphate contains not less than 1.5 and not more than 2.0 Units of bleomycin per mg and the content of bleomycins is: bleomycin $A_2$, between 55 per cent and 70 per cent;bleomycin B2, between 25 per cent and 32 per cent; sum of bleomycin $A_2$ and bleomycin $B_2$, not less than 85 per cent; demethylbleomycin A2, not more than 5.5 per cent; other related substances, not more than 9.5 per cent.

**Category**. Anticancer.

**Dose**. By injection, the equivalent of 15 to 30 Units of bleomycin weekly, in divided doses.

**Description**. A white or cream-coloured, amorphous powder.

*CAUTION — Bleomycin Sulphate must be handled with care, avoiding contact with the skin and inhalation of airborne particles.*



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### Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *bleomycin sulphate RS*.

B. Gives the reactions of sulphates (2.3.1).

### Tests

**pH** (2.4.24). 4.5 to 6.0, determined in a solution containing 10 Units per ml.

**Copper**. Not more than 0.02 per cent determined by Method A or by Method B.

A. Weigh accurately about 50 mg, transfer to a 60-ml separator and dissolve in 10.0 ml of *0.1 M hydrochloric acid*. Add 10 ml of a 0.01 per cent w/v solution of *zinc bis (diphenyl dithiocarbamate)* in *carbon tetrachloride* and shake vigorously for 1 minute. Allow the layers to separate, filter the lower layer through 1 g of *anhydrous sodium sulphate*. Treat similarly 10.0 ml of *copper standard solution (10 ppm Cu)* and measure the absorbances (2.4.7) of the two solutions at the maximum at about 435 nm, using *carbon tetrachloride* as the blank.

B. Determine by atomic absorption spectrophotometry (2.4.2) measuring at 324.7 nm using an air-acetylene flame and a solution prepared in the following manner: Dissolve about 50 mg of the substance under examination in *water* and dilute to 10.0 ml with the same solvent. Use *copper solution AAS* suitably diluted with *water*, for preparing the reference solutions.

**Content of bleomycins**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve the substance under examination in freshly boiled and cooled *water* so as to give a solution containing about 2.5 Units per ml. *This solution should be stored at 2° to 8° until just before use.*

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Nucleosil C18),
- mobile phase: Transfer 0.96 g of *sodium 1-pentanesulphonate* to a 1000-ml volumetric flask, add 5.0 ml of *glacial acetic acid* and 900 volumes of *water*. Mix and adjust the pH to 4.3 with *strong ammonia solution* (1.86 g of *disodium edetate* may be included if needed for satisfactory chromatography). Adjust the volume with *water*, mix well, filter and degas before use. Use a linear gradient of 10 per cent to 40 per cent *methanol*, which also is filtered and degassed before use, mixed with this solution,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

After the final conditions are reached (about 60 minutes) allow the chromatography to proceed with the final gradient mixture for an additional 20 minutes or until demethylbleomycin $A_2$ is eluted.

Inject the test solution and proceed with gradient elution, pumping the mobile phase mixture under the conditions mentioned above for about 80 minutes or until the demethylbleomycin $A_2$ is eluted. The usual order of elution is bleomycinic acid, bleomycin $A_2$ (first principal peak), bleomycin $A_5$, bleomycin $B_2$ (second principal peak), bleomycin $B_4$ and demethylbleomycin $A_2$ (retention time relative to bleomycin $A_2$, between 1.5 and 2.5).

Measure the peak responses of all peaks. Calculate the percentage contents of each bleomycin component by comparing the ratios of the individual areas of the peaks with that of the total area of all bleomycins.

**Loss on drying** (2.4.19). Not more than 3.0 per cent, determined on 50 mg by drying in an oven over *phosphorous pentoxide* at 60° at a pressure not exceeding 0.25 kPa for 3 hours.

**Assay**. Determine by the microbiological assay of antibiotics, Method A or B (2.2.10), and express the result in Units per mg.

*Bleomycin Sulphate intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.*

**Bacterial endotoxins** (2.2.3). Not more than 10.0 Endotoxin Units per unit of bleomycin.

*Bleomycin Sulphate intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.*

**Sterility** (2.2.11). Complies with the test for sterility.

**Storage**. If the material is sterile, it should be stored in sterile, tamper-evident containers and sealed so as to exclude micro-organisms.

**Labelling**. The label states (1) the strength with respect to Bleomycin Sulphate as the number of bleomycin Units per mg; (2) whether or not the contents are intended for use in the manufacture of parenteral preparations.

## Bleomycin Injection

Bleomycin sulphate injection

Beomycin injection is a sterile freeze dried material consisting of Bleomycin sulphate with or without excipients. It is filled in a sealed container.



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The injection is constituted by dissolving the contents of the sealed container in the requisite amount of the liquid stated on the label before use.

*The constituted solution complies with the tests for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).*

**Storage**. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Bleomycin injection contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of bleomycin and the content of bleomycins is: bleomycin $A_2$, between 55 per cent and 70 per cent;bleomycin $B_2$, between 25 and 32 per cent; sum of bleomycin $A_2$ and bleomycin $B_2$, not less than 85 per cent; demethylbleomycin $A_2$, not more than 5.5 per cent; other related substances, not more than 9.5 per cent.

*The contents of the sealed container comply with the tests stated under Parenteral Preparations (Powders for Injection) and with the following requirements.*

**Usual strength**. 15 mg per ml.

## Identification

A. Determine by infra-red absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *bleomycin sulphate RS*.

B. Gives the reactions of sulphates (2.3.1).

## Tests

**pH** (2.4.24). 4.5 to 6.0, determined in a solution containing 10 Units per ml.

**Copper**. Not more than 0.02 per cent, determined by Method A or Method B

A. Weigh accurately a quantity containing about 50 mg of bleomycin, transfer to a 60-ml separator and dissolve in 10.0 ml of *0.1 M hydrochloric acid*. Add 10 ml of a 0.01 per cent w/v solution of *zinc bis (diphenyl dithiocarbamate)* in *carbon tetrachloride* and shake vigorously for 1 minute. Allow the layers to separate, filter the lower layer through 1 g of *anhydrous sodium sulphate*. Treat similarly 10.0 ml of *copper standard solution (10 ppm Cu)* and measure the absorbances (2.4.7) of the two solutions at the maximum at about 435 nm, using *carbon tetrachloride* as the blank.

B. Determine by atomic absorption spectrophotometry (2.4.2) measuring at 324.7 nm using an air-acetylene flame and a solution prepared in the following manner: Weigh accurately a quantity containing about 75 mg of bleomycin, dissolve in *water* and dilute to 10 .0 ml with the same solvent. Use *copper solution AAS* suitably diluted with *water*, for preparing the reference solutions.

**Content of bleomycin**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh accurately a suitable quantity dissolve in freshly boiled and cooled *water* and dilute to obtain a solution containing about 2.5 Units per ml. *This solution should be stored at 2° to 8° until just before use.*

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Nucleosil 7C18),
- mobile phase: Transfer 0.96 g of *sodium 1-pentanesulphonate* to a 1000-ml volumetric flask, add 5.0 ml of *glacial acetic acid* and 900 volumes of *water*. Mix and adjust the pH to 4.3 with *strong ammonia solution* (1.86 g of *disodium edetate* may be included if needed for satisfactory chromatography). Adjust the volume with *water*, mix well, filter and degas before use. Use a linear gradient of 10 per cent to 40 per cent *methanol*, which also is filtered and degassed before use, mixed with this solution,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

After the final conditions are reached (about 60 minutes) allow the chromatography to proceed with the final gradient mixture for an additional 20 minutes or until demethylbleomycin $A_2$ is eluted.

Inject the test solution and proceed with gradient elution, pumping the mobile phase mixture under the conditions mentioned above for about 80 minutes or until the demethylbleomycin $A_2$ is eluted. The usual order of elution is bleomycinic acid, bleomycin $A_2$ (first principal peak), bleomycin $A_5$, bleomycin $B_2$ (second principal peak), bleomycin $B_4$ and demethylbleomycin $A_2$ (retention time relative to bleomycin $A_2$, between 1.5 and 2.5).

Measure the peak responses of all the peaks. Calculate the percentage contents of each bleomycin component by comparing the ratios of the individual areas of the peaks with that of the total area of all bleomycins.

**Bacterial endotoxins** (2.2.3). Not more than 10.0 Endotoxin Units per unit of bleomycin.

**Loss on drying** (2.4.19). Not more than 6.0 per cent, determined by drying the combined contents of two containers in an oven at 60° at a pressure not exceeding 0.7 kPa for 3 hours.

**Assay**. Determine the weight of the contents of 10 containers. Mix the contents of the containers and determine by the microbiological assay of antibiotics, Method A or B (2.2.10) and express the results in Units per vial.

**Storage**. The sealed container should be protected from light.



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**Labelling**. The label states the total number of units contained in the sealed container.

## Boric Acid

$H_3BO_3$ Mol. Wt. 61.8

Boric Acid contains not less than 99.5 per cent and not more than 100.5 per cent of $H_3BO_3$, calculated on the dried basis.

**Category**. Local antiinfective.

**Description**. A white, crystalline powder or colourless shiny plates unctuous to the touch or white crystals; odourless.

### Identification

A. Dissolve 0.1 g by gently warming with 5 ml of *methanol* to which a few drops of *sulphuric acid* have been added. Ignite the solution; the flame has a green border.

B. Dissolve 3.0 g in 90 ml of boiling *distilled water*, cool; the solution is faintly acid.

### Tests

**Appearance of solution**. A 3.5 per cent w/v solution in boiling *water* is clear (2.4.1), and colourless (2.4.1).

**pH** (2.4.24). 3.8 to 4.8, determined in the solution obtained in Identification test B.

**Solubility in ethanol**. Dissolve 1.0 g in 10 ml of boiling *ethanol (95 per cent)*; the solution is not more opalescent than opalescence standard OS2 (2.4.1), and colourless (2.4.1).

**Arsenic** (2.3.10). Dissolve 1.0 g in 50 ml of *water* containing 2 g of *citric acid* and add 0.1 ml of *stannous chloride solution AsT* and 10 ml of *hydrochloric acid*. The resulting solution complies with the limit test for arsenic (10 ppm).

**Heavy metals** (2.3.13). A solution produced by dissolving 1.0 g in 2 ml of *dilute acetic acid* and diluting with sufficient *water* to produce 25 ml complies with the limit test for heavy metals, Method A (20 ppm).

**Sulphates** (2.3.17). Dissolve 0.33 g in 10 ml of boiling *water*, and dilute to 15 ml with *water*. The solution complies with the limit test for sulphates (450 ppm).

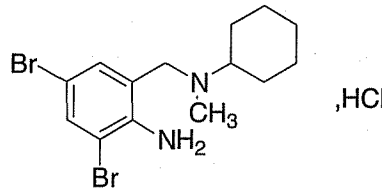
**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying over *silica gel* for 5 hours.

**Assay**. Weigh accurately about 2.0 g, dissolve in a mixture of 50 ml of *water* and 100 ml of *glycerin*, previously neutralised to *phenolphthalein solution*. Titrate with *1 M sodium hydroxide* using *phenolphthalein solution* as indicator.

1 ml of *1 M sodium hydroxide* is equivalent to 0.06183 g of $H_3BO_3$.

**Labelling**. The label states that it is not meant for internal use.

## Bromhexine Hydrochloride




$C_{14}H_{20}Br_2N_2,HCl$ Mol. Wt. 412.6

Bromhexine Hydrochloride is 2-amino-3,5-dibromobenzyl(cyclohexyl)methylamine hydrochloride.

Bromhexine Hydrochloride contains not less than 98.5 per cent and not more than 101.5 per cent of $C_{14}H_{20}Br_2N_2,HCl$, calculated on the dried basis.

**Category**. Expectorant.

**Dose**. 8 to 16 mg three to four times daily.

**Description**. A white or almost white, crystalline powder; odourless or almost odourless.

### Identification

*Test A may be omitted if tests B, C, D and E are carried out. Tests B, C and D may be omitted if tests A and E are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *bromhexine hydrochloride RS* or with the reference spectrum of bromhexine hydrochloride.

B. In the test for Related Substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (c).

C. Dissolve about 25 mg in a mixture of 1 ml of *1 M sulphuric acid* and 50 ml of *water*, add 2 ml of *dichloromethane* and 5 ml of a freshly prepared 2 per cent w/v solution of *chloramine T* and shake; a brownish yellow colour is produced in the lower layer.

D. A solution prepared by dissolving about 1 mg in 3 ml of *0.1 M hydrochloric acid* gives the reaction for primary aromatic amines (2.3.1).

E. Dissolve about 20 mg in 1 ml of *methanol* and add 1 ml of *water*. The solution gives reaction A of chlorides (2.3.1).

### Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 50 mg of the substance under examination in 10 ml of *methanol*.



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*Reference solution (a).* Dissolve 5 mg of *bromhexine impurity C RS* in *methanol*, add 1.0 ml of the test solution and dilute to 10.0 ml with the same solvent.

*Reference solution (b).* Dilute 1.0 ml of the test solution to 100.0 ml with *methanol*. Dilute 1.0 ml of this solution to 10.0 ml with the same solvent.

Chromatographic system
- a stainless steel column 12 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (3 µm),
- mobile phase: a mixture of 20 volumes of buffer solution prepared by dissolving 0.5 ml of *orthophosphoric acid* to 950.0 ml of *water*, adjust the pH to 7.0 with *triethylamine*, dilute to 1000 ml and 80 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 248 nm,
- injection volume. 10 µl.

The relative retention time with reference to bromhexine hydrochloride for bromhexine hydrochloride impurity A is about 0.1, for bromhexine hydrochloride impurity B is about 0.2, for bromhexine hydrochloride impurity C is about 0.4 and for bromhexine hydrochloride impurity D is about 0.5.

Inject reference solution (a). The test is not valid unless the resolution between the peak due to bromhexine hydrochloride and bromhexine hydrochloride impurity C is not less than 12.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent), the area of one such peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and sum of areas of all secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.3 g, dissolve in 70 ml of *ethanol (95 per cent)*, add 1 ml of *0.1 M hydrochloric acid* and titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25). Record the volume added between the two inflections.

1ml of *0.1 M sodium hydroxide* is equivalent to 0.04126 g of $C_{14}H_{20}Br_2N_2,HCl$.

**Storage**. Store protected from light.

# Bromhexine Tablets

Bromhexine Hydrochloride Tablets

Bromhexine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of bromhexine hydrochloride, $C_{14}H_{20}Br_2N_2,HCl$.

**Usual strengths**. 4 mg; 8 mg.

## Identification

A. When examined in the range 230 nm to 360 nm (2.4.7), the solution obtained in the Assay shows an absorption maximum only at about 317 nm.

B. Suspend a quantity of the powdered tablets containing 0.1 g of Bromhexine Hydrochloride in 5 ml of *dilute ammonia solution* and extract with two quantities, each of 20 ml, of *chloroform*. Wash the combined extracts with 5 ml of *water*, filter through *anhydrous sodium sulphate* and evaporate the filtrate to dryness using a rotary evaporator. If necessary, scratch the inside of the flask with a glass rod to induce crystallisation. Mix the residue with 1 g of *sodium carbonate*, heat at a dull red heat for 10 minutes, allow to cool, extract with *water* and filter. The filtrate, after acidification with *2 M nitric acid*, yields reaction A of bromides (2.3.1).

C. Shake a quantity of the powdered tablets containing 20 mg of Bromhexine Hydrochloride with 10 ml *methanol* and filter. The filtrate gives reaction A of chlorides (2.3.1).

## Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Disperse a quantity of the powdered tablets containing 50 mg of Bromhexine Hydrochloride in 10 ml of *methanol*.

*Reference solution (a).* Dissolve 5 mg of *bromhexine impurity C RS* in *methanol*, add 1.0 ml of the test solution and dilute to 10.0 ml with the same solvent.

*Reference solution (b).* Dilute 1.0 ml of the test solution to 100.0 ml with *methanol*. Dilute 1.0 ml of this solution to 10.0 ml with the same solvent.

Chromatographic system
- a stainless steel column 12 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (3 µm),
- mobile phase: a mixture of 20 volumes of buffer solution prepared by dissolving 0.5 ml of *orthophosphoric acid* to 950.0 ml of *water*, adjust the pH to 7.0 with *triethylamine*, dilute to 1000 ml and 80 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,



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– spectrophotometer set at 248 nm,
– injection volume. 10 µl.

The relative retention time with reference to bromhexine hydrochloride for bromhexine hydrochloride impurity A is about 0.1, for bromhexine hydrochloride impurity B is about 0.2, for bromhexine hydrochloride impurity C is about 0.4 and for bromhexine hydrochloride impurity D is about 0.5.

Inject reference solution (a). The test is not valid unless the resolution between the peak due to bromhexine hydrochloride and bromhexine hydrochloride impurity C is not less than 12.0.

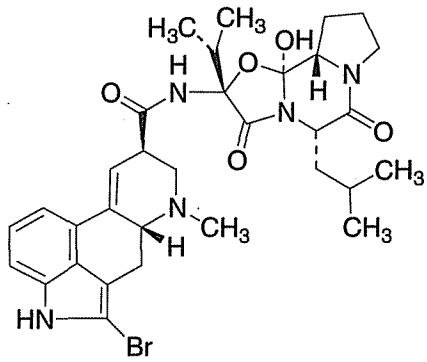
Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent), the area of one such peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and sum of areas of all secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 8 mg of Bromhexine Hydrochloride, shake with 50 ml of *0.1 M methanolic hydrochloric acid* for 30 minutes, add sufficient *0.1 M methanolic hydrochloric acid* to produce 100.0 ml and filter. Measure the absorbance of the filtrate at the maximum at about 317 nm (2.4.7). Calculate the content of $C_{14}H_{20}Br_2N_2$,HCl taking 87 as the specific absorbance at 317 nm.

**Storage**. Store protected from light.

# Bromocriptine Mesylate




, $CH_3SO_3H$

$C_{32}H_{40}BrN_5O_5,CH_4O_3S$ Mol. Wt. 750.7

Bromocriptine Mesylate is (5'*S*)-2-bromo-12'-hydroxy-2'-(1-methylethyl)-5'-(2-methylpropyl)ergotaman-3',6',18-trione methanesulphonate

Bromocriptine Mesylate contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{32}H_{40}BrN_5O_5,CH_4O_3S$, calculated on the dried basis.

**Category**. Antiparkinsonian.

**Dose**. The equivalent of 2.5 mg to 20 mg of bromocriptine daily, in divided doses.

**Description**. A white or slightly coloured, fine crystalline powder; very sensitive to light.

*NOTE — Carry out the tests as rapidly as possible without exposure to daylight and with minimum exposure to artificial light.*

## Identification

*Test A may be omitted if tests B, C, D and E are carried out. Tests B, C, D and E may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry in a mineral oil dispersion (2.4.6). Compare the spectrum with that obtained with *bromocriptine mesylate RS* or with the reference spectrum of bromocriptine mesylate.

B. Dissolve 5 mg in 5 ml of *methanol* and dilute to 100 ml with *0.01 M hydrochloric acid*. The resulting solution, when examined in the range 230 nm to 360 nm (2.4.7) shows an absorption maximum at about 305 nm and a minimum at about 270 nm; absorbance at about 305 nm, 0.60 to 0.68.

C. In the test for Related substances, the principal band in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (d).

D. To about 0.1 g add 5 ml of *2 M hydrochloric acid*, shake for 5 minutes, filter and add 1 ml of a 6 per cent w/v solution of *barium chloride* to the filtrate; it remains clear. Mix another 0.1 g with 0.5 g of *anhydrous sodium carbonate* and ignite until a white residue is obtained. After cooling, dissolve the residue in 5 ml of water (solution A); solution A gives the reactions of sulphates (2.3.1).

E. Solution A gives reaction A of bromides (2.3.1).

## Tests

**Appearance of solution**. A 1.0 per cent w/v solution in *methanol* is clear (2.4.1), and not more intensely coloured than reference solution BS5, YS5 *or* BYS5 (2.4.1).

**pH** (2.4.24). 3.1 to 3.8, determined in a 1.0 per cent w/v solution in a mixture of 2 volumes of *methanol* and 8 volumes of *water*.



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**Specific optical rotation** (2.4.22). + 95° to + 105°, determined in a 1.0 per cent w/v solution in a mixture of equal volumes of *methanol* and *dichloromethane*.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. A mixture of 50 volumes of buffer solution pH 2.0 and 50 volumes of *methanol*.

*Test solution*. Dissolve 50 mg of the substance under examination in 5.0 ml of *methanol* and dilute to 10.0 ml with buffer solution pH 2.0.

*Reference solution (a)*. Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture.

*Reference solution (b)*. Dilute 1.0 ml of the reference solution (a) to 10.0 ml with the solvent mixture.

*Reference solution (c)*. Dissolve the contents of a vial of *bromocriptine mesilate for system suitability RS* (containing bromocriptine mesilate impurity A and B) in 1.0 ml of the solvent mixture.

Chromatographic system

- a stainless steel column 12 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5µm),
- mobile phase: A. a mixture of 0.8 per cent w/v solution of 0.079 per cent w/v solution of *ammonium carbonate*, B. *acetonitrile*,
- a linear gradient programme using the conditions given below,
- flow rate. 2 ml per minute,
- spectrophotometer set at 300 nm,
- injection volume. 20 µl.

| Time (in min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0-30 | 90→40 | 10→60 |
| 30-45 | 40 | 60 |

Inject reference solution (b). The relative retention time with reference to bromocriptine for bromocriptine mesilate impurity A is about 1.2.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to 2-bromodehydro-á-ergocriptine (bromocriptine mesilate impurity A) and á-ergocriptine (bromocriptine mesilate impurity B) is not less than 1.1.

Inject the test solution and reference solutions (b). In the chromatogram obtained with the test solution, the area of any secondary peak corresponding to bromocriptine mesilate impurity A is not more than 0.2 times the area of corresponding peak in the chromatogram obtained with reference solution (b) (0.02 per cent), the area of secondary peak corresponding to bromocriptine mesilate impurity C is not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent), the area of secondary peak corresponding to bromocriptine mesilate impurity B, D, E, F, G is not more than twice the area of corresponding peak in the chromatogram obtained with reference solution (a) (0.2 per cent) and sum of areas of all other secondary peaks is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent) (except the peak due to bromocriptine mesilate impurity A).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 3.0 per cent, determined on 0.5 g by drying in an oven over *phosphorus pentoxide* at 80° at a pressure of 1.5 to 2.5 kPa for 5 hours.

**Assay**. Weigh accurately about 0.5 g, dissolve in 80 ml of a mixture of 10 volumes of *anhydrous glacial acetic acid* and 70 volumes of *acetic anhydride*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25) Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.07507 g of $C_{32}H_{40}BrN_5O_5,CH_4O_3S$.

**Storage**. Store protected from light in a deep freezer (temperature not exceeding –15°).

# Bromocriptine Capsules

## Bromocriptine Mesylate Capsules

Bromocriptine Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of bromocriptine, $C_{32}H_{40}BrN_5O_5$.

**Usual strengths**. 5 mg; 10 mg.

*NOTE — Carry out the tests as rapidly as possible without exposure to daylight and with minimum exposure to artificial light.*

### Identification

A. Shake a quantity of the contents of the capsules containing 10 mg of bromocriptine with 50 ml of *methanol* for 30 minutes, centrifuge and dilute 5 ml of the supernatant liquid to 20 ml with *methanol*. When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum at about 305 nm and a minimum at about 270 nm.

B. In the test for Related substances, the principal band in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (d).



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## Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture.* A mixture of 50 volumes of buffer solution pH 2.0 and 50 volumes of *methanol.*

*Test solution.* Shake a quantity of the contents of the capsules containing 50 mg of bromocriptine with 5.0 ml of *methanol* and dilute to 10.0 ml with buffer solution pH 2.0.

*Reference solution (a).* Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture.

*Reference solution (b).* Dilute 1.0 ml of the reference solution (a) to 10.0 ml with the solvent mixture.

*Reference solution (c).* Dissolve the contents of a vial of *bromocriptine mesilate for system suitability RS* (containing bromocriptine mesilate impurity A and B) in 1.0 ml of the solvent mixture.

Chromatographic system

- a stainless steel column 12 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5µm),
- mobile phase: A. a mixture of 0.8 per cent w/v solution of 0.079 per cent w/v solution of *ammonium carbonate,* B. *acetonitrile,*
- a linear gradient programme using the conditions given below,
- flow rate. 2 ml per minute,
- spectrophotometer set at 300 nm,
- injection volume. 20 µl.

| Time (in min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0-30 | 90→401 | 10→60 |
| 30-45 | 40 | 60 |

Inject reference solution (b). The relative retention time with reference to bromocriptine for bromocriptine mesilate impurity A is about 1.2.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to 2-bromodehydro-á-ergocriptine (bromocriptine mesilate impurity A) and á-ergocriptine (bromocriptine mesilate impurity B) is not less than 1.1.

Inject the test solution and reference solutions (b). In the chromatogram obtained with the test solution, the area of any secondary peak corresponding to bromocriptine mesilate impurity A is not more than 0.2 times the area of corresponding peak in the chromatogram obtained with reference solution (b) (0.02 per cent), the area of secondary peak corresponding to bromocriptine mesilate impurity C is not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent), the area of secondary peak corresponding to bromocriptine mesilate impurity B, D, E, F, G is not more than twice the area of corresponding peak in the chromatogram obtained with reference solution (a) (0.2 per cent) and sum of areas of all other secondary peaks is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent) (except the peak due to bromocriptine mesilate impurity A).

**Uniformity of content**. Comply with the test stated under Capsules.

Empty the contents of one capsule, crush, if necessary, add 10.0 ml of *methanol*, shake vigorously and centrifuge. If necessary, dilute the solution appropriately and carry out the procedure described under the Assay beginning at the words "measure the absorbance.....".

**Other tests**. Comply with the tests stated under Capsules.

**Assay**. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 25 mg of bromocriptine and shake vigorously with 30 ml of *methanol*. Dilute to 100.0 ml with *methanol* and filter. Dilute further with *methanol* to yield a final concentration of about 50 mcg per ml and measure the absorbance of the resulting solution at the maximum at about 305 nm (2.4.7). Calculate the content of $C_{32}H_{40}BrN_5O_5$ from the absorbance obtained by repeating the operation using *bromocriptine mesylate RS* equivalent to 25 mg of bromocriptine instead of the substance under examination.

**Storage**. Store protected from light.

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

# Bromocriptine Tablets

## Bromocriptine Mesylate Tablets

Bromocriptine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of bromocriptine, $C_{32}H_{40}BrN_5O_5$.

**Usual strengths**. 1 mg; 2.5 mg.

*NOTE — Carry out the tests as rapidly as possible without exposure to daylight and with minimum exposure to artificial light.*

## Identification

A. Shake a quantity of powdered tablets containing about 20 mg of bromocriptine with 20 ml of *methanol*, filter, evaporate



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the filtrate to dryness on a water-bath and dry at 105° for 1 hour.The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *bromocriptine mesylate RS* or with the reference spectrum of bromocriptine mesylate.

B. Shake a quantity of the powdered tablets containing about 10 mg of bromocriptine with 50 ml of *methanol* for 30 minutes, centrifuge and dilute 5 ml of the supernatant liquid to 20 ml with *methanol*. When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum at about 305 nm and a minimum at about 270 nm.

C. In the test for Related substances, the principal band in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (d).

## Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture.* A mixture of 50 volumes of buffer solution pH 2.0 and 50 volumes of *methanol.*

*Test solution*. Shake a quantity of the powdered tablets containing 50 mg of bromocriptine with 5.0 ml of *methanol* and dilute to 10.0 ml with buffer solution pH 2.0.

*Reference solution (a)*. Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture.

*Reference solution (b)*. Dilute 1.0 ml of the reference solution (a) to 10.0 ml with the solvent mixture.

*Reference solution (c)*. Dissolve the contents of a vial of *bromocriptine mesilate for system suitability RS* (containing bromocriptine mesilate impurity A and B) in 1.0 ml of the solvent mixture.

Chromatographic system

- a stainless steel column 12 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5µm),
- mobile phase: A. a mixture of 0.8 per cent w/v solution of 0.079 per cent w/v solution of *ammonium carbonate,*
  B. *acetonitrile,*
- a linear gradient programme using the conditions given below,
- flow rate. 2 ml per minute,
- spectrophotometer set at 300 nm,
- injection volume. 20 µl.

| Time (in min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0-30 | 90→40 | 10→60 |
| 30-45 | 40 | 60 |

Inject reference solution (b). The relative retention time with reference to bromocriptine for bromocriptine mesilate impurity A is about 1.2.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to 2-bromodehydro-á-ergocriptine (bromocriptine mesilate impurity A) and á-ergocriptine (bromocriptine mesilate impurity B) is not less than 1.1.

Inject the test solution and reference solutions (b). In the chromatogram obtained with the test solution, the area of any secondary peak corresponding to bromocriptine mesilate impurity A is not more than 0.2 times the area of corresponding peak in the chromatogram obtained with reference solution (b) (0.02 per cent), the area of secondary peak corresponding to bromocriptine mesilate impurity C is not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent), the area of secondary peak corresponding to bromocriptine mesilate impurity B, D, E, F, G is not more than twice the area of corresponding peak in the chromatogram obtained with reference solution (a) (0.2 per cent) and sum of areas of all other secondary peaks is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent) (except the peak due to bromocriptine mesilate impurity A).

**Uniformity of content**. Comply with the test stated under Tablets.

Finely crush one tablet, add 10.0 ml of *methanol*, shake vigorously and centrifuge. If necessary, dilute the solution appropriately and carry out the procedure described under the Assay beginning at the words "Measure the absorbance.....".

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 2.5 mg of bromocriptine and shake vigorously with 30 ml of *methanol*. Dilute to 50.0 ml with *methanol* and filter. Measure the absorbance of the resulting solution at the maximum at about 305 nm (2.4.7). Calculate the content of $C_{32}H_{40}BrN_5O_5$ from the absorbance obtained by repeating the operation with *bromocriptine mesylate RS* equivalent to 25 mg of bromocriptine in 50 ml *methanol* and diluting 5.0 ml to 50.0 ml with *methanol*.

**Storage**. Store protected from light.

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

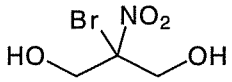


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# Bronopol




$C_3H_6BrNO_4$ Mol. Wt. 200.0

Bronopol is 2-bromo-2-nitropropane-1,3-diol.

Bronopol contains not less than 99.0 per cent and not more than 101.0 per cent of $C_3H_6BrNO_4$, calculated on the anhydrous basis.

**Category**. Antiseptic; local anaesthetic.

**Description**. White or almost white crystals or crystalline powder.

## Identification

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

B. Dissolve 0.1 g in 10 ml of *water*, add 10 ml of *7.5 M sodium hydroxide* and carefully with constant stirring and cooling, 0.5 g of *nickel-aluminium alloy*. Allow the reaction to subside, filter and carefully neutralise with *nitric acid*. The resulting solution gives reaction A of bromides (2.3.1).

## Tests

**pH** (2.4.24). 5.0 to 7.0, determined on 1.0 per cent w/v solution.

**Related substances**. Determine by liquid chromatography (2.4.14)

*Test solution*. Dissolve 0.2 g of the substance under examination in 100 ml of mobile phase.

*Reference solution (a)*. Dilute 5 ml of the test solution to 50 ml with the mobile phase. Further, dilute 1 ml of the solution to 100 ml with the mobile phase.

*Reference solution (b)*. A solution containing 0.001 per cent w/v each of *2-methyl-2-nitropropan-1,3-diol* and *tris(hydroxymethyl)nitromethane* in the mobile phase.

*Reference solution (c)*. A solution containing 0.0002 per cent w/v each of *2-methyl-2-nitropropane-1,3-diol, 2-nitroethanol, sodium bromide* and *tris(hydroxymethyl)-nitromethan* in the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 35°,
- mobile phase: a mixture of 189 volumes of *water*, 10 volumes of *acetonitrile* and 1 volume of a 10 per cent v/v solution of *orthophosphoric acid*, adjusting the pH to 3.0 using *2 M sodium hydroxide*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 214 nm,
- injection volume. 20 µl.

Inject reference solution (c). The test is not valid unless the resolution between the peaks corresponding to sodium bromide and tris (hydroxymethyl) nitromethane is at least 1.0 and the resolution between the peaks corresponding to tris(hydroxymethyl)nitromethane and 2-nitroethanol is at least 1.5.

Inject the test solution, reference solution (a) and reference solution (b). Continue the chromatography for 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any peaks corresponding to 2-methyl-2-nitropropane-1,3-diol and tris (hydroxymethyl) nitromethane are not more than the area of the corresponding peaks in the chromatogram obtained with reference solution (b) (0.5 per cent each) and the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

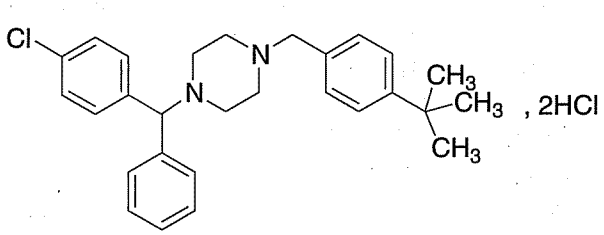
**Water** (2.3.43). Not more than 0.5 per cent, determined on 5.0 g.

**Assay**. In a flask fitted with a reflux condenser dissolve 0.4 g in 15 ml of *water* and add 15 ml of *7.5 M sodium hydroxide*. Slowly, with caution, add 2 g of *nickel-aluminium alloy* through the reflux condenser, agitating the flask whilst cooling under running *water*. Allow the mixture to stand for 10 minutes and boil for 1 hour. Cool and filter under reduced pressure, washing the condenser, flask and residue with 150 ml of *water*. Combine the filtrate and washings, add 25 ml of *nitric acid* and 40 ml of *0.1 M silver nitrate*, shake vigorously and titrate with *0.1 M ammonium thiocyanate* using *ammonium iron(III) sulphate solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M silver nitrate* is equivalent to 0.020 g of $C_3H_6BrNO_4$.

**Storage**. Store protected from light.

# Buclizine Hydrochloride




$C_{28}H_{33}ClN_2,2HCl$ Mol. Wt. 506.0

Buclizine Hydrochloride is (*RS*)-1-(4-*tert*-butylbenzyl)-4-(4-chlorobenzhydryl)piperazine dihydrochloride



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Buclizine Hydrochloride contains not less than 99.0 per cent and not more than 100.5 per cent of $C_{28}H_{33}ClN_2,2HCl$, calculated on the dried basis.

**Category**. Antihistaminic; antiemetic.

**Description**. A white or slightly yellowish, crystalline powder.

### Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *buclizine dihydrochloride RS* or with the reference spectrum of buclizine dihydrochloride.

B. A 0.25 per cent w/v solution in *ethanol (50 per cent)* gives reaction A of chlorides (2.3.1).

### Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution (a)*. Dissolve 0.5 g of the substance under examination in 100 ml of the initial mobile phase.

*Test solution (b)*. Dissolve 10 mg of the substance under examination in 100 ml of the initial mobile phase and mix. Dilute 10 ml of this solution to 100 ml with the same mobile phase.

*Reference solution (a)*. A 0.1 per cent w/v solution of *buclizine dihydrochloride RS* in the initial mobile phase.

*Reference solution (b)*. A 0.001 per cent w/v solution of *1,4-bis(4-chlorobenzylhydryl) piperazine RS* (*buclizine impurity A RS*) in the initial mobile phase.

Chromatographic system

- a stainless steel column 20 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (10 µm) (Such as Nucleosil C 18),
- mobile phase, initial: *0.01 M sodium heptanesulphonate* in a mixture of 55 volumes of *water* and 45 volumes of *acetonitrile*, with the pH adjusted to 4.0 with *1 M orthophosphoric acid*; final: *0.01 M sodium heptanesulphonate* in a mixture of 20 volumes of *water* and 80 volumes of *acetonitrile*, with the pH adjusted to 4.0 with *1 M orthophosphoric acid*, a linear gradient elution programme for 30 minutes with the initial mobile phase and 10 minutes with the final mobile phase,
- flow rate. 2 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 20 µl.

Inject reference solution (a). The test is not valid unless the relative standard deviation is not more than 2.0 per cent.

Inject test solution (a), test solution (b) and reference solution (b). In the chromatogram obtained, the area of any peak corresponding to impurity A is not greater than the area of the peak obtained in the chromatogram obtained with reference solution (b) and the area of any other secondary peak is not greater than the area of the peak in the chromatogram obtained with test solution (b)

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

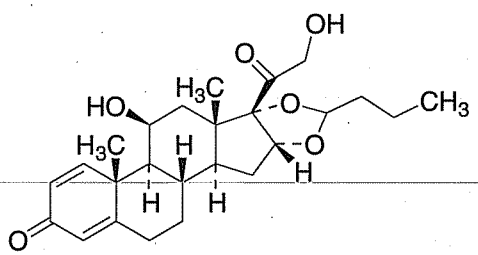
**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.4 g, dissolve in 50 ml of *anhydrous acetic acid*, add 10 ml of mercuric acetate solution. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.0253 g of $C_{28}H_{33}ClN_2,2HCl$.

**Storage**. Store protected from light and moisture.

## Budesonide




$C_{25}H_{34}O_6$ Mol. Wt. 430.5

Budesonide is a mixture of the C-22*S* (epimer A) and the C-22*R* (epimer B) epimers of 16α,17-[(1*RS*)-butylidenebis(oxy)]-11β,21-dihydroxypregna-1,4-diene-3,20-dione.

Budesonide contains not less than 98.0 per cent and not more than 102.0 per cent of a mixture of epimers A and B, $C_{25}H_{34}O_6$, calculated on the dried basis.

**Category**. Glucocorticoid.

**Description**. A white or almost white, crystalline powder.

### Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

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

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.



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*Mobile phase*. Add a mixture of 1.2 volumes of *water* and 8 volumes of *methanol* to a mixture of 15 volumes of *ether* and 77 volumes of *dichloromethane*.

*Solvent mixture*. 1 volume of *methanol* and 9 volumes of *methylene chloride*.

*Test solution*. Dissolve 25 mg of the substance under examination in 10 ml of the solvent mixture.

*Reference solution (a)*. A 0.25 per cent w/v solution of *budesonide RS* in the solvent mixture.

*Reference solution (b)*. A solution containing 0.25 per cent w/v of *triamcinolone acetonide RS* and 0.25 per cent w/v of *budesonide RS* in the solvent mixture.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

C. Dissolve about 2 mg in 2 ml of *sulphuric acid*. A yellow colour appears in 5 minutes and the colour changes to brown or reddish-brown in 30 minutes. Add cautiously the solution to 10 ml of *water* and mix. The colour fades and a clear solution remains.

D. Dissolve about 1 mg in 2 ml of a solution containing 2 g of *phosphomolybdic acid* in a mixture of 10 ml of *dilute sodium hydroxide solution*, 15 ml of *water* and 25 ml of *glacial acetic acid*. Heat for 5 minutes on a water-bath. Cool in iced water for 10 minutes and add 3 ml of *dilute sodium hydroxide solution*. The solution turns blue.

## Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 50 mg of the substance under examination in 30 ml of *acetonitrile*. Add about 60 ml of *phosphate buffer pH 3.2* and, if necessary, disperse with the aid of ultrasound to dissolve. Dilute with *phosphate buffer pH 3.2* to 100 ml and allow to stand for at least 15 minutes before use and filter.

*Reference solution (a)*. Dissolve 50 mg of *budesonide RS* in 30 ml of *acetonitrile*. Add about 60 ml of *phosphate buffer pH 3.2* and disperse, if necessary, with the aid of ultrasound to dissolve. Dilute to 100 ml *with phosphate buffer pH 3.2* and allow to stand for at least 15 minutes before use and filter.

*Reference solution (b)*. Dilute reference solution (a) with the mobile phase to get a 0.00025 per cent w/v solution of budesonide.

Use the chromatographic system described in the Assay.

Inject reference solution (a). The test is not valid unless the resolution between epimer B peak and epimer A peak is not less than 1.5, the tailing factor for epimer B peak is not more than 1.5 and the relative standard deviation of sum of epimer A and epimer B peaks for replicate injections is not more than 2.0 per cent.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution: the area of any peak, other than the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of the areas of all the peaks, other than the principal peak, is not greater than thrice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent).

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1 g by drying in an oven at 105°.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 50 mg of the substance under examination in 30 ml of *acetonitrile* and dilute to 100.0 ml with *phosphate buffer solution pH 3.2* and filter.

*Reference solution*. Dissolve 50 mg of *budesonide RS* in 30 ml of *acetonitrile* and dilute to 100.0 ml with *phosphate buffer solution pH 3.2*.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 34 volumes of *acetonitrile* and 66 volumes of a buffer solution prepared by adding 100 ml of 0.25 per cent w/v solution of *orthophosphoric acid* to 900 ml of 0.4 per cent w/v solution of *sodium dihydrogen phosphate* and adjusting the pH to 3.2,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the resolution between epimer B peak and epimer A peak is not less than 1.5, the tailing factor for epimer B peak is not more than 1.5, the column efficiency determined for epimer B peak is not less than 4000 theoretical plates and the relative standard deviation for the sum of epimer A and B peaks for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{25}H_{34}O_6$.

**Storage**. Store protected from light.

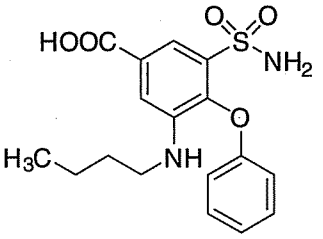


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# Bumetanide




$C_{17}H_{20}N_2O_5S$ Mol. Wt. 364.4

Bumetanide is 3-(aminosulphonyl)-5-(butylamino)-4-phenoxybenzoic acid

Bumetanide contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{17}H_{20}N_2O_5S$, calculated on the dried basis.

**Category**. Diuretic.

**Description**. A white crystalline powder.

## Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *bumetanide RS* or with the reference spectrum of bumetanide.

## Tests

**Appearance of solution**. A 0.5 per cent w/v solution in 0.6 per cent w/v solution of *potassium hydroxide* is clear (2.4.1) and colourless (2.4.1).

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 50 mg of the substance under examination in 25.0 ml of the mobile phase.

*Reference solution (a)*. Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Further dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

*Reference solution (b)*. Dissolve 2 mg each of *3-nitro-4-phenoxy-5-sulphamoylbenzoic acid (bumetanide impurity A RS)* and *3-amino-4-phenoxy-5-sulphamoylbenzoic acid (bumetanide impurity B RS)* in 10.0 ml of the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with encapped octylsilane bonded to porous silica (3.5 µm),
- mobile phase: a mixture of 70 volumes of *methanol*, 25 volumes of *water* and 5 volumes of a 2.72 per cent w/v solution of *potassium dihydrogen phosphate*, previously adjusted to pH 7.0 with 28 per cent w/v solution of *potassium hydroxide*. Add *tetrahexylammonium bromide* to this mixture to obtain a 0.22 per cent w/v solution,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to bumetanide impurity A and bumetanide impurity B is not less than 2.0. The relative retention time with reference to bumetanide for bumetanide impurity B is about 0.4, for bumetanide impurity A is about 0.6, for 3-[[(2*RS*)-2-ethylhexyl]amino]-4-phenoxy-5-sulphamoyl benzoic acid (bumetanide impurity D) is about 2.5 and for butyl 3-(butylamino)-4-phenoxy-5-sulphamoylbenzoate (bumetanide impurity C) is about 4.4.

Inject reference solution (a) and the test solution. Run the chromatogram 5 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of secondary peak corresponding to bumetanide impurity A, B, C and D is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of the areas of all secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 4 hours.

**Assay**. Weigh accurately about 0.3 g and dissolve in 50 ml of *ethanol (95 per cent)*. Add 0.1 ml of *phenol red solution* and titrate with *0.1 M sodium hydroxide* until a violet-red colour is obtained. Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.03644 g of $C_{17}H_{20}N_2O_5S$.

**Storage**. Store protected from light.

# Bumetanide Injection

Bumetanide Injection is a sterile solution of Bumetanide in Water for Injections.

*The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.*



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Bumetanide Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of bumetanide, $C_{17}H_{20}N_2O_5S$.

**Usual strength**. 0.25 mg per ml.

### Identification

A. Shake a quantity of the injection containing 10 mg of Bumetanide with 20 ml of *ether*, filter the ether layer through *anhydrous sodium sulphate* and evaporate. The residue complies with the following tests. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *bumetanide RS* or with the reference spectrum of bumetanide.

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

### Tests

**pH** (2.4.24). 6.0 to 7.8.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 2.5 volumes of *methanol*, 10 volumes of *glacial acetic acid*, 10 volumes of *cyclohexane* and 80 volumes of *chloroform*.

*Test solution (a)*. To a quantity of the injection containing 5.0 mg of Bumetanide, adjust the pH to 12 with *0.1 M sodium hydroxide* and extract with two 20 ml quantities of *ether*. Discard the ether, adjust the pH to 4 using *1 M acetic acid*, extract with two further 20 ml quantities of *ether*, dry the ether by filtering through *anhydrous sodium sulphate*, wash the filter with 5 ml of *ether* and evaporate the combined filtrate and washings to dryness. Dissolve the residue in 5 ml of *methanol* and centrifuge. Evaporate the supernatant liquid to dryness using a rotary evaporator and dissolve the residue in 0.5 ml of *methanol*.

*Test solution (b)*. Dilute 1 ml of test solution (a) to 10 ml with *methanol*. Dilute 1 ml of this solution to 30 ml with *methanol*.

*Test solution (c)*. Dilute 1 ml of test solution (b) to 3 ml with *methanol*.

*Reference solution*. A 0.005 per cent w/v solution of *3-amino-4-phenoxy-5-sulphamoylbenzoic acid RS (bumetanide impurity A RS)* in *methanol*.

Apply to the plate 25 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in a current of warm air and examine in ultraviolet light at 365 nm. Any secondary spot in the chromatogram obtained with test solution (a) corresponding to 3-amino-4-phenoxy-5-sulphamoylbenzoic acid is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent), any other secondary spot is not more intense than the spot in the chromatogram obtained with test solution (b) (0.3 per cent) and not more than two other such spots are more intense than the spot in the chromatogram obtained with test solution (c) (0.1 per cent).

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. 2 volumes of *glacial acetic acid*, 5 volumes of *tetrahydrofuran* and 45 volumes of *methanol*.

*Test solution*. Dilute a quantity of the injection containing 2.5 mg of Bumetanide to 25.0 ml with the solvent mixture.

*Reference solution (a)*. A 0.025 per cent w/v solution of *bumetanide RS* in the solvent mixture. Dilute 10.0 ml of the solution to 25.0 ml with *water*.

*Reference solution (b)*. A 0.0125 per cent w/v solution of *3-amino-4-phenoxy-5-sulphamoylbenzoic acid RS* in reference solution (a).

Chromatographic system
- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (10 µm) (such as Bondapak ODS),
- mobile phase: a mixture of 2 volumes of *glacial acetic acid*, 5 volumes of *tetrahydrofuran*, 45 volumes of *water* and 50 volumes of *methanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to bumetanide and 3-amino-4-phenoxy-5-sulphamoylbenzoic acid is not less than 15.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{17}H_{20}N_2O_5S$ in the injection.

## Bumetanide Oral Solution

Bumetanide Oral Solution is a solution of Bumetanide in a suitable flavoured vehicle.

*The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.*

Bumetanide Oral Solution contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of bumetanide, $C_{17}H_{20}N_2O_5S$.

**Usual strength**. 0.25 mg per ml.

### Identification

A. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).



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

### Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 2.5 volumes of *methanol*, 10 volumes of *glacial acetic acid*, 10 volumes of *cyclohexane* and 80 volumes of *chloroform*.

*Test solution (a)*. Mix a quantity of the oral solution containing 2 mg of Bumetanide with 10 ml of *water* and 0.6 ml of *1 M hydrochloric acid*, add 5 ml of *ethyl acetate*, shake for 15 minutes, centrifuge and decant the *ethyl acetate*. Add a further 5 ml of *ethyl acetate* to the residue, shake for 15 minutes, centrifuge and decant the *ethyl acetate*. Evaporate the combined ethyl acetate extracts to dryness and dissolve the residue in 0.5 ml of *methanol*.

*Test solution (b)*. Dilute 1.0 ml of test solution (a) to 10.0 ml with *methanol*.

*Test solution (c)*. Dilute 1.0 ml of test solution (b) to 10.0 ml with *methanol*. Dilute 1 ml of this solution to 3 ml with *methanol*.

*Test solution (d)*. Dilute 1.0 ml of test solution (c) to 100.0 ml with *methanol*.

*Reference solution (a)*. A 0.04 per cent w/v solution of *bumetanide RS* in *methanol*.

*Reference solution (b)*. A 0.002 per cent w/v solution of *3-amino-4-phenoxy-5-sulphamoylbenzoic acid RS* in *methanol*.

Apply to the plate 25 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 365 nm. Any secondary spot in the chromatogram obtained with test solution (a) corresponding to 3-amino-4-phenoxy-5-sulphamoyl-benzoic acid is not more intense than the spot in the chromatogram obtained with test solution (c) (0.5 per cent), any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.3 per cent) and not more than two other such spots are more intense than the spot in the chromatogram obtained with test solution (d) (0.1 per cent).

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. 2 volumes of *glacial acetic acid*, 5 volumes of *tetrahydrofuran* and 45 volumes of *methanol*.

*Test solution*. Mix a quantity of the oral solution containing 2.5 mg of Bumetanide with 12.5 ml of *water* and 0.8 ml of *1 M hydrochloric acid*, add 10 ml of *ethyl acetate*, shake for 15 minutes, centrifuge and decant the *ethyl acetate*. Repeat the extraction procedure twice using a further two 10 ml quantities of *ethyl acetate* and beginning at the words 'add 10 ml of ... '. Evaporate the combined ethyl acetate extracts to dryness, dissolve the residue in 10 ml of the solvent mixture and dilute to 20 ml with *water*.

*Reference solution (a)*. A 0.025 per cent w/v solution of *bumetanide RS* in the solvent mixture. Dilute 5 ml of the solution to 10.0 ml with *water*.

*Reference solution (b)*. A 0.0125 per cent w/v solution of *3-amino-4-phenoxy-5-sulphamoyl-benzoic acid RS* in reference solution (a).

Chromatographic system
- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (10 µm) (such as µBondapak ODS),
- mobile phase: a mixture of 2 volumes of *glacial acetic acid*, 5 volumes of *tetrahydrofuran*, 45 volumes of *water* and 50 volumes of *methanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to bumetanide and 3-amino-4-phenoxy-5-sulphamoylbenzoic acid is not less than 15.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{17}H_{20}N_2O_5S$ in oral solution.

Determine the weight per ml of the oral solution (2.4.29) and calculate the content of $C_{17}H_{20}N_2O_5S$, weight in volume.

## Bumetanide Tablets

Bumetanide Tablets contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of bumetanide, $C_{17}H_{20}N_2O_5S$.

**Usual strengths**. 0.5 mg; 1 mg; 2 mg.

### Identification

A. Shake a quantity of the powdered tablets containing 50 mg of Bumetanide with 25 ml of *ether*, filter through *anhydrous sodium sulphate* and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *bumetanide RS* or with the reference spectrum of bumetanide.

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



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## Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 2.5 volumes of *methanol*, 10 volumes of *glacial acetic acid*, 10 volumes of *cyclohexane* and 80 volumes of *chloroform*.

*Test solution*. Dissolve 0.125 g of substance under examination in 20 ml of a mixture of equal volumes of *acetonitrile* and *methanol*, shake for 20 minutes. Centrifuge for 10 minutes, decant and reserve the supernatant liquid. Extract the residue with 5 ml of a mixture of equal volumes of *acetonitrile* and *methanol*, shaking mechanically for 30 seconds, centrifuge for 10 minutes, decant and combine the extracts. Evaporate the combined extracts to dryness under reduced pressure, dissolve the residue in 0.5 ml of *methanol* and centrifuge for 10 minutes.

*Reference solution (a)*. Dilute 0.3 ml of the test solution to 100 ml with *methanol*.

*Reference solution (b)*. Dilute 0.1 ml of the test solution to 100 ml with *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.3 per cent) and not more than three such spots are more intense than the spot in the chromatogram obtained with reference solution (b) (0.1 per cent).

**Uniformity of content**. *(for tablets containing 10 mg or less)*. Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14).

*Test solution*. Disperse 1 tablet in 10.0 ml of the solvent mixture, shake with the aid of ultrasound for 5 minutes, dilute to 20.0 ml with *water* and filter.

*Reference solution*. A 0.01 per cent w/v solution of *bumetanide RS* in the solvent mixture. Dilute 10.0 ml of this solution to 20.0 ml with *water*.

Use the chromatographic system as described under Assay.

Calculate the content of $C_{17}H_{20}N_2O_5S$ in the tablet.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. 2 volumes of *glacial acetic acid*, 5 volumes of *tetrahydrofuran* and 45 volumes of *methanol*.

*Test solution*. Dissolve 2.5 mg of the substance under examination in 10 ml of the solvent mixture, shake for 5 minutes and dilute to 25.0 ml with *water*.

*Reference solution (a)*. A 0.025 per cent w/v solution of *bumetanide RS* in the solvent mixture. Dilute 10.0 ml of the solution to 25.0 ml with *water*.

*Reference solution (b)*. A 0.0125 per cent w/v solution of *3-amino-4-phenoxy-5-sulphamoylbenzoic acid RS* in reference solution (a).

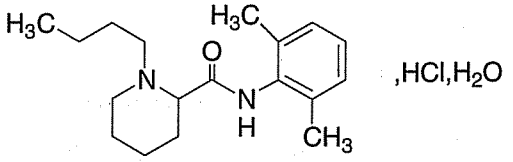
Chromatographic system
- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 2 volumes of *glacial acetic acid*, 5 volumes of *tetrahydrofuran*, 45 volumes of *water* and 50 volumes of *methanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to bumetanide and 3-amino-4-phenoxy-5-sulphamoylbenzoic acid is not less than 15.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{17}H_{20}N_2O_5S$ in the tablets.

# Bupivacaine Hydrochloride




$C_{18}H_{28}N_2O,HCl,H_2O$ Mol. Wt. 342.9

Bupivacaine Hydrochloride is (*RS*)-1-butyl-*N*-(2,6-dimethylphenyl)-2-piperidinecarboxamide hydrochloride monohydrate.

Bupivacaine Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{18}H_{28}N_2O,HCl$, calculated on the dried basis.

**Category**. Local anaesthetic.

**Dose**. By infiltration anaesthesia, the equivalent of 2 mg of anhydrous bupivacaine hydrochloride per kg of body weight.

**Description**. A white, crystalline powder or colourless crystals; almost odourless.

## Identification

*Test A may be omitted if tests B, C, D and E are carried out. Tests B, C and D may be omitted if tests A and E are carried out.*



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A. Determine by infrared absorption spectrphotometry (2.4.6). Compare the spectrum with that obtained with *bupivacaine hydrochloride RS* or with the reference spectrum of bupivacaine hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.05 per cent w/v solution in *0.01 M hydrochloric acid* shows two absorption maxima at about 263 nm and 271 nm; absorbance at about 263 nm, about 0.70 and at about 271 nm, about 0.57.

C. Dissolve 0.1 g in 10 ml of *water*, add 2 ml of *2 M sodium hydroxide* and shake with two quantities, each of 15 ml, of *ether*. Dry the combined ether extracts over *anhydrous sodium sulphate*, filter, evaporate the ether, recrystallise the residue from *ethanol (90 per cent)* and dry the residue at a pressure of 1.5 to 2.5 kPa. The melting range (2.4.21) of the residue is between 105° and 108° (2.4.21).

D. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

E. A 10 per cent w/v solution gives reaction A of chlorides (2.3.1).

### Tests

**Acidity or alkalinity**. To 10 ml of a 2.0 per cent w/v solution in *carbon dioxide-free water* add 0.2 ml of *0.01 M sodium hydroxide*; the pH is not less than 4.7. Add 0.4 ml of *0.01 M hydrochloric acid*; the pH is not more than 4.7 (2.4.24).

**Appearance of solution**. A 2.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and colourless (2.4.1).

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase.* A mixture of 100 volumes of *methanol* and 0.1 volume of *strong ammonia solution.*

*Test solution (a).* Dissolve 5.0 g of the substance under examination in 100 ml of *methanol.*

*Test solution (b).* Dilute 10 ml of test solution (a) to 100 ml with *methanol.*

*Reference solution (a).* Dilute 5 ml of test solution (b) to 100 ml with *methanol.*

*Reference solution (b).* A 0.5 per cent w/v solution of bupivacaine *hydrochloride RS* in *methanol.*

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with *dilute potassium iodobismuthate solution*. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a).

**2,6-Dimethylaniline**. To 2.0 ml of a 5.0 per cent w/v solution in *methanol* (solution A) add 1 ml of a freshly prepared 1 per cent w/v solution of *4-dimethylaminobenzaldehyde* in *methanol* and 2 ml of *glacial acetic acid* and allow to stand for 10 minutes. Any yellow colour produced is not more intense than that obtained with a solution prepared at the same time and in the same manner using 2 ml of a 0.0005 per cent w/v solution of *2,6-dimethylaniline* in *methanol* in place of solution A (100 ppm).

**Heavy metals** (2.3.13). A 10.0 per cent w/v solution in a mixture of 85 volumes of *methanol* and 15 volumes of *water* complies with the limit test for heavy metals Method D (10 ppm).Prepare the standard using *lead standard solution* (1 ppm Pb) obtained by diluting *lead standard solution* (*20 ppm Pb*) with a mixture of 85 volumes of *methanol* and 15 volumes of *water.*

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). 4.5 to 6.0 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.25 g, dissolve in a mixture of 5.0 ml of *0.01 M hydrochloric acid* and 50 ml of *ethanol (95 per cent)* and titrate with *0.01 M ethanolic sodium hydroxide*, determining the end-point potentiometrically (2.4.25). Note the volume added between the inflections.

1 ml of *0.01 M ethanolic sodium hydroxide* is equivalent to 0.03249 g of $C_{18}H_{28}N_2O$,HCl.

**Storage**. Store protected from light.

## Bupivacaine Injection

Bupivacaine Hydrochloride Injection

Bupivacaine Injection is a sterile solution of Bupivacaine Hydrochloride in Water for Injection.

Bupivacaine Injection contains not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of anhydrous bupivacaine hydrochloride, $C_{18}H_{28}N_2O$,HCl.

**Usual strengths**. The equivalent of 25, 50 and 75 mg of anhydrous bupivacaine hydrochloride in 10 ml.

### Identification

A. To a volume containing 25 mg of anhydrous bupivacaine hydrochloride add 2 ml of *strong ammonia solution*, shake and filter. Wash the precipitate with *water* and dry at 60° at a pressure of 2 kPa for 16 hours. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *bupivacaine*



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*hydrochloride RS* treated in the same manner or with the reference spectrum of bupivacaine.

B. To a volume containing 50 mg of anhydrous bupivacaine hydrochloride add 15 ml of *picric acid solution*; the precipitate, after rapid washing with a small quantity of *water* followed by successive quantities, each of 2 ml, of *methanol* and *ether* melts at about 194° (2.4.21).

C. To a volume containing 50 mg of anhydrous bupivacaine hydrochloride add 2 ml of a 10 per cent w/v solution of *disodium hydrogen phosphate* and sufficient *iodine solution* to produce a distinct brown colour. Remove the excess iodine by adding *0.1 M sodium thiosulphate*; no pink colour is produced.

### Tests

**pH** (2.4.24). 4.0 to 6.5.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 100 volumes of *methanol* and 0.1 volume of *strong ammonia solution*.

*Test solution*. Evaporate almost to dryness a volume containing 0.1 g of anhydrous bupivacaine hydrochloride using a rotary evaporator, add sufficient *methanol* to the residue to produce 2 ml, mix well, centrifuge and use the supernatant liquid.

*Reference solution*. Dilute 1 volume of the test solution to 100 volumes with *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with *dilute potassium iodobismuthate solution*. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**2,6-Dimethylaniline**. To a volume containing 25 mg of anhydrous bupivacaine hydrochloride add *water*, if necessary, to produce 10 ml and sufficient *2 M sodium hydroxide* to make the solution just alkaline. Extract with three quantities, each of 5 ml, of *chloroform*. Dry the combined extracts over anhydrous *sodium sulphate*, filter, wash the filter with 5 ml of *chloroform* and evaporate the filtrate to dryness using a rotary evaporator. Dissolve the residue in 2 ml of *methanol*. Add 1 ml of a freshly prepared 1 per cent w/v solution of *4-dimethylaminobenzaldehyde* in *methanol* and 2 ml of *glacial acetic acid* and allow to stand for 10 minutes. Any yellow colour produced is not more intense than that obtained with a solution prepared at the same time and in the same manner using 2 ml of a 0.0005 per cent w/v solution of *2,6-dimethylaniline* in *methanol* in place of the injection under examination.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

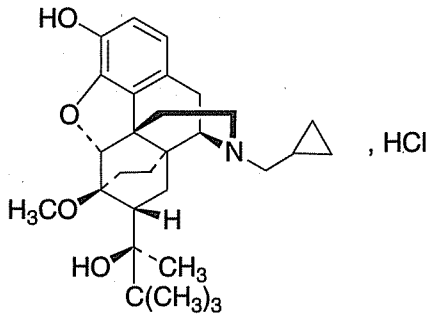
**Assay**. To an accurately measured volume containing about 0.5 g of anhydrous bupivacaine hydrochloride add 5 ml of *water* and 2 ml of *1 M sodium hydroxide* and extract with three quantities, each of 15 ml, of *chloroform*. Combine the chloroform extracts, wash with two quantities, each of 5 ml, of *water*, extract the aqueous solutions with 5 ml of *chloroform* and evaporate the combined chloroform extracts to dryness on a water-bath. Add two successive quantities, each of 5 ml, of *acetone* and evaporate. Dissolve the residue in 50 ml of *anhydrous glacial acetic acid*. Add 15 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03249 g of $C_{18}H_{28}N_2O,HCl$.

**Storage**. Store in single dose or multiple dose containers, preferably of Type 1 glass.

**Labelling**. The label states the strength in terms of the equivalent amount of anhydrous bupivacaine hydrochloride in a suitable dose-volume.

## Buprenorphine Hydrochloride




$C_{29}H_{41}NO_4,HCl$ Mol. Wt. 504.1

Buprenorphine Hydrochloride is (6*R*,7*R*,14*S*)-17-cyclopropylmethyl-7,8-dihydro-7-[(1*S*)-1-hydroxy-1,2,2-trimethylpropyl]-6-*O*-methyl-6,14-ethano-17-normorphine hydrochloride.

Buprenorphine Hydrochloride contains not less than 97.0 per cent and not more than 102.0 per cent of $C_{29}H_{41}NO_4,HCl$, calculated on the dried basis.

**Category**. Narcotic analgesic.

**Dose**. By intramuscular or slow intravenous injection, the equivalent of 300 to 600 µg of buprenorphine every 6 to



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8 hours; sublingually, the equivalent of upto 400 µg of buprenorphine every 6 to 8 hours. (108 µg of buprenorphine hydrochloride is approximately equivalent to 100 µg of buprenorphine)

**Description**. A white to off-white, crystalline powder.

### Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *buprenorphine hydrochloride RS*.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.01 per cent w/v solution in *0.01 M hydrochloric acid* shows an absorption maximum at about 286 nm; absorbance at about 286 nm, about 0.33.

C. Dissolve about 5 mg in 5 ml of hot *water*, add 2 ml of *dilute hydrochloric acid* and 2 ml of a 2 per cent w/v solution of *sodium nitrite* and allow to stand for 10 minutes; a yellow colour is produced.

D. Dissolve 10 mg in 10 ml of hot *water*; add 2 ml of *dilute nitric acid*, shake and add 1 ml of *silver nitrate solution*; a white precipitate is produced.

### Tests

**Related substances.** Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 25 mg of the substance under examination in 10.0 ml of the mobile phase.

*Reference solution (a)*. Dissolve 5 mg of the substance under examination in 2 ml of *methanol*, add 0.25 ml of *2 M hydrochloric acid.*

*Reference solution (b)*. Dilute 0.5 ml of the test solution to 200.0 ml with the mobile phase.

*Reference solution (c)*. Dilute 0.65 ml of the test solution to 100.0 ml with the mobile phase.

*Reference solution (d)*. Dilute 4.0 ml of the reference solution (b) 10.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5µm),
- column temperature 40°,
- mobile phase: a mixture of 10 volumes of 1 per cent w/ v solution of *ammonium acetate* and 60 volumes of *methanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 288 nm,
- injection volume. 20 µl.

Inject reference solution (a). The relative retention time for first peak with reference to buprenorphine is about 0.93.

Inject the test solution, reference solution (a) and (b). Run the chromatogram 2.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent) and the sum of areas of all secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.65 per cent). Ignore any secondary peak with an area less than the area of the principal peak in the chromatogram obtained with reference solution (d).

**Heavy metals** (2.3.13). Moisten the residue obtained in the test for Sulphated ash with a few drops of *hydrochloric acid* and evaporate almost to dryness on a water-bath. Dissolve the residue in 10 ml of *water* by warming, cool, transfer to a test-tube with the aid of 10 ml of *water* and add 2 ml of *dilute acetic acid*. The solution complies with the limit test for heavy metals, Method A (20 ppm). Prepare the standard using 5 ml of *lead standard solution (2 ppm Pb)*.

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 0.5 g by drying in an oven at 105° for 4 hours.

**Assay**. Weigh accurately about 0.5 g, dissolve in 60 ml of *anhydrous glacial acetic acid*, add 10 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, using 0.1 ml of *crystal violet solution* as indicator to a green end-point. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.05041 g of $C_{29}H_{41}NO_4$,HCl.

## Buprenorphine Injection

Buprenorphine Hydrochloride Injection

Buprenorphine Injection is a sterile solution of Buprenorphine Hydrochloride in Water for Injection.

Buprenorphine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of buprenorphine, $C_{29}H_{41}NO_4$.

**Usual strength**. The equivalent of 300 µg of buprenorphine per ml.

### Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254*.

*Mobile phase*. A mixture of 85 volumes of *toluene*, 15 volumes of *methanol* and 0.5 volume of *strong ammonia solution*.



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*Test solution.* Transfer a volume of the injection containing 1.5 mg of Buprenorphine Hydrochloride to a 125-ml separator, add 0.5 ml of *dilute ammonia solution*, shake and extract with three quantities, each of 10 ml, of *chloroform*, washing each chloroform extract with the same 10 ml of *water* and discard the *water.* Evaporate the combined chloroform extracts to dryness on a water-bath and dissolve the residue in 1.5 ml of *chloroform.*

*Reference solution.* Dissolve 1.5 mg of *buprenorphine hydrochloride RS* in 5 ml of *0.01 M hydrochloric acid*, transfer the solution to a 125-ml separator and repeat the above procedure beginning at the words "add 0.5 ml of *dilute ammonia solution.....*".

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of air and examine in ultraviolet light at 254 nm or expose to iodine vapours. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. To a volume containing about 5 mg of Buprenorphine Hydrochloride in a 125-ml separator, add 1 ml of *dilute ammonia solution* and shake with three quantities, each of 10 ml, of *chloroform.* Wash each chloroform extract with the same 10 ml of *water* and discard the washings. Evaporate the combined chloroform extracts to dryness on a water-bath and dissolve the residue in 50 ml of *0.1 M hydrochloric acid.* When examined in the range 230 to 360 nm (2.4.7) the resulting solution shows an absorption maximum only at about 286 nm.

### Tests

**pH** (2.4.24). 3.5 to 6.5.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. Measure accurately a volume containing 1.5 mg of buprenorphine and transfer to a 25-ml volumetric flask. Add 1 ml of *1 M hydrochloric acid*, 2 ml of a 2 per cent w/v solution of *sodium nitrite* and shake well. Stopper the flask and allow to stand for 15 minutes. Dilute the solution to volume with *dilute ammonia solution* and measure the absorbance of the resulting solution at the maximum at about 460 nm (2.4.7), using as the blank a solution prepared in the same manner by treating 5 ml of *water* instead of the preparation under examination.

Calculate the content of $C_{29}H_{41}NO_4$ from the absorbance obtained by repeating the procedure with 5 ml of a solution containing *buprenorphine hydrochloride RS* equivalent to 0.03 per cent w/v of buprenorphine.

**Labelling**. The label states the strength in terms of the equivalent amount of buprenorphine in a suitable dose-volume.

## Buprenorphine Tablets

Buprenorphine Hydrochloride Tablets

Buprenorphine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of buprenorphine, $C_{29}H_{41}NO_4$.]

**Usual strengths**. The equivalent of 200 µg and 400 µg of buprenorphine .

### Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254.*

*Mobile phase.* A mixture of 85 volumes of *toluene*, 15 volumes of *methanol* and 0.5 volume of *strong ammonia solution.*

*Test solution.* Extract a quantity of the powdered tablets containing 1 mg of Buprenorphine Hydrochloride with three quantities, each of 10 ml, of *methanol*, filtering each extract through a sintered-glass filter (porosity No. 4). Evaporate the filtrate to dryness and dissolve the residue in 1 ml of *methanol.*

*Reference solution.* Dissolve 1 mg of *buprenorphine hydrochloride RS* in 1 ml of *methanol.*

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of air and examine in ultraviolet light at 254 nm or expose to iodine vapours. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. Shake vigorously a quantity of the powdered tablets containing 2 mg of Buprenorphine Hydrochloride with 20 ml of hot *water*, filter and cool. The filtrate, when examined in the range 230 nm to 360 nm (2.4.7), shows an absorption maximum at about 286 nm; absorbance at about 286 nm, about 0.33.

### Tests

**Disintegration**. The requirement of Disintegration does not apply.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 200 mcg of buprenorphine and transfer to a 125-ml separator. Add 10 ml of hot *water*, shake, add 1 ml of a 10 per cent w/v solution of *sodium bicarbonate* and shake well. Add 3 ml of a 10 per cent v/v solution of *acetic acid*, shake, add 3 ml of a 0.2 per cent w/v solution of *metanil yellow* and again shake well. Shake with 100 ml of *chloroform* for about 5 minutes and allow the two layers to separate over a period of 45 minutes. Collect the chloroform layer into another 250-ml separator and extract the chloroform layer with 50.0 ml of *1 M hydrochloric acid.* Discard the chloroform layer, centrifuge the red acid layer and measure the absorbance at the maximum at about 530 nm (2.4.7),



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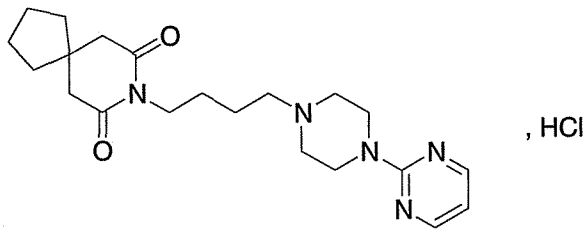
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using *1 M hydrochloric acid* as the blank. Calculate the content of $C_{29}H_{41}NO_4$ from the absorbance obtained by repeating the procedure with 10.0 ml of a solution containing *buprenorphine hydrochloride RS* equivalent to 0.002 per cent w/v solution of buprenorphine beginning at the words "transfer to a 125-ml separator...." .

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

# Buspirone Hydrochloride




$C_{21}H_{31}N_5O_2,HCl$ Mol. Wt. 422.0

Buspirone Hydrochloride is 8-[4-[4-(pyrimidin-2-yl) piperazin-1-yl]butyl]-8-azaspiro[4.5]decane-7,9-dione hydrochloride.

Buspirone Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{21}H_{31}N_5O_2,HCl$, calculated on the dried basis.

**Category**. Anxiolytic.

**Description**. A white or almost white, crystalline powder.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *buspirone hydrochloride RS*. If the spectra obtained show differences, dissolve the substance under examination and the reference substance separately in *methanol*, evaporate to dryness on a water-bath and record new spectra using the residues.

B. Gives reaction A of chlorides (2.3.1).

## Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 25 mg of the substance under examination in 25.0 ml of mobile phase A.

*Reference solution (a)*. Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A. Dilute 1.0 ml of this solution to 10.0 ml with mobile phase A.

*Reference solution (b)*. A 0.05 per cent w/v solution of *2,2' (piperazine-1, 4-diyl) dipyrimidine RS* (*buspirone impurity A RS*) in the test solution.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature 40°,
- mobile phase: A. 95 volumes of a solution containing 0.68 per cent w/v of *potassium dihydrogen phosphate* and 0.093 per cent w/v of *sodium hexanesulphonate monohydrate*, previously adjusted to pH 3.4 with *orthophosphoric acid* and 5 volumes of *acetonitrile*,
  B. 25 volumes of a solution containing 0.34 per cent w/v of *potassium dihydrogen phosphate* and 0.352 per cent w/v of *sodium hexanesulphonate monohydrate*, previously adjusted to pH 2.2 with *orthophosphoric acid* and 75 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 240 nm and at 210 nm,
- injection volume. 20 µl.

| Time (in min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0 | 90 | 10 |
| 6 | 90 | 10 |
| 34 | 42 | 58 |
| 45 | 42 | 58 |
| 55 | 0 | 100 |
| 56 | 100 | 0 |
| 60 | 100 | 0 |
| 61 | 90 | 10 |

Inject reference solution (b). The test is not valid unless the peak-to-valley ratio at 240 nm is not less than 5.0, where $H_p$ = height above the baseline of the peak due to impurity A and $H_v$ = height above the baseline of the lowest point of the curve separating this peak from the peak due to buspirone.

Inject the test solution and reference solution (a) and set the spectrophotometer at 240 nm. In the chromatogram obtained with the test solution, the area of any secondary peak obtained is not more than 3 times the area obtained with reference solution (a) (0.3 per cent). The sum of the areas of all the secondary peaks is not more than 5 times the area obtained with reference solution (a) (0.5 per cent ). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Inject the test solution and reference solution (a) and set the spectrophotometer at 210 nm. In the chromatogram obtained with the test solution, the area of any secondary peak obtained is not more than 3 times the area obtained with reference solution (a) (0.3 per cent). The sum of the areas of all the secondary peaks is not more than 5 times the area obtained with reference solution (a) (0.5 per cent). Ignore any peak with



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an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.15 g, dissolve in 10 ml of *glacial acetic acid* and add 50 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid,* determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.021 g of $C_{21}H_{32}ClN_5O_2$.

**Storage**. Store protected from light.

## Buspirone Tablets

Buspirone Hydrochloride Tablets

Buspirone Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of buspirone, $C_{21}H_{31}N_5O_2$.

**Usual strengths**. 5 mg; 10 mg.

### Identification

A. Extract a quantity of the powdered tablets containing 50 mg of buspirone with 50 ml of *chloroform*, filter and evaporate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *buspirone hydrochloride RS* or with the reference spectrum of buspirone hydrochloride.

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

### Tests

**Dissolution** (2.5.2).

Apparatus No. 1

Medium. *0.01 M hydrochloric acid.*

Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 235 nm (2.4.7). Calculate the content of $C_{21}H_{31}N_5O_2$ in the medium from the absorbance obtained from a solution of known concentration of *buspirone hydrochloride RS* in the same medium.

D. Not less than 80 per cent of the stated amount of $C_{21}H_{31}N_5O_2$.

**Uniformity of content**. Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), using the chromatographic conditions and the reference solution as described in the Assay.

*Test solution*. Disperse one tablet in the minimum amount of *1 M hydrochloric acid* and dilute to 25.0 ml with *water*, and filter.

Calculate the content of $C_{21}H_{31}N_5O_2$ in the tablet.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh and powder 20 tablets. Weigh accurately a quantity of powder containing 25 mg of buspirone, disperse in 15 ml of *1 M hydrochloric acid* and dilute to 50.0 ml with *water*, filter. Dilute 10.0 ml of filtrate to 100.0 with *water*.

*Reference solution*. Dissolve 30 mg of *buspirone hydrochloride RS* in 15 ml of *1 M hydrochloric acid* and dilute to 50.0 ml with *water*. Dilute 10.0 ml of the solution to 100.0 ml with *water*.

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 65 volumes of *methanol* and 35 volumes of *0.067 M monobasic potassium phosphate* with the pH adjusted to 4.0 with *orthophosphoric acid*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

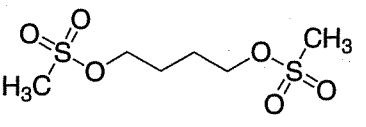
Inject the test solution and the reference solution.

Calculate the content of $C_{21}H_{31}N_5O_2$ in the tablets.

**Storage**. Store protected from moisture.

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

## Busulphan



$C_6H_{14}O_6S_2$ Mol. Wt. 246.3

Busulphan is 1,4-butanediol dimethanesulphonate.



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Busulphan contains not less than 99.0 per cent and not more than 100.5 per cent of $C_6H_{14}O_6S_2$, calculated on the dried basis.

**Category**. Anticancer.

**Dose**. 2 to 4 mg daily; maintenance dose, 0.5 to 2 mg daily.

**Description**. A white or almost white, crystalline powder.

### Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

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

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of equal volumes of *acetone* and *toluene*.

*Test solution*. Dissolve 1 g of the substance under examination in 100 ml of *acetone*.

*Reference solution*. A 1 per cent w/v solution of *busulphan RS* in *acetone*.

Apply to the plate 5 µl of each solution. After development, dry the plate in a current of hot air, spray with *anisaldehyde solution* and heat at 120°. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

C. Heat 0.1 g with 5 ml of *1 M sodium hydroxide* until a clear solution is obtained and allow to cool. To 2 ml of the solution add 0.1 ml of a 3 per cent w/v solution of *potassium permanganate*; the purple colour changes to violet, then to blue and finally to green. Filter and add 1 ml of *ammoniacal silver nitrate solution*; a precipitate is produced.

D. Fuse 0.1 g with 0.1 g of *potassium nitrate* and 0.25 g of *potassium hydroxide*, cool and dissolve the residue in 5 ml of *water*. Acidify with *dilute hydrochloric acid* and add a few drops of *barium chloride solution*; a white precipitate is produced.

### Tests

**Appearance of solution**. Dissolve 0.25 g in 20.0 ml of *acetonitrile*, dilute to 25 ml with *water* and examine immediately. The solution is clear (2.4.1), and not more intensely coloured than reference solution BS6 (2.4.1).

**Acidity**. Dissolve 0.2 g in 50 ml of warm *ethanol* previously neutralised to *methyl red solution* and titrate with *0.1 M sodium hydroxide* using *methyl red solution* as indicator; not more than 0.05 ml of *0.1 M sodium hydroxide* is required.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 2.0 per cent, determined on 1.0 g by drying in an oven over *phosphorus pentoxide* at 60° at a pressure of 1.5 to 2.5 kPa.

**Assay**. Weigh accurately about 0.25 g and shake with 50 ml of *water*. Boil under a reflux condenser for 30 minutes and, if necessary, restore the initial volume with *water*. Allow to cool and titrate with *0.1 M sodium hydroxide*, using 0.3 ml of *dilute phenolphthalein solution* as indicator, until a pink colour is produced.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.01232 g of $C_6H_{14}O_6S_2$.

**Storage**. Store protected from light.

## Busulphan Tablets

Busulphan Tablets contain not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of busulphan, $C_6H_{14}O_6S_2$. The tablets are coated.

**Usual strength**. 2 mg.

### Identification

A. Warm a quantity of the powdered tablets containing 10 mg of Busulphan with 10 ml of *acetone*, filter and evaporate the filtrate to dryness. Dry the residue at 60° at a pressure not exceeding 0.7 kPa for 1 hour. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *busulphan RS* or with the reference spectrum of busulphan.

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

### Tests

**Disintegration** (2.5.1). Maximum time, 15 minutes.

**Uniformity of content**. Comply with the test stated under Tablets.

Determine by gas chromatography (2.4.13).

*Test solution*. Add 1 ml of *water* to one tablet in a 50-ml volumetric flask and place in an ultrasonic bath until completely dispersed. Add 30 ml of *acetone*, shake for 15 minutes and dilute to 50.0 ml with *acetone*. Centrifuge and



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dilute a quantity of the supernatant liquid with *acetone* to produce a solution containing 0.0001 per cent w/v of Busulphan. To 5.0 ml of the resulting solution add 5 ml of a 30 per cent w/v solution of *sodium iodide* in *acetone*, stopper the flask lightly and heat in a water-bath at 50° for 90 minutes. Cool, add 10 ml of a 0.0001 per cent w/v solution of *1,5-di-iodopentane* (internal standard) in *acetone*, mix, add 10 ml of *water* and 20.0 ml of *hexane*, shake vigorously for 1 minute and allow to separate. Use the hexane layer.

*Reference solution (a)*. Add 5 ml of a 30 per cent w/v solution of *sodium iodide* in *acetone* to 5.0 ml of a 0.0001 per cent w/v solution of *busulphan RS* in *acetone*, stopper the flask lightly and heat in a water-bath at 50° for 90 minutes. Cool, add 10 ml of the internal standard solution, mix, add 10 ml of *water* and 20.0 ml of *hexane*, shake vigorously for 1 minute and allow to separate. Use the hexane layer.

*Reference solution (b)*. Prepare in the same manner as reference solution (a) but using 10 ml of *acetone* in place of internal standard solution.

Chromatographic system

- a glass column 1.5 m x 4 mm, packed with acid-washed, diatomaceous support (80 to 100 mesh) coated with 3 per cent w/w of phenyl methyl silicone fluid (50 per cent phenyl),
- temperature:
  column. 140°,
  inlet port and detector at 240°,
- electron capture detector,
- flow rate. 30 ml per minute of the carrier gas.

Calculate the content of $C_6H_{14}O_6S_2$ in the tablet.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Determine by gas chromatography (2.4.13) as given under the test for Uniformity of content using the following test solution.

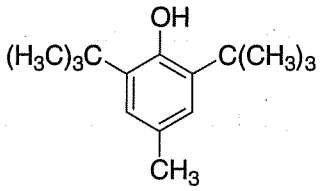
*Test solution*. Weigh accurately a quantity of the powdered tablets containing about 2.5 mg of Busulphan, add 5 ml of *water* and place in an ultrasonic bath until completely dispersed. Add 150 ml of *acetone*, shake for 15 minutes and dilute to 250.0 ml with *acetone*. Centrifuge and dilute 10.0 ml of the supernatant liquid to 100.0 ml with *acetone*. To 5.0 ml of the resulting solution add 5 ml of a 30 per cent solution of *sodium iodide* in *acetone*, stopper the flask lightly and heat in a water-bath at 50° for 90 minutes. Cool, add 10 ml of the internal standard solution, mix, add 10 ml of *water* and 20.0 ml of *hexane*, shake vigorously for 1 minute and allow to separate. Use the hexane layer.

Calculate the content of $C_6H_{14}O_6S_2$ in the tablets.

**Storage**. Store protected from light.

# Butylated Hydroxytoluene

BHT




$C_{15}H_{24}O$ Mol. Wt. 220.4

Butylated Hydroxytoluene is 2,6-bis(1,1-dimethylethyl)- 4-methylphenol.

**Category**. Pharmaceutical aid (antioxidant).

**Description**. A white to yellowish white, crystalline powder.

## Identification

*Test A may be omitted if tests B, C, D and E are carried out. Tests B and C may be omitted if tests A, D and E are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *butylated hydroxytoluene RS*.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.005 per cent w/v solution in *ethanol* shows an absorption maximum only at about 278 nm; absorbance at about 278 nm, between 0.40 and 0.45.

C. Dissolve about 10 mg in 2 ml of *ethanol (95 per cent)*, add 1 ml of a 0.1 per cent w/v solution of *testosterone propionate* in *ethanol (95 per cent)* and 2 ml of *2 M sodium hydroxide*, heat in a water-bath at 80° for 10 minutes and allow to cool; a blue colour is produced.

D. Dissolve about 0.1 g in 10 ml of *ethanol (95 per cent)*, add 2 ml of a 2.0 per cent w/v solution of *sodium tetraborate* and a few crystals of *2,6-dichloroquinone-4-chlorimide*; not more than a faint blue colour is produced (distinction from butylated hydroxyanisole).

E. Dissolve a few crystals in 10 ml of *ethanol (95 per cent)*, add 0.5 ml of a 0.2 per cent w/v solution of *potassium ferricyanide* and 0.5 ml of a 0.2 per cent per cent w/v solution of *ferric ammonium sulphate* in *0.5 M sulphuric acid*; a green to blue colour is produced.

## Tests

**Freezing point** (2.4.11). 69° to 70°.

**Appearance of solution**. A 10.0 per cent w/v solution in *methanol* is clear (2.4.1), and not more intensely coloured than reference solution YS5 or BYS5 (2.4.1).



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**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

*Mobile phase. Dichloromethane.*

*Test solution.* Dissolve 2 g of the substance under examination in 100 ml of *methanol.*

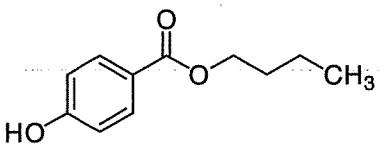
*Reference solution.* Dilute 1 ml of the test solution to 200 ml with *methanol.*

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with a freshly prepared mixture of 70 volumes of *water*, 20 volumes of a 10.5 per cent w/v solution of *ferric chloride* and 10 volumes of *potassium ferricyanide solution*. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

# Butylparaben

Butyl Hydroxybenzoate




$C_{11}H_{14}O_3$ Mol. Wt. 194.2

Butylparaben is *n*-butyl *p*-hydroxybenzoate.

Butylparaben contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{11}H_{14}O_3$.

**Category.** Pharmaceutical aid.

**Description.** A white or almost white, crystalline powder or colourless crystals.

## Identification

*Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.*

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

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

C. To about 10 mg in a test-tube, add 1 ml of *sodium carbonate solution*, boil for 30 second and cool (solution A). To a further 10 mg in a similar test-tube add 1 ml of *sodium carbonate solution*; the substance partly dissolves (solution B). Add at the same time to solution A and solution B, add 5 ml of *aminopyrazolone solution* and 1 ml of *potassium ferricyanide solution*. Solution B is yellow to orange-brown. Solution A is orange to red, the color being clearly more intense than any similar color which may be obtained with solution B.

## Tests

**Appearance of solution.** A 10.0 w/v solution in *ethanol (95 per cent)* is clear (2.4.1) and not more intensely colored than reference solution BYS7 (2.4.1).

**Acidity.** To 2 ml of solution A, add 3 ml of *ethanol (95 per cent)*, 5 ml of *carbon dioxide-free water and* 0.1 ml of *bromocresol green solution.* Not more than 0.1 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to blue.

**Related substances.** Determine by thin-layer chromatography (2.4.17). Coating the plate with *silica gel GF254.*

*Mobile phase.* A mixture of 1 volume of *glacial acetic acid*, 30 volumes of *water* and 70 volumes of *methanol.*

*Test solution (a).* Dissolve 0.1 g of the substance under examination in 10 ml of *acetone.*

*Test solution (b).* Dilute 1 ml of test solution (a) to 10 ml with *acetone.*

*Reference solution (a).* Dilute 0.5 ml of test solution (a) to 100 ml with *acetone.*

*Reference solution (b).* A 0.1 per cent w/v solution of *butyl parahydroxybenzoate RS* in *acetone.*

*Reference solution (c).* Dissolve 10 mg of *propyl parahydroxybenzoate* in 1 ml of test solution (a) and dilute to 10 ml with *acetone.*

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the principal spot in the chromatogram obtained with reference solution (a) (0.5 per cent).



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**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Assay**. To 1.0 g add 20.0 ml of *1 M sodium hydroxide*. Heat at about 70° for 1 hour, cool rapidly in an ice bath. Titrate the excess sodium hydroxide with *0.5 M sulphuric acid* to the second point of inflexion, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *1 M sodium hydroxide* is equivalent to 0.1942 g of $C_{11}H_{14}O_3$.



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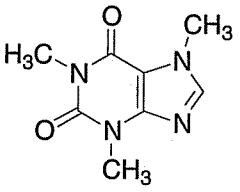
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# Caffeine



$C_8H_{10}N_4O_2$ Mol. Wt. 194.2 (anhydrous)

$C_8H_{10}N_4O_2,H_2O$ Mol. Wt. 212.2 (monohydrate)

Caffeine is 3,7-dihydro-1,3,7-trimethyl-1*H*-purine-2,6-dione or its monohydrate.

Caffeine contains not less than 98.5 per cent and not more than 101.5 per cent of $C_8H_{10}N_4O_2$, calculated on the dried basis.

**Category**. Central nervous system stimulant.

**Dose**. 300 to 600 mg.

**Description**. Silky white crystals, white glistening needles or a white crystalline powder; odourless; sublimes readily.

## Identification

*Test A may be omitted if tests B and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6), after drying the substance under examination at 100° for 1 hour. Compare the spectrum with that obtained with *caffeine RS* or with the reference spectrum of caffeine.

B. To 10 mg in a porcelain dish, add 1 ml of *hydrochloric acid* and 0.1 g of *potassium chlorate* and evaporate to dryness on a water-bath. Expose the residue to the vapours of *dilute ammonia solution*; a purple colour is produced which disappears on addition of a solution of a fixed alkali.

C. To a saturated solution add a few drops of *tannic acid solution*; a white precipitate is produced which is soluble in excess of the reagent.

D. To 5 ml of saturated solution add 1.5 ml of *0.05 M iodine*, the solution remains clear. Add a few drops of *dilute hydrochloric acid*; a brown precipitate is formed which dissolves on neutralisation with *sodium hydroxide solution*.

## Tests

**Appearance of solution**. A 1.0 per cent w/v solution is clear (2.4.1) and colourless (2.4.1).

**Acidity or alkalinity**. Dissolve 0.2 g in 10 ml of boiling *water* and cool. Add 0.1 ml of *bromothymol blue solution*. The solution is coloured green or yellow. Titrate with *0.02 M sodium hydroxide* to a blue colour; not more than 0.1 ml is required.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 40 volumes of *1-butanol*, 30 volumes of *chloroform*, 10 volumes of *strong ammonia solution* and 3 volumes of *acetone*.

*Test solution*. A 2.0 per cent w/v solution of the substance under examination in a mixture of 3 volumes of *chloroform* and 2 volumes of *methanol*.

*Reference solution*. A 0.01 per cent w/v solution of the substance under examination in a mixture of 3 volumes of *chloroform* and 2 volumes of *methanol*.

Apply to the plate 10 µl of each solution. After development, dry in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Arsenic** (2.3.10). Mix 3.3 g with 3 g of *anhydrous sodium carbonate*, add 10 ml of *bromine solution* and mix thoroughly. Evaporate to dryness on a water-bath, gently ignite and dissolve the cooled residue in 16 ml of *brominated hydrochloric acid* and 45 ml of *water*. Remove the excess of bromine with 2 ml of *stannous chloride solution AsT*. The resulting solution complies with the limit test for arsenic (3 ppm).

**Heavy metals** (2.3.13). Mix 2.0 gm with 5 ml of *0.1 M hydrochloric acid* and 45 ml of *water*, warm gently until solution is complete and cool to room temperature. The solution complies with the limit test for heavy metals, Method A (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent (for the anhydrous form) and 8.5 per cent (for the monohydrate form), determined on 1.0 g by drying in an oven at 100° for 1 hour.

**Assay**. Weigh accurately about 0.18 g and dissolve with warming in 5 ml of *anhydrous glacial acetic acid*. For Caffeine Hydrate, use material previously dried at 100° to 105°. Cool, add 10 ml of *acetic anhydride* and 20 ml of *toluene*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25).

1 ml *0.1 M perchloric acid* is equivalent to 0.01942 g of $C_8H_{10}N_4O_2$.

**Storage**. Store protected from light and moisture.

**Labelling**. The label states whether it is anhydrous or monohydrate.

# Calamine

## Prepared Calamine

Calamine is Zinc Oxide with a small proportion of ferric oxide.

Calamine contains not less than 98.0 per cent and not more than 100.5 per cent of ZnO, calculated on the ignited basis.



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**Category**. Topical protectant.

**Description**. A fine, amorphous, impalpable, pink or reddish-brown powder.

### Identification

A. Shake 1 g with 10 ml of *dilute hydrochloric acid* and filter; the filtrate gives the reactions of zinc salts (2.3.1).

B. To 1 g add 10 ml of *dilute hydrochloric acid*, heat to boiling and filter. To the filtrate add a few drops of *ammonium thiocyanate solution*; a reddish colour is produced.

### Tests

**Acid-insoluble substances**. Not more than 1 per cent w/w, determined by the following method. Dissolve 1.0 g in 25 ml of warm *dilute hydrochloric acid*. If any insoluble residue remains, filter, wash with *water*, dry to constant weight at 105°, cool and weigh.

**Alkaline substances**. Digest 1.0 g with 20 ml of warm *water*, filter and add 2 drops of *phenolphthalein solution* to the filtrate. If a red colour is produced, not more than 0.2 ml of *0.05 M sulphuric acid* is required to decolorise it.

**Water-soluble dyes**. Shake 1.0 g with 10 ml of *water* and filter; the filtrate is colourless.

**Ethanol-soluble dyes**. Shake 1.0 g with 10 ml of *ethanol (90 per cent)* and filter; the filtrate is colourless.

**Arsenic** (2.3.10). Dissolve 1.25 g in 15 ml of *brominated hydrochloric acid AsT*, add 45 ml of *water* and remove the excess of bromine with a few drops of *stannous chloride solution AsT*. The resulting solution complies with the limit test for arsenic (8 ppm).

**Lead**. Dissolve 2.0 g in a mixture of 20 ml of *water* and 5 ml of *glacial acetic acid*, filter and add 0.25 ml of *potassium chromate solution*; the solution remains clear for 5 minutes.

**Calcium**. Dissolve 0.5 g in a mixture of 10 ml of *water* and 2.5 ml of *glacial acetic acid* by warming on a water-bath, if necessary and filter. To 0.5 ml of the filtrate, add 15 ml of *dilute ammonia solution* and 2 ml of a 2.5 per cent w/v solution of *ammonium oxalate* and allow to stand for 2 minutes; the solution remains clear.

**Soluble barium salts**. To the remainder of the filtrate obtained in the test for Calcium add 2 ml of *1 M sulphuric acid* and allow to stand for 5 minutes; the solution remains clear.

**Chlorides** (2.3.12). Dissolve 0.33 g in *water* with the addition of 1 ml of *nitric acid* and dilute to 30 ml with *water*. The resulting solution complies with the limit test for chlorides (750 ppm).

**Sulphates** (2.3.17). Dissolve 0.1 g in *water* with the addition of 3 ml of *2 M hydrochloric acid*, filter and dilute to 60 ml with *water*. The resulting solution complies with the limit test for sulphates (0.6 per cent).

**Loss on ignition** (2.4.20). Not more than 2.0 per cent, determined on 2.0 g by igniting to constant weight at a temperature not less than 900°.

**Assay**. Weigh accurately about 1.5 g and digest with 50.0 ml of *0.5 M sulphuric acid*, applying gentle heat until no further solution occurs. Filter and wash the residue with hot *water* until the last washing is neutral to *litmus paper*. To the combined filtrate and washings, add 2.5 g of *ammonium chloride*, cool and titrate with *1 M sodium hydroxide* using *methyl orange solution* as indicator.

1 ml of *0.5 M sulphuric acid* is equivalent to 0.04068 g of ZnO.

**Storage**. Store protected from light and moisture.

## Aqueous Calamine Cream

| | |
|---|---|
| Calamine | 40 g |
| Zinc Oxide | 30 g |
| Liquid Paraffin | 200 g |
| Self-Emulsifying Glyceryl Monostearate | 50 g |
| Cetostearyl Alcohol | 40 g |
| Cetomacrogol 1000 | 10 g |
| Phenoxyethanol | 5 g |
| Purified Water, freshly boiled and cooled | 625 g |

Melt together the Cetostearyl Alcohol and Cetomacrogol 1000, stir until cold and dissolve this mixture and the Self-Emulsifying Glyceryl Monostearate in the Liquid Paraffin at 60°. Add with rapid stirring to a solution of the Phenoxyethanol in 450 g of the Purified Water at the same temperature and stir until cold. Triturate the Calamine and the Zinc Oxide with the remainder of the Purified Water and incorporate in the cream with stirring.

Aqueous Calamine Cream contains not less than 6.30 per cent and not more than 7.67 per cent w/w of ZnO.

### Identification

The residue obtained in the Assay is yellow when hot and white when cool.

### Tests

**Other tests**. Comply with the tests stated under Creams.

**Assay**. Weigh accurately about 4 g. Heat carefully, taking care to avoid spurting, until the liquid is completely evaporated and the solid is charred. Ignite the residue to constant weight at a temperature of not less than 900°.

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



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**Labelling.** The label states (1) the concentrations of Calamine and Zinc Oxide in the preparation; (2) that the preparation is intended for external use only; (3) the storage conditions.

## Calamine Lotion

| | |
|---|---|
| Calamine | 150 g |
| Zinc Oxide | 50 g |
| Bentonite | 30 g |
| Sodium Citrate | 5 g |
| Liquefied Phenol | 5 ml |
| Glycerin | 50 ml |
| Purified Water, freshly boiled and cooled sufficient to produce | 1000 ml |

Triturate the Calamine, the Zinc Oxide and the Bentonite with a solution of the Sodium Citrate in about 700 ml of Purified Water and add the Liquified Phenol, the Glycerin and sufficient Purified Water to produce 1000 ml.

### Identification

A. To 2 ml add 2 ml of *periodic acid reagent*, shake, centrifuge and add 0.5 ml of the supernatant liquid to 2 ml of *ammonical silver nitrate solution* in a test-tube; a silver mirror is produced on the walls of the tube.

B. Mix 2 ml with 50 ml of *water*, centrifuge and decant the supernatant liquid. Suspend the residue in 20 ml of *water*, add 1 ml of *hydrochloric acid*, mix and filter. 5 ml of the filtrate, after neutralisation by dropwise addition of *2 M sodium hydroxide*, gives the reactions of zinc salts (2.3.1).

### Tests

**Microbial contamination** (2.2.9). 1 g is free from *Staphylococcus aureus* and 10 g is free from *Pseudomonas aeruginosa.*

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

**Labelling.** The label states (1) the concentrations of Calamine and Zinc Oxide in the preparation; (2) that the preparation is intended for external use only; (3) that the contents should be shaken before use; (4) the conditions under which the preparation should be stored.

## Calamine Ointment

| | |
|---|---|
| Calamine | 150 g |
| White Soft Paraffin | 850 g |

Triturate the calamine with part of the White Soft Paraffin until smooth and gradually incorporate the remainder of the White Soft Paraffin.

Calamine Ointment contains not less than 7.8 per cent and not more than 9.4 per cent w/w of Zn.

### Identification

The residue obtained in the Assay is yellow when hot and white when cool.

### Tests

**Other tests.** Complies with the tests stated under Ointments.

**Assay.** Weigh accurately about 1.0 g. Heat gently until the base is completely volatalised or charred. Increase the heat until all the carbon is removed and ignite the residue until, after further ignition, two successive weighings do not differ by more than 0.2 per cent of the weight of the residue.

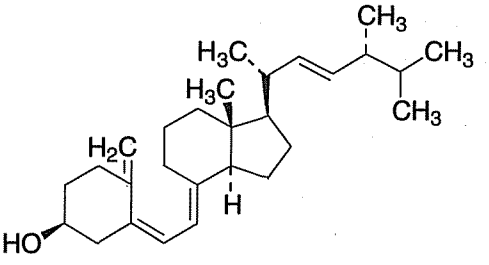
1g of the residue is equivalent to 0.8034 g of Zn.

**Storage.** Store in well-closed containers, at a temperature not exceeding 30°.

**Labelling.** The label states (1) the concentration of Calamine in the preparation; (2) that the preparation is intended for external use only; (3) the storage conditions.

## Calciferol

Ergocalciferol; Vitamin $D_2$




$C_{28}H_{44}O$ Mol. Wt. 396.7

Calciferol is (5*Z*,7*E*,22*E*)-(3*S*)-9,10-secoergosta-5,7,10(19),22-tetraen-3-ol.

Calciferol contains not less than 97.0 per cent and not more than 103.0 per cent of $C_{28}H_{44}O$.

**Category.** Vitamin D (antirachitic).

**Dose.** Orally, in prevention of rickets, not more than 20 µg (800 Units) daily, allowance being made for vitamin D obtained from other sources; in the treatment of rickets and osteomalacia, 125 µg to 1.25 mg (5,000 to 50,000 Units) daily; in the treatment of hypocalcaemia of hypoparathyroidism, 1.25 to 5 mg (50,000



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to 200,000 Units) daily. By intramuscular injection, 5 to 10 mg.

[Calciferol contains 40,000 Units of antirachitic activity (vitamin D) in each mg].

**Description**. White or almost white crystals or a white or slightly yellowish, crystalline powder. It is sensitive to air, heat and light. A reversible isomerisation to pre-ergocalciferol may occur in solution, depending on temperature and time.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ergocalciferol RS*.

B. Dissolve 1 mg in 1 ml of *1,2-dichloroethane* and 4 ml of *antimony trichloride solution*; a yellowish-orange colour is produced.

C. In the test for Ergosterol, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution (a).

D. To a solution of about 0.5 mg in 5 ml of *chloroform* add 0.3 ml of *acetic anhydride* and 0.1 ml of *sulphuric acid* and shake vigorously; a bright red colour is produced which rapidly changes through violet and blue to green.

## Tests

**Specific optical rotation** (2.3.22). +103° to +107°, determined within 30 minutes of preparation, in a solution prepared by dissolving 0.2 g rapidly and without heating in sufficient *aldehyde-free ethanol (95 per cent)* to produce 25.0 ml.

**Light absorption**. Dissolve 10 mg rapidly and without heating in sufficient *aldehyde-free ethanol (95 per cent)* to produce 100.0 ml. Dilute 5.0 ml of this solution to 50.0 ml with *aldehyde-free ethanol (95 per cent)*. Absorbance of the resulting solution at the maximum at about 265 nm (2.4.7), measured within 30 minutes of preparation, 0.45 to 0.50.

**Reducing substances**. To 10 ml of a 1 per cent w/v solution in *aldehyde-free ethanol (95 per cent)* add 0.5 ml of a 0.5 per cent w/v solution of *blue tetrazolium* in *aldehyde-free ethanol (95 per cent)* and 0.5 ml of a solution prepared by diluting 1 volume of *tetramethylammonium hydroxide solution (10 per cent)* with *aldehyde-free ethanol (95 per cent)* to make 10 volumes. Allow to stand for exactly 5 minutes and add 1 ml of *glacial acetic acid*. Measure the absorbance of the resulting solution at the maximum at about 525 nm (2.4.7), using as the blank a solution prepared by treating 10 ml of *aldehyde-free ethanol (95 per cent)* in the same manner. The absorbance is not more than that obtained by carrying out the procedure described above simultaneously using a solution containing 0.2 µg per ml of *hydroquinone* in *aldehyde-free ethanol (95 per cent)* and beginning at the words "add 0.5 ml of a 0.5 per cent w/v solution....".

**Ergosterol**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A 0.01 per cent w/v solution of *butylated hydroxytoluene* in a mixture of equal volumes of *cyclohexane* and *peroxide-free ether*.

*Test solution*. Dissolve 0.25 g of the substance under examination in sufficient *1,2-dichloroethane* containing 1 per cent w/v of squalane and 0.1 per cent w/v *butylated hydroxytoluene* (solvent A) to produce 5 ml.

*Reference solution (a)*. A 5.0 per cent w/v solution of *ergocalciferol RS* in solvent A.

*Reference solution (b)*. A 0.01 per cent w/v solution of *ergosterol RS* in solvent A.

*Reference solution (c)*. Mix equal volumes of reference solution (a) and (b).

Apply to the plate 10 µl of each solution. Develop the chromatograms immediately, protected from light. After development dry the plate in air and spray three times with *antimony trichloride reagent*. Examine the chromatograms for not more than 4 minutes after spraying. The principal spot in the chromatogram obtained with the test solution is initially orange-yellow but becomes brown later. In the chromatogram obtained with the test solution any violet spot with an $R_f$ value slightly lower than that of the principal spot (due to ergosterol and appearing slowly) is not more intense than the spot in the chromatogram obtained with reference solution (b). The chromatogram obtained with the test solution shows no spot that does not correspond to one of the spots in the chromatograms obtained with reference solution (a) and (b). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated principal spots.

**Assay**. *Carry out the following procedure as rapidly as possible in subdued light and protected from air.*

Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh accurately about 50.0 mg of the substance under examination, dissolve in 10 ml of *toluene* without heating and dilute to 100.0 ml with the mobile phase; dilute 5.0 ml of this solution to 50.0 ml with the mobile phase; further dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

*Reference solution (a)*. Dissolve 50.0 mg of *ergocalciferol RS* in 10 ml of *toluene* without heating and dilute to 100.0 ml with the mobile phase; dilute 5.0 ml of this solution to 50.0 ml with the mobile phase; further dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.



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*Reference solution (b).* Dissolve 50.0 mg of *cholecalciferol RS* in 10 ml of *toluene* without heating and dilute to 100.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.Heat under a reflux condenser 5.0 ml of this solution, under nitrogen, using a water-bath for 60 minutes to obtain a solution of cholecalciferol, precholecalciferol and *trans*-cholecalciferol. Cool and dilute the refluxed solution to 50.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with porous silica or ceramic microparticles (3 to 10 µm) (Such as Nucleosil 50-S 5 µm),
- mobile phase: a mixture of 997 volumes of *hexane* and 3 volumes of *1-pentanol*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 or 20 µl.

Inject a suitable volume of reference solution (b) and adjust the sensitivity so that the height of the peak due to cholecalciferol is more than 50 per cent of full-scale deflection.The approximate relative retention times calculated with reference to cholecalciferol are 0.4 for precholecalciferol and 0.5 for *trans*-cholecalciferol. The resolution between precholecalciferol and *trans*-cholecalciferol should not be less than 1.0; if necessary adjust the proportions of the constituents and flow rate of the mobile phase to obtain the required resolution.

Inject a suitable volume of reference solution (a) and adjust the sensitivity so that height of the peak due to ergocalciferol is more than 50 per cent of full-scale deflection. Inject the same volume of the test solution. Measure the areas of the major peaks.

Calculate the content of $C_{28}H_{44}O$.

**Storage**. Store protected from light in hermetically-sealed containers under nitrogen in a refrigerator (2° to 8°). The contents of an opened container should be used immediately.

# Calciferol Capsules

Calciferol Capsules contain Cholecalciferol or Ergocalciferol usually as a vegetable oil solution contained in soft gelatin capsules.

Calciferol Capsules contain not less than 90.0 per cent and not more than 125.0 per cent of the stated amount of cholecalciferol, $C_{27}H_{44}O$ or ergocalciferol, $C_{28}H_{44}O$.

**Usual strengths**. 250 µg; 1.25 mg.
[Cholecalciferol and Ergocalciferol contain 40,000 Units of antirachitic activity (vitamin D) in each mg].

## Identification

Extract a capsule with 5 ml of *ethanol-free chloroform*, filter and to 1 ml of the filtrate add 9 ml of *antimony trichloride solution*. The light absorption of the resulting solution shows an absorption maximum at about 500 nm (2.4.7).

## Tests

**Other tests**. Comply with the tests stated under Capsules.

**Assay**. Determine by liquid chromatography (2.4.14).

*Carry out the following procedure as rapidly as possible in subdued light and protected from air.*

*Test solution.* Mix the content of 20 capsules. Weigh accurately a quantity containing about 250 µg of Calciferol, disperse in 4 ml of *water*. Add 12 ml of *dimethyl sulphoxide*, mix, extract with 100 ml of *hexane* by shaking for 30 minutes, centrifuge the hexane layer and use the clear supernatant liquid.

*Reference solution (a).* A 0.001 per cent w/v solution of *cholecalciferol RS* or *ergocalciferol RS*, as appropriate.

*Reference solution (b).* Dissolve 50.0 mg of *cholecalciferol RS* or *ergocalciferol RS* as appropriate in 10 ml of *toluene* without heating and dilute with the mobile phase to 100.0 ml; dilute 5.0 ml of this solution to 50.0 ml with the mobile phase. Reflux 5.0 ml of this solution, under nitrogen, using a water-bath, for 60 minutes to obtain a solution of cholecalciferol, precholecalciferol and *trans*-cholecalciferol. Cool and dilute the refluxed solution to 50.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with porous silica or ceramic microparticles (3 to 10 µm) (Such as Nucleosil 50-S 5 µm),
- mobile phase: a mixture of 997 volumes of *hexane* and 3 volumes of *1-pentanol*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 or 20 µl.

Inject a suitable volume of reference solution (b). Adjust the sensitivity so that the height of the peak due to cholecalciferol is more than 50 per cent of the full-scale deflection. Record the chromatograms after five more injections. The approximate relative retention times calculated with reference to cholecalciferol are 0.4 for precholecalciferol and 0.5 for *trans*-cholecalciferol. The resolution between precholecalciferol and *trans*-cholecalciferol should be not less than 1.0; if necessary adjust the proportions of the constituents and flow rate of the mobile phase to obtain the required resolution.

Inject reference solution (a). Adjust the sensitivity so that the height of the peak due to cholecalciferol or ergocalciferol is more than 50 per cent of the full-scale deflection.

Inject the test solution and reference solution (a).



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Calculate the content of cholecalciferol, $C_{27}H_{44}O$, or ergocalciferol, $C_{28}H_{44}O$ in the capsules.

**Storage**. Store protected from light and moisture at a temperature not exceeding 30°.

## Calciferol Injection

Calciferol Injection is a sterile solution of Cholecalciferol or Ergocalciferol in Ethyl Oleate.

Calciferol Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cholecalciferol, $C_{27}H_{44}O$ or ergocalciferol, $C_{28}H_{44}O$.

**Usual strength**. 7.5 mg of Cholecalciferol or Ergocalciferol per ml.
[Cholecalciferol or Ergocalciferol contains 40,000 Units of antirachitic activity (vitamin D) in each mg].

**Description**. A pale yellow, oily liquid.

### Identification

To 1 ml of a 0.2 per cent v/v solution of the injection in *ethanol-free chloroform* add 9 ml of *antimony trichloride solution*. The resulting solution shows an absorption maximum at about 500 nm (2.4.7).

### Tests

**Other tests**. Comply with the tests stated under Parenteral Preparations (Injections).

**Assay**. Determine by liquid chromatography (2.4.14).

*Carry out the following procedure as rapidly as possible in subdued light and protected from air.*

*Test solution*. Weigh accurately a volume of injection containing about 250 µg of Calciferol, disperse in 4 ml of *water*. Add 12 ml of *dimethyl sulphoxide*, mix, extract with 100 ml of *hexane* by shaking for 30 minutes, centrifuge the hexane layer and use the clear supernatant liquid.

*Reference solution (a)*. A 0.001 per cent w/v solution of *cholecalciferol RS* or *ergocalciferol RS*, as appropriate.

*Reference solution (b)*. Dissolve 50.0 mg of *cholecalciferol RS* or *ergocalciferol RS* as appropriate in 10 ml of *toluene* without heating and dilute with the mobile phase to 100.0 ml; dilute 5.0 ml of this solution to 50.0 ml with the mobile phase. Reflux 5.0 ml of this solution, under nitrogen, using a water-bath, for 60 minutes to obtain a solution of cholecalciferol, precholecalciferol and *trans*-cholecalciferol. Cool and dilute the refluxed solution to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with porous silica or ceramic microparticles (3 to 10 µm) (such as Nucleosil 50-S 5 µm),
- mobile phase: a mixture of 997 volumes of *hexane* and 3 volumes of *1-pentanol*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 or 20 µl.

Inject a suitable volume of reference solution (b). Adjust the sensitivity so that the height of the peak due to cholecalciferol is more than 50 per cent of the full-scale deflection. Record the chromatograms after five more injections. The approximate relative retention times calculated with reference to cholecalciferol are 0.4 for precholecalciferol and 0.5 for *trans*-cholecalciferol. The resolution between precholecalciferol and *trans*-cholecalciferol should be not less than 1.0; if necessary adjust the proportions of the constituents and flow rate of the mobile phase to obtain the required resolution.

Inject reference solution (a). Adjust the sensitivity so that the height of the peak due to cholecalciferol or ergocalciferol is more than 50 per cent of the full-scale deflection.

Inject the test solution and reference solution (a).

Calculate the content of cholecalciferol, $C_{27}H_{44}O$, or ergocalciferol, $C_{28}H_{44}O$ in the injection.

**Storage**. Store in a single dose container protected from light at a temperature not exceeding 30°.

**Labelling**. The label states (1) that the preparation is for intramuscular use only; (2) the number of Units of antirachitic activity (vitamin D) per ml.

## Calciferol Oral Solution

Calciferol Oral Drops; Calciferol Solution

Calciferol Oral Solution is a solution of Cholecalciferol or Ergocalciferol in a suitable vegetable oil and may be prepared by warming to 40° a 1 per cent w/v suspension of Cholecalciferol or Ergocalciferol in a suitable vegetable oil, such as Arachis Oil, carbon dioxide being bubbled through it to facilitate solution, and adding a sufficient quantity of the oil to produce a solution containing the stated amount of Cholecalciferol or Ergocalciferol.

Calciferol Oral Solution contains not less than 85.0 per cent and not more than 120.0 per cent of the stated amount of cholecalciferol, $C_{27}H_{44}O$ or ergocalciferol, $C_{28}H_{44}O$.

**Usual strength**. 75 µg of Cholecalciferol or Ergocalciferol per ml.

[Cholecalciferol or Ergocalciferol contains 40,000 Units of antirachitic activity (vitamin D) in each mg].

**Description**. A pale yellow, oily liquid; odour, slight but not rancid.



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### Identification

To 1 ml of a 20 per cent v/v solution in *ethanol-free chloroform* add 9 ml of *antimony trichloride solution*. The light absorption of the resulting solution shows an absorption maximum at about 500 nm (2.4.7).

### Tests

**Other tests**. Complies with the tests stated under Oral Liquids.

**Assay**. Determine by liquid chromatography (2.4.14).

*Carry out the following procedure as rapidly as possible in subdued light and protected from air.*

*Test solution*. Weigh accurately a quantity containing about 250 µg of Calciferol, disperse in 4 ml of *water*. Add 12 ml of *dimethyl sulphoxide*, mix, extract with 100 ml of *hexane* by shaking for 30 minutes, centrifuge the hexane layer and use the clear supernatant liquid.

*Reference solution (a)*. A 0.001 per cent w/v solution of *cholecalciferol RS* or *ergocalciferol RS*, as appropriate.

*Reference solution (b)*. Dissolve 50.0 mg of *cholecalciferol RS* or *ergocalciferol RS* as appropriate in 10 ml of *toluene* without heating and dilute with the mobile phase to 100.0 ml; dilute 5.0 ml of this solution to 50.0 ml with the mobile phase. Reflux 5.0 ml of this solution, under nitrogen, using a water-bath, for 60 minutes to obtain a solution of cholecalciferol, precholecalciferol and *trans*-cholecalciferol. Cool and dilute the refluxed solution to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with porous silica or ceramic microparticles (3 to 10 µm) (such as Nucleosil 50-S 5 µm),
- mobile phase: a mixture of 997 volumes of *hexane* and 3 volumes of *1-pentanol*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 or 20 µl.

Inject a suitable volume of reference solution (b). Adjust the sensitivity so that the height of the peak due to cholecalciferol is more than 50 per cent of the full-scale deflection. Record the chromatograms after five more injections. The approximate relative retention times calculated with reference to cholecalciferol are 0.4 for precholecalciferol and 0.5 for *trans*-cholecalciferol. The resolution between precholecalciferol and *trans*-cholecalciferol should be not less than 1.0; if necessary adjust the proportions of the constituents and flow rate of the mobile phase to obtain the required resolution.

Inject reference solution (a). Adjust the sensitivity so that the height of the peak due to cholecalciferol or ergocalciferol is more than 50 per cent of the full-scale deflectison.

Inject the test solution and reference solution (a).

Determine the weight per ml (2.4.29) of the oral solution and calculate the content of $C_{27}H_{44}O$ or $C_{28}H_{44}O$, weight in volume.

**Storage**. Store protected from light and moisture at a temperature not exceeding 30°.

**Labelling**. The label states the number of Units of antirachitic activity (vitamin D) per ml.

## Calciferol Tablets

Calciferol Tablets contain Cholecalciferol or Ergocalciferol

Calciferol Tablets contain not less than 90.0 per cent and not more than 125.0 per cent of the stated amount of cholecalciferol, $C_{27}H_{44}O$ or ergocalciferol, $C_{28}H_{44}O$.

**Usual strengths**. 250 µg; 1.25 mg.
[Cholecalciferol or Ergocalciferol contains 40,000 Units of antirachitic activity (vitamin D) in each mg].

### Identification

Powder a tablet, extract with 5 ml of *ethanol-free chloroform*, filter and to 1 ml of the filtrate add 9 ml of *antimony trichloride solution*; a brownish-red colour is produced.

### Tests

**Uniformity of content**. Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay using the following test solution.

*Test solution*. For tablets containing less than 250 µg, add 2 ml of *water* to one tablet in an amber-coloured flask and disperse with the aid of ultrasound. Add 6 ml of *dimethyl sulphoxide*, mix, extract with 25 ml of *hexane* by shaking for 30 minutes, centrifuge the hexane layer and use the clear supernatant liquid. For tablets containing more than 250 µg, prepare the solution in the same manner but using 4 ml of *water*, 12 ml of *dimethyl sulphoxide* and 100 ml of *hexane*.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Carry out the following procedure as rapidly as possible in subdued light and protected from air.*

*Test solution*. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 250 µg of Calciferol, disperse in 4 ml of *water*. Add 12 ml of *dimethyl sulphoxide*, mix, extract with 100 ml of *hexane* by shaking for 30 minutes, centrifuge the hexane layer and use the clear supernatant liquid.

*Reference solution (a)*. A 0.001 per cent w/v solution of *cholecalciferol RS* or *ergocalciferol RS*, as appropriate.

*Reference solution (b)*. Dissolve 50.0 mg of *cholecalciferol RS* or *ergocalciferol RS* as appropriate in 10 ml of *toluene*



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without heating and dilute with the mobile phase to 100.0 ml; dilute 5.0 ml of this solution to 50.0 ml with the mobile phase. Reflux 5.0 ml of this solution, under nitrogen, using a water-bath, for 60 minutes to obtain a solution of cholecalciferol, precholecalciferol and *trans*-cholecalciferol. Cool and dilute the refluxed solution to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with porous silica or ceramic microparticles (3 to 10 µm) (Such as Nucleosil 50-S 5 µm),
- mobile phase: a mixture of 997 volumes of *hexane* and 3 volumes of *1-pentanol*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 or 20 µl.

Inject a suitable volume of reference solution (b). Adjust the sensitivity so that the height of the peak due to cholecalciferol is more than 50 per cent of the full-scale deflection. Record the chromatograms after five more injections. The approximate relative retention times calculated with reference to cholecalciferol are 0.4 for precholecalciferol and 0.5 for *trans*-cholecalciferol. The resolution between precholecalciferol and *trans*-cholecalciferol should be not less than 1.0; if necessary adjust the proportions of the constituents and flow rate of the mobile phase to obtain the required resolution.

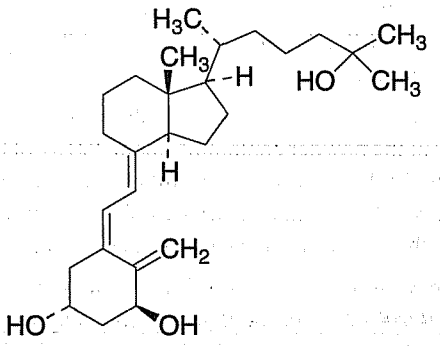
Inject reference solution (a). Adjust the sensitivity so that the height of the peak due to cholecalciferol or ergocalciferol is more than 50 per cent of the full-scale deflection.

Inject the test solution and reference solution (a).

Calculate the content of cholecalciferol, $C_{27}H_{44}O$, or ergocalciferol, $C_{28}H_{44}O$ in the tablets.

**Storage**. Store protected from light and moisture, at a temperature not exceeding 30°.

# Calcitriol




$C_{27}H_{44}O_3$ Mol. Wt. 416.6

Calcitriol is (5*Z*,7*E*)-9,10-secocholesta-5,7,10(19)-triene-1α,3β,25-triol.

Calcitriol contains not less than 97.0 per cent and not more than 103.0 per cent of $C_{27}H_{44}O_3$.

**Category**. Vitamin D analogue.

**Dose**. 0.5 µg.

**Description**. A white or almost white crystals.

A reversible isomerisation to pre-calcitriol takes place in solution, depending on temperature and time. The activity is due to both compounds.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *calcitriol RS* or with the reference specturm of calcitriol.

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

## Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

Carry out the test as described under Assay.

In the chromatogram obtained with the test solution, the area of any secondary peak, apart from pre-calcitriol, eluted within twice the retention time of calcitriol, is not more than 0.5 times the area of the peak obtained with reference solution (b) ( 0.5 per cent) and the sum of areas of all the secondary peaks is not more than the area of the peaks obtained with reference solution (b) (1.0 per cent ). Ignore any peak with an area less than 0.1 times that of the peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

**Assay**. Determine by liquid chromatography (2.4.14).

*NOTE — Carry out the assay as rapidly as possible, avoiding exposure to actinic light and air.*

*Test solution*. Dissolve 1.0 mg of the substance under examination in 10.0 ml of the mobile phase.

*Reference solution (a)*. A 0.01 per cent w/v solution of *calcitriol RS* in the mobile phase.

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

*Reference solution (c)*. Keep 2 ml of reference solution (a) at 80° for 30 minutes.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with (5 µm),
- column temperature. 40°,



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- mobile phase: a mixture of 45 volumes of a solution containing 0.1 per cent w/v of *tris(hydroxymethyl) aminomethane* with the pH adjusted to 7.3 with *orthophosphoric acid* and 55 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 50 µl.

Inject reference solution (c). The relative retention times with reference to calcitriol for precalcitriol is about 0.9 and the resolution between the peaks due to calcitriol and pre-calcitriol is not less than 3.5. Inject reference solution (a) in replicate. The relative standard deviation is not more than 2.0 per cent and the column efficiency for calcitriol peak is not less than 10000 theoretical plates.

Inject the test solution and reference solution (a). Run the chromatogram for twice the retention time of calcitriol.

Calculate the content of $C_{27}H_{44}O_3$.

**Storage.** Store under nitrogen, protected from light and moisture, in a refrigerator (2° to 8°). The contents of an opened container are to be used immediately.

## Calcitriol Capsules

Calcitriol Capsules contain a solution of Calcitriol in a suitable fixed oil.

Calcitriol Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of calcitriol, $C_{27}H_{44}O_3$.

### Identification

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

### Tests

**Other tests.** Comply with the tests stated under Capsules.

**Assay.** Determine by liquid chromatography (2.4.14).

*Note—Carry out the test in subdued light.*

*Test solution.* Dilute a quantity of the mixed contents of capsules containing 1.5 µg of Calcitriol to 1 ml with the mobile phase.

*Reference solution.* A 0.00015 per cent w/v solution of *calcitriol RS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with silica (5 µm) (Such as Lichrosorb Si60),
- mobile phase: a mixture of 1 volume of *propanol*, 2 volumes of *methanol*, 40 volumes of *hexane* and 60 volumes of *ethyl acetate*,
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{27}H_{44}O_3$ in the capsules.

## Calcium Carbonate

Precipitated Chalk

$CaCO_3$ Mol. Wt. 100.1

Calcium Carbonate contains not less than 98.0 per cent and not more than 100.5 per cent of $CaCO_3$, calculated on the dried basis.

**Category.** Fine, white, microcrystalline powder.

**Dose.** 1 to 5 g.

**Description.** A fine, white, microcrystalline powder.

### Identification

A. Dissolve 5.0 g in 80 ml of *2 M acetic acid*. When effervescence ceases, boil the solution for 2 minutes, allow to cool, dilute to 100 ml with *2 M acetic acid* and filter, if necessary, through a sintered-glass filter reserving any residue for the test for Substances insoluble in *acetic acid*; 0.2 ml of the filtrate (solution A) gives reactions A and B of calcium salts (2.3.1).

B. Gives reaction A of carbonates (2.3.1).

### Tests

**Substances insoluble in acetic acid.** Wash any residue obtained in Identification test A with four quantities, each of 5 ml of hot *water* and dry at 100° for 1 hour; the residue weighs not more than 10 mg (0.2 per cent).

**Arsenic** (2.3.10). Dissolve 2.5 g in 15 ml of *brominated hydrochloric acid* and 45 ml of *water* and remove the excess of bromine with a few drops of *stannous chloride solution AsT*. The resulting solution complies with the limit test for arsenic (4 ppm).

**Heavy metals** (2.3.13). To 1.0 g add 5 ml of *water*, and 8 ml of *dilute hydrochloric acid*, the latter being added slowly, shake



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and evaporate to dryness on a water-bath. Dissolve the residue in 20 ml of *water*, filter, add to the filtrate 3 ml of *dilute acetic acid* and *water* to make 25 ml. The solution complies with the limit test for heavy metals, Method A (20 ppm).

**Barium**. Dissolve 0.6 g in 10 ml of *2 M acetic acid* by boiling, cool and add 10 ml of *calcium sulphate solution*; the solution remains clear for not less than 15 minutes.

**Iron** (2.3.14). Dissolve 0.2 g in 5 ml *water* and 0.5 ml of *iron-free hydrochloric acid*, boil and dilute to 40 ml with *water*, the solution complies with the limit test for iron (200 ppm).

**Magnesium and alkali metals**. Dissolve 1.0 g in 10 ml of *dilute hydrochloric acid*, neutralise the solution by adding *dilute ammonia solution*, heat the solution to boiling and add 50 ml of hot *ammonium oxalate solution*. Cool, dilute to 100 ml with *water* and filter. To 50 ml of the filtrate add 1.5 ml of *dilute sulphuric acid*, evaporate to dryness on a water-bath, heat the residue to redness, allow to cool and weigh. The residue weighs not more than 5 mg (1.0 per cent).

**Chlorides** (2.3.12). 1.0 g dissolved in *water* by the addition of 3 ml of *nitric acid* complies with the limit test for chlorides (250 ppm).

**Sulphates** (2.3.17). Suspend 50.0 mg in 5 ml of *water* and add dropwise sufficient *dilute hydrochloric acid* to effect solution. Add 2 ml of *dilute hydrochloric acid*; the resulting solution complies with the limit test for sulphates (0.3 per cent).

**Loss on drying** (2.4.19). Not more than 2.0 per cent, determined on 1.0 g by drying in an oven at 200°.

**Assay**. Weigh accurately about 0.1 g and dissolve in 3 ml of *dilute hydrochloric acid* and 10 ml of *water*. Boil for 10 minutes, cool and dilute to 50 ml with *water*. Titrate with *0.05 M disodium edetate* to within a few ml of the expected end-point, add 8 ml of *sodium hydroxide solution* and 0.1 g of *calcon mixture* and continue the titration until the colour of the solution changes from pink to a full blue colour.

1 ml of *0.05 M disodium edetate* is equivalent to 0.005004 g of $CaCO_3$.

# Calcium Chloride

Calcium Chloride Dihydrate

$CaCl_2,2H_2O$ Mol. Wt. 147.0

Calcium Chloride contains not less than 97.0 per cent and not more than 103.0 per cent of $CaCl_2,2H_2O$.

**Category**. Calcium replenisher.

**Dose**. Orally,1 to 2 g; by slow intravenous injection, 5 to 10 ml of a 10 per cent w/v solution.

**Description**. A white, crystalline powder or fragments or granules; odourless; hygroscopic.

## Identification

A. Gives reactions A and B of calcium salts (2.3.1).

B. A 10 per cent w/v solution in *carbon dioxide-free water* prepared from *distilled water* (solution A) gives reaction A of chlorides (2.3.1).

## Tests

**Appearance of solution**. Solution A is clear (2.4.1) and not more intensely coloured than reference solution YS6 (2.4.1).

**Acidity or alkalinity**. To 10 ml of a freshly prepared 10 per cent w/v solution add 2 drops of *phenolphthalein solution*. Titrate with *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide*; not more than 0.2 ml is required.

**Arsenic** (2.3.10). Dissolve 3.33 g in 15 ml of *brominated hydrochloric acid* and 45 ml of *water* and remove the excess of bromine with a few drops of *stannous chloride solution AsT*. The resulting solution complies with the limit test for arsenic (3 ppm).

**Aluminium and phosphate**. To 10 ml of a 5.0 per cent w/v solution, add 2 drops of *dilute hydrochloric acid* and 1 drop of *phenolphthalein solution*. Add *ammonium chloride-ammonium hydroxide solution* dropwise until the solution is faintly pink, add a few drops in excess and heat the liquid to boiling; no turbidity or precipitate is produced.

**Barium**. To 10 ml of solution A add 1 ml of *calcium sulphate solution*. After not less than 15 minutes the solution is not more opalescent than a mixture of 10 ml of solution A and 1 ml of *distilled water*.

**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals, Method A (10 ppm).

**Iron** (2.3.14). Dissolve 2.0 g in 0.5 ml of *hydrochloric acid* and 25 ml of *water*; the resulting solution complies with the limit test for iron (20 ppm).

**Magnesium and alkali salts**. Dissolve 1.0 g in 50 ml of *water*, add 0.5 g of *ammonium chloride* heat the solution to boiling and add 50 ml of hot *ammonium oxalate solution*. Cool, dilute to 100 ml with *water* and filter. To 50 ml of the filtrate add 1.5 ml of *dilute sulphuric acid*, evaporate to dryness on a water-bath, heat the residue to redness, allow to cool and weigh. The residue weighs not more than 5 mg (1.0 per cent).

**Sulphates** (2.3.17). 0.5 g dissolved in 15 ml of *distilled water* complies with the limit test for sulphates (300 ppm).

**Assay**. Weigh accurately about 0.15 g and dissolve in 50 ml of *water*. Titrate with *0.05 M disodium edetate* to within a few ml of the expected end-point, add 8 ml of *sodium hydroxide*



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*solution* and 0.1 g of *calcon mixture* and continue the titration until the colour of the solution changes from pink to a full blue colour.

1 ml of *0.05 M disodium edetate* is equivalent to 0.007351 g of $CaCl_2,2H_2O$.

**Storage**. Store protected from moisture.

## Calcium Chloride Injection

Calcium Chloride Dihydrate Injection

Calcium Chloride Injection is a sterile solution of Calcium Chloride Dihydrate in Water for Injections.

Calcium Chloride Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of calcium chloride dihydrate, $CaCl_2, 2H_2O$.

**Usual strengths**. Each gram of calcium chloride dihydrate represents approximately 6.8 mmol (13.6 mEq) calcium and 13.6 mmol (13.6 mEq) chloride. Each ml of the 5 ml ampoule contains 1.01 mmol (2.20 mEq) calcium. Each ml of the 10 ml ampoule contains 0.68 mmol (1.36 mEq) calcium.

### Identification

A. Gives reaction A of calcium salts (2.3.1).

B. Dilute 1 volume of the injection to 50 ml with *water*. Gives the reactions of chlorides (2.3.1).

### Tests

**pH** (2.4.24). 5.0 to 8.0.

**Appearance of solution**. The injection is not more intensely coloured than reference solution BYS6 (2.4.1).

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

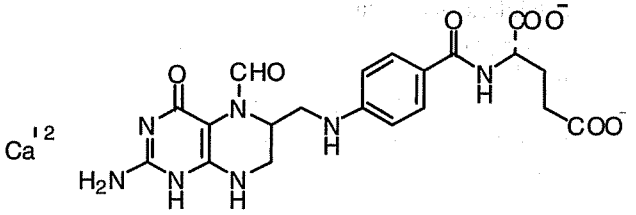
**Assay**. Measure a volume of the injection containing about 0.3 g of Calcium Chloride Dihydrate, add 300 ml of *water*, 6.0 ml of *sodium hydroxide solution* (40 per cent w/v) and 15 mg of *calconecarboxylic acid triturate*. Titrate with *0.1 M disodium edetate* until the colour changes from violet to blue.

1 ml of *0.1 M disodium edetate* is equivalent to 0.004008 g of Ca.

**Labelling**. The label states (1) the percentage w/v of Calcium Chloride Dihydrate; (2) the concentration of calcium ion as millimoles in a suitable volume; (3) the concentration of chloride ion as millimoles in a suitable volume; (4) that the injection should be used in accordance with the manufacturer's instructions; (5) that solutions containing visible solid particles must not be used.

## Calcium Folinate

Leucovorin Calcium



$C_{20}H_{21}CaN_7O_7$ Mol. Wt. 511.5

Calcium Folinate is calcium *N*-[4-(2-amino-5-formyl-1,4,5,6,7,8-hexahydro-4-oxo-6-pteridinyl)methylaminobenzoyl]-L-glutamate.

Calcium Folinate contains not less than 95.0 per cent and not more than 105.0 per cent of $C_{20}H_{21}CaN_7O_7$, calculated on the anhydrous basis.

**Category**. Antidote to folate antagonists.

**Dose**. Upto 120 mg in divided doses over 12 to 24 hours by intramuscular or intravenous injection or infusion, followed by 12 to 15 mg intramuscularly or 15 mg orally every 6 hours for the next 48 hours.

**Description**. A yellowish white or yellow powder; odourless.

### Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *calcium folinate RS*.

### Tests

**Heavy metals** (2.3.13). 4.0 g complies with the limit test for heavy metals, Method B (5 ppm).

**Water** (2.3.43). Not more than 17.0 per cent determined on 0.5 g.

**Assay**. *Use only freshly deionised water wherever water is specified throughout this procedure. Protect the solutions from unnecessary exposure to light and complete the Assay without prolonged interruption.*

Determine by liquid chromatography (2.4.14).

*Solvent mixture*. Add 15 ml of a 25 per cent w/v solution of *tetrabutylammonium hydroxide* in *methanol* to 900 ml of *water*, adjust the pH to 7.5 ± 0.1 with *0.67 M sodium dihydrogen phosphate* and dilute with *water* to 1000 ml.

*Test solution*. A 0.02 per cent w/v solution of the substance under examination in the solvent mixture.



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*Reference solution.* A solution containing 0.0175 per cent w/v each of *calcium folinate RS* and *folic acid RS* in the solvent mixture.

Chromatographic system

- a stainless steel column 30 cm x 4 mm, packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a mixture of 15 ml of a 25 per cent w/v solution of *tetrabutylammonium hydroxide*, 825 ml of *water* and 125 ml of *acetonitrile*, previously adjusted to pH 7.5 ± 0.1 with *0.67 M sodium dihydrogen phosphate*, diluted with *water* to 1000 ml,
- flow rate. 1 to 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject the reference solution. The relative retention times for calcium folinate and folic acid are 1.0 and about 1.6 respectively. The test is not valid unless the relative standard deviation for replicate injections is not more than 3.6 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{20}H_{21}CaN_7O_7$.

**Storage.** Store protected from light and moisture.

# Calcium Folinate Injection

Leucovorin Calcium Injection

Calcium Folinate Injection is a sterile solution of Calcium Folinate in Water for Injection.

Calcium Folinate Injection contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of folinic acid, $C_{20}H_{23}N_7O_7$.

**Usual strength.** The equivalent of 3 mg of folinic acid per ml. (3.25 mg of calcium folinate is approximately equivalent to 3 mg of folinic acid).

**Description.** A clear, yellowish solution.

## Identification

Transfer a volume containing about 6 mg of folinic acid to a glass-stoppered, 50-ml centrifuge tube, add about 40 ml of *acetone*, mix, centrifuge for a few minutes and decant the liquid phase. Repeat the washing with an additional 40 ml of *acetone*. Dry the precipitate obtained with a stream of dry nitrogen. The precipitate complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *calcium folinate RS*.

## Tests

**pH** (2.4.24). 6.5 to 8.5.

**Other tests.** Complies with the tests stated under Parenteral Preparations (Injections).

**Assay.** *Use only freshly deionised water wherever water is specified throughout this procedure. Protect the solutions from unnecessary exposure to light and complete the Assay without prolonged interruption.*

Determine by liquid chromatography (2.4.14).

*Solvent mixture.* Add 15 ml of a 25 per cent w/v solution of *tetrabutylammonium hydroxide* in *methanol to 900 ml of water*, adjust the pH to 7.5 ± 0.1 with *0.67 M sodium dihydrogen phosphate* and dilute with *water* to 1000 ml.

*Test solution.* Transfer an accurately measured volume of the Injection containing about 9 mg of folinic acid to a 50 ml volumetric flask, dilute to volume with a solution prepared by adding 15 ml of a 25 per cent w/v solution of *tetrabutylammonium hydroxide* in *methanol* to 900 ml of *water*, adjusting the pH to 7.5 ± 0.1 with *0.67 M sodium dihydrogen phosphate* and diluting with *water* to 1000 ml. Transfer 25.0 ml of this solution into a 60-ml separator, add 25 ml of *dichloromethane*, shake the mixture, allow the layers to separate and discard the dichloromethane extract. Repeat the extraction with two more quantities, each of 25ml, of *dichloromethane*, discarding the dichloromethane extracts. Filter the aqueous layer, discarding the first 5 ml of the filtrate, and collect the remaining filtrate in a glass-stoppered conical flask.

*Reference solution.* A solution containing 0.0175 per cent w/v each of *calcium folinate RS* and *folic acid RS* in the solvent mixture.

Chromatographic system

- a stainless steel column 30 cm x 4 mm, packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a mixture of 15 ml of a 25 per cent w/v solution of *tetrabutylammonium hydroxide*, 825 ml of *water* and 125 ml of *acetonitrile*, previously adjusted to pH 7.5 ± 0.1 with *0.67 M sodium dihydrogen phosphate*, diluted with *water* to 1000 ml,
- flow rate. 1 to 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject the reference solution. The relative retention times for calcium folinate and folic acid are 1.0 and about 1.6 respectively. The test is not valid unless the relative standard deviation for replicate injections is not more than 3.6 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{20}H_{23}N_7O_7$ in the injection.

1 mg calcium folinate is approximately equivalent to 0.93 mg of folinic acid.



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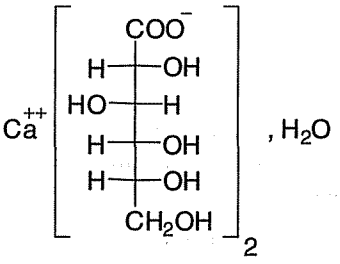
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**Storage**. Store in single dose containers preferably of type I glass, protected from light.

**Labelling**. The label states the strength in terms of the equivalent amount of folinic acid.

## Calcium Gluconate



$C_{12}H_{22}CaO_{14},H_2O$ Mol. Wt. 448.4

Calcium Gluconate is calcium D-gluconate monohydrate.

Calcium Gluconate contains not less than 98.5 per cent and not more than 102.0 per cent of $C_{12}H_{22}CaO_{14},H_2O$.

**Category**. Calcium replenisher.

**Dose**. Orally, upto 15 g daily, in divided doses. By intramuscular or slow intravenous injection, 1 to 2 g. (500 mg of calcium gluconate is approximately equivalent to 2.3 mmol of $Ca^{++}$).

**Description**. A white, crystalline powder or granules.

### Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 50 volumes of *ethanol (95 per cent)*, 30 volumes of *water*, 10 volumes of *strong ammonia solution* and 10 volumes of *ethyl acetate*.

*Test solution*. A 2.0 per cent w/v solution of the substance under examination in *water*, heating if necessary, to 60° in a water-bath to effect solution.

*Reference solution*. A 2.0 per cent w/v solution of *calcium gluconate RS* in *water*, heating if necessary, to 60° in a water-bath to effect solution.

Apply to the plate 5 µl of each solution. After development, dry the plate at 100° for 20 minutes, cool and spray with a 5 per cent w/v solution of *potassium dichromate* in a 40 per cent w/w solution of *sulphuric acid*. After 5 minutes the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. To 1 ml of a 3 per cent w/v solution add 0.05 ml of *ferric chloride test solution*; a yellow colour is produced.

C. A 2.0 per cent w/v solution gives reactions A and B of calcium salts (2.3.1).

### Tests

**Appearance of solution**. A 2.0 per cent w/v solution at 60° is not more intensely coloured than reference solution YS6 (2.4.1). On cooling to room temperature the solution is not more opalescent than opalescence standard OS2 (2.4.1).

**Acidity and alkalinity**. Dissolve 0.5 g in 20 ml of *water*, add 0.1 ml of *0.01 M hydrochloric acid* and 0.1 ml of *phenolphthalein solution*; no colour is produced. Add 0.3 ml of *0.01 M sodium hydroxide*; a pink colour is produced.

**Arsenic** (2.3.10). Dissolve 5.0 g in 50 ml of *water* and 12 ml of *stannated hydrochloric acid AsT*. The resulting solution complies with the limit test for arsenic (2 ppm).

**Heavy metals** (2.3.13). 1.0 g dissolved in 4 ml of *dilute hydrochloric acid* and sufficient *water* to produce 25 ml complies with the limit test for heavy metals, Method A (20 ppm).

**Chlorides** (2.3.12). 1.0 g complies with the limit test for chlorides (250 ppm).

**Sulphates** (2.3.17). 1.0 g complies with the limit test for sulphates (150 ppm).

**Sucrose and reducing sugars**. To 10 ml of 5 per cent w/v solution in hot *water* add 2 ml of *dilute hydrochloric acid* and boil for 2 minutes. Cool, add 15 ml of *sodium carbonate solution*, allow to stand for 5 minutes and filter. Add 5 ml of the clear filtrate to 2 ml of *potassium cupri-tartrate solution* and boil for 2 minutes; no red precipitate is formed.

**Assay**. Weigh accurately about 0.5 g and dissolve in 50 ml of warm *water*; cool, add 5.0 ml of *0.05 M magnesium sulphate* and 10 ml of *strong ammonia solution* and titrate with *0.05 M disodium edetate* using *mordant black II mixture* as indicator. From the volume of *0.05 M disodium edetate* required subtract the volume of the magnesium sulphate solution added.

1 ml of the remainder of *0.05 M disodium edetate* is equivalent to 0.02242 g of $C_{12}H_{22}CaO_{14},H_2O$.

## Calcium Gluconate Injection

Calcium Gluconate Injection is a sterile solution of Calcium Gluconate in Water for Injections. Not more than 5.0 per cent of the Calcium Gluconate may be replaced with a suitable calcium salt as a stabilising agent.

Calcium Gluconate Injection contains a quantity of calcium equivalent to not less than 8.5 per cent and not more than



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9.4 per cent of the stated amount of calcium gluconate, $C_{12}H_{22}O_{14}Ca, H_2O$.

**Usual strengths**. The equivalent of 500 mg and 1 g of calcium gluconate in 5 ml; the equivalent of 1 g of calcium gluconate in 10 ml. (A 10 per cent w/v solution of Calcium Gluconate contains approximately 0.45 mmol of $Ca^{++}$ per ml).

### Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 50 volumes of *ethanol (95 per cent)*, 30 volumes of *water*, 10 volumes of *strong ammonia solution* and 10 volumes of *ethyl acetate*.

*Test solution*. Dilute a suitable volume of the substance under examination to obtain a solution containing 2 per cent w/v of Calcium Gluconate.

*Reference solution*. A 2 per cent w/v solution of *calcium gluconate RS* in *water*, heating if necessary, to 60° in a water-bath to effect solution.

Apply to the plate 5 µl of each solution. After development, dry the plate at 100° for 20 minutes, cool and spray with a 5 per cent w/v solution of *potassium dichromate* in a 40 per cent w/w solution of *sulphuric acid*. After 5 minutes the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. To 1 ml add 0.05 ml of *ferric chloride test solution*; an intense yellow colour is produced.

C. Gives the reactions of calcium salts (2.3.1).

### Tests

**pH** (2.4.24). 6.0 to 8.2.

**Bacterial endotoxins** (2.2.3). Not more than 0.17 Endotoxin Unit per mg of calcium gluconate.

**Other tests**. Complies with the tests stated under Parenteral preparations (Injections).

**Assay**. To an accurately measured volume equivalent to 0.5 g of Calcium Gluconate add 50 ml of *water*; cool, add 5.0 ml of *0.05 M magnesium sulphate* and 10 ml of *strong ammonia solution* and titrate with *0.05 M disodium edetate* using *mordant black II mixture* as indicator. From the volume of *0.05 M disodium edetate* required subtract the volume of the magnesium sulphate solution added.

1 ml of the remainder of *0.05 M disodium edetate* is equivalent to 0.002004 g of Ca.

**Labelling**. The label states (1) the strength as a percentage w/v of calcium gluconate equivalent to the total amount of calcium present; (2) that solutions containing visible solid particles must not be used; (3) the percentage of any added stabilising agent.

## Calcium Gluconate Tablets

Calcium Gluconate Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of calcium gluconate, $C_{12}H_{22}O_{14}Ca,H_2O$.

**Usual strengths**. 325 mg; 500 mg; 650 mg; 1 g.

### Identification

A warm filtered solution of the powdered tablets equivalent to a 10 per cent w/v solution of Calcium Gluconate complies with the following tests.

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 50 volumes of *ethanol (95 per cent)*, 30 volumes of *water*, 10 volumes of *strong ammonia solution* and 10 volumes of *ethyl acetate*.

*Test solution*. A 2 per cent w/v solution of the substance under examination in *water*, heating if necessary, to 60° in a water-bath to effect solution.

*Reference solution*. A 2 per cent w/v solution of *calcium gluconate RS* in *water*, heating if necessary, to 60° in a water-bath to effect solution.

Apply to the plate 5 µl of each solution. After development, dry the plate at 100° for 20 minutes, cool and spray with a 5 per cent w/v solution of *potassium dichromate* in a 40 per cent w/w solution of *sulphuric acid*. After 5 minutes the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. To 1 ml of a 3 per cent w/v solution add 0.05 ml of *ferric chloride test solution*; a yellow colour is produced.

C. A 2 per cent w/v solution gives reactions A and B of calcium salts (2.3.1).

### Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *water*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter. Measure the absorbance of the filtrate, diluted if necessary at 422.7 nm by atomic absorption spectrophotometry (2.4.2) using a calcium hollow-cathode



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lamp as the radiation source, an air-acetylene flame. Calculate the total content of calcium in the medium using from the absorbance obtained from a solution of 100 ppm Calcium concentration in *calcium RS*.

Calculate the content of calcium gluconate.

1 mg of calcium is equivalent to 11.21 mg of $C_{12}H_{22}CaO_{14},H_2O$.

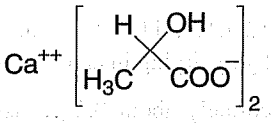
D. Not less than 70 per cent of the stated amount of $C_{12}H_{22}CaO_{14},H_2O$.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.5 g of Calcium Gluconate and ignite, gently at first, until free from carbon. Cool, add 10 ml of *water* and sufficient *dilute hydrochloric acid*, dropwise, to effect complete solution of the residue. Neutralise with *dilute ammonia solution*, add 5.0 ml of *0.05 M magnesium sulphate* and 10 ml of *strong ammonia solution* and titrate with *0.05 M disodium edetate* using *mordant black II mixture* as indicator. From the volume of *0.05 M disodium edetate* required subtract the volume of the magnesium sulphate solution added.

1 ml of the remainder of *0.05 M disodium edetate* is equivalent to 0.02242 g of $C_{12}H_{22}O_{14}Ca,H_2O$.

# Calcium Lactate



$C_6H_{10}CaO_6,xH_2O$ Mol. Wt. 218.2 (anhydrous)

Calcium Lactate is hydrated calcium *(RS)*-2-hydroxypropionate or mixtures of the calcium salts of *(R)*-, *(S)*- and *(RS)*-2-hydroxypropionic acid.

Calcium Lactate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_6H_{10}CaO_6$, calculated on the dried basis.

**Category**. Calcium replenisher.

**Dose**. Upto 8 g daily, in divided doses.

**Description**. White granules or powder; odourless or with slight but not unpleasant odour. The pentahydrate is somewhat efflorescent.

## Identification

A. A solution acidified with *sulphuric acid* and warmed with *potassium permanganate* develops the odour of acetaldehyde.

B. Gives the reactions of calcium salts and of lactates (2.3.1).

## Tests

**Acidity or alkalinity**. To 10 ml of a 5.0 per cent w/v solution in *carbon dioxide-free water* add 0.1 ml of *0.1 M hydrochloric acid* and 0.1 ml of *phenolphthalein solution*; no colour is developed. Add 0.6 ml of *0.1 M sodium hydroxide*; a pink colour is produced.

**Arsenic** (2.3.10). Dissolve 5.0 g in 50 ml of *water* and 12 ml of *stannated hydrochloric acid*. The resulting solution complies with the limit test for arsenic (2 ppm).

**Heavy metals** (2.3.13). 1.0 g dissolved in 2.5 ml of *dilute hydrochloric acid* and sufficient *water* to produce 25 ml complies with the limit test for heavy metals, Method A (20 ppm).

**Iron** (2.3.14). 0.5 g complies with the limit test for Iron (80 ppm).

**Chlorides** (2.3.12). Dissolve 1.25 g in 10 ml *water*, add 2 ml of *nitric acid* and sufficient *water* to produce 50 ml; the resulting solution complies with the limit test for chlorides (200 ppm).

**Sulphates** (2.3.17). Dissolve 0.1 g in 10 ml of *water*, add 2 ml of *hydrochloric acid* and sufficient *water* to produce 15 ml; the resulting solution complies with the limit test for sulphates (0.15 per cent).

**Reducing sugars**. Dissolve 1 g in 10 ml of *water*, add 5 ml of *potassium cupri-tartrate solution* and boil; not more than a slight brick-red precipitate is produced.

**Loss on drying** (2.4.19). Not more than 30 per cent, determined on 0.5 g by drying in an oven at 120° for 4 hours.

**Assay**. Weigh accurately about 0.3 g, dissolve in 50 ml of *water*, add 5.0 ml of *0.05 M magnesium sulphate* and 10 ml of *strong ammonia solution* and titrate with *0.05 M disodium edetate* using *mordant black II mixture* as indicator. From the volume of *0.05 M disodium edetate* required substract the volume of the magnesium sulphate solution added.

1 ml of the remainder of *0.05 M disodium edetate* is equivalent to 0.01091 g of $C_6H_{10}CaO_6$.

**Storage**. Store protected from moisture.

# Calcium Lactate Tablets

Calcium Lactate Tablets contain Calcium Lactate equivalent to not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of calcium lactate pentahydrate, $C_6H_{10}CaO_6,5H_2O$.

**Usual strengths**. The equivalent of 300 mg and 600 mg of calcium lactate pentahydrate (300 mg of calcium lactate pentahydrate is approximately equivalent to 1 mmol of $Ca^{++}$).



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### Identification

A. Extract a quantity of the powdered tablets with *water*, filter and acidify the filtrate with *sulphuric acid*, add *potassium permanganate* and warm; the odour of acetaldehyde is produced.

B. The powdered tablets, when moistened with *hydrochloric acid* and introduced on a platinum wire into the flame of a bunsen burner, gives a brick-red colour to the flame.

### Tests

**Disintegration** (2.5.1). 30 minutes.

**Other tests.** Comply with the tests stated under Tablets.

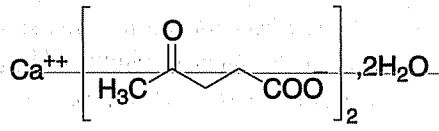
**Assay.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.3 g of calcium lactate pentahydrate, dissolve as completely as possible in 50 ml of *water*, add 5.0 ml of *0.05 M magnesium sulphate* and 10 ml of *strong ammonia solution* and titrate with *0.05 M disodium edetate* using *mordant black II mixture* as indicator. From the volume of *0.05 M disodium edetate* required subtract the volume of the magnesium sulphate solution added.

1 ml of the remainder of *0.05 M disodium edetate* is equivalent to 0.01542 g of $C_6H_{10}CaO_6,5H_2O$.

**Storage.** Store protected from moisture.

**Labelling.** The label states the strength in terms of the equivalent amount of calcium lactate pentahydrate.

## Calcium Levulinate




$C_{10}H_{14}CaO_6,2H_2O$ Mol. Wt. 306.3

Calcium Levulinate is calcium di(4-oxopentanoate) dihydrate.

Calcium Levulinate contains not less than 97.5 per cent and not more than 100.5 per cent of $C_{10}H_{14}CaO_6$, calculated on the dried basis.

**Category.** Calcium replenisher.

**Dose.** By intramuscular or intravenous injection, 1 g once a day.

**Description.** A white, crystalline or amorphous powder; odour, faint and suggestive of burnt sugar.

### Identification

A. Dissolve 0.5 g in 5 ml of *water*, add 5 ml of *1 M sodium hydroxide* and filter. To the filtrate add 5 ml of *iodine solution*; a precipitate of iodoform is produced.

B. Dissolve 0.1 g in 2 ml of *water*, add 5 ml of *dinitrophenylhydrazine solution* and allow the mixture to stand in an ice-bath for 1 hour. Collect the precipitate on a filter, wash well with cold *water* and dry at 105° for 1 hour; the hydrazone so obtained melts between 198° and 206° (2.4.21).

C. Gives the reactions of calcium salts (2.3.1).

D. Melting range (2.4.21) 119° to 125°.

### Tests

**pH** (2.4.24). 7.0 to 8.5, determined in a 10.0 per cent w/v solution.

**Arsenic** (2.3.10). Dissolve 3.3 g in 50 ml of *water* and 12 ml of *stannated hydrochloric acid*. The resulting solution complies with the limit test for arsenic (3 ppm).

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

**Reducing sugars.** Dissolve 0.5 g in 10 ml of *water*, add 2 ml of *3 M hydrochloric acid*, boil for about 10 minutes and cool. Add 5 ml of *sodium carbonate solution* allow to stand for 5 minutes, dilute with *water* to 20 ml and filter. Add 5 ml of the clear filtrate to about 2 ml of *potassium cupri-tartrate solution* and boil for 1 minute; no red precipitate is formed immediately.

**Loss on drying** (2.4.19). 11.0 per cent to 12.5 per cent, determined on 0.2 g by drying in an oven at 105°.

**Assay.** Weigh accurately about 0.2 g, dissolve in 50 ml of *water*, add 5.0 ml of *0.05 M magnesium sulphate* and 10 ml of *strong ammonia solution* and titrate with *0.05 M disodium edetate* using *mordant black II mixture* as indicator. From the volume of *0.05 M disodium edetate* required subtract the volume of the magnesium sulphate solution added.

1 ml of the remainder of *0.05 M disodium edetate* is equivalent to 0.01351 g of $C_{10}H_{14}CaO_6$.

## Calcium Levulinate Injection

Calcium Levulinate Injection is a sterile solution of Calcium Levulinate in Water for Injections.

Calcium Levulinate Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of calcium levulinate, $C_{10}H_{14}CaO_6,2H_2O$.

**Usual strength.** 100 mg per ml.



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### Identification

A. To a volume of the injection containing 0.5 g Calcium Levulinate add 5 ml of *1 M sodium hydroxide* and filter. To the filtrate add 5 ml of *iodine solution*; a precipitate of iodoform is produced.

B. To a volume of the injection containing 0.1 g of Calcium Levulinate add 5 ml of *dinitrophenylhydrazine solution* and allow the mixture to stand in an ice-bath for 1 hour. Collect the precipitate on a filter, wash well with cold *water* and dry at 105° for 1 hour; the hydrazone so obtained melts between 198° and 206° (2.4.21).

C. Gives the reactions of calcium salts (2.3.1).

### Tests

**pH** (2.4.24). 7.0 to 8.5.

**Bacterial endotoxins** (2.2.3). Not more than 35.7 Endotoxin Units per mg of calcium levulinate.

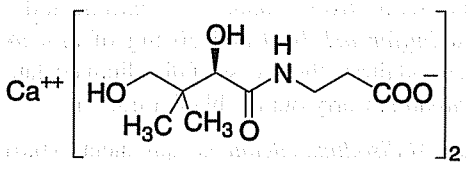
**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. To a volume containing 0.2 g of Calcium Levulinate add 50 ml of *water*, add 5.0 ml of *0.05 M magnesium sulphate* and 10 ml of *strong ammonia solution* and titrate with *0.05 M disodium edetate* using *mordant black II mixture* as indicator. From the volume of *0.05 M disodium edetate* required subtract the volume of the magnesium sulphate solution added.

1 ml of the remainder of *0.05 M disodium edetate* is equivalent to 0.01532 g of $C_{10}H_{14}CaO_6,2H_2O$.

**Storage**. Store in single dose containers.

## Calcium Pantothenate




$C_{18}H_{32}CaN_2O_{10}$ Mol. Wt. 476.5

Calcium Pantothenate is the calcium salt of (*R*)-3-(2,4-dihydroxy-3,3-dimethylbutyramido)propionic acid.

Calcium Pantothenate contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{18}H_{32}CaN_2O_{10}$, calculated on the dried basis.

**Category**. B-group vitamin (enzyme co-factor).

**Dose**. 10 to 100 mg daily, in divided doses.

**Description**. A white powder; slightly hygroscopic.

### Identification

A. In the test for β-Alanine, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

B. Boil 50 mg in 5 ml of *1 M sodium hydroxide* for 1 minute, cool, and add 5 ml of *1 M hydrochloric acid* and 2 drops of *ferric chloride test solution*; a strong yellow colour is produced.

C. To 50 mg in 2 ml of *1 M sodium hydroxide* add 0.1 ml of *copper sulphate solution*; a blue colour is produced.

D. Gives reaction A of calcium salts (2.3.1).

### Tests

**Appearance of solution**. A 5.0 per cent w/v solution in *carbon dioxide-free water* is clear, (2.4.1) and colourless (2.4.1).

**pH** (2.4.24). 6.8 to 8.0, determined in a 5.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). +25.0° to +27.5°, determined at 20° in a 5.0 per cent w/v solution.

**β-Alanine**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 65 volumes of *ethanol* and 35 volumes of *water*.

*Test solution (a)*. A 4 per cent w/v solution of the substance under examination in *water*.

*Test solution (b)*. A 0.4 per cent w/v solution of the substance under examination in *water*.

*Reference solution (a)*. A 0.4 per cent w/v solution of *calcium pantothenate RS* in *water*.

*Reference solution (b)*. A 0.02 per cent w/v solution of b-*alanine* in *water*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of air, spray with *ethanolic ninhydrin solution* and heat at 110° for 10 minutes. Any spot corresponding to β-alanine in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b).

**Heavy metals** (2.3.13). 1.0 g dissolved in 25 ml of *water* complies with the limit test for heavy metals, Method A (20 ppm).

**Loss on drying** (2.4.19). Not more than 3.0 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.18 g and dissolve in 50 ml of *anhydrous glacial acetic* acid. Titrate *0.1 M perchloric acid*,



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determining the end point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02383 g of $C_{18}H_{32}CaN_2O_{10}$.

**Storage**. Store protected from moisture.

# Dibasic Calcium Phosphate

Calcium Hydrogen Phosphate

$CaHPO_4$ Mol. Wt. 136.1 (anhydrous)

$CaHPO_4,2H_2O$ Mol. Wt. 172.1 (dihydrate)

Dibasic Calcium Phosphate is anhydrous or contains two molecules of water of hydration.

Dibasic Calcium Phosphate contains not less than 98.0 per cent and not more than 105.0 per cent of $CaHPO_4$ (for anhydrous material) or of $CaHPO_4,2H_2O$ (for the dihydrate).

**Category**. Calcium supplement; pharmaceutical aid (excipient).

**Dose**. As calcium supplement, 1 to 5 g.

**Description**. A white, crystalline powder; odourless.

## Identification

A. Gives reaction B of calcium salts (2.3.1).

B. Dissolve 0.1 g in a mixture of 5 ml of *2 M nitric acid* and 5 ml of *water*; the solution gives reaction C of phosphates (2.3.1).

## Tests

**Acid-insoluble substances**. Heat 5.0 g with a mixture of 40 ml of *water* and 10 ml of *hydrochloric acid* and dilute to 100 ml with *water*. Filter, wash with hot *water* until the last washing is free from chloride and dry the residue at 105° for 1 hour (0.1 per cent).

**Arsenic** (2.3.10). Dissolve 1.0 g in 15 ml of *brominated hydrochloric acid*, add 45 ml of *water* and remove the excess of bromine with a few drops of *stannous chloride solution AsT*. The resulting solution complies with the limit test for arsenic (10 ppm).

**Heavy metals** (2.3.13). Dissolve 2.5 g in 20 ml of *2 M hydrochloric acid*, filter if necessary, and add *6 M ammonia* until a precipitate is formed. Add *2 M hydrochloric acid* just enough to dissolve the precipitate and dilute to 50 ml with *distilled water* (solution A). 10 ml of this solution complies with the limit test for heavy metals, Method A (40 ppm).

**Barium**. To 10 ml of solution A add 0.5 ml of *1 M sulphuric acid*, mix and set aside for 15 minutes. The solution is not more opalescent than a mixture of 10 ml of solution A and 0.5 ml of *distilled water* treated in the same manner.

**Iron** (2.3.14). 2.0 ml of solution A diluted to 10 ml with *water* complies with the limit test for Iron (400 ppm).

**Carbonate**. Suspend 1 g in 5 ml of *water* and add 2 ml of *hydrochloric acid*; no effervescence is produced.

**Chlorides** (2.3.12). Dissolve 0.2 g in *water* by the addition of 2 ml of *nitric acid*. The solution complies with the limit test for chlorides (0.125 per cent).

**Sulphates** (2.3.17). Dissolve 30.0 mg in 25 ml of *water* by the addition of 2 ml of *hydrochloric acid*. The solution complies with the limit test for sulphates (2.3.17) (0.5 per cent).

**Nitrate**. To 0.1 g add 10 ml of *water*, 10 ml of *nitrogen-free sulphuric acid* and 1 ml of *indigo carmine solution* and heat to boiling; the blue colour does not disappear.

**Reducing substances**. Shake 1.0 g with 5 ml *of water* and 5 ml of *3 M sulphuric acid* for 1 minute. Add 0.1 ml of *0.005 M potassium permanganate* and shake for 20 seconds. The slight pink colour is not less intense than that produced by treating 1 g of *calcium carbonate* in the same manner.

**Proteinous impurities**. Heat 0.5 g gently in a dry test-tube; no change in colour is observed and no unpleasant odour is emitted.

**Monocalcium and tricalcium phosphates**. Dissolve 2.0 g in 30.0 ml of *1 M hydrochloric acid*, add 20 ml of *water* and 0.05 ml of *methyl orange solution* and titrate the excess of acid with *1 M sodium hydroxide*. Not less than 14.0 ml and not more than 15.5 ml of *1 M hydrochloric acid* (for anhydrous material) and not less than 11.0 ml and not more than 12.5 ml of *1 M hydrochloric acid* (for the dihydrate) is required.

**Loss on ignition** (2.4.20). 6.5 to 8.5 per cent (for anhydrous material) and 24.5 to 26.5 per cent (for the dihydrate), determined on 1.0 g by igniting at 500°.

**Assay**. Weigh accurately about 0.3 g and dissolve in a mixture of 5 ml of *water* and 1 ml of *7 M hydrochloric acid*, add 25.0 ml of *0.1 M disodium edetate* and dilute to 200 ml with *water*. Neutralise with *strong ammonia solution*, add 10 ml of *ammonia buffer pH 10.0* and 50 mg of *mordant black 11 mixture* and titrate the excess of disodium edetate with *0.1 M zinc sulphate*. Carry out the blank titration.

1 ml of *0.1 M disodium edetate* is equivalent to 0.01361 g of $CaHPO_4$ or 0.01721 g of $CaHPO_4,2H_2O$.

# Tribasic Calcium Phosphate

Calcium Hydroxide Phosphate; Calcium Phosphate

Tribasic Calcium Phosphate consists mainly of tricalcium diorthophosphate, $Ca_3(PO_4)_2$, together with calcium phosphates of more acidic or basic character.



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Tribasic Calcium Phosphate contains not less than 90.0 per cent and not more than 100.5 per cent of calcium phosphates, calculated as $Ca_3(PO_4)_2$.

**Category**. Pharmaceutical aid (excipient).

**Description**. A white, amorphous powder; odourless or almost odourless.

### Identification

A. Gives reaction B of calcium salts (2.3.1).

B. Dissolve 0.1 g in a mixture of 5 ml of *2 M nitric acid* and 5 ml of *water*; the solution gives reaction C of phosphates (2.3.1).

### Tests

**Acid-insoluble substances**. Heat 5.0 g with a mixture of 40 ml of *water* and 10 ml of *hydrochloric acid* and dilute to 100 ml with *water*. Filter, wash with hot *water* until the last washing is free from chloride and dry the residue at 105° for 1 hour (0.3 per cent).

**Water-soluble substances**. Digest 2.0 g with 100 ml of *water* for 30 minutes on a water-bath, cool, add sufficient *water* to restore the original volume, stir well and filter. Evaporate 50 ml of the filtrate to dryness and dry the residue at 105° to constant weight (0.5 per cent).

**Arsenic** (2.3.10). Dissolve 2.0 g in a mixture of 15 ml of *brominated hydrochloric acid*, add 45 ml of *water* and remove the excess of bromine with a few drops of *stannous chloride solution AsT*. The resulting solution complies with the limit test for arsenic (5 ppm).

**Heavy metals** (2.3.13). Warm 1.0 g with 4 ml of *dilute hydrochloric acid*, add sufficient *water* to produce 50 ml and filter. 25 ml of this solution complies with the limit test for heavy metals, Method A (40 ppm).

**Iron** (2.3.14). Dissolve 0.2 g in a mixture of 5 ml of *water* and 0.5 ml of *iron-free hydrochloric acid* with the addition of 1 g of citric acid. Dilute the solution to 40 ml with *water*. The solution complies with the limit test for iron (200 ppm).

**Carbonate**. Suspend 1 g in 10 ml of *water* and add 2 ml of *hydrochloric acid*; no effervescence is produced.

**Chlorides** (2.3.12). Dissolve 0.25 g in 25 ml of *water* by the addition of 1 ml of *nitric acid*. The solution complies with the limit test for chlorides (0.1 per cent ).

**Sulphates** (2.3.17). Dissolve 100.0 mg in *water* with the aid of 3 ml of *1 M hydrochloric acid* and dilute to 60 ml with *water*. 15 ml of the resulting solution complies with the limit test for sulphates (0.6 per cent).

**Proteinous impurities**. Heat 0.5 g gently in a dry test-tube; no change in colour is observed and no unpleasant odour is emitted.

**Loss on ignition** (2.4.20). Not more than 8.0 per cent, determined on 1.0 g by igniting at 800° for 30 minutes.

**Water** (2.3.43). Not more than 2.5 per cent, determined on 1.0 g.

**Assay**. Weigh accurately about 1.0 g and dissolve in 10 ml of *hydrochloric acid* by heating on a water-bath, add 50 ml of *water*, cool and dilute to 250.0 ml with *water*. To 25.0 ml of the resulting solution add 30.0 ml of *0.05 M disodium edetate*, 10.0 ml of *ammonia buffer pH 10.9* and 100 ml of *water* and titrate the excess of disodium edetate with *0.05 M zinc chloride* using *mordant black 11 solution* as indicator. Carry out the blank titration.

1 ml of *0.05 M disodium edetate* is equivalent to 0.00517 g of $Ca_3(PO_4)_2$.

## Calcium Stearate

Octadecanoic acid, calcium salt

Calcium Stearate, is a compound of calcium with a mixture of solid organic acids obtained from fats and consists chiefly of variable proportions of calcium stearate and calcium palmitate.

Calcium Stearate contains the equivalent of not less than 9.0 per cent and not more than 10.5 per cent of calcium oxide (CaO).

**Category**. Pharmaceutical aid.

### Identification

A. Heat 1 g with a mixture of 25 ml of *water* and 5 ml of *hydrochloric acid*; fatty acids are liberated and appear as an oily layer floating on the surface of the liquid. The water layer gives the tests for calcium (2.3.1).

B. Mix 25 g with 200 ml of hot *water*, add 60 ml of *2 M sulphuric acid*, and heat the mixture, with frequent stirring, until the separated fatty acid layer is clear. Wash the fatty acids with boiling *water* until free from sulphate, collect them in a small beaker, and warm on a steam bath until the *water* has separated and the fatty acids are clear. Allow the acids to cool, pour off the water layer, melt the acids, filter into a dry beaker, and dry at 105° for 20 minutes; the fatty acids so obtained congeal at a temperature not below 54° (2.4.10).

### Tests

**Compositions of fatty acids**. Determine by gas chromatography (2.4.13).

*Test solution*. Dissolve 0.1 g of the substance under examination in 5 ml of *boron trifluoride- methanol solution*. Boil under a reflux condenser for 10 minutes. Add 4 ml of



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*heptane* through the condenser. Boil under a reflux condenser for 10 minutes. Allow to cool. Add 20 ml of a saturated *sodium chloride solution*. Shake and allow the layers to separate. Remove about 2 ml of the organic layer and dry over 0.2 g of *anhydrous sodium sulphate*. Dilute 1.0 ml of this solution to 10.0 ml with *heptane*.

*Reference solution.* Dissolve 50 mg each of *palmitic acid RS* and *stearic acid RS* in 5 ml of *boron trifluoride- methanol solution.* Boil under a reflux condenser for 10 minutes. Add 4 ml of *heptane* through the condenser. Boil under a reflux condenser for 10 minutes. Allow to cool. Add 20 ml of a saturated *sodium chloride solution*. Shake and allow the layers to separate. Remove about 2 ml of the organic layer and dry over 0.2 g of *anhydrous sodium sulphate*. Dilute 1.0 ml of this solution to 10.0 ml with *heptane*.

Chromatographic system

- a glass column 30 m x 0.32 mm packed with silanised diatomaceous support coated with macrogol 20000 (film thickness 0.5 µm),
- temperature:

| column | time (min) | temperature (°) |
|---|---|---|
| | 0-2 | 70 |
| | 2-36 | 70-240 |
| | 36-41 | 240 |

- Inlet port at 220° and detector at 260°,
- flame ionization detector,
- flow rate. 2.4 ml per minute using nitrogen as carrier gas.

Inject 1 µl of the reference solution. The test is not valid unless the resolution between the peaks due to methyl stearate and methyl palmitate is not less than 5.0. The relative retention time with reference to methyl stearate for methyl palmitate is about 0.88.

Inject 1 µl of the test solution and the reference solution.

Calculate the content of palmitic acid and stearic acid.

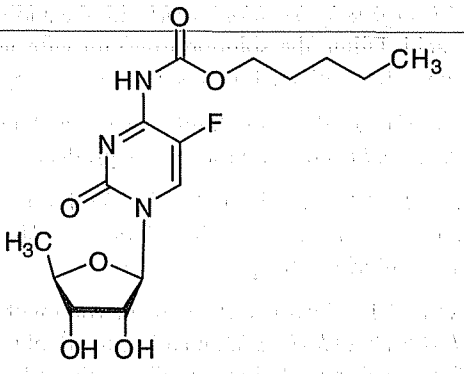
**Loss on drying** (2.4.19). Not more than 4.0 per cent, determined on 1.0 g by drying in an oven at 105°.

**Heavy metals** (2.3.13).Place 2.5 g in a porcelain dish, place a 500 mg portion in a second dish to provide the control, and to each add 5 ml of a 1 in 4 solution of *magnesium nitrate* in *alcohol*. Cover the dishes with 7.5-cm short-stem funnels so that the stems are straight up. Heat on a hot plate at low heat for 30 minutes, then heat at medium heat for 30 minutes, and cool. Remove the funnels, add 2 ml of *standard lead solution* (*20 ppm Pb*) to the control, and heat each dish over a suitable burner until most of the carbon is burned off. Cool, add 10 ml of *nitric acid*, and transfer the solutions into 250 ml beakers. Add 5 ml of 70 per cent *perchloric acid*, cautiously evaporate to dryness, add 2 ml of *hydrochloric acid* to the residues, and wash down the insides of the beakers with *water*. Evaporate carefully to dryness again, swirling near the dry point to avoid spattering. Repeat the hydrochloric acid treatment, then cool, and dissolve the residues in about 10 ml of *water*. To each solution add 1 drop of *phenolphthalein solution* and add *sodium hydroxide solution* until the solutions just turn pink, then add *3 M hydrochloric acid* until the solutions become colourless. Add 1 ml of *1 M acetic acid* and a small amount of charcoal to each solution, and filter through filter paper into 50 ml Nessler cylinders. Wash with water, dilute with *water* to 40 ml, add 1.2 ml of *thioacetamide reagent* and 2 ml of *pH* 3.5 *acetate buffer* to each tube, and allow to stand for 5 minutes; the color of the test solution does not exceed that of the control *(10 ppm Pb)*.

**Assay**. Boil about 1.2 g accurately weighed, with 50 ml of *1 M sulphuric acid* for about 3 hours using a watch glass cover to avoid splattering, or until the separated fatty acid layer is clear, adding *water*, if necessary to maintain the original volume. *[Note-Stirring may be helpful in obtaining a clear layer and decreasing extraction time.]* Cool, filter, and wash the filter and the flask thoroughly with *water* until the last washing is not acid to *litmus*. Neutralize the filtrate with 1 *M sodium hydroxide* to *litmus*. While stirring, preferably with a magnetic stirrer, titrate with *0.05 M disodium edetate* as follows. Add about 30 ml from a 50-ml burette, then add 1 ml of *1 M sodium hydroxide* and 300 mg of *hydroxy naphthol blue*, and continue the titration to a blue end-point.

1 ml of *0.05 M disodium edetate* is equivalent to 0.002804 g of calcium.

## Capecitabine




$C_{15}H_{22}FN_3O_6$ Mol. Wt. 359.4

Capecitabine is 5′-deoxy-5-fluoro-*N*-[(pentyloxy)carbonyl] cytidine.



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Capecitabine contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{15}H_{22}FN_3O_6$, calculated on the anhydrous basis.

**Category**. Anticancer.

**Description.** A white to off-white crystalline powder.

### Identification

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

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

### Tests

**Specific optical rotation** (2.4.22). + 96° to + 100°, determined on 1.0 per cent w/v solution in *methanol*, at 20°.

**Related substances**. Determine by liquid chromatography (2.4.14), as described under Assay.

Inject the reference solution and the test solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any secondary peak is not more than 1.0 per cent the area of the principal peak. The sum of all the secondary peaks is not more than 2.0 per cent the area of the principal peak

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

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 0.3 per cent, determined on 0.5 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture.* 60 volumes of *water*, 35 volumes of *methanol* and 5 volumes of *acetonitrile.*

*Test solution.* Dissolve about 60 mg of the substance under examination in 100 ml of the solvent mixture.

*Reference solution.* A 0.06 per cent w/v solution of *capecitabine RS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Inertsil ODS-3),
- column temperature. 40°,
- sample temperature. 5°,
- mobile phase: A. a mixture of 60 volumes of 0.1 per cent v/v solution of *acetic acid*, 35 volumes of *methanol* and 5 volumes of *acetonitrile,*
  B. a mixture of 80 volumes of *methanol*, 15 volumes of 0.1 per cent v/v solution of *acetic acid* and 5 volumes of *acetonitrile,*
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 250 nm,
- injection volume.10 µl.

| Time (in min) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0-5 | 100 | 0 |
| 5-20 | 100 → 49 | 0 → 51 |
| 20-30 | 49 | 51 |
| 30-31 | 49 → 100 | 51 → 0 |
| 31 – 40 | 100 | 0 |

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{15}H_{22}FN_3O_6$.

**Storage**. Store protected from moisture.

## Capecitabine Tablets

Capecitabine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of capecitabine, $C_{15}H_{22}FN_3O_6$.

**Usual strength**. 500 mg.

### Identification

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

### Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *phosphate buffer pH 6.8*,
Speed and time. 50 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

*Test solution.* Dilute the filtrate, if necessary, with the dissolution medium.

*Reference solution.* Dissolve an accurately weighed quantity of *capecitabine RS* in the mobile phase and dilute with dissolution medium to obtain a solution having a known concentration similar to the test solution.



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Use chromatographic system as described under Assay.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{15}H_{22}FN_3O_6$.

D. Not less than 80 per cent of the stated amount of $C_{15}H_{22}FN_3O_6$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution.* Disperse a quantity of powdered tablets containing about 100 mg of Capecitabine in the mobile phase, sonicate for 30 minutes and dilute to 100.0 ml with the mobile phase.

*Reference solution.* A 0.001 per cent w/v solution of *capecitabine RS* in the mobile phase.

Use chromatographic system as described under Assay.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 5.0 per cent.

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

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Disperse a quantity of powdered tablets containing about 100 mg of Capecitabine in the mobile phase, sonicate for 30 minutes and dilute to 100.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

*Reference solution.* A 0.01 per cent w/v solution of *capecitabine RS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm) (such as YMC-pack),
- mobile phase: a mixture of 60 volumes of a buffer solution prepared by dissolving 2.5 g of *ammonium acetate* in 1000 ml of *water*, adjusted to pH 4.5 with *trifluoroacetic acid*, 20 volumes of *acetonitrile*, and 20 volumes of *methanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the theoretical plates are not less than 2000, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

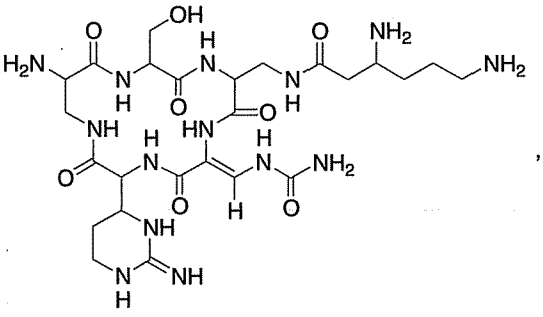
Inject the reference solution and the test solution.

Calculate the content of $C_{15}H_{22}FN_3O_6$.

**Storage**. Store at a temperature not exceeding 30°.

**Labelling**. The label states the strength in terms of the amount of Capecitabine.

# Capreomycin Sulphate




, $2H_2SO_4$

$C_{25}H_{44}N_{14}O_8,2H_2SO_4$ Mol. Wt. 864.7

Capreomycin Sulphate is the disulphate salt of capreomycin, a polypeptide mixture produced by certain strains of *Streptomyces capreolus*.

It has a potency equivalent to not less than 700 µg and not more than 1050 µg of capreomycin per mg.

**Category**. Antituberculosis.

**Description**. A white or almost white powder.

## Identification

A. When examined in the range 230 nm to 350 nm (2.4.7), a 0.004 per cent w/v solution in *0.1 M hydrochloric acid* shows an absorption maximum only at 268 nm. Absorbance at 268 nm, about 1.2.

B. When examined in the range 230 nm to 350 nm (2.4.7), a 0.004 per cent w/v solution in *0.1 M sodium hydroxide* shows an absorption maximum only at 287 nm. Absorbance at 287 nm, about 0.8.

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



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### Tests

**Appearance of solution**. A 10.0 per cent w/v solution in *water* is clear (2.4.1), when examined immediately after preparation.

**pH** (2.4.24). 4.5 to 7.5, determined in a 3.0 per cent w/v solution.

**Capreomycin I content**. Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve 25 mg of the substance under examination in 100 ml of *water*.

*Reference solution.* A 0.025 per cent w/v solution of *capreomycin sulphate RS* in *water*.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with nitrile groups chemically bonded to porous silica particles (5 µm) (such as Spherisorb CN),
- mobile phase: 55 volumes of the solution prepared by dissolving 0.5 g of *ammonium bisulphate* in 1000 ml of *water,* filter and 45 volumes of *methanol,*
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 268 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the resolution between the two principal peaks is at least 1.5.

In the chromatogram obtained with the test solution, the sum of the areas of the two principal peaks, due to capreomycins 1A and 1B, is not less than 90 per cent of the total areas of all the peaks.

**Sulphated ash** (2.3.18). Not more than 3.0 per cent.

**Loss on drying** (2.4.19). Not more than 10.0 per cent, determined on 0.1 g by drying in an oven for 4 hours at 100° at a pressure not exceeding 0.7 kPa.

**Assay**. Determine by the microbiological assay of antibiotics (2.2.10).

*Caproemycin Suphate intended for use in the manufacture of parenteral preparations complies with the following additional requirements.*

**Bacterial endotoxins** (2.2.3). Not more than 0.35 Endotoxin Unit per mg of capreomycin.

**Sterility** (2.2.11). Complies with the test for sterility.

**Storage**. Store protected from moisture.

## Capreomycin Injection

Capreomycin Injection is a sterile material consisting of Capreomycin Sulphate with or without auxiliary agents. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

*The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).*

**Usual strength**. 1 g.

**Storage**. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Capreomycin injection contains an amount of Capreomycin Sulphate equivalent to not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of capreomycin.

*The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.*

### Identification

A. When examined in the range 230 nm to 350 nm (2.4.7), a solution containing 0.004 per cent w/v of capreomycin in *0.1 M hydrochloric acid* shows an absorption maximum only at 268 nm. Absorbance at 268 nm, about 1.2.

B. When examined in the range 230 nm to 350 nm (2.4.7), a solution containing 0.004 per cent w/v of capreomycin in *0.1 M sodium hydroxide* shows an absorption maximum only at 287 nm. Absorbance at 287 nm, about 0.8.

### Tests

**Appearance of solution**. A 10.0 per cent w/v solution in *water* is clear (2.4.1), when examined immediately after preparation.

**pH** (2.4.24). 4.5 to 7.5, determined in a 3.0 per cent w/v solution.

**Capreomycin I content**. Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve a quantity of the injection containing about 25 mg of capreomycin in 100 ml of *water*.

*Reference solution.* A 0.025 per cent w/v solution of *capreomycin sulphate RS* in *water*.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with nitrile groups chemically bonded to porous silica particles (5 µm) (such as Spherisorb CN),
- mobile phase: a mixture of 55 volumes of a solution prepared by dissolving 0.5 g of *ammonium bisulphate* in 1000 ml of *water,* filtered and 45 volumes of *methanol,*
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 268 nm,
- injection volume. 20 µl.



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Inject the reference solution. The test is not valid unless the resolution between the two principal peaks is at least 1.5.

In the chromatogram obtained with the test solution, the sum of the areas of the two principal peaks, due to capreomycins 1A and 1B, is not less than 90 per cent of the total areas of all the peaks.

**Loss on drying** (2.4.19). Not more than 10.0 per cent, determined on 0.1 g by drying in an oven at 100°at a pressure not exceeding 0.7 kPa for 4 hours.

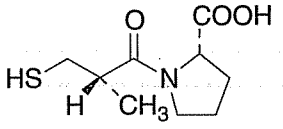
**Bacterial endotoxins** (2.2.3). Not more than 0.35 Endotoxin Unit per mg of capreomycin.

**Assay**. Determine by the microbiological assay of antibiotics (2.2.10).

**Storage**. Store protected from moisture, at a temperature not exceeding 25°.

**Labelling**. The label states the quantity of Capreomycin Sulphate in terms of the equivalent amount of capreomycin.

# Captopril




$C_9H_{15}NO_3S$ Mol. Wt. 217.3

Captopril is 1-[(2*S*)-3-mercapto-2-methylpropionyl]-L-proline.

Captopril contains not less than 97.5 per cent and not more than 102.0 per cent of $C_9H_{15}NO_3S$, calculated on the dried basis.

**Category**. Antihypertensive.

**Dose**. Initially, 12.5 to 50 mg twice daily; usual maintenance dose, 25 mg twice daily; maximum, 50 mg twice daily.

**Description**. A white to off-white, crystalline powder; odour, characteristic, sulphide-like.

## Identification

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

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 75 volumes of *toluene*, 25 volumes of *glacial acetic acid* and 1 volume of *methanol*.

*Test solution*. A 0.4 per cent w/v solution of the substance under examination in *methanol*.

*Reference solution*. A 0.4 per cent w/v solution of *captopril RS* in *methanol*.

Apply to the plate, in the form of 1-cm bands, 50 µl of each solution. Allow the mobile phase to rise 12 cm. Dry in air and spray with a freshly prepared mixture of 1 volume of *strong ammonia solution* and 6 volumes of a 0.04 per cent w/v solution of *5,5'-dithiobis(2-nitrobenzoic acid)* in *methanol* and allow to stand for 5 minutes. The principal band in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

C. Melting range (2.4.21) 104° to 110°.

## Tests

**Specific optical rotation** (2.4.22). –125° to –134°, determined in a 1.0 per cent w/v solution in *ethanol*.

**Related substances.** Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 50 mg of the substance under examination in 100 ml of the mobile phase.

*Reference solution (a)*. Dilute 2.0 ml of the test solution to 100 ml with the mobile phase.

*Reference solution (b)*. Dissolve 10 mg of the substance under examination in the mobile phase, add 0.25 ml of *0.05 M iodine* and dilute to 100.0 ml with the mobile phase. Dilute 10 ml of this solution to 100 ml with the mobile phase.

Chromatographic system

- a stainless steel column 12.5 cm x 4.0 mm packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 0.05 volume of *orthophosphoric acid*, 50 volumes of *methanol* and 50 volumes of *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 20 µl.

Inject reference solution (b). The chromatogram shows three peaks. The test is not valid unless the resolution between the last 2 eluting peaks is not less than 2.0.

Inject the test solution and reference solution (a). Run the chromatogram three times the retention time of the principal peak. In the chromatogram obtained with test solution, the area of any secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent) and sum of areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent). Ignore any peak with a retention time less than 1.4 minutes.



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**Heavy metals** (2.3.13). 0.66 g complies with the limit test for heavy metals, Method B (30 ppm).

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa.

**Assay**. Weigh accurately about 0.3 g, dissolve in 100 ml of *water* in a stoppered-flask, add 10 ml of *1.8 M sulphuric acid* and 1 g of *potassium iodide*. Titrate with *0.025 M potassium iodate* using 3 ml of *starch solution*, added towards the end-point, as indicator.

1ml of *0.025 M potassium iodate* is equivalent to 0.03308 g of $C_9H_{15}NO_3S$.

**Storage**. Store protected from moisture.

## Captopril Tablets

Captopril Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of captopril, $C_9H_{15}NO_3S$.

**Usual strengths**. 12.5 mg; 25 mg; 50 mg.

### Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 75 volumes of *toluene*, 25 volumes of *glacial acetic acid* and 1 volume of *methanol*.

*Test solution*. Extract a quantity of the powdered tablets containing 100 mg of Captopril with 25 ml of *methanol* and centrifuge. Use the clear supernatant liquid.

*Reference solution*. A 0.4 per cent w/v solution of *captopril RS* in *methanol*.

Apply to the plate, in the form of 1-cm bands, 50 µl of each solution. Allow the mobile phase to rise 12 cm. Dry in air and spray with a freshly prepared mixture of 1 volume of *strong ammonia solution* and 6 volumes of a 0.04 per cent w/v solution of *5,5'-dithiobis(2-nitrobenzoic acid)* in *methanol* and allow to stand for 5 minutes. The principal band in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

### Tests

**Dissolution** (2.5.2).

Apparatus No. 2,
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm and 20 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance (2.4.7) of the filtrate, suitably diluted if necessary, at the maximum at about 212 nm.

Calculate the content of $C_9H_{15}NO_3S$ in the medium from the absorbance obtained from a solution of known concentration of *captopril RS*.

D. Not less than 80 per cent of the stated amount of $C_9H_{15}NO_3S$.

**Other tests**. Complies with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*NOTE—Protect the solutions from exposure to air and use within 8 hours of preparation.*

*Test solution*. Dissolve a quantity of the finely powdered tablets containing about 25 mg of Captopril in 25 ml of the mobile phase with the aid of ultrasound for 15 minutes, centrifuge and use the clear supernatant liquid.

*Reference solution*. A 0.1 per cent w/v solution of *captopril RS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a mixture of 55 volumes of *methanol* and 45 volumes of *water* containing 0.05 volumes of *phosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 20 µl.

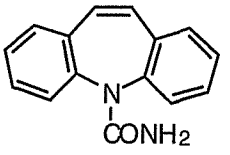
Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_9H_{15}NO_3S$ in the tablets.

**Storage**. Store protected from moisture.

## Carbamazepine




$C_{15}H_{12}N_2O$ Mol. Wt. 236.3

Carbamazepine is 5*H*-dibenz[*b*,*f*]azepine-5-carboxamide.

Carbamazepine contains not less than 97.0 per cent and not more than 103.0 per cent of $C_{15}H_{12}N_2O$, calculated on the dried basis.



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**Category**. Anticonvulsant.

**Dose**. 200 mg daily, increasing to 1.2 g daily, in divided doses, in accordance with the needs of the patient.

**Description**. A white or yellowish-white, crystalline powder; almost odourless; exhibits polymorphism.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *carbamazepine RS*.

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

## Tests

**Acidity or alkalinity**. Stir 1.0 g with 20 ml of *carbon dioxide-free water* for 15 minutes and filter. Titrate 10 ml of the filtrate with *0.01 M sodium hydroxide* using 0.05 ml of *phenolphthalein solution* as indicator; not more than 0.5 ml is required. Add 0.15 ml of a 0.05 per cent w/v solution of *methyl red* and titrate with *0.01 M hydrochloric acid* until the colour changes to red; not more than 1.0 ml is required.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution (a)*. Dissolve 0.15 g of the substance under examination in *methanol* and dilute to 50 ml with the same solvent. Mix with the aid of ultrasound and dilute 10 ml of this solution to 20 ml with *water*.

*Test solution (b)*. Dilute 10 ml of test solution (a) to 50 ml with a mixture of equal volumes of *methanol* and *water*.

*Reference solution (a)*. Dissolve 7.5 mg of *carbamazepine RS*, 7.5 mg of *10,11-dihydrocarbamazepine RS* and 7.5 mg of *iminodibenzyl* in *methanol* and dilute to 100 ml with the same solvent. Dilute 1.0 ml of this solution to 50 ml with a mixture of equal volumes of *methanol* and *water*.

*Reference solution (b)*. Dissolve 0.15 g of *carbamazepine RS* in *methanol* and dilute to 50 ml with the same solvent. Dilute 5 ml of this solution to 50 ml with a mixture of equal volumes of *methanol* and *water*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with nitrile groups chemically bonded to porous silica particles (10 µm),
- mobile phase: a mixture of 3 volumes of *tetrahydrofuran*, 12 volumes of *methanol* and 85 volumes of *water* adding 0.2 ml of *formic acid* and 0.5 ml of *triethylamine* to 1000 ml of this solution,
- flow rate. 2 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 20 µl.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to carbamazepine and 10,11-dihydrocarbamazepine is more than 1.7.

Inject test solution (a). Run the chromatograms for 6 times the retention time of carbamazepine (about 10 minutes). The areas of any peaks corresponding to 10,11-dihydrocarbamazepine and iminodibenzyl are not more than the areas of the corresponding peaks in the chromatogram obtained with reference solution (a) (0.1 per cent each). The area of any other secondary peak is not more than the area of the peak due to carbamazepine (0.1 per cent) and the sum of all the secondary peaks is not more than 5 times the area of the peak due to carbamazepine ( 0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

**Chlorides** (2.3.12). 1.5 g complies with the limit test for chlorides (165 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 2 hours.

**Assay**. Determine by liquid chromatography (2.4.14) as given under the test for Related substances using test solution (b) and reference solution (b).

Inject reference solution (b). The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately test solution (b) and reference solution (b).

Calculate the content of $C_{15}H_{12}N_2O$.

**Storage**. Store protected from moisture.

# Carbamazepine Extended-release Tablets

"*Carbamazepine Extended-release Tablets manufactured by different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable.*"

Carbamazepine Extended-release Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of carbamazepine, $C_{15}H_{12}N_2O$.

**Usual strengths**. 100 mg; 200 mg; 400 mg.



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### Identification

Boil a quantity of the powdered tablets containing 0.2 g of Carbamazepine with 15 ml of *acetone*, filter the hot solution, wash the filtrate with two quantities, each of 5 ml, of hot *acetone*, cool in ice, evaporate the combined filtrates to dryness. The residue complies with the following test

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *carbamazepine RS*.

### Tests

**Dissolution** (2.5.2). Complies with the test stated under tablets.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution.* Shake a quantity of the powdered tablets containing 0.3 g of Carbamazepine with 100 ml of *methanol* for 15 minutes. Dilute to 200 ml with *water*, mix and filter.

*Reference solution.* Dissolve 7.5 mg each of *carbamazepine RS*, *10,11-dihydrocarbamazepine* and *iminodibenzyl* in *methanol* and dilute to 100 ml with the same solvent. Dilute 1 ml of the resulting solution to 50 ml with *methanol (50 per cent)*.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with porous silica particles (10 µm) with chemically bonded nitrile groups (such as Nucleosil 10 CN),
- mobile phase: a mixture of 30 volumes of *tetrahydrofuran*, 120 volumes of *methanol* and 850 volumes of *water*, to which is added 0.2 ml of *anhydrous formic acid* and 0.5 ml of *triethylamine*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to carbamazepine and 10, 11-dihydrocarbamazepine is at least 1.7.

Inject the test solution and continue the chromatography for 6 times the retention time of carbamazepine (about 10 minutes). In the chromatogram obtained with the test solution, the areas of any peaks corresponding to 10,11-dihydrocarbamazepine and iminodibenzyl are not greater than the areas of the corresponding peaks in the chromatogram obtained with the reference solution (0.1 per cent). The area of any other secondary peak is not greater than the area of the peak due to carbamazepine (0.1 per cent) and the sum of the areas of any such peaks is not greater than 5 times the area of the peak due to carbamazepine (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the peak due to carbamazepine in the chromatogram obtained with the reference solution (0.05 per cent).

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14) as given under the test for Related substances using the following solutions.

*Test solution.* Shake a quantity of the powdered tablets containing about 0.3 g of Carbamazepine with 100.0 ml of *methanol* for 15 minutes. Dilute to 200.0 ml with *water*, mix, filter and further dilute 1 volume of the filtrate to 5 volumes with *methanol (50 per cent)*.

*Reference solution.* A 0.03 per cent w/v solution of *carbamazepine RS* in *methanol (50 per cent)*.

Inject the test solution and the reference solution.

Calculate the content of $C_{15}H_{12}N_2O$ in the tablets.

**Storage**. Store protected from moisture.

## Carbamazepine Tablets

Carbamazepine Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of carbamazepine, $C_{15}H_{12}N_2O$.

**Usual strength**. 200 mg.

### Identification

Boil a quantity of the powdered tablets containing 0.2 g of Carbamazepine with 15 ml of *acetone*, filter the hot solution, wash the filtrate with two 5 ml quantities of hot acetone, cool in ice, evaporate the combined filtrates to dryness. The residue complies with the following test

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *carbamazepine RS*.

### Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution.* Shake a quantity of the powdered tablets containing 0.3 g of Carbamazepine with 100 ml of *methanol* for 15 minutes. Dilute to 200 ml with *water*, mix and filter.

*Reference solution.* Dissolve 7.5 mg each of *carbamazepine RS*, *10,11-dihydrocarbamazepine* and *iminodibenzyl* in *methanol* and dilute to 100 ml with the same solvent. Dilute 1 ml of the resulting solution to 50 ml with *methanol (50 per cent)*.



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Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with nitrile groups chemically bonded to porous silica particles (10 µm) (such as Nucleosil 10 CN),
- mobile phase: a mixture of 30 volumes of *tetrahydrofuran*, 120 volumes of *methanol* and 850 volumes of *water*, adding 0.2 ml of *anhydrous formic acid* and 0.5 ml of *triethylamine* to 1000 ml of the solution,
- flow rate. 2 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to carbamazepine and 10, 11-dihydrocarbamazepine is at least 1.7.

Inject the test solution and continue the chromatography for 6 times the retention time of carbamazepine which is about 10 minutes.

In the chromatogram obtained with the test solution, the areas of any peaks corresponding to 10,11-dihydrocarbamazepine and iminodibenzyl are not greater than the areas of the corresponding peaks in the chromatogram obtained with the reference solution (0.1 per cent). The area of any other secondary peak is not greater than the area of the peak due to carbamazepine (0.1 per cent) and the sum of the areas of any such peaks is not greater than 5 times the area of the peak due to carbamazepine (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the peak due to carbamazepine in the chromatogram obtained with the reference solution (0.05 per cent).

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14) as given under the test for Related substances using the following solutions.

*Test solution*. Shake a quantity of the powdered tablets containing about 0.3 g of Carbamazepine with 100.0 ml of *methanol* for 15 minutes. Dilute to 200.0 ml with water, mix, filter and further dilute 1 volume of the filtrate to 5 volumes with *methanol (50 per cent)*.

*Reference solution*. A 0.03 per cent w/v solution of *carbamazepine RS* in *methanol (50 per cent)*.

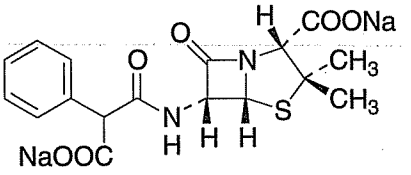
Inject alternately the test solution and the reference solution.

Calculate the content of $C_{15}H_{12}N_2O$ in the tablets.

**Storage**. Store protected from moisture.

# Carbenicillin Sodium

Carbenicillin Disodium




$C_{17}H_{16}N_2Na_2O_6S$ Mol. Wt. 422.4

Carbenicillin Sodium is the disodium (6*R*)-6-[(2*RS*)-2-carboxylato-2-phenylacetamido]penicillinate.

Carbenicillin Sodium contains the equivalent of not less than 770 µg of carbenicillin per mg, calculated on the anhydrous basis.

**Category**. Antibacterial.

**Dose**. By intravenous injection, the equivalent of 12 to 30 g of carbenicillin daily, in divided doses; by intramuscular injection, the equivalent of 4 to 8 g of carbenicillin daily, in divided doses.

**Description**. A white or slightly yellowish powder; odourless; hygroscopic.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *carbenicillin sodium RS* or with the reference spectrum of carbenicillin.

B. Heat 0.5 g in a small sealed container on a water-bath for 3 minutes, remove the seal, and immediately replace by a cork fitted with a platinum loop carrying a drop of a solution freshly prepared by mixing 1 ml of a 0.5 per cent w/v solution of *sodium carbonate*, 1 ml of *phenolphthalein solution* and 10 ml of *water*; the reagent is decolourised within 2 minutes.

C. A 5 per cent w/v solution gives the reactions of sodium salts (2.3.1).

## Tests

**pH** (2.4.24). 6.5 to 8.0, determined in a 10.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). +182° to +196°, determined at 20° in a 1.0 per cent w/v solution.

**Iodine-absorbing substances**. Not more than 8.0 per cent, calculated on the anhydrous basis, determined by the following method. Weigh accurately about 0.13 g and dissolve in sufficient mixed *phosphate buffer pH 7.0* to produce 25.0 ml. To 10.0 ml add 10 ml of *mixed phosphate buffer pH 4.0* and 10.0 ml of *0.01 M iodine* and titrate immediately with *0.01 M sodium thiosulphate* using *starch solution*, added towards the end of the titration, as indicator. Repeat the operation



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without the substance under examination. The difference between the titration represents the amount of iodine-absorbing substances present.

1 ml of *0.01M sodium thiosulphate* is equivalent to 0.000489 g of iodine-absorbing substances.

**Bacterial endotoxins** (2.2.3). Not more than 0.05 Endotoxin Unit per mg of carbenicillin.

**Water** (2.3.43). Not more than 6.0 per cent, determined on 0.15 g.

**Assay**. Determine by the microbiological assay of antibiotics, Method A (2.2.10) and express the result in µg of carbenicillin per mg.

**Storage**. Store in sterile containers, sealed so as to exclude micro-organisms, in a refrigerator (2° to 8°).

## Carbenicillin Sodium Injection

Carbenicillin Injection; Carbenicillin Disodium Injection

Carbenicillin Sodium Injection is a sterile material consisting of Carbenicillin Sodium, with or without auxilliary substances. It is filled in sealed containers.

The injection is constituted by dissolving the contents of a sealed container in the requisite amount of Water for Injections immediately before use.

*The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).*

**Storage**. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Carbenicillin Sodium Injection contains the equivalent of not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of carbenicillin, $C_{17}H_{18}N_2O_6S$.

**Usual strengths**. The equivalent of 1 g and 5 g of carbenicillin.

**Description**. A white or almost white powder; odourless; hygroscopic.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

### Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *carbenicillin sodium RS* or with the reference spectrum of carbenicillin.

B. Heat 0.5 g in a small sealed container on a water-bath for 3 minutes, remove the seal, and immediately replace by a cork fitted with a platinum loop carrying a drop of a solution freshly prepared by mixing 1 ml of a 0.5 per cent w/v solution of *sodium carbonate*, 1 ml of *phenolphthalein solution* and 10 ml of *water*; the reagent is decolourised within 2 minutes.

C. A 5 per cent w/v solution gives the reactions of sodium salts (2.3.1).

### Tests

**pH** (2.4.24). 6.5 to 8.0, determined in a 10.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). +182° to +196°, determined at 20° in a 1.0 per cent w/v solution.

**Iodine-absorbing substances**. Not more than 8.0 per cent, calculated on the anhydrous basis, determined by the following method. Weigh accurately about 0.13 g and dissolve in sufficient mixed *phosphate buffer pH 7.0* to produce 25.0 ml. To 10.0 ml add 10 ml of *mixed phosphate buffer pH 4.0* and 10.0 ml of *0.01 M iodine* and titrate immediately with *0.01 M sodium thiosulphate* using *starch solution*, added towards the end of the titration, as indicator. Repeat the operation without the substance under examination. The difference between the titration represents the amount of iodine-absorbing substances present.

1 ml of 0.01M *sodium thiosulphate* is equivalent to 0.000489 g of iodine-absorbing substances.

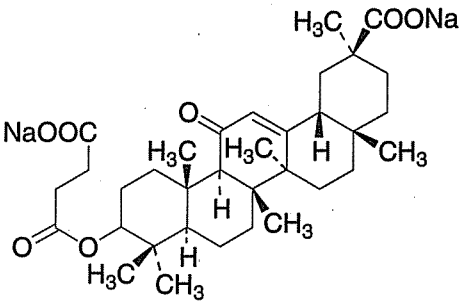
**Bacterial endotoxins** (2.2.3). Not more than 0.05 Endotoxin Unit per mg of carbenicillin.

**Water** (2.3.43). Not more than 6.0 per cent, determined on 0.15 g.

**Assay**. Determine the weight of the contents of 10 containers and determine by the microbiological assay of antibiotics, Method A (2.2.10) using the mixed contents of the 10 containers.

**Labelling**. The label states the quantity of Carbenicillin Sodium contained in the sealed container in terms of the equivalent amount of carbenicillin.

## Carbenoxolone Sodium




$C_{34}H_{48}Na_2O_7$ Mol. Wt. 614.7

Carbenoxolone Sodium is disodium 3β-(3-carboxylatopropionyloxy)-11-oxoolean-12-en-30-oate.



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Carbenoxolone Sodium contains not less than 97.0 per cent and not more than 103.0 per cent of $C_{34}H_{48}Na_2O_7$, calculated on the anhydrous basis.

**Category**. Antiulcer.

**Dose**. 300 mg daily, in divided doses for 1 week; subsequently, upto 150 mg daily, in divided doses.

**Description**. A white or pale cream powder; hygroscopic; irritant to nasal membranes.

### Identification

A. Dissolve 0.1 g in 5 ml of *water*, just acidify with *2 M hydrochloric acid*, stir well and filter. Wash the residue with *water* until the washings are no longer acidic and dry to constant weight at 105°. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *carbenoxolone sodium RS* treated in the same manner or with the reference spectrum of carbenoxolone.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.0025 per cent w/v solution in a mixture of equal volumes of methanol and *0.02 M sodium carbonate* shows an absorption maximum only at about 256 nm; absorbance at about 256 nm, about 0.5.

C. Mix 5 mg with 50 mg of resorcinol and 2 ml of *sulphuric acid (80 per cent)*. Heat at 200° for 10 minutes, cool, pour into 200 ml of water and add sufficient *5 M sodium hydroxide* to make the mixture just alkaline; an intense green fluorescence is produced.

D. A 5 per cent w/v solution gives the reactions of sodium salts (2.3.1).

### Tests

**pH** (2.4.24). 8.0 to 9.2, determined in a 10.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). +132° to +140°, determined in a 1.0 per cent w/v solution in a mixture of equal volumes of *methanol* and *0.02 M sodium carbonate*.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel F254* (such as precoated Merck silica gel 60 F254 plates).

*Mobile phase*. A mixture of 60 volumes of *ethyl acetate*, 20 volumes of *methanol*, 11 volumes of *water* and 1 volume of *strong ammonia* solution.

*Test solution*. A 1.5 per cent w/v solution of the substance under examination in *methanol*.

*Reference solution*. A 0.03 per cent w/v solution of the substance under examination in *methanol*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Spray with a 1.5 per cent w/v solution of *vanillin* in *sulphuric acid (60 per cent)* and heat at 105° for 10 to 15 minutes. By both methods of visualisation, any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Water** (2.3.43). Not more than 4.0 per cent, determined on 0.6 g.

**Assay**. Weigh accurately about 1.0 g and dissolve in 30 ml of *water*. Add 30 ml of *chloroform* and 15 ml of a mixture of 10 volumes of *2 M hydrochloric acid* and 90 volumes of *water*, shake and allow to separate. Add the *chloroform layer* to 40 ml of a 20 per cent w/v solution of *sodium chloride*, shake and allow to separate. Repeat the extraction with four quantities, each of 15 ml, of *chloroform*, combine the chloroform extracts and add sufficient *chloroform* to produce 100.0 ml. Evaporate 25.0 ml, dry the residue at 100° at a pressure of 2 kPa and dissolve in 10 ml of *dimethylformamide*. Titrate with *0.1 M tetrabutylammonium hydroxide* using *thymol blue solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.03073 g of $C_{34}H_{48}Na_2O_7$.

## Carbenoxolone Tablets

Carbenoxolone Sodium Tablets

Carbenoxolone Sodium Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of carbenoxolone sodium, $C_{34}H_{48}Na_2O_7$.

**Usual strength**. 50 mg.

### Identification

A. Shake a quantity of the powdered tablets containing 0.2 g of Carbenoxolone Sodium with 10 ml of *methanol*, filter and evaporate to dryness. The residue complies with the following tests.

1. When examined in the range 230 nm to 360 nm (2.4.7), a 0.0025 per cent w/v solution in a mixture of equal volumes of *methanol* and *0.02 M sodium carbonate* shows an absorption maximum only at about 256 nm; absorbance at about 256 nm, about 0.5.

2. Mix 5 mg with 50 mg of *resorcinol* and 2 ml of *sulphuric acid (80 per cent)*. Heat at 200° for 10 minutes, cool, pour into 200 ml of *water* and add sufficient *5 M sodium hydroxide* to make the mixture just alkaline; an intense green fluorescence is produced.

B. A 5 per cent w/v solution of the residue obtained in test A gives the reactions of sodium salts (2.3.1).



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### Tests

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel F254* (such as precoated Merck silica gel 60 F254 plates).

*Mobile phase*. A mixture of 60 volumes of *ethyl acetate*, 20 volumes of *methanol*, 11 volumes of *water* and 1 volume of *strong ammonia* solution.

*Test solution*. Triturate a quantity of the powdered tablets containing 0.1 g of Carbenoxolone Sodium with 20 ml of *methanol*, filter, evaporate the filtrate to low volume and add sufficient *methanol* to produce 10 ml.

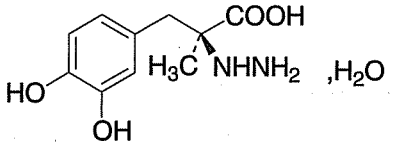
*Reference solution.* Dilute 3 volumes of the test solution to 100 volumes with *methanol.*

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Spray with a 1.5 per cent w/v solution of *vanillin* in *sulphuric acid (60 per cent)* and heat at 105° for 10 to 15 minutes. By both methods of visualisation, any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Other tests**. Complies with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Triturate a quantity of the powdered tablets containing about 75 mg of Carbenoxolone Sodium with a small volume of *methanol*, filter and add sufficient *methanol* to produce 250.0 ml. To 10.0 ml add 10 ml of *0.02 M sodium carbonate* and sufficient of a mixture of equal volumes of *methanol* and *0.02 M sodium carbonate* to produce 100.0 ml and measure the absorbance of the resulting solution at the maximum at about 256 nm (2.4.7). Calculate the content of $C_{34}H_{48}Na_2O_7$ taking 199 as the specific absorbance at the maximum at about 256 nm.

## Carbidopa




$C_{10}H_{14}N_2O_4,H_2O$ Mol. Wt. 244.2

Carbidopa is (*S*)-3-(3,4-dihydroxyphenyl)-2-hydrazino-2-methylpropionic acid monohydrate.

Carbidopa contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{10}H_{14}N_2O_4$, calculated with on the dried basis.

**Category**. Antiparkinsonian.

**Dose**. 10 to 25 mg in combination with Levodopa.

**Description**. A white to creamy white powder; odourless or practically odourless.

### Identification

*Tests A and C may be omitted if tests B, D and E are carried out. Tests B, D and E may be omitted if tests A and C are carried out.*

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

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.004 per cent w/v solution in a 1 per cent v/v solution of *hydrochloric acid* in *methanol* shows an absorption maximum only at about 282 nm; absorbance at about 282 nm, about 0.52.

C. Complies with the test for Specific optical rotation.

D. Shake vigorously about 5 mg with 10 ml of *water* for 1 minute and add 0.3 ml of *ferric chloride solution*; an intense green colour is produced, which quickly becomes reddish brown.

E. Suspend 20 mg in 5 ml of *water* and add 5 ml of *cupri-tartaric solution* and heat; the colour of the solution changes to dark brown and a red precipitate is produced.

### Tests

**Appearance of solution**. Dissolve 0.25 g in 25 ml of *1 M hydrochloric acid*. The solution is clear (2.4.1) and not more intensely coloured than reference solution BYS6 or BS6 (2.4.1).

**Specific optical rotation** (2.4.22). -22.5° to -26.5°, determined in a solution prepared by dissolving 0.25 g in 25 ml of *aluminium chloride solution.*

**Hydrazine**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silanised silica gel G.*

*Mobile phase*. A mixture of 2 volumes of *methanol* and 1 volume of *water.*

*Test solution*. Place 25 g of *strongly basic anion exchange resin* into each of two stoppered conical flasks, add 150 ml of *carbon dioxide-free water* to each flask and allow to stand for 30 minutes shaking occasionally. Decant the liquid from both flasks and repeat the process with further quantities, each of 150 ml, of *carbon dioxide-free water.* Separately transfer the resin portions into two 100-ml measuring cylinders, 3.5 to 4.5 cm in internal diameter, using 60 ml of *carbon dioxide-free water* for one portion (A) and 20 ml of *carbon dioxide-free water* for the other portion (B). Into each cylinder, insert a gas-inlet tube, 2 to 3 mm in internal diameter at the end and reaching almost to the bottom of the cylinder, and pass a rapid current of *nitrogen for chromatography* through each mixture



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so that homogeneous suspensions are produced. After 30 minutes, without interrupting the gas flow, add 1 ml of a solution prepared by dissolving 0.5 g of the substance under examination in sufficient *2 M hydrochloric acid* to produce 2 ml to cylinder A. After 1 minute stop the gas flow to cylinder A and transfer the contents, through a moistened filter paper, into cylinder B. After 1 minute, stop the gas flow to cylinder B and immediately pour the solution through a moistened filter paper into a freshly prepared mixture of 1 ml of a 20 per cent w/v solution of *salicylaldehyde* in *methanol* and 20 ml of *phosphate buffer solution pH 5.5*, shake thoroughly for 1 minute and heat in a water-bath at 60° for 15 minutes; the liquid becomes clear. Allow to cool, add 2 ml of *toluene,* shake vigorously for 2 minutes and centrifuge. Vigorously shake the toluene layer with two quantities, each of 20 ml, of a 20 per cent w/v solution of *sodium metabisulphite* and then with two quantities, each of 50 ml, of *water* and use the toluene layer.

*Reference solution*. Prepare at the same time and in the same manner but using 1 ml of a 0.002 per cent w/v solution of *hydrazine sulphate* in *2 M hydrochloric acid* in place of 1 ml of the solution of the substance under examination.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 365 nm. Any secondary spot in the chromatogram obtained with the test solution showing a yellow fluorescence is not more intense than the corresponding spot in the chromatogram obtained with the reference solution.

**Methyldopa and 3-*O*-methylcarbidopa**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 0.1 g in sufficient *0.1 M hydrochloric acid* to produce 10 ml.

*Reference solution (a)*. Dissolve 5 mg of *methyldopa RS* and 5 mg of *3-O-methylcarbidopa RS* in sufficient *0.1 M hydrochloric acid* to produce 100 ml.

*Reference solution (b)*. Dissolve 5 mg of *carbidopa RS* and 5 mg of *methyldopa RS* in sufficient *0.1 M hydrochloric acid* to produce 10 ml.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 98 volumes of a 1.4 per cent w/v solution of *potassium dihydrogen phosphate* and 2 volumes of *methanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 282 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to methyldopa and carbidopa is greater than 4.0.

Inject the test solution and reference solution (a)

In the chromatogram obtained with the test solution, the areas of any peaks corresponding to methyldopa and 3-O-methylcarbidopa are not greater than the areas of the corresponding peaks in the chromatogram obtained with reference solution (a).

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

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

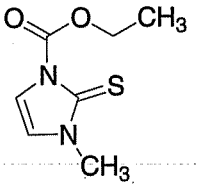
**Loss on drying** (2.4.19). 6.9 to 7.9 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.15 g and dissolve in 75 ml of *anhydrous glacial acetic acid* with the aid of gentle heat. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02262 g of $C_{10}H_{14}N_2O_4$.

**Storage**. Store protected from light.

# Carbimazole



$C_7H_{10}N_2O_2S$ Mol. Wt. 186.2

Carbimazole is ethyl 3-methyl-2-thioxo-4-imidazoline-1-carboxylate.

Carbimazole contains not less than 98.0 per cent and not more than 102.0 per cent of $C_7H_{10}N_2O_2S$, calculated on the dried basis.

**Category**. Antithyroid.

**Dose**. Controlling dose, 30 to 60 mg daily, in divided doses; maintenance dose, 5 to 20 mg daily.

**Description**. A white or creamy-white, crystalline powder; odour, characteristic.

## Identification

*Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.*

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



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B. In the test for Thiamazole and other related substances, the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (b).

C. To a solution prepared by dissolving about 10 mg in a mixture of 50 ml of *water* and 0.05 ml of *dilute hydrochloric acid*, add 1 ml of *potassium iodobismuthate solution*; a red precipitate is produced.

### Tests

**Thiamazole and other related substances.** Determine by liquid chromatography (2.4.14).

*Note —Use freshly prepared solutions.*

*Solvent mixture*. 20 volumes of *acetonitrile* and 80 volumes of *water*.

*Test solution*. Dissolve 5 mg of the substance under examination in 10.0 ml of the solvent mixture.

*Reference solution (a)*. A solution containing 0.005 per cent w/v of *thiamazole (carbimazole impurity A)* and 0.0001 per cent w/v of *carbimazole RS* in the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

*Reference solution (b)*. Dissolve 5 mg of *thiamazole* in 10.0 ml of the solvent mixture. Dilute 1.0 ml of this solution to 100 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 3.9 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 10 volumes of *acetonitrile* and 90 volumes of *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to carbimazole impurity A and carbimazole is not less than 5.0.

Inject the test solution and reference solution (b). Run the chromatogram 1.5 times the retention time of the principal peak. In the chromatogram obtained with test solution, the area of secondary peak corresponding to carbimazole impurity A is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the areas of any other secondary peak is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 24 hours.

**Assay**. Weigh accurately about 50 mg and dissolve in sufficient *water* to produce 500.0 ml. To 10.0 ml of the solution add 10 ml of *1 M hydrochloric acid* and sufficient *water* to produce 100.0 ml and measure the absorbance of the resulting solution at the maximum at about 291 nm (2.4.7). Calculate the content of $C_7H_{10}N_2O_2S$ taking 557 as the specific absorbance at 291 nm.

**Storage**. Store protected from light.

## Carbimazole Tablets

Carbimazole Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of carbimazole, $C_7H_{10}N_2O_2S$.

**Usual strengths**. 5 mg; 20 mg.

### Identification

A. Shake a quantity of the powdered tablets containing 50 mg of Carbimazole with two quantities, each of 5 ml of *chloroform*. Combine the chloroform extracts, filter and evaporate the filtrate to dryness. Dry the residue at 60° at a pressure not exceeding 0.7 kPa for 30 minutes The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *carbimazole RS* or with the reference spectrum of carbimazole.

B. To a small quantity of the powdered tablets add 1 drop of *dilute potassium iodobismuthate solution*; a scarlet colour is produced.

### Tests

**Thiamazole and other related substances.** Determine by liquid chromatography (2.4.14).

*Note —Use freshly prepared solutions and protect from light.*

*Test solution*. Disperse a quantity of the powdered tablets containing about 20 mg of Carbimazole in 10 ml of *acetonitrile* with the aid of ultrasound for 5 minutes, filter. Dilute 1 ml of this solution to 4 ml with 5 per cent v/v of *acetonitrile*.

*Reference solution (a)*. A 0.00025 per cent w/v of *carbimazole RS* in 5 per cent v/v of *acetonitrile*.

*Reference solution (b)*. A 0.0005 per cent w/v of *thiamazole* in 5 per cent v/v of *acetonitrile*.

*Reference solution (c)*. A solution containing 0.01 per cent w/v of *carbimazole RS* and 0.0005 per cent w/v of *thiamazole* in 5 per cent v/v of *acetonitrile*.

Chromatographic system
- a stainless steel column 15 cm x 3.9 mm packed with octadecylsilane bonded to porous silica (5 µm),



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- mobile phase: A. 5.0 per cent v/v solution of *acetonitrile*,
  B. 20.0 per cent v/v solution of *acetonitrile*,
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

| Time (min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0 | 100 | 0 |
| 4.5 | 100 | 0 |
| 4.6 | 0 | 100 |
| 30 | 0 | 100 |
| 30.1 | 100 | 0 |
| 40 | 100 | 0 |

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to thiamazole (carbimazole impurity A) and carbimazole is not less than 5.0.

Inject the test solution, reference solution (a) and (b). In the chromatogram obtained with test solution, the area of secondary peak corresponding to thiamazole is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent).

**Uniformity of content**. Comply with the test stated under Tablets.

*Test solution*. Powder one tablet, add 300 ml of *water* warmed to a temperature not exceeding 35°, shake for a few minutes and add sufficient *water* to produce 500.0 ml. Mix well, filter and dilute further, if necessary with *water*. Complete the Assay beginning at the words "Measure the absorbance....".

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 40 mg of Carbimazole, add 300 ml of *water* warmed to a temperature not exceeding 35°, shake for a few minutes and add sufficient water to produce 500.0 ml. Mix well and filter. Dilute 5.0 ml of the filtrate to 50.0 ml with *water* and mix well. Measure the absorbance of the resulting solution at the maximum at about 291 nm (2.4.7). Calculate the content of $C_7H_{10}N_2O_2S$ taking 557 as the specific absorbance at the maximum at about 291 nm.

**Storage**. Store protected from light and moisture at a temperature not exceeding 30°.

# Carbomers

Carbomers are high molecular mass polymers of acrylic acid cross-linked with polyalkenyl ethers of sugars or polyalcohols.

Carbomers contains not less than 56.0 per cent and not more than 68.0 per cent of carboxylic acid (-COOH) groups, calculated on the dried basis.

**Category**. Excipient.

**Description**. A white, fluffy powder, hygroscopic.

## Identification

*Test A may be omitted if tests B, C, D and E are carried out. Tests B, C, D may be omitted if tests A and E are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *carbomers RS*.

B. Adjust a 1 per cent w/v dispersion to about pH 7.5 with *1 M sodium hydroxide*. A highly viscous gel is formed.

C. Add 2 ml of a 10 per cent w/v solution of *calcium chloride* with continuous stirring to 10 ml of the gel obtained in test B. A white precipitate is immediately produced.

D. Add 0.5 ml of *thymol blue solution* to 10 ml of a 1 per cent w/v dispersion. An orange colour is produced. Add 0.5 ml of *cresol red solution* to 10 ml of a 1 per cent w/v dispersion. A yellow colour is produced.

E. It complies with the test for viscosity (2.4.28).

## Tests

**Apparent viscosity**. The nominal apparent viscosity is in the range 300 mPa s to 115 000 mPa s. For a product with a nominal apparent viscosity of 20000 mPa s or greater, the apparent viscosity is 70.0 per cent to 130.0 per cent of the value stated on the label; for a product with a nominal apparent viscosity less than 20 000 mPa s, the apparent viscosity is 50.0 per cent to 150.0 per cent of the value stated on the label.

Dry the substance under examination in vacuum at 80° for 1 hour. Carefully add 2.5 g of the previously dried substance under examination to 500 ml of *water* in a 1000 ml beaker while stirring continuously at 1000 ± 50 rpm, with the stirrer shaft set at an angle of 60° to one side of the beaker. Add the previously dried substance over a period of 45 to 90 seconds, at a uniform rate, ensuring that loose aggregates of powder are broken up and continue stirring at 1000 ± 50 rpm for 15 minutes. Remove the stirrer, and place the beaker containing the dispersion in a water-bath at 25 ± 0.2° for 30 minutes. Insert the stirrer to a depth necessary to ensure that air is not drawn into the dispersion, and while stirring at 300 ± 25 rpm, titrate with a glass-calomel electrode system to pH 7.3 to 7.8 by adding a 18 per cent w/v solution of *sodium hydroxide*



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below the surface, determining the end-point potentiometrically (2.4.25). The total volume of the 18 per cent w/v solution of *sodium hydroxide* used is about 6.2 ml. Allow 2-3 minutes before the final pH determination. If the final pH exceeds 7.8, discard the preparation, and prepare another using a smaller amount of sodium hydroxide for titration. Return the neutralised preparation to the water-bath at 25° for 1 hour, then perform the viscosity determination without delay to avoid slight viscosity changes that occur 75 minutes after neutralisation. Determine the viscosity (2.4.28) with a rotating viscometer with a spindle rotating at 20 rpm, using a spindle suitable for the expected apparent viscosity.

**Free acrylic acid**. Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve 0.125 g of the substance under examination in 25 ml of a 2.5 per cent w/v solution of *aluminium potassium sulphate*. Heat the suspension at 50° for 20 minutes with shaking. Then shake the suspension at room temperature for 60 minutes. Centrifuge and use the clear supernatant solution.

*Reference solution.* Dissolve 62.5 mg of *acrylic acid RS* in 100 ml of a 2.5 per cent w/v solution of *aluminium potassium sulphate*. Dilute 1.0 ml of this solution to 50.0 ml with 2.5 per cent w/v solution of *aluminium potassium sulphate*.

Chromatographic system

- a stainless steel column 12 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. dissolve 0.136 g in 100 ml of *potassium dihydrogen phosphate*, adjust to pH 2.5 using *dilute phosphoric acid*,
  B. equal volumes of a solution of 0.136 g of *potassium dihydrogen phosphate* in 100 ml of water and *acetonitrile*,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 205 nm,
- injection volume. 20 µl.

| Time (in min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) | Comments |
|---|---|---|---|
| 0 – 8 | 100 → 0 | 0 | isocratic |
| 9 – 20 | 0 → 100 | 100 | linear gradient |
| 21 – 30 | 100 → 0 | 0 | isocratic |

Inject alternately the test solution and the reference solution. The retention time for acrylic acid is about 6.0 minutes. The area of the peak in the chromatogram obtained with the test solution is not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.25 per cent).

**Benzene**. Determine by gas chromatography (2.4.13).

*Diluent.* Dissolve 0.1 g of *benzene* in 100 ml of *dimethyl sulphoxide*. Further dilute 1.0 ml of the solution to 100.0 ml with *water*. Further dilute 1.0 ml of this solution to 100.0 ml with *water*.

*Test solution.* Weigh 50.0 mg of the substance under examination, add 5.0 ml of *water* and 1.0 ml of *dimethyl sulphoxide*.

*Reference solution.* Weigh 50.0 mg of the substance under examination, add 4.0 ml of *water*, 1.0 ml of *dimethyl sulphoxide* and 1.0 ml of the diluent.

*Close the vials with a tight rubber membrane stopper coated with polytetrafluoroethylene and secure with an aluminium crimped cap. Shake to obtain a homogeneous dispersion.*

Chromatographic system

- a capillary column 30 m x 0.53 mm, packed with cyanopropyl phenyl polysiloxane,
- temperature :
  column at 130°,
  inlet port and detector at 240°,
- flow rate. 30 ml per minute of the carrier gas.
  *Stratic head-space conditions which may be used:*
- equilibration temperature 80°,
- equilibration time 60 minutes,
- transfer line temperature 90°.

Inject 1 ml of the gaseous phase of the test solution and 1 ml of the gaseous phase of the reference solution; repeat these injections twice more. Maximum relative standard deviation of the differences in area between the analyte peaks obtained from the 3 replicate pair injections of the reference solution and the test solution is 15 per cent. The test is not valid unless the relative standard deviation for replicate injections is not more than 15 per cent.

The mean area of the peak corresponding to benzene in the chromatograms obtained with the test solution is not more than half the mean area of the peak corresponding to benzene in the chromatograms obtained with the reference solution (2 ppm).

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method C (20 ppm). Use 2 ml of *lead standard solution (10 ppm Pb).*

**Sulphated ash** (2.3.18). Not more than 4.0 per cent, determined on 1.0 g.

**Loss on drying** (2.4.19). Not more than 3.0 per cent, determined on 1.0 g by drying in vacuum at 80° for 60 minutes.

**Assay**. Weigh accurately about 0.12 g, add 50 ml of *water* slowly with stirring and heating at 60° for 15 minutes. Stop heating, add 150 ml of *water* and continue stirring for



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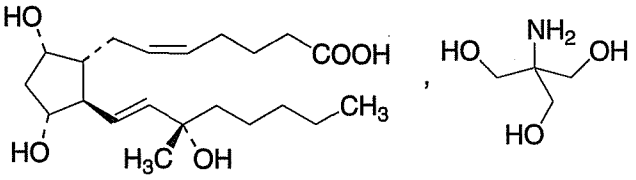
30 minutes. Add 2 g of *potassium chloride* and titrate with *0.2 M sodium hydroxide* determining the end-point potentiometrically (2.4.25).

1 ml of *0.2 M sodium hydroxide* is equivalent to 0.009 g of carboxylic acid (-COOH) groups.

**Storage**. Store protected from moisture.

**Labelling**. The label states the nominal apparent viscosity.

# Carboprost Tromethamine




$C_{21}H_{36}O_5,C_4H_{11}NO_3$ Mol. Wt. 489.70

Carboprost Tromethamine is a salt of (5*Z*,13*E*)-(8*R*,9*S*,11*R*,12*R*,15*S*)-9,11,15-trihydroxy-15-methylprosta-5,13-dienoic acid with 2-amino-2-hydroxymethyl-1,3-propanediol.

Carboprost Tromethamine contains not less than 95.0 per cent and not more than 105.0 per cent of $C_{21}H_{36}O_5,C_4H_{11}NO_3$, calculated on the dried basis.

*Great care should be taken to prevent inhaling particles of Carboprost Tromethamine and exposing the skin to it.*

**Category**. Uterine stimulant; abortifacient.

**Dose**. By deep intramuscular injection, 250 µg repeated if necessary at 1.5-hour intervals, the total dose not exceeding 12 mg. Continuous administration should not be longer than 2 days.

**Description**. A white powder.

## Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *carboprost tromethamine RS*. Examine the substances as mulls.

## Tests

**Specific optical rotation**. (2.4.22) + 18.0° to + 24.0°, determined in a 1.0 per cent w/v solution in *ethanol (95 per cent)*.

**15*R*-Epimer and 5-*trans* isomer**. Determine by liquid chromatography (2.4.14).

Follow the method described under Assay but using injection volume 25 µl. The usual order of elution is guaiphenesin, the 2-naphthacyl ester of 15*R*-epimer, the 2-naphthacyl ester of carboprost and the 2-naphthacyl ester of the *5-trans* isomer with retention times of about 7, 8, 11 and 13 minutes respectively. Measure the peak areas for the four components and calculate the contents of the 15R-epimer and *5-trans* isomer. The percentages of I5*R*-epimer (as tromethamine salt) and *5-trans* isomer are not more than 2.0 per cent and 4.0 per cent respectively.

**Loss on drying**. Not more than 1.0 per cent, determined on 20 mg by Method B.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test Solution*. Weigh accurately about 5 mg of the substance under examination, transfer to a stoppered 50-ml centrifuge tube. Add 20.0 ml of *dichloromethane* and 2 ml of citrate buffer prepared by dissolving 10.5 g of *citric acid monohydrate* in about 75 ml of *water*, adjusting the pH of the solution to 4.0 by addition of *sodium hydroxide solution* slowly and diluting to 100 ml with *water*. Shake the stoppered tube for about 10 minutes and centrifuge. Transfer 4.0 ml of the lower dichloromethane layer to a suitable vial and evaporate the solvent with the aid of a stream of nitrogen. To the dried material add 100 µl of a freshly prepared 2 per cent w/v solution of *α-bromo-2'-acetonaphthone* in *acetonitrile* and swirl to wash down the sides of the vial. Add 50 ml of a freshly prepared 1 per cent v/v solution of *diisopropylethylamine* in *acetonitrile*, swirl again and place the vial at a temperature of 30° to 35° for not less than 15 minutes. Evaporate the acetonitrile from the vial with the aid of a stream of nitrogen, add 2.0 ml of a 0.7 per cent w/v solution of guaiphenesin (internal standard) in the mobile phase, mix and filter the resulting solution through a fine porosity filter.

*Reference solution*. Prepare in the same manner but using *caroboprost tromethamine RS* in place of the substance under examination.

Chromatographic system

- stainless steel column 30 cm x 4 mm, packed with porous silica particles (3 to 10 µm),
- mobile phase: a mixture of 7 ml of *1,3-butanediol*, 0.5 ml of *water* and 992 ml of *dichloromethane*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set 254 nm,
- injection volume. 10 µl.

Inject the reference solution. The retention times for guaiphenesin and the 2-naphthacyl ester of carboprost are about 7 minutes and 11 minutes respectively. The test is not valid unless the resolution between these two peaks is greater than 4.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution. Calculate the content of $C_{21}H_{36}O_5,C_4H_{11}NO_3$.



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**Storage**. Store in a refrigerator (2° to 8°).

# Carboprost Tromethamine Injection

Carboprost Tromethamine Injection is a sterile solution of Carboprost Tromethamine in Water for Injections. It may contain Benzyl alcohol, Sodium Chloride and Tromethamine.

Carboprost Tromethamine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of carboprost, $C_{21}H_{36}O_5$.

**Usual strengths**. the equivalent of 250 µg and 500 µg of carboprost in 1 ml.

**Description**. A colourless solution.

## Identification

Extract a volume of the injection containing 2.5 mg of Carboprost Tromethamine with 1.5 to 2 times its volume of *chloroform.* Discard the chloroform Iayer and acidify the aqueous layer with 3 to 5 drops of *hydrochloric acid.* Extract the acidified solution with an equivalent volume of *chloroform.* Filter the chloroform layer through a pledget of cotton and concentrate the filtrate to a volume of less than 1 ml. To the resulting solution add 150 mg to 180 mg of *potassium bromide IR* and mix well. Dry the potassium bromide mixture in vacuum overnight and prepare a disc from the dried mixture.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *carboprost tromethamine RS* treated in the same manr.rost.

## Tests

**pH** (2.4.24). 7.0 to 8.0.

**Bacterial endotoxins**. Not more than 714.3 Endotoxin Units per mg of carboprost tromethamine.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Transfer a volume of the injection containing 500 µg of carboprost to a stoppered 50-ml centrifuge tube. Add 20.0 ml of *dichloromethane* and 1.0 ml of citrate buffer prepared by dissolving 10.5 g of *citric acid monohydrate* in about 75 ml of *water,* adjusting the pH of the solution to 4.0 by addition of *sodium hydroxide solution* slowly and diluting to 100.0 ml with *water.* Shake the stoppered tube for about 10 minutes and centrifuge. Transfer 8.0 ml of the lower dichloromethane layer to a suitable vial and evaporate the solution with the aid of a stream of nitrogen (The residue may not evaporate to dryness because of the presence of benzyl alcohol). Add 100 µl of a freshly prepared 2 per cent w/v solution of *α-bromo-2'-acetonaphthone in acetonitrile* and swirl to wash down the sides of the vial. Add 50 µl of a freshly prepared 1 per cent v/v solution of *diisopropylethylamine* in *acetonitrile,* swirl again and place the vial at a temperature of 30° to 35° for not less than 15 minutes. Evaporate the acetonitrile from the vial with the aid of a stream of nitrogen, add 1.0 ml of a 0.3 per cent w/v solution of *guaiphenesin* (internal standard) in the mobile phase, mix and filter the resulting solution through a fine porosity filter.

*Reference solution.* Prepare an aqueous solution containing about 0.332 mg of *carboprost tromethamine RS* and 9 mg of *benzyl alcohol* per ml. Transfer 2.0 ml of the resulting solution to a stoppered 50-ml centrifuge tube and proceed as given under the test solution beginning at the words "Add 20.0 ml *of dichloromethane....* ".

Chromatographic system

- a stainless steel column 30 cm x 4 mm, packed with porous silica particles (3 to 10 µm),
- mobile phase: a mixture of 7 ml of *1,3-butanediol,* 0.5 ml of *water* and 992 ml of *dichloromethane,*
- flow rate. 1.5 ml per minute,
- spectrophotometer set 254 nm,
- injection volume. 10 µl.

Inject the reference solution. The retention times for guaiphenesin and the 2-naphthacyl ester of carboprost are about 7 minutes and 11 minutes respectively. The test is not valid unless the resolution between these two peaks is greater than 4.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the quantity, in µg, of carboprost $C_{21}H_{36}O_5$ per ml of the injection from the ratios of the peak response of the 2-naphthacyl ester of carboprost and the internal standard obtained with the test solution, the ratios of the peak response of the 2-naphthacyl ester of carboprost and the internal standard obtained with the reference solution and the concentration, in µg per ml, of carboprost in *carboprost tromethamine RS* in the reference solution.

**Storage**. Store in a refrigerator (2° to 8°).

**Labelling**. The label states the strength in terms of the equivalent amount of carboprost in a suitable dose-volume.

# Sodium Carboxymethyl Cellulose

Carmellose Sodium

Sodium Carboxymethylcellulose is the sodium salt of a partially-substituted poly(carboxymethyl) ether of cellulose.



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Sodium Carboxymethylcellulose contains not less than 6.5 per cent and not more than 10.8 per cent of sodium, Na, calculated on the dried basis.

**Category**. Pharmaceutical aid (suspending agent; viscosity-increasing agent; excipient).

**Description**. A white or almost white, granular powder; odourless or almost odourless; hygroscopic.

### Identification

A. Sprinkle a quantity containing 1.0 g of the dried substance on to 90 ml of *carbon dioxide-free water* at 40° to 50°, stir vigorously until a colloidal solution is produced, cool and dilute to 100 ml with *carbon dioxide-free water* (solution A). To 10 ml of solution A add 1 ml of *copper sulphate solution*; a blue, cotton-like precipitate is produced.

B. Boil 5 ml of solution A for a few minutes; no precipitate is produced.

C. Solution A gives the reactions of sodium salts (2.3.1).

### Tests

**Appearance of solution**. Solution A is not more opalescent than opalescence standard OS4 (2.4.1), and not more intensely coloured than reference solution YS6 (2.4.1).

**pH** (2.4.24). 6.0 to 8.0, determined in solution A.

**Apparent viscosity**. 75 to 140 per cent of the declared value, determined by the following method. To 50 ml of water heated to 90° add, with stirring, a quantity containing 2 g of the dried substance under examination or, for a product of low viscosity, use the quantity required to give the concentration on the label. Allow to cool, dilute to 100 ml with *water* and continue stirring until solution is complete. Determine the viscosity by Method C (2.4.28), at 20° using a shear rate of 10 $s^{-1}$. If necessary, use rates slightly below and slightly above 10 $s^{-1}$ and interpolate.

**Arsenic** (2.3.10). Place 5.0 g in a dry Kjeldahl flask, add 20 ml of *nitric acid*, and warm cautiously until the reaction commences. Allow the reaction to subside without further heating, then add a mixture of 20 ml of *nitric acid* and 5 ml of *sulphuric acid* and heat until brown fumes cease to be evolved. Add 0.5 ml of *perchloric acid (60 per cent)*, heat until white fumes appear, and if the liquid is still dark add further small quantities of *nitric acid* and heat until the liquid becomes pale yellow. Heat again until the white fumes appear and continue heating for a further 15 minutes. Add 0.5 ml of *perchloric acid (60 per cent)* and continue heating for a few minutes. Allow the solution to cool add 10 ml of *water*, and heat until white fumes appear. Repeat the heating with a further 5 ml of *water*, cool and add 40 ml of *water* and 10 ml of *stannated hydrochloric acid AsT*. The resulting solution complies with the limit test for arsenic (1 ppm). Prepare the standard using 0.5 ml of *arsenic standard solution (10 ppm As)*.

**Heavy metals** (2.3.13). To the residue obtained in the test for Sulphated ash add 1 ml of *hydrochloric acid*, evaporate to dryness on a water-bath and dissolve the residue in 20 ml of *water*. 12 ml of the solution complies with the limit test for heavy metals, Method D ( 20 ppm). Prepare the standard using *lead standard solution (1 ppm Pb)*.

**Chlorides** (2.3.12). 10 ml of solution A complies with the limit test for chlorides (0.25 per cent).

**Sulphated ash** (2.3.18). 20.0 to 33.3 per cent, calculated on the dried basis, determined on 1.0 g dispersed in a mixture of equal volumes of *sulphuric acid* and *water*.

**Loss on drying** (2.4.19). Not more than 10.0 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.2 g and disperse in 80 ml of *anhydrous glacial acetic acid*. Heat on a water-bath for 2 hours, cool. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.002299 g of Na.

**Storage**. Store protected from light.

**Labelling**. The label states (1) the apparent viscosity in millipascal seconds of a 2 per cent w/v solution or, where the viscosity is low, the concentration of the solution to be used and the apparent viscosity in mPa s; (2) that the contents are not intended for use in the manufacture of an injectable preparation.

## Carnauba Wax

Carnauba Wax is obtained from the leaves of *Copernicia cerifera* Mart. (Fam. Palmae) after purification to remove foreign matter.

**Category**. Pharmaceutical aid (tablet coating agent).

**Description**. A pale yellow to light brown coarse powder, flakes or lumps of hard brittle wax; odour, characteristic and free from rancidity.

### Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 98 volumes of *chloroform* and 2 volumes of *ethyl acetate*.

*Test solution*. Dissolve 0.1 g of the substance under examination, with warming, in 5 ml of *chloroform* and use the warm solution.



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*Reference solution.* Dissolve 5 mg of (+)-*menthol*, 5 µl of *menthyl acetate* and 5 mg of *thymol* in 10 ml of *toluene*.

Apply separately to the plate, as bands 20 mm x 3 mm, 30 µl of test solution and 10 µl of reference solution.

After development, dry the plate in air and spray with a freshly prepared 20 per cent w/v solution of *phosphomolybdic acid* in *ethanol (95 per cent)* and heat at 105° for 15 minutes. The chromatogram obtained with the reference solution shows in the lower part a dark blue band due to menthol, a reddish band above it due to thymol and a dark blue band in the upper part due to menthyl acetate. The chromatogram obtained with the test solution shows a large blue band due to triacontanol (melissyl alcohol) at an $R_f$ value between those of the bands due to menthol and thymol in the chromatogram obtained with the reference solution and blue bands at $R_f$ values between those of the bands due to menthyl acetate and thymol in the chromatogram obtained with the reference solution. In addition, the chromatogram obtained with the test solution shows further bands at higher $R_f$ values than menthyl acetate, that with the highest $R_f$ value being very pronounced, and a number of faint bands below that due to triacontanol; a band on the line of application is blue.

### Tests

**Melting range** (2.4.21). 78° to 88°, determined by Method II.

**Heavy metals** (2.3.13). 0.5 g complies with the limit test for heavy metals, Method B (40 ppm).

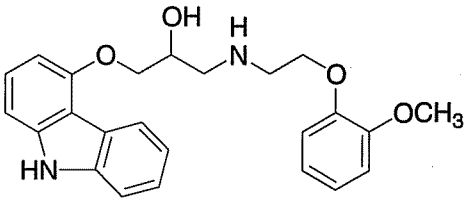
**Acid value.** Not more than 12.0, determined by the following method. Weigh accurately about 2.0 g *(w)* in a flask fitted with a reflux condenser, add 40 ml of *xylene* and heat until the substance has dissolved. Add 20 ml of *ethanol (95 per cent)* and titrate the hot solution with *0.5 M ethanolic potassium hydroxide*, using *phenolphthalein solution* as indicator, until a pink colour persists for at least 10 seconds ($n_1$ ml). Repeat the operation without the substance under examination ($n_2$ ml). Calculate the acid value from the expression $28.05(n_2–n_1)/w$.

**Saponification value.** Between 78 and 95, determined by the following method. To the titrated solution from the determination of the Acid value, add 20.0 ml of *0.5 M ethanolic potassium hydroxide* and boil under a reflux condenser for 3 hours. Titrate the hot solution immediately with *0.5 M hydrochloric acid*, using 1 ml of *phenolphthalein solution* as indicator, until the red colour is discharged. Reheat the solution to boiling and continue the titration, if necessary, until the red colour no longer reappears on heating ($n_3$ ml). Repeat the operation without the substance under examination ($n_4$ ml). Calculate the saponification value from the expression $a+[28.05(n_4-n_3)/w]$ where $a$ is the acid value.

**Sulphated ash** (2.3.18). Not more than 0.25 per cent, determined on 2.0 g.

**Storage.** Store protected from light and moisture.

## Carvedilol




$C_{24}H_{26}N_2O_4$ Mol. Wt. 406.5

Carvedilol is (*RS*)-1-(9*H*-carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy)ethyl]amino]propan-2-ol.

Carvedilol contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{24}H_{26}N_2O_4$, calculated on the dried basis.

Category. Antihypertensive.

**Description.** A white or almost white, crystalline powder.

### Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *carvedilol RS* or with the reference spectrum of carvedilol.

### Tests

**Related substances.** Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve 25.0 mg of the substance under examination in sufficient mobile phase to produce 25.0 ml.

*Reference solution (a).* Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

*Reference solution (b).* Dissolve 0.005 g of *2RS-1-[benzyl[2-(2-methoxyphenoxy)ethyl]-amino]3-(9H-carbazol-4-yloxy)propan-2-ol) RS* (*carvedilol impurity A RS*) in 5.0 ml of the test solution and dilute to 100.0 ml with the mobile phase.

*Reference solution (c).* Dilute 1.0 ml of reference solution (b) to 100.0 ml with the mobile phase. Dilute 2.0 ml of this solution to 10.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 12.5 cm x 4.6 mm packed with octylsilane bonded to porous silica (5 µm) (Such as YMC- Pack pro C8),
- column temperature. 55°,
- mobile phase: dissolve 1.77 g of *potassium dihydrogen phosphate* in *water* and dilute to 650 ml with the same solvent, adjust the pH to 2.0 with dilute *orthophosphoric acid* and add 350 ml of *acetonitrile,*



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- flow rate. 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to carvedilol and carvedilol impurity A is not less than 1.7.

Inject the test solution and the reference solutions. Run the chromatograms for eight times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of the peak due to carvedilol impurity A is not more than twice the area of the peak in the chromatogram obtained with reference solution (c) (0.02 per cent), the area of the peak due to any other impurity is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent) and the sum of the areas of all the secondary peaks is not more than five times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.01 per cent).

**Heavy metals** (2.3.13). 2.0 g complies with limit test for heavy metals, Method B (10 ppm Pb).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.35 g and dissolve in 60 ml of *glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04065 g of $C_{24}H_{26}N_2O_4$.

**Storage**. Store protected from light, at a temperature not exceeding 30°.

## Carvedilol Tablets

Carvedilol Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of carvedilol, $C_{24}H_{26}N_2O_4$.

**Usual strengths**. 3.125 mg; 6.25 mg; 12.5 mg.

### Identification

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

### Tests

**Dissolution** (2.5.2).

Apparatus No. 1,

Medium. 900 ml of gastric buffer pH 1.3 prepared by dissolving 2 g of *sodium chloride* in 7 ml of *hydrochloric acid* and 500 ml of *water* and diluting to 1000 ml with *water*,

Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14) using the chromatographic conditions as described in the Assay.

*Test solution*. Dilute the filtrate, if necessary, with the dissolution medium.

*Reference solution*. Prepare a solution using *carvedilol RS* in the dissolution medium to obtain the same concentration as expected in the test solution.

Calculate the content of $C_{24}H_{26}N_2O_4$.

D. Not less than 70 per cent of the stated amount of $C_{24}H_{26}N_2O_4$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Shake a quantity of the powdered tablets containing 25 mg of Carvedilol with 15 ml of the mobile phase, dilute to 25 ml with the mobile phase and filter.

*Reference solution (a)*. A 0.1 per cent w/v solution of *carvedilol RS* in the mobile phase.

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

Chromatographic system

- a stainless steel column 12.5 cm x 4.6 mm packed with octylsilane bonded to porous silica (5 µm) (such as YMC-Pack pro C8),
- column temperature 55°,
- mobile phase: dissolve 1.77 g of *potassium dihydrogen phosphate* in *water*, dilute to 650 ml with the same solvent, adjust the pH to 2.0 with dilute *orthophosphoric acid* and add 350 ml of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume. 20 µl.

Inject reference solution (a). The test is not valid unless the column efficiency in not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). Run the chromatograms for eight times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with reference solution (b) (2.0 per cent).

**Uniformity of content**. Comply with the test stated under Tablets.



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Determine by liquid chromatography (2.4.14), using the chromatographic conditions as described under Assay.

*Test solution.* Disperse one tablet in 5 ml of water and dilute to 25 ml with the mobile phase and filter.

*Reference solution.* Prepare a solution using *carvedilol RS* in the mobile phase to obtain the same concentration as expected in the test solution.

Calculate the content of $C_{24}H_{26}N_2O_4$ in the tablet.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 50 mg of Carvedilol, disperse in 10 ml of *water* and dilute to 50 ml with the mobile phase and filter. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

*Reference solution.* A 0.01 per cent w/v solution of *carvedilol RS* in the mobile phase.

Chromatographic system

- a stainless steel column 12.5 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm) (such as YMC-Pack pro C8),
- mobile phase: a mixture of 50 volumes of 2 per cent w/v solution of *sodium heptane sulphonate* in *water*, 25 volumes of *acetonitrile* and 25 volumes of *methanol* with the pH adjusted to 3.1 with *orthophosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 285 nm,
- injection volume. 10 µl.

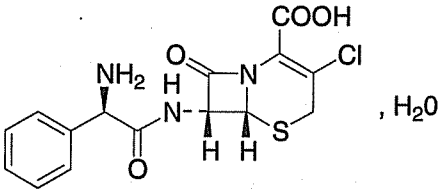
Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, the column efficiency is not less than 2000 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{24}H_{26}N_2O_4$ in the tablets.

**Storage**. Store protected from light, at a temperature not exceeding 30°.

# Cefaclor




$C_{15}H_{14}ClN_3O_4S,H_2O$ Mol. Wt 385.8

Cefaclor is (6*R*,7*R*)-7-[[(2*R*)-2-amino-2-phenylacetyl]amino]-3-chloro-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid monohydrate.

Cefaclor contains not less than 96.0 per cent and not more than 102.0 per cent of $C_{15}H_{14}ClN_3O_4S$, calculated on the anhydrous basis.

**Category**. Antibacterial.

**Description**. A white or slightly yellow powder.

## Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cefaclor RS* or with the reference spectrum of cefaclor.

## Tests

**pH** (2.4.24). 3.0 to 4.5, determined in a suspension, prepared by dispersing 0.25 g in 10 ml of *carbon dioxide-free water*.

**Specific optical rotation** (2.4.22). +101° to +111°, determined in 1.0 per cent w/v solution in a 1.0 per cent w/v solution of *hydrochloric acid*.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture.* A 0.27 per cent w/v solution of *sodium dihydrogen phosphate*, adjusted to pH 2.5 with *phosphoric acid*.

*Test solution.* Dissolve 50 mg of the substance under examination in 10 ml of the solvent mixture.

*Reference solution (a)* A solution containing 0.0025 per cent w/v *cefaclor RS* and 0.005 per cent w/v *delta-3-cefaclor RS* in the solvent mixture.

*Reference solution (b)* Dilute 1 ml of the test solution to 100 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a 0.78 per cent w/v solution of *sodium dihydrogen phosphate* adjusted to pH 4.0 with *phosphoric acid*,
  B. mix 450 ml of *acetonitrile* with 550 ml of mobile phase A,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 220 nm,
- injection volume. 20 µl.

Increase the concentration of mobile phase B continuously and linearly by 0.67 per cent v/v per minute for 30 minutes (25 per cent v/v). Then increase the concentration of mobile phase B continuously and linearly by 5 per cent v/v per minute for 15 minutes (100 per cent v/v). Finally elute with mobile phase B for 10 minutes.



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Equilibrate the column with a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A for at least 15 minutes between each analysis. Inject the solutions. At the end of the programme change the composition of the mobile phase to a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A to re-equilibrate the column.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to cefaclor and delta-3-cefaclor is not less than 2.0 and the tailing factor of the cefaclor peak is not more than 1.2. If necessary, adjust the acetonitrile content of the mobile phase.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any peak, other than the principal peak and any peaks due to the mobile phase, is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of the areas of all such peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b).

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

**Water** (2.3.43). 3.0 to 6.5 per cent, determined on 0.2 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 15 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the mobile phase.

*Reference solution (a)*. A 0.03 per cent w/v solution of *cefaclor RS* in the mobile phase.

*Reference solution (b)*. A solution containing 0.03 per cent w/v each of *cefaclor RS* and *delta-3-cefaclor RS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture prepared by adding 220 volumes of *methanol* to a mixture of 780 volumes of *water*, 10 volumes of *triethylamine* and 1 g of *sodium pentanesulphonate*, adjusted to pH 2.5 with *orthophosphoric acid*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to cefaclor and delta-3-cefaclor is not less than 2.5. Adjust the concentration of methanol in the mobile phase, if necessary. The test is not valid unless the tailing factor of the cefaclor peak is not more than 1.5.

Inject reference solution (a) 6 times. The test is not valid unless the relative standard deviation of the peak area of cefaclor is not more than 1.0 per cent.

Inject alternately the test solution and reference solution (a).

Calculate the content of $C_{15}H_{14}ClN_3O_4S$.

**Storage**. Store protected from moisture.

# Cefaclor Capsules

Cefaclor Capsules contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of cefaclor, $C_{15}H_{14}ClN_3O_4S$.

**Usual strengths**. 250 mg; 500 mg.

## Identification

A. Shake a quantity of the contents of the capsules containing 0.3 g of anhydrous cefaclor with 100 ml of *water*, filter and dilute 1 ml of the filtrate to 100 ml with *water*.

When examined in the range 190 nm to 310 nm (24.7), the resulting solution shows an absorption maximum only at about 264 nm.

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

## Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *water*,
Speed and time. 50 rpm and 45 minute.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 264 nm (2.4.7). Calculate the content of $C_{15}H_{14}ClN_3O_4S$ in the medium from the absorbance obtained from a solution of known concentration of *cefaclor RS* in the same medium.

D. Not less than 70 per cent of the stated amount of $C_{15}H_{14}ClN_3O_4S$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. A 0.27 per cent w/v solution of *sodium dihydrogen orthophosphate*, adjusted pH to 2.5, if necessary, with *orthophosphoric acid*.



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*Test solution*. Shake a quantity of the contents of the capsules containing 0.5 g of anhydrous cefaclor with 200 ml of the solvent mixture, dilute to 250 ml with the solvent mixture and filter.

*Reference solution (a)*. A 0.002 per cent w/v solution of *cefaclor RS* in the solvent mixture.

*Reference solution (b)*. A solution containing 0.0025 per cent w/v of *cefaclor RS* and 0.005 per cent w/v of *delta-3-cefaclor RS* in the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Spherisorb ODS-2),
- mobile phase: A. a 0.78 per cent w/v solution of *sodium dihydrogen orthophosphate* adjusted to pH 4.0 with *orthophosphoric acid*,
  B. a mixture of 450 volumes of *acetonitrile* and 550 volumes of mobile phase A,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 220 nm,
- injection volume. 20 µl.

| Time (in min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) | Comments |
|---|---|---|---|
| 0 – 30 | 95 – 75 | 5 – 25 | linear gradient |
| 30 – 45 | 75 – 0 | 25 – 100 | |
| 45 – 55 | 0 | 100 | isocratic |
| 55 – 70 | 0 – 95 | 100 – 5 | re-equilibration |

Equilibrate the column with a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A for at least 15 minutes.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to cefaclor and delta-3-cefaclor is not less than 2.0. If necessary, adjust the proportion of acetonitrile in the mobile phase.

Inject the test solution and reference solution (a). In the chromatogram obtained with the test solution the area of any secondary peak is not greater than half the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent) and the sum of the areas of any such peaks is not greater than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Shake a quantity of the contents of capsules containing about 75 mg of anhydrous cefaclor with 200 ml of the mobile phase, dilute to 250.0 ml with the mobile phase and filter.

*Reference solution (a)*. A 0.03 per cent w/v solution of *cefaclor RS* in the mobile phase.

*Reference solution (b)*. A solution containing 0.03 per cent w/v each of *cefaclor RS* and *delta-3-cefaclor RS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Beckman Ultrasphere ODS and Supelcosil LC-18-DB),
- mobile phase: a solution prepared by dissolving 1 g of *sodium pentanesulphonate* in a mixture of 780 volumes of *water* and 10 volumes of *triethylamine*, adjusting the pH to 2.5 with *orthophosphoric acid* adding 220 volumes of *methanol* and mixing,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to cefaclor and delta-3-cefaclor is not less than 2.5.

Inject alternately the test solution and reference solution (a).

Calculate the content of $C_{15}H_{14}ClN_3O_4S$ in the capsules.

**Storage**. Store protected from moisture.

**Labelling**. The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous cefaclor.

## Cefaclor Oral Suspension

Cefaclor Oral Suspension is a mixture consisting of Cefaclor with buffering agents and other excipients. It contains a suitable flavouring agent. It is filled in a sealed container.

The suspension is constituted by dispersing the contents of the sealed container in the specified volume of Water just before use.

Cefaclor Oral Suspension contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of cefaclor, $C_{15}H_{14}ClN_3O_4S$.

When stored at the temperature and for the period stated on the label during which the constituted suspension may be



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expected to be satisfactory for use, it contains not less than 80.0 per cent of the stated amount of cefaclor, $C_{15}H_{14}ClN_3O_4S$.

**Usual strengths.** 15 mg per 5 ml; 375 mg per 5 ml.

**Storage.** Store protected from moisture at a temperature not exceeding 30°.

The constituted suspension complies with the tests stated under Oral liquids and with the following tests.

## Identification

A. Shake a quantity of the oral suspension containing 0.3 g of anhydrous cefaclor with 500 ml of *water* and filter.

When examined in the range 190 nm to 310 nm (2.4.7), the filtrate shows an absorption maximum only at about 264 nm.

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

## Tests

**Related substances.** Determine by liquid chromatography (2.4.14).

*Solvent mixture.* A 0.27 per cent w/v solution of *sodium dihydrogen orthophosphate,* adjusted to pH 2.5 with *orthophosphoric acid.*

*Test solution.* Shake a quantity of the oral suspension containing about 0.25 g of anhydrous cefaclor with 200 ml of the solvent mixture, dilute to 250 ml with the solvent mixture and filter.

*Reference solution (a).* A 0.001 per cent w/v solution of *cefaclor RS* in the solvent mixture.

*Reference solution (b).* A solution containing 0.0025 per cent w/v of *cefaclor RS* and 0.005 per cent w/v of *delta-3-cefaclor RS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Spherisorb ODS-2),
- mobile phase: A. a 0.78 per cent w/v solution of *sodium dihydrogen orthophosphate,* djusted to pH 4.0 with *orthophosphoric acid,*
  B. a mixture of 450 volumes of *acetonitrile* and 550 volumes of mobile phase A,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 220 nm,
- injection volume. 20 µl.

| Time (in min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) | Comments |
|---|---|---|---|
| 0 – 30 | 95 – 75 | 5 – 25 | linear gradient |
| 30 – 45 | 75 – 0 | 25 – 100 | linear gradient |
| 45 – 55 | 0 | 100 | isocratic |
| 55 – 70 | 0 – 95 | 100 – 5 | re-equilibration |

Equilibrate the column with a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A for at least 15 minutes.

Inject reference solution (b). The test is not valid unless resolution between the peaks due to cefaclor and delta-3-cefaclor is not less than 2.0. If necessary, adjust the proportion of acetonitrile in the mobile phase.

Inject the test solution and reference solution (a). In the chromatogram obtained with the test solution the area of any secondary peak is not greater than the area of the principal peak in the chromatogram obtained with reference solution (a) (1 per cent) and the sum of the areas of any such peaks is not greater than three times the area of the principal peak in the chromatogram obtained with reference solution (a) (3 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

**Other tests.** Complies with the tests stated under Oral Liquids.

**Assay.** Determine by liquid chromatography (2.4.14).

*Test solution.* Shake a quantity of the oral suspension containing about 75 mg of anhydrous cefaclor with 200 ml of the mobile phase, dilute to 250.0 ml with the mobile phase and filter.

*Reference solution (a).* A 0.03 per cent w/v solution of *cefaclor RS* in the mobile phase.

*Reference solution (b).* A solution containing 0.03 per cent w/v each of *cefaclor RS* and *delta-3-cefaclor RS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Beckman Ultrasphere ODS and Supelcosil LC-18-DB),
- mobile phase: a solution prepared by dissolving 1 g of *sodium pentanesulphonate* in a mixture of 780 ml of water and 10 ml of *triethylamine,* adjusting the pH to 2.5 using *orthophosphoric acid,* adding 220 ml of *methanol* and mixing,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume. 20 µl.



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Inject reference solution (b). The test is not valid unless the resolution between the peaks due to cefaclor and delta-3-cefaclor is not less than 2.5 and the tailing factor of the peak due to cefaclor is not more than 1.5.

Inject alternately the test solution and reference solution (a).

Determine the weight per ml of the oral suspension (2.4.29) and calculate the content of $C_{15}H_{14}ClN_3O_4S$, weight in volume.

Repeat the procedure using a portion of the constituted suspension that has been stored at the temperature and for the period stated on the label.

**Storage**. Store at the temperature and use within the period stated on the label.

**Labelling**. The label states the quantity in terms of the equivalent amount of anhydrous cefaclor.

## Cefaclor Sustained-release Tablets

Cefaclor Sustained-release Tablets are prolonged-release tablets containing Cefaclor. The appropriate release of the active ingredient is demonstrated by a suitable dissolution test

Cefaclor Tablets contains not less than 90.0 per cent and not more than 105.0 per cent of the stated amount of cefaclor, $C_{15}H_{14}ClN_3O_4S$.

**Usual strengths**. 125 mg; 250 mg.

### Identification

A. Shake a quantity of the powdered tablets containing 0.3 g of anhydrous cefaclor with 100 ml of *water*, filter and dilute 1 ml of the filtrate to 100 ml with *water*.

When examined in the range 190 nm to 310 nm (2.4.7), the resulting solution shows an absorption maximum only at about 264 nm.

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

### Tests

**Dissolution** (2.5.2). Complies with the test stated under tablets.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. A 0.27 per cent w/v solution of *sodium dihydrogen orthophosphate*, adjusted pH to 2.5, if necessary, with *orthophosphoric acid*.

*Test solution*. Shake a quantity of the powdered tablets containing 0.75 g of anhydrous cefaclor with 200 ml of the solvent mixture, dilute to 250 ml with the solvent mixture and filter.

*Reference solution (a)*. A 0.003 per cent w/v solution of *cefaclor RS* in the solvent mixture.

*Reference solution (b)*. A solution containing 0.0025 per cent w/v of *cefaclor RS* and 0.005 per cent w/v of *delta-3-cefaclor RS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Spherisorb ODS-2),
- mobile phase: A. a 0.78 per cent w/v solution of *sodium dihydrogen orthophosphate* adjusted to pH 4.0 with *orthophosphoric acid*
  B. a mixture of 450 volumes of *acetonitrile* and 550 volumes of mobile phase A.
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 220 nm,
- injection volume. 20 µl.

| Time (in min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) | Comment |
|---|---|---|---|
| 0 – 30 | 95 – 75 | 5 – 25 | linear gradient |
| 30 – 45 | 75 – 0 | 25 – 100 | linear gradient |
| 45 – 55 | 0 | 100 | isocratic |
| 55 – 70 | 0 – 95 | 100 – 5 | re-equilibration |

Equilibrate the column with a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A for at least 15 minutes.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to cefaclor and delta-3-cefaclor is not less than 2.0. If necessary, adjust the proportion of acetonitrile in the mobile phase.

Inject the test solution and reference solution (a). In the chromatogram obtained with test solution the area of any secondary peak is not greater than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.6 per cent) and the sum of the areas of any such peaks is not greater than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (2 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 75 mg of anhydrous



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cefaclor, disperse in the mobile phase, shake, dilute to 250.0 ml with the mobile phase and filter.

*Reference solution (a).* A 0.03 per cent w/v solution of *cefaclor RS* in the mobile phase.

*Reference solution (b).* A solution containing 0.03 per cent w/v each of *cefaclor RS* and *delta-3-cefaclor RS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Beckman Ultrasphere ODS and Supelcosil LC-18-DB),
- mobile phase: a solution prepared by dissolving 1 g of *sodium pentanesulphonate* in a mixture of 780 volumes of *water* and 10 volumes of *triethylamine*, adjusting the pH to 2.5 with *orthophosphoric acid*, adding 220 volumes of *methanol* and mixing,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to cefaclor and delta-3-cefaclor is not less than 2.5.

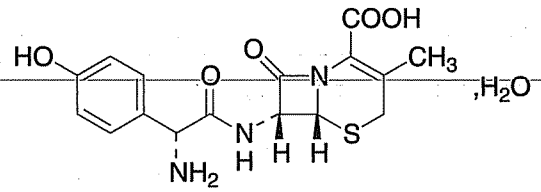
Inject the test solution and reference solution (a).

Calculate the content of $C_{15}H_{14}ClN_3O_4S$ in the tablets.

**Storage.** Store protected from moisture.

**Labelling.** The label states the strength in terms of the equivalent amount of anhydrous cefaclor.

# Cefadroxil




$C_{16}H_{17}N_3O_5S,H_2O$ Mol. Wt. 381.4

Cefadroxil is 7-[(*R*)-2-amino-2-(4-hydroxyphenyl)acetamido]-3-methyl-3-cephem-4-carboxylic acid monohydrate.

Cefadroxil contains not less than 95.0 per cent and not more than 101.0 per cent of $C_{16}H_{17}N_3O_5S$, calculated on the anhydrous basis.

**Category.** Antibacterial.

**Dose.** 500 mg to 2 g daily, in divided doses.

**Description.** A white to off-white, crystalline powder.

## Identification

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

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H* and impregnating the dry plate by placing it in a tank containing a shallow layer of about 1 cm of a mixture of 95 volumes of *n-hexane* and 5 volumes of *1-tetradecane,* allowing the solvent to ascend to the top, removing the plate and allowing the solvent to evaporate.

*Mobile phase.* A mixture of 60 volumes of *0.1 M citric acid,* 40 volumes of *0.1 M disodium hydrogen phosphate* and 1.5 volumes of a 6.66 per cent w/v solution of *ninhydrin* in *acetone.*

*Test solution.* A 0.2 per cent w/v solution of the substance under examination in *water.*

*Reference solution* (a). A 0.2 per cent w/v solution of *cefadroxil RS* in *water.*

*Reference solution (b).* A mixture of equal volumes of the test solution and reference solution (a).

Apply to the plate 20 µl of each solution. After development, dry the plate in air, spray with a 0.2 per cent w/v solution of *ninhydrin* in *ethanol,* dry at 110° for 10 minutes and examine. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single compact spot.

## Tests

**pH** (2.4.24). 4.0 to 6.0, determined in a 5.0 per cent w/v suspension.

**Specific optical rotation** (2.4.22). +165° to +178°, determined in a 1.0 per cent w/v solution.

**Related substances** Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve 50 mg of the substance under examination in 50.0 ml of mobile phase A.

*Reference solution (a).* Dissolve 10 mg of *D-α-(4-hydroxyphenyl)glycine RS (cefadroxil monohydrate impurity A RS)* in 10.0 ml of the mobile phase A.

*Reference solution (b).* Dissolve 10 mg of *7-aminodesacetoxycephalosporanic acid RS (cefadroxil monohydrate impurity B RS)* in 10.0 ml of the *phosphate buffer pH 7.0.*

*Reference solution (c).* Dilute 1.0 ml each of reference solution (a) and (b) to 100.0 ml with the mobile phase A.



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*Reference solution (d).* Dissolve 10 mg each of *dimethylformamide* and *dimethylacetamide* in 10.0 ml of the mobile phase A. Dilute 1.0 of this solution to 100.0 ml with the mobile phase A.

*Reference solution (e).* Dilute 1.0 ml of the reference solution (c) to 25.0 ml with the mobile phase A.

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. *phosphate buffer pH 5.0*, B. *methanol*,
- a linear gradient programme using the conditions given below,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 20 µl.

| Time (in min) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0-1 | 98 | 2 |
| 1-20 | 98-70 | 2-30 |
| 20-23 | 70-98 | 30-2 |
| 23-30 | 98 | 2 |

Inject reference solution (c). The relative retention time with reference to cefadroxil for dimethylformamide is about 0.4 and for dimethylacetamide is about 0.75. The test is not valid unless the resolution between the peaks due to cefadroxil impurity A and C is not less than 5.0. In the chromatogram obtained with reference solution (e) signal- to- noise ratio for the second peak is not less than 10.

Inject the test solution and reference solutions (c). In the chromatogram obtained with the test solution, the area of secondary peak due to cefadroxil impurity A is not more than the area of the first peak in the chromatogram obtained with reference solution (c) (1.0 per cent), the area of any secondary peak is not more than the area of the second peak in the chromatogram obtained with reference solution (c) (1.0 per cent). The sum of areas of all the secondary peaks is not more than 3 times the area of the second peak in the chromatogram obtained with reference solution (c) (3.0 per cent). Ignore any peak with an area less than 0.05 times the area of the second peak in the chromatogram obtained with reference solution (c) (0.05 per cent). Ignore the peaks due to dimethylformamide and dimethylacetamide.

***N,N*-Dimethylaniline** (2.3.21). Not more than 20 ppm, determined by Method B.

**Water** (2.3.43). 4.2 to 6.0 per cent, determined on 0.5 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* A freshly prepared 0.1 per cent w/v solution of the substance under examination in *phosphate buffer pH 5.0.*

*Reference solution.* A freshly prepared 0.1 per cent w/v solution of *cefadroxil RS* in *phosphate buffer pH 5.0.*

Chromatographic system
- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a mixture of 96 volumes of *phosphate buffer pH 5.0* and 4 volumes of *acetonitrile*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_{16}H_{17}N_3O_5S$.

**Storage**. Store protected from moisture at a temperature not exceeding 30°.

# Cefadroxil Capsules

Cefadroxil Capsules contain not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of anhydrous cefadroxil, $C_{16}H_{17}N_3O_5S$.

**Usual strength**. The equivalent of 500 mg of anhydrous cefadroxil.

## Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H* and impregnating the dry plate by placing it in a tank containing a shallow layer of about 1 cm of a mixture of 95 volumes of *n-hexane* and 5 volumes of *1-tetradecane,* allowing the solvent to ascend to the top, removing the plate and allowing the solvent to evaporate.

*Mobile phase.* A mixture of 60 volumes of *0.1 M citric acid*, 40 volumes of *0.1 M disodium hydrogen phosphate* and 1.5 volumes of a 6.66 per cent w/v solution of *ninhydrin* in *acetone.*

*Test solution.* Shake a quantity of the contents of a capsule with sufficient *water* to produce a solution containing 0.2 per cent w/v of Cefadroxil.

*Reference solution (a).* A 0.2 per cent w/v solution of *cefadroxil RS* in *water.*

*Reference solution (b).* A mixture of equal volumes of the test solution and reference solution (a).

Apply to the plate 20 µl of each solution. After development, dry the plate in air, spray with a 0.2 per cent w/v solution of *ninhydrin* in *ethanol,* dry at 110° for 10 minutes and examine.



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The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single compact spot.

### Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *water,*
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance (2.4.7) of the filtrate, suitably diluted if necessary, at the maximum at about 263 nm.

Calculate the content of $C_{16}H_{17}N_3O_5S$ in the medium from the absorbance obtained from a solution of known concentration of *cefadroxil RS.*

D. Not less than 75 per cent of the stated amount of $C_{16}H_{17}N_3O_5S$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve a quantity of content of capsules containing 0.5 g of anhydrous cefadroxil in 50 ml of the mobile phase, mix for 10 minutes and filter.

*Reference solution (a).* A 0.01 per cent w/v solution of *cefadroxil RS* in the mobile phase.

*Reference solution (b).* A 0.01 per cent w/v solution of *D-α-(4-hydroxyphenyl) glycine RS (cefadroxil impurity A RS)* in the mobile phase.

*Reference solution (c).* A 0.01 per cent w/v solution of *7-aminodesacetoxycephalosporanic acid RS (cefadroxil impurity B RS)* in the mobile phase.

Chromatographic system
- a stainless steel column 30 cm x 3.9 mm packed with octadecylsilane bonded to porous silica (10 µm) (such as Bondapak C18),
- column temperature. 40°,
- mobile phase: add 200 volumes of *1M potassium hydroxide*, 40 volumes of *0.4 M tetrabutylammonium hydroxide* and 80 volumes of *methanol* in 1600 volumes of *water* and dilute to 2000 volume with *water*, adjust the pH to 7.0 with *orthophosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 50 µl.

Inject reference solution (a). The test is not valid unless the theoretical plates are not less than 1500 and symmetry factor is not more than 1.6 and the relative standard deviation for replicate injection is not more than 2.0 per cent.

Inject the test solution, reference solution (a), (b) and (c). Run the chromatogram 6 times the retention times of the principal peak. In the chromatogram obtained with the test solution, the area of peak corresponding to cefadroxil impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent), the area of peak corresponding to cefadroxil impurity B is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent) and the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

**Water** (2.3.43). Not more than 7.0 per cent, determined on 0.5 g of the mixed contents of 20 capsules.

**Other tests**. Comply with the tests stated under Capsules.

**Assay**. Determine by liquid chromatography (2.4.14).

*NOTE — Use freshly prepared solutions.*

*Test solution*. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 0.2 g of Cefadroxil, add sufficient *phosphate buffer pH 5.0*, shake for 30 minutes, dilute to 200.0 ml with the same solvent and filter.

*Reference solution.* A 0.1 per cent w/v solution of *cefadroxil RS* in *phosphate buffer pH 5.0.*

Chromatographic system
- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 96 volumes of *phosphate buffer pH 5.0* and 4 volumes of *acetonitrile*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_{16}H_{17}N_3O_5S$ in the capsules.

**Storage**. Store protected from moisture at a temperature not exceeding 30°.

**Labelling**. The label states the strength in terms of anhydrous cefadroxil.

## Cefadroxil Oral Suspension

Cefadroxil Mixture

Cefadroxil Oral Suspension is a mixture of Cefadroxil with buffering agents and other excipients. It contains a suitable flavouring agent. It is filled in a sealed container.



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The suspension is constituted by dispersing the contents of the sealed container in the specified volume of water just before use.

Cefadroxil Oral Suspension contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of $C_{16}H_{17}N_3O_5S$.

When stored at the temperature and for the period stated on the label during which the constituted suspension may be expected to be satisfactory for use, it contains not less than 80.0 per cent of the stated amount of cefadroxil.

**Usual strengths**. The equivalent of 125 mg and 250 mg of anhydrous cefadroxil per 5 ml after reconstitution.

### Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H* and impregnating the dry plate by placing it in a tank containing a shallow layer of about 1 cm of a mixture of 95 volumes of *n-hexane* and 5 volumes of *1-tetradecane,* allowing the solvent to ascend to the top, removing the plate and allowing the solvent to evaporate.

*Mobile phase*. A mixture of 60 volumes of *0.1 M citric acid*, 40 volumes of *0.1 M disodium hydrogen phosphate* and 1.5 volumes of a 6.66 per cent w/v solution of *ninhydrin* in *acetone.*

*Test solution.* Dilute a suitable quantity of the freshly prepared suspension with water to obtain a solution containing 0.2 per cent w/v of cefadroxil. Filter the solution.

*Reference solution* (a). A 0.2 per cent w/v solution of *cefadroxil RS* in *water.*

*Reference solution (b).* A mixture of equal volumes of the test solution and reference solution (a).

Apply to the plate 20 µl of each solution After development, dry the plate in air, spray with a 0.2 per cent w/v solution of *ninhydrin* in *ethanol,* dry at 110° for 10 minutes and examine. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single compact spot.

**Water** (2.3.43). Not more than 2.0 per cent, determined on 1.0 g, using a mixture of 2 volumes of *carbon tetrachloride*, 2 volumes of *chloroform* and 1 volume of *methanol* in place of *methanol* in the titration vessel.

*The constituted suspension complies with the tests stated under Oral liquids and with the following tests.*

### Tests

**pH** (2.4.24). 4.5 to 6.0.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Transfer an accurately weighed quantity of the suspension containing about 0.1g of cefadroxil to a 100-ml volumetric flask, add *phosphate buffer pH 5.0,* shake for 30 minutes, dilute to 100.0 ml with the same solvent and filter.

*Reference solution.* A 0.1 per cent w/v solution of *cefadroxil RS* in *phosphate buffer pH 5.0.*

Chromatographic system
- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 96 volumes of *phosphate buffer pH 5.0* and 4 volumes of *acetonitrile*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Determine the weight per ml (2.4.29) of the suspension and calculate the content of $C_{16}H_{17}N_3O_5S$, weight in volume.

Repeat the procedure using a portion of the suspension that has been stored at the temperature and for the period stated on the label during which it may be expected to be satisfactory for use.

**Storage**. Store protected from moisture, at a temperature not exceeding 30°.

**Labelling**. The label states the quantity of active ingredient in terms of anhydrous cefadroxil.

## Cefadroxil Tablets

Cefadroxil Tablets contain not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of anhydrous cefadroxil, $C_{16}H_{17}N_3O_5S$.

**Usual strengths**. The equivalent of 500 mg and 1 g of anhydrous cefadroxil.

### Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H* and impregnating the dry plate by placing it in a tank containing a shallow layer of about 1 cm of a mixture of 95 volumes of *n-hexane* and 5 volumes of *1-tetradecane,* allowing the solvent to ascend to the top, removing the plate and allowing the solvent to evaporate.

*Mobile phase*. A mixture of 60 volumes of *0.1 M citric acid*, 40 volumes of *0.1 M disodium hydrogen phosphate* and



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1.5 volumes of a 6.66 per cent w/v solution of *ninhydrin* in *acetone.*

*Test solution.* Shake a quantity of the powdered tablets with sufficient water to produce a solution containing 0.2 per cent w/v of cefadroxil. Filter the solution.

*Reference solution* (a). A 0.2 per cent w/v solution of *cefadroxil RS* in *water.*

*Reference solution (b).* A mixture of equal volumes of the test solution and reference solution (a).

Apply to the plate 20 µl of each solution After development, dry the plate in air, spray with a 0.2 per cent w/v solution of *ninhydrin* in *ethanol,* dry at 110° for 10 minutes and examine. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single compact spot.

## Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *water,*
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted if necessary, at the maximum at about 263 nm (2.4.7). Calculate the content of $C_{16}H_{17}N_3O_5S$ in the medium from the absorbance obtained from a solution of known concentration of *cefadroxil RS.*

D. Not less than 75 per cent of the stated amount of $C_{16}H_{17}N_3O_5S$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve a quantity of powdered tablets containing about 0.5 g of anhydrous cefadroxil in 50 ml of the mobile phase, mix for 10 minutes and filter.

*Reference solution (a).* A 0.01 per cent w/v solution of *cefadroxil RS* in the mobile phase.

*Reference solution (b).* A 0.01 per cent w/v solution of *D-α-(4-hydroxyphenyl) glycine RS (cefadroxil impurity A RS)* in the mobile phase.

*Reference solution (c).* A 0.01 per cent w/v solution of *7-aminodesacetoxycephalosporanic acid RS (cefadroxil impurity B RS)* in the mobile phase.

Chromatographic system
- a stainless steel column 30 cm x 3.9 mm packed with octadecylsilane bonded to porous silica (10 µm) (such as µBondpak C18),
- column temperature. 40°,
- mobile phase: add 200 volumes of *1M potassium hydroxide*, 40 volumes of *0.4 M tetrabutylammonium hydroxide* and 80 volumes of *methanol* in 1600 volumes of *water* and dilute to 2000 volume with *water*, adjust the pH to 7.0 with *orthophosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 50 µl.

Inject reference solution (a). The test is not valid unless the theoretical plates are not less than 1500 and symmetry factor is not more than1.6 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution, reference solution (a), (b) and (c). Run the chromatogram 6 times the retention times of the principal peak. In the chromatogram obtained with the test solution, the area of peak corresponding to cefadroxil impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent), the area of peak corresponding to cefadroxil impurity B is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent) and the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

**Other tests**. Comply with the tests stated under Tablets.

**Water** (2.3.43). Not more than 8.0 per cent, determined on 0.5 g of the powdered tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*NOTE—Prepare the following solutions freshly.*

*Test solution.* Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.2 g of cefadroxil, dissolve in *phosphate buffer pH 5.0* by shaking for 30 minutes and dilute to 200.0 ml the same solvent. Filter the solution.

*Reference solution.* A 0.1 per cent w/v solution of *cefadroxil RS* in *phosphate buffer pH 5.0.*

Chromatographic system
- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a mixture of 96 volumes of *phosphate buffer pH 5.0* and 4 volumes of *acetonitrile*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.



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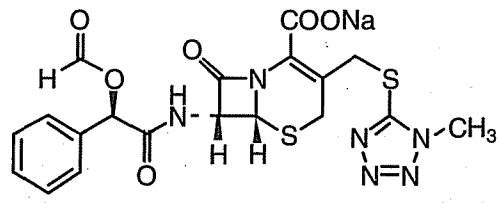
Inject alternately the test solution and the reference solution.

Calculate the content of $C_{16}H_{17}N_3O_5S$ in the tablets.

**Storage**. Store protected from moisture at a temperature not exceeding 30°.

**Labelling**. The label states the strength in terms of anhydrous cefadroxil.

## Cefamandole Nafate



$C_{19}H_{17}N_6NaO_6S_2$ Mol. Wt. 512.5

Cefamandole Nafate is 7-D-mandelamido-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-3-cephem-4-carboxylic acid

Cefamandole Nafate contains not less than 93.0 per cent and not more than 102.0 per cent of $C_{19}H_{17}N_6NaO_6S_2$, calculated on the anhydrous and sodium carbonate-free basis, for the sum of the content of cefamandole nafate, and cefamandole sodium expressed as cefamandole nafate.

Cefamandole Sodium contains not more than 10.0 per cent of $C_{18}H_{17}N_6NaO_5S_2$, calculated on the anhydrous and sodium carbonate-free basis.

Sodium Carbonate contains not less than 4.8 per cent and not more than 6.4 per cent of $Na_2CO_3$.

**Category**. Antibacterial.

**Description**. A white or almost white powder.

### Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cefamandole nafate RS* or with the reference spectrum of cefamandole nafate.

B. Gives the reactions of sodium salt (2.3.1).

### Tests

**Appearance of solution**. A 10.0 per cent w/v solution in *carbon dioxide-free water* (solution A) is clear (2.4.1) and its absorbance at 475 nm (2.4.7) is not more than 0.03.

**pH** (2.4.24). 6.0 to 8.0, measured after 30 minutes, determined in solution A.

**Specific optical rotation** (2.4.24). – 35.0° to – 45.0°, determined in a 10.0 per cent w/v solution in *acetate buffer* pH 4.7 calculated on anhydrous and sodium carbonate-free basis.

**Related substances**. Determine by liquid chromatography (2.4.14).

*NOTE—Prepare the solutions immediately before use.*

*Solvent mixture.* 18 volumes of *acetonitrile* and 75 volumes of a 10 per cent v/v solution of *triethylamine*, adjusted to pH 2.5 with *orthophosphoric acid.*

*Test solution.* Dissolve 100 mg of the substance under examination in 10.0 ml of the solvent mixture.

*Reference solution (a).* Dilute 1.0 ml of the test solution to 10.0 ml with the solvent mixture, then heat at 60° for 30 minutes.

*Reference solution (b).* Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 1 volume of *triethylamine phosphate buffer* prepared by dissolving 2.0 g of *sodium pentanesulphonate* in 350 ml of *water,* add 40 ml of *triethylamine*, adjusted to pH 2.5 with *orthophosphoric acid* and dilute to 700 ml with *water,* and 2 volumes of *water,*

  B. a mixture of equal volumes of *triethylamine phosphate buffer*, *methanol* and *acetonitrile,*
- a linear gradient programme using the conditions given below,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

| Time (in min) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0-1 | 100 | 0 |
| 1-35 | 100→0 | 0→100 |
| 35-45 | 0 | 100 |
| 45-50 | 0→100 | 100→0 |

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to cefamandole and cefamandole nafate is not less than 5.0. The relative retention time with reference to cefamandole nafate for cefamandole is about 0.8.

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



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is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (5.0 per cent). Ignore any peaks with an area less than 0.1 times the area of the principle peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

**2-Ethylhexanoic acid**. Not more than 2.0 per cent.

Determine by gas chromatography (2.4.13).

*Test solution*. Prepare a 1.0 per cent w/v solution of *valeric acid* (internal standard) in *hexane* (solution A). Dissolve 1.0 g of the substance under examination in 5 ml of *water* in a glass-stoppered flask, add 3 ml of *2 M hydrochloric acid*, 1 ml of solution A and 5 ml of *hexane*, shake vigorously for 1 minute, centrifuge if necessary and use the clear supernatant layer.

*Reference solution (a)*. Prepare in the same manner as the test solution but using an extra 1 ml of *hexane* in place of solution A.

*Reference solution (b)*. Prepare in the same manner as the test solution but using 20 mg of *2-ethylhexanoic acid* suspended in 5 ml of *water* in place of the substance under examination.

Chromatographic system
- a glass column 1.8 m x 4 mm, packed with a support impregnated with a stationary phase suitable for the separation of free fatty acids (such as a column containing 10 per cent of SP 1200 and 1 per cent of *phosphoric acid* on Chromosorb W AW, 80-100 mesh),
- temperature:
  column. 145°,
- inlet port and detector. 150°,
- flow rate. 45 ml per minute of the carrier gas.

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

**Water** (2.3.43). Not more than 2.0 per cent, determined on 0.5 g.

*Cefamandole Nafate intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.*

**Bacterial endotoxins** (2.2.3). Not more than 0.15 Endotoxin Unit per mg of cefamandole nafate.

**Assay**. Determine by liquid chromatography (2.4.14).

*NOTE—Prepare the solutions immediately before use.*

*Test solution.* Dissolve 50 mg of the substance under examination in 100.0 ml of the mobile phase.

*Reference solution (a)*. A 0.05 per cent w/v solution of *cefamandole nafate RS* in the mobile phase.

*Reference solution (b)*. Dilute 1.0 ml of the test solution to 10.0 ml with the mobile phase, then heat at 60° for 30 minutes.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed, with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 25 volumes of *acetonitrile* and 75 volumes of a 10 per cent v/v solution of *triethylamine*, adjusted to pH 2.5 with *orthophosphoric acid,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is not less than 7.0. The relative standard deviation for replicate injections is not more than 3.0 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{19}H_{17}N_6NaO_6S_2$ as the sum of the areas of the two peaks corresponding to cefamandole nafate and cefamandole sodium expressed as cefamandole nafate.

1 mg of $C_{18}H_{17}N_6Na0_5S_2$ is equivalent to 1.0578 mg of $C_{19}H_{17}N_6NaO_6S_2$.

**Sodium carbonate**. Dissolve 500 mg of the substance under examination in 50 ml of *water*. Titrate with *0.1 M hydrochloric acid,* determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M hydrochloric acid* is equivalent to 0.0053 g of $Na_2CO_3$.

**Storage**. Store protected from light and moisture, if the substance is sterile, store in a sterile, air tight, tamper proof container.

**Labelling**. The label states that the substance contains sodium carbonate.

## Cefamandole Injection

### Cefamandole Nafate Injection

Cefamandole Injection is a sterile material consisting of Cefamandole Nafate with or without buffering agents and other excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

*The constituted solution complies with the requiremens for the Clarity of Solution and Particulate matter stated under Parental Preparations (Injections).*

**Usual strengths**. The equivalent of 1 g; 2 g and 10 g of cefamandole.



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**Storage.** The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Cefamandole Injection contains not less than 90.0 per cent and not more than 115.0 per cent of $C_{18}H_{17}N_6NaO_5S_2$.

## Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

*Mobile phase.* A mixture of 50 volumes of *ethyl acetate*, 20 volumes of *acetone*, 10 volumes of *glacial acetic acid* and 10 volumes of *water.*

*Test solution.* Dilute a volume of the injection containing about 100 mg of Cefamandole in 10.0 ml of the mobile phase.

*Reference solution.* A 1.0 per cent w/v solution of *cefamandole nafate RS* in the mobile phase.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 8.0 cm. Dry the plate in air and examine in ultraviolet light. The principal spot in the chromatogram obtained with the test solution corresponds to the spot in the chromatogram obtained with the reference solution.

## Tests

**pH** (2.4.24). 6.0 to 8.0, determined in a 1.0 per cent w/v solution of cefamandole.

**Bacterial endotoxins** (2.2.3). Not more than 0.15 Endotoxin Unit per mg of cefamandole.

**Sterility** (2.2.11). Complies with the tests for sterility.

**Water** (2.3.43). Not more than 3.0 per cent, determined on 0.1 g.

**Other tests.** Complies with the tests stated under Parenteral Preparations (Injections).

**Assay.** Determine by liquid chromatography (2.4.14).

*NOTE—Prepare the solutions immediately before use.*

*Test solution.* Dilute a quantity of the injection containing about 50 mg of Cefamandole Nafate with 100.0 ml of the mobile phase.

*Reference solution (a).* A 0.05 per cent w/v solution of *cefamandole nafate RS* in the mobile phase.

*Reference solution (b).* Dilute 1.0 ml of the test solution to 10.0 ml with the mobile phase, then heat at 60 ° for 30 minutes.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 25 volumes of *acetonitrile* and 75 volumes of a 10 per cent v/v solution of *triethylamine*, adjusted to pH 2.5 with *orthophosphoric acid,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is not less than 7.0. The relative standard deviation for replicate injections is not more than 3.0 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{19}H_{17}N_6NaO_6S_2$ as the sum of the areas of the two peaks corresponding to cefamandole nafate and cefamandole sodium expressed as cefamandole nafate.

1 mg of $C_{18}H_{17}N_6NaO_5S_2$ is equivalent to 1.0578 mg of $C_{19}H_{17}N_6NaO_6S_2$.

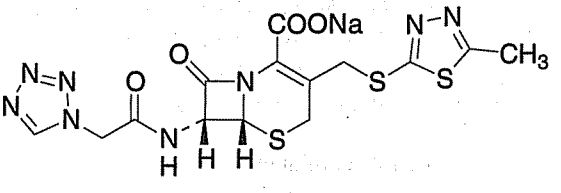
**Sodium carbonate.** Dilute a quantity of the injection containing about 500 mg of Cefamandole Nafate with 50 ml of *water.* Titrate with *0.1 M hydrochloric acid,* determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M hydrochloric acid* is equivalent to 0.0053 g of $Na_2CO_3$.

**Storage.** Store protected from moisture, in a sterile, tamper evident sealed container so as to exclude micro-organism, at a temperature not exceeding 30°.

# Cefazolin Sodium

Cephazolin Sodium




$C_{14}H_{13}N_8NaO_4S_3$ Mol. Wt. 476.5

Cefazolin Sodium is sodium 7-[(1*H*)-tetrazol-1-ylacetamido]-3-(5-methyl-1,3,4-thiadiazol-2-ylthiomethyl)-3-cephem-4-carboxylate.

Cefazolin Sodium contains not less than 85.0 per cent and not more than 105.0 per cent of cefazolin $C_{14}H_{14}N_8O_4S_3$, calculated on the anhydrous basis.

**Category.** Antibacterial.

**Dose.** By intramuscular or intravenous injection or infusion, 1 to 4 g daily, in divided doses.



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**Description**. A white to off-white, crystalline powder; odourless.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cefazoline sodium RS* or with the reference spectrum of cefazolin sodium.

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

## Tests

**pH** (2.4.24). 4.0 to 6.0, determined in a 10.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). -10.0° to -24.0°, determined in a 5.5 per cent w/v solution in *0.1 M sodium bicarbonate*.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 50 mg of the substance under examination in 20.0 ml of mobile phase A.

*Reference solution (a)*. Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A.

*Reference solution (b)*. Dissolve 20 mg of the substance under examination in 10 ml of 0.2 per cent w/v solution of *sodium hydroxide*, allow to stand for 30 minutes. Dilute 1.0 ml of this solution to 20.0 ml with mobile phase A.

Chromatographic system

- a stainless steel column 12.5 cm x 4 mm packed with octadecylsilane bonded to porous silica (3 µm),
- column temperature 45°,
- mobile phase: A. a solution containing 1.45 per cent w/v of *disodium hydrogen phosphate* and 0.35 per cent w/v of *potassium dihydrogen phosphate*,
  B. *acetonitrile*,
  a linear gradient programme using the conditions given below,
- flow rate.1.2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 5 µl.

| Time (min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0 - 2 | 98 | 2 |
| 2 - 4 | 98→85 | 2→15 |
| 4 - 10 | 85→60 | 15→40 |
| 10 - 11.5 | 60→35 | 40→65 |
| 11.5 – 12 | 35 | 65 |
| 12 - 15 | 35→98 | 65→2 |
| 15 - 21 | 98 | 2 |

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to cefazolin and cefazolin impurity L is not less than 2.0.

Inject the test solution and reference solution (a). In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent), the sum of area of all the secondary peaks is not more than 3.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (3.5 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

**Water** (2.3.43). Not more than 6.0 per cent, determined on 0.15 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solution A*. Prepared by dissolving 0.75 g of *salicylic acid* (internal standard) in 5 ml of *methanol* and diluting to 100.0 ml with *mixed phosphate buffer pH 7.0*.

*Test solution*. A 0.1 per cent w/v solution of the substance under examination in *mixed phosphate buffer pH 7.0*. to 5.0 ml of this solution add 5.0 ml of solution A and add sufficient volume of *mixed phosphate buffer pH 7.0* to produce 100.0 ml and mix.

*Reference solution*. A 0.1 per cent w/v solution of *cefazolin sodium RS* in *mixed phosphate buffer pH 7.0*. To 5.0 ml of this solution add 5.0 ml of solution A and add sufficient volume of *mixed phosphate buffer pH 7.0* to produce 100.0 ml and mix.

Chromatographic system

- a stainless steel column 30 cm x 4 mm, packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a mixture of 9 volumes of *phosphate buffer pH 3.6* and 1 volume of *acetonitrile*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative retention times of salicylic acid and cefazolin are 0.7 and 1.0 respectively.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_{14}H_{14}N_8O_4S_3$.

*Cefazolin Sodium intended for use in the manufacture of parenteral preparations complies with the following additional requirements.*

**Bacterial endotoxins** (2.2.3). Not more than 0.15 Endotoxin Unit per mg of cefazolin.

**Sterility** (2.2.11). Complies with the test for sterility.



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**Storage**. Store in sterile containers, sealed so as to exclude micro-organisms protected from moisture at a temperature not exceeding 30°.

**Labelling**. The label states the quantity of Cefazolin Sodium contained in the sealed container in terms of the equivalent amount of cefazolin.

## Cefazolin Sodium Injection

Cefazolin Injection; Cephazolin Sodium Injection; Cephazolin Injection

Cefazolin Sodium Injection is a sterile material consisting of Cefazolin Sodium with or without excipients. It is filled in sealed containers.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

*The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).*

**Usual strengths**. The equivalent of 125 mg; 250 mg; 500 mg; and 1 g of cefazolin.

**Storage**. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Cefazolin Sodium Injection contains not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of cefazolin, $C_{14}H_{14}N_8O_4S$.

**Description**. A white to off-white, crystalline powder; odourless.

*The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements*

### Identification

A. Determine by infra-red absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cefazoline sodium RS* or with the reference spectrum of cefazolin sodium.

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

### Tests

**pH** (2.4.24). 4.0 to 6.0, determined in a 10.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). -10.0° to -24.0°, determined in a 5.5 per cent w/v solution in *0.1 M sodium bicarbonate*.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve an accurately weighed quantity of powder containing 0.25 g of cefazolin in 100 ml of mobile phase A.

*Reference solution (a)*. Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A.

*Reference solution (b)*. Dissolve 20 mg of *cefazolin RS* in 10 ml of 0.2 per cent w/v solution of *sodium hydroxide*, allow to stand for 30 minutes. Dilute 1.0 ml of this solution to 20.0 ml with mobile phase A.

Chromatographic system

- a stainless steel column 12.5 cm x 4 mm packed with endcapped octadecylsilane bonded to porous silica (3 µm) (such as Nucleosil C18),
- column temperature 45°,
- mobile phase: A. a solution containing 1.45 per cent w/v of *disodium hydrogen phosphate* and 0.35 per cent w/v of *potassium dihydrogen phosphate*,
  B. *acetonitrile*,
- a linear gradient programme using the conditions given below,
- flow rate.1.2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 5 µl.

| Time (min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0-2 | 98 | 2 |
| 2-4 | 98→85 | 2→15 |
| 4-10 | 85→60 | 15→40 |
| 10-11.5 | 60→35 | 40→65 |
| 11.5-12 | 35 | 65 |
| 12-15 | 35→98 | 65→2 |
| 15-21 | 98 | 2 |

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to cefazolin and cefazolin impurity L is not less than 2.0.

Inject the test solution and reference solution (a). In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent), the sum of areas of all the secondary peaks is not more than 3.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (3.5 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

**Bacterial endotoxins** (2.2.3). Not more than 0.15 Endotoxin Unit per mg of cefazolin.

**Water** (2.3.43). Not more than 6.0 per cent, determined on 0.15 g.



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**Assay**. Determine by liquid chromatography (2.4.14).

*Solution A*. Prepare by dissolving 0.75 g of *salicylic acid* (internal standard) in 5 ml of *methanol* and diluting to 100.0 ml with *mixed phosphate buffer pH 7.0*.

*Test solution*. Determine the weight of the contents of 10 containers. Weigh accurately a quantity of the mixed contents of the 10 containers, dissolve in the *mixed phosphate buffer pH 7.0* and dilute to obtain a solution containing 0.1 per cent w/v of cefazolin. To 5.0 ml of this solution add 5.0 ml of solution A and add sufficient volume of *mixed phosphate buffer pH 7.0* to produce 100.0 ml and mix.

*Reference solution*. A 0.1 per cent w/v solution of *cefazolin sodium RS* in *mixed phosphate buffer pH 7.0*. To 5.0 ml of this solution add 5.0 ml of solution A and sufficient volume of *mixed phosphate buffer pH 7.0* to produce 100.0 ml and mix.

Chromatographic system
- a stainless steel column 30 cm x 4 mm, packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a mixture of 9 volumes of *phosphate buffer pH 3.6* and 1 volume of *acetonitrile*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative retention times of salicylic acid and cefazolin are 0.7 and 1.0 respectively.

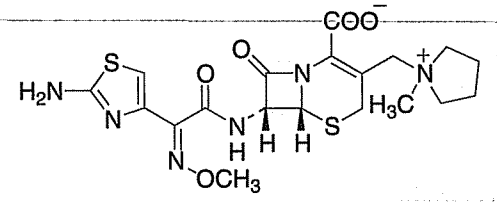
Inject alternately the test solution and the reference solution.

Calculate the content of $C_{14}H_{14}N_8O_4S_3$ in the injection.

**Storage**. Store protected from moisture at a temperature not exceeding 30°. The constituted solution should be stored protected from light and used within 24 hours when stored at a temperature not exceeding 30° or within 4 days when stored between 2° and 8°.

**Labelling**. The label states the quantity of Cefazolin Sodium contained in the sealed container in terms of the equivalent amount of cefazolin.

# Cefepime Hydrochloride




, HCl, $H_2O$

$C_{19}H_{25}ClN_6O_5S_2,HCl,H_2O$ Mol. wt. 571.5

Cefepime Hydrochloride is 1-[[(6*R*, 7*R*)-7-[[(2*Z*)-(2-aminothiazol-4-yl) (methoxyimino)acetyl]amino]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methylpyrrolidinium chloride monohydrochloride monohydrate.

Cefepime Hydrochloride contains not less than 825 µg and not more than 911 µg of cefepime, $C_{19}H_{24}N_6O_5S_2$, per mg, calculated on the anhydrous basis.

**Category**. Antibacterial.

**Description**. A white to off-white, crystalline powder.

## Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cefepime hydrochloride RS* or with the reference spectrum of cefepime hydrochloride.

## Tests

**Appearance of solution**. A 10 per cent w/v solution is clear (2.4.1) and is not more intensely coloured than reference solution YS3 (2.4.1).

***N*-methylpyrrolidine**. Not more than 0.3 per cent.

Determine by liquid chromatography (2.4.14).

*Note — Prepare the solutions immediately before use.*

*Test solution*. Dissolve 0.1g of the substance under examination in 10.0 ml of *0.01 M nitric acid*.

*Reference solution (a)*. Dilute 0.15 g of *N-methylpyrrolidine* to 100.0 ml with *water* and mix. Dilute 2.0 ml of this solution to 100.0 ml with *0.01 M nitric acid*.

*Reference solution (b)*. Dilute 0.15 g of *pyrrolidine* to 100.0 ml with *water* and mix. Dilute 2.0 ml of this solution to 100.0 ml with *0.01 M nitric acid*. Mix 5.0 ml of this solution with 5.0 ml of reference solution (a).

Chromatographic system
- a stainless steel column 5 cm × 4.6 mm, packed with a strong cation exchange resin (5 µm),
- mobile phase: a mixture of 100 volumes of *0.01 M nitric acid* and 1 volume of *acetonitrile*,
- flow rate. 1 ml per minute,
- conductivity detector,
- injection volume. 100 µl.

Inject reference solutions (a) and (b). In the chromatogram obtained with reference solution (a) the symmetry factor for the peak due to N-methylpyrrolidine is not more than 2.5 and the relative standard deviation for replicate injections of reference solution (a) is not more than 5.0 per cent. In the chromatogram obtained with reference solution (b) the peak to valley ratio between the peaks due to pyrrolidine and N-methylpyrrolidine is not less than 3.



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Inject the test solution. Continue the chromatography for 1.1 times the retention time of cefepime (about 50 minutes), eluting as a broadened peak.

Calculate the content of *N-methylpyrrolidine.*

**Related substances**. Determine by liquid chromatography (2.4.14).

*Note — Prepare the solutions immediately before use.*

*Test solution*. Dissolve 70 mg of the substance under examination in mobile phase A, stir with the aid of ultrasound for about 5 minutes and add sufficient mobile phase to produce 50.0 ml

*Reference solution (a)*. A 0.14 per cent w/v solution of *cefepime hydrochloride RS* in mobile phase A.

*Reference solution (b)*. Dilute 1.0 ml of reference solution (a) to 10.0 ml with mobile phase A. Dilute 2.0 ml of this solution to 100.0 ml with mobile phase A.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 10 volumes of *acetonitrile* and 90 volumes of a 0.068 per cent w/v solution of *potassium dihydrogen phosphate,* adjusted to pH 5.0 with *dilute phosphoric acid,*

  B. a mixture of equal volumes of *acetonitrile* and a 0.068 per cent w/v solution of *potassium dihydrogen phosphate,* adjusted to pH 5.0 with *dilute orthophosphoric acid,*
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

| Time (in min.) | mobile phase A ( per cent v/v) | mobile phase B ( per cent v/v) |
|---|---|---|
| 0-10 | 100 | 0 |
| 10-30 | 100→50 | 0→50 |
| 30-35 | 50 | 50 |
| 35-36 | 50→100 | 50→0 |
| 36-45 | 100 | 0 |

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 4000 theoretical plates and the tailing factor is not more than 1.5. The relative retention times with reference to cefepime are 2.5 for [(6*R*,7*R*)-7-[[(2*E*)-(2-aminothiazol-4-yl)(methoxyimino)acetyl]amino]-3-[(1-methylpyrrolidinio) methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0] oct-2-ene-2-carboxylate] (cefepime impurity A) and about 4.1 for [(6*R*,7*R*)-7-[[(2*Z*)-(2-aminothiazol-4-yl)(methoxyimino) acetyl]amino]thiazol-4-yl](methoxyimino)acetyl]amino]-3-[(1-methylpyrrolidinio) methyl-8-oxo-5-thia-1-azabicyclo[4.2.0] oct-2-ene-2-carboxylate] (cefepime impurity B).

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak due to cefepime impurity A is not more than 1.5 times the area of the principal peak in the chromatogram obtained with the reference solution (b) (0.3 per cent), the area of any secondary peak due to cefepime impurity B is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent) and the sum of the areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with the reference solution (b) (1.0 per cent). Ignore any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

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

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). 3.0 per cent to 4.5 per cent, determined on 0.4 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 70 mg of the substance under examination in 50.0 ml of the mobile phase.

*Reference solution*. A 0.14 per cent w/v solution of *cefepime hydrochloride RS* in the mobile phase.

Chromatographic system

- a stainless steel column 30 cm × 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 94 volumes of a solution prepared by dissolving 5.76 g of *sodium 1-pentanesulfonate* in 2000 ml of *water*, adjusting the pH to 3.4 with *glacial acetic acid* and then pH 4.0 with *potassium hydroxide*, and 6 volumes of *acetonitrile*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0. The column efficiency is not less than 1500 theoretical plates. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{19}H_{24}N_6O_5S_2$.

*Cefepime Hydrochloride intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.*



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**Bacterial endotoxins** (2.2.3). Not more than 0.04 Endotoxin Unit per mg.

*Cefepime Hydrochloride intended for use in the manufacture of parenteral preparations without a further appropriate sterilization procedure complies with the following additional requirement.*

**Sterility** (2.2.11). Complies with the test for sterility.

**Storage**. Store protected from light and moisture. If it is intended for use in the manufacture of parenteral preparations, the container should be sterile and sealed so as to exclude micro-organisms.

**Labelling**. The label states whether or not the contents are intended for use in the manufacture of parenteral preparations.

## Cefepime Injection

Cefepime Injection is sterile mixture of Cefepime Hydrochloride and Arginine. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

*The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).*

**Storage**. The constituted solution should be used immediately after preparation but, in any case, within the period not exceeding 7 days, recommended by the manufacturer provided the solution is stored in a refrigerator (2° to 8°).

Cefepime Injection contains Cefepime Hydrochloride equivalent to not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of cefepime, $C_{19}H_{24}N_6O_5S_2$.

**Usual strengths**. 225 mg; 500 mg; 1 g.

**Description**. A white to pale yellow powder.

*The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for injection) and with the following requirements.*

### Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 70 volumes of *n-propyl alcohol*, 50 volumes of *water* and 40 volumes of *strong ammonia solution*.

*Test solution*. Weigh accurately a quantity equivalent to about 0.4 g of cefepime and dissolve in sufficient *water* to produce 10 ml.

*Reference solution*. Weigh accurately about 0.2 g of *L-arginine RS* and dissolve in 10 ml of *water*.

Apply to the plate 5 µl of each solution. After development, dry the plate at 100° until the ammonia disappears completely. Spray the plate with a 0.2 per cent w/v solution of *ninhydrin* in a mixture of 95 volumes of *butyl alcohol* and 5 volumes of *2 M acetic acid*. Heat the plate at 105° for 15 minutes. Cool and examine in daylight. The dark red spot due to arginine in the chromatogram obtained with the test solution corresponds to the spot in the chromatogram obtained with the reference solution.

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

### Tests

**pH** (2.4.24). 4.0 to 6.0, determined in a solution containing about 100 mg of cefepime per ml.

***N*-methylpyrrolidine**. Not more than 1.0 per cent.

Determine by liquid chromatography (2.4.14).

*Note — Prepare the solutions immediately before use.*

*Test solution*. Dissolve 1.0 g of the substance under examination in 100.0 ml of *0.01 M nitric acid*.

*Reference solution (a)*. Dilute 0.15 g of *N-methylpyrrolidine* to 100.0 ml with *water* and mix. Dilute 2.0 ml of this solution to 100.0 ml with *0.01 M nitric acid*.

*Reference solution (b)*. Dilute 0.15 g of *pyrrolidine* to 100.0 ml with *water* and mix. Dilute 2.0 ml of this solution to 100.0 ml with *0.01 M nitric acid*. Mix 5.0 ml of this solution with 5.0 ml of reference solution (a).

Chromatographic system

- a stainless steel column 5 cm × 4.6 mm, packed with a strong cation exchange resin (5 µm),
- mobile phase: a mixture of 100 volumes of *0.01 M nitric acid* and 1 volume of *acetonitrile*,
- flow rate. 1 ml per minute,
- conductivity detector,
- injection volume. 100 µl.

Inject reference solution (a) and (b). In the chromatogram obtained with reference solution (a) the symmetry factor for the peak due to N-methylpyrrolidine is not more than 2.5 and the relative standard deviation for replicate injections of reference solution (a) is not more than 5.0 per cent. In the chromatogram obtained with reference solution (b) the peak to valley ratio between the peaks due to pyrrolidine and *N*-methylpyrrolidine is not less than 3.



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Inject the test solution. Continue the chromatography for 1.1 times the retention time of cefepime (about 50 minutes), eluting as a broadened peak.

Calculate the content of *N-methylpyrrolidine.*

**Related substances.** Determine by liquid chromatography (2.4.14).

*Note — Prepare the solutions immediately before use.*

*Test solution.* Dissolve 70 mg of the substance under examination in mobile phase A, stir with the aid of ultrasound for about 5 minutes and add sufficient mobile phase to produce 50.0 ml.

*Reference solution (a).* A 0.14 per cent w/v solution of *cefepime hydrochloride RS* in mobile phase A.

*Reference solution (b).* Dilute 1.0 ml of reference solution (a) to 10.0 ml with mobile phase A. Dilute 2.0 ml of this solution to 100.0 ml with mobile phase A.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 10 volumes of *acetonitrile* and 90 volumes of 0.068 per cent w/v solution of *potassium dihydrogen phosphate,* adjusted to pH 5.0 with *dilute orthophosphoric acid,*

  B. a mixture of equal volumes of *acetonitrile* and a 0.068 per cent w/v solution of *potassium dihydrogen phosphate,* adjusted to pH 5.0 with *dilute orthophosphoric acid,*
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

| Time (in min.) | mobile phase A ( per cent v/v) | mobile phase B ( per cent v/v) |
|---|---|---|
| 0-10 | 100 | 0 |
| 10-30 | 100→50 | 0→50 |
| 30-35 | 50 | 50 |
| 35-36 | 50→100 | 50→0 |
| 36-45 | 100 | 0 |

Inject the reference solution (a). The test is not valid unless the column efficiency is not less than 4000 theoretical plates and the tailing factor is not more than 1.5. The relative retention times with reference to cefepime are 2.5 for (6*R*,7*R*)-7-[[(2*E*)-(2-aminothiazol-4-yl)(methoxyimino) acetyl]amino]-3-[(1-methylpyrrolidinio)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0] oct-2-ene-2-carboxylate (cefepime impurity A) and about 4.1 for (6*R*,7*R*)-7-[[(2*Z*)-(2-aminothiazol-4-yl)(methoxyimino) acetyl]amino]-4-yl](methoxyimino acetyl]amino]-4-yl] (methoxyimino)acetyl]amino]-3-[(1-methylpyrrolidinio) methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (cefepime impurity B).

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any peak due to cefepime impurity A is not more than 2.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent), the area of any peak due to cefepime impurity B is not more than 2.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than 7.5 times the area of the principal peak in the chromatogram obtained with the reference solution (b) (1.5 per cent). Ignore any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

**Bacterial Endotoxins** (2.2.3). Not more than 0.06 Endotoxin Unit per mg of cefepime.

**Water** (2.3.43). Not more than 4.0 per cent, determined on 0.5 g

**Assay.** Determine by liquid chromatography (2.4.14).

*Test solution.* Determine the weight of the contents of 10 containers. Dissolve, with shaking, a quantity of the mixed contents of the 10 containers containing about 70 mg of cefepime in 50 ml of the mobile phase.

*Reference solution.* A 0.14 per cent w/v solution of *cefepime hydrochloride RS* in the mobile phase.

Chromatographic system

- a stainless steel column 30 cm × 3.9 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 94 volumes of a solution prepared by dissolving 5.76 g of *sodium 1-pentanesulphonate* in 2000 ml of *water*, adjusting the pH to 3.4 with *glacial acetic acid* and then pH 4.0 with *potassium hydroxide*, and 6 volumes of *acetonitrile*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0. The column efficiency is not less than 1500 theoretical plates. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{19}H_{24}N_6O_5S_2$ in the injection.

**Storage.** Store protected in sterile containers so as to exclude micro-organisms, at a temperature not exceeding 30°. Protect from light.

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

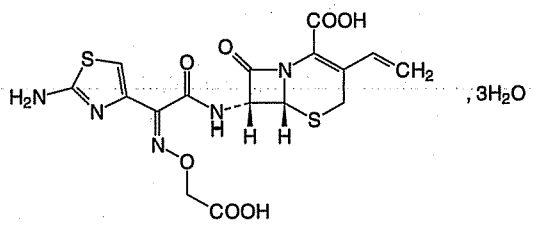


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# Cefixime



$C_{16}H_{15}N_5O_7S_2, 3H_2O$ Mol. Wt. 507.5

Cefixime is (6*R*,7*R*)-7-[[(2*Z*)-2-(2-aminothiazol-4-yl)-[(carboxymethoxy)imino]acetyl]amino]-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid trihydrate.

Cefixime contains not less than 95.0 per cent and not more than 101.0 per cent of $C_{16}H_{15}N_5O_7S_2$, calculated on the anhydrous basis.

**Category**. Antibacterial.

**Description**. A white to light yellow, crystalline powder.

## Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cefixime RS*. If the spectra obtained show differences, dissolve the substance under examination and the reference substance separately in *methanol*, evaporate to dryness and record new spectra using the residues.

## Tests

**pH** (2.4.24). 2.6 to 4.1, determined in a 5.0 per cent w/v suspension in *carbon dioxide-free water*.

**Related substances**. Determine by liquid chromatography (2.4.14) as described under Assay.

Inject reference solution (b). Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained is not less than 50 per cent of the full scale of the recorder. Inject the test solution and continue the chromatography for 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak, other than the principal peak, is not greater than half the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent); the sum of the areas of all the peaks, other than the principal peak, is not greater than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3 per cent). Ignore any peak with an area less than 0.1 times that of the principal peak in the chromatogram obtained with reference solution (b).

**Water** (2.3.43). 9.0 per cent to 12.0 per cent, determined on 0.20 g.

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Assay**. Determine by liquid chromatography (2.4.14).

*Phosphate buffer pH 7.0*. Dissolve 7.1 g *dibasic sodium phosphate* in *water* and dilute to 500 ml with *water*. Adjust the pH of the solution to 7.0 with *monobasic potassium phosphate solution*.

*Monobasic potassium phosphate solution*. Dissolve 6.8 g of *monobasic potassium phosphate* in *water* and dilute to 500 ml with *water*.

*Test solution*. Disperse about 25 mg of the substance under examination in 25.0 ml of *phosphate buffer pH 7.0*.

*Reference solution (a)*. Dissolve 25 mg of *cefixime RS* in 25.0 ml of *phosphate buffer pH 7.0*.

*Reference solution (b)*. Dilute 1.0 ml of reference solution (a) to 100.0 ml with *phosphate buffer pH 7.0*.

*Reference solution (c)*. Dissolve 10 mg of *cefixime RS* in 10 ml of *water*. Heat on a water-bath for 45 minutes. Cool and inject immediately.

Chromatographic system

- a stainless steel column 12.5 cm × 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature 40°,
- mobile phase: a mixture of 25 volumes of *acetonitrile* and 75 volumes of a *tetrabutylammonium hydroxide* solution prepared by diluting 25 ml of *0.4 M tetrabutylammonium hydroxide solution* to 1000 ml with *water* and adjusting the pH to 6.5 with *1.5 M orthophosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Inject reference solution (c). The relative retention times are about 0.9 for cefixime *E*-isomer and 1.0 for cefixime and the resolution between cefixime and cefixime *E*-isomer is not less than 2.0.

Inject reference solution (a). The column efficiency is not less than 4000 theoretical plates, the tailing factor is not less than 0.9 and not more than 2.0.

Inject the test solution and reference solution (a).

Calculate the content of $C_{16}H_{15}N_5O_7S_2$.

**Storage**. Store protected from light.

# Cefixime Oral Suspension

Cefixime Oral Suspension is a mixture consisting of Cefixime with buffering agents and other excipients. It contains a suitable flavouring agent. It is filled in a sealed container.



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*The suspension is constituted by dispersing the contents of the sealed container in the specified volume of Water just before use.*

Cefixime Oral Suspension contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of cefixime $C_{16}H_{15}N_5O_7S_2$.

When stored at the temperature and for the period stated on the label during which the constituted suspension may be expected to be satisfactory for use, it contains not less than 80.0 per cent of the stated amount of cefixime $C_{16}H_{15}N_5O_7S_2$.

**Storage**. Store protected from moisture, at a temperature not exceeding 30°.

**Usual strengths**. 100 mg per 5 ml; 200 mg per 5 ml; 400 mg per 5 ml.

### Identification

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

**Water** (2.3.43). Not more than 2.0 per cent.

*The constituted suspension complies with the tests stated under Oral Liquids and with the following tests.*

### Tests

**pH** (2.4.24). 2.5 to 4.5.

**Assay**. Determine by liquid chromatography (2.4.14).

*Phosphate buffer pH 7.0.* Dissolve 7.1 g *dibasic sodium phosphate* in *water* and dilute to 500 ml with *water*. Adjust the pH of the solution to 7.0 with *monobasic potassium phosphate solution*.

*Monobasic potassium phosphate solution.* Dissolve 6.8 g of *monobasic potassium phosphate* in *water* and dilute to 500 ml with *water*.

*Test solution.* Dilute an accurately weighed quantity of the oral suspension with *phosphate buffer pH 7.0* to obtain a solution having a concentration of 0.2 mg of cefixime per ml.

*Reference solution (a).* A 0.02 per cent w/v solution of *cefixime RS* in *phosphate buffer pH 7.0*.

*Reference solution (b).* Dissolve 10 mg of *cefixime RS* in 10 ml of *water*. Heat this solution at 95° for 45 minutes. Cool and inject immediately.

Chromatographic system
- a stainless steel column 12.5 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm),
- column temperature 40°,
- mobile phase: a mixture of 30 volumes of *tetrabutylammonium hydroxide solution* prepared by diluting 25 ml of *0.4 M tetrabutylammonium hydroxide solution* to 1000 ml with *water* and adjusting the pH to 6.5 with *1.5 M orthophosphoric acid*, and 10 volumes of *acetonitrile*,
- flow rate adjusted so that the retention time of cefixime is about 10 minutes,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Inject reference solution (b).The relative retention times are about 0.9 for cefixime *E*-isomer and 1.0 for cefixime and the resolution between cefixime and cefixime *E*-isomer is not less than 2.0.

Inject reference solution (a). The column efficiency is not less than 4000 theoretical plates, the tailing factor is not less than 0.9 and not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and reference solution (a).

Determine the weight per ml (2.4.29) of the oral suspension and calculate the content of $C_{16}H_{15}N_5O_7S_2$ weight in volume.

Repeat the procedure using a portion of the constituted suspension that has been stored at a temperature not exceeding 30°, for the period stated on the label. Calculate the content of $C_{16}H_{15}N_5O_7S_2$ weight in volume.

**Storage**. Store protected from moisture, at a temperature not exceeding 30°.

**Labelling**. The label states (1) the quantity of active ingredient in terms of the equivalent amount of cefixime; (2) the temperature of storage and the period during which the constituted suspension may be expected to be satisfactory for use.

## Cefixime Tablets

Cefixime Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cefixime, $C_{16}H_{15}N_5O_7S_2$.

**Usual strengths**. 50 mg; 100 mg; 200 mg.

### Identification

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

### Tests

**Dissolution** (2.5.2).

Apparatus No. 2,

Medium. *0.05 M potassium phosphate buffer pH 7.2*, prepared by dissolving 6.8 g of *monobasic potassium phosphate* in 1000 ml of *water*, adjusted to pH 7.2 with *1 M sodium hydroxide*,

Speed and time. 100 rpm and 45 minutes.



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Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 288 nm (2.4.7). Calculate the content of $C_{16}H_{15}N_5O_7S_2$ in the medium from the absorbance obtained from a solution of known concentration of *cefixime RS* in the same medium.

*Note — A small amount of methanol not exceeding 0.1 per cent of the total volume may be used to dissolve cefixime and the solution may be mixed with the aid of ultrasound to assure complete dissolution.*

D. Not less than 75 per cent of the stated amount of $C_{16}H_{15}N_5O_7S_2$.

**Other tests**. Comply with the tests stated under Tablets.

**Water** (2.3.43). Not more than 10.0 per cent, determined on 0.5 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*Phosphate buffer pH 7.0.* Dissolve 7.1 g *dibasic sodium phosphate* in *water* and dilute to 500 ml with *water*. Adjust the pH of the solution to 7.0 with *monobasic potassium phosphate solution*.

*Monobasic potassium phosphate solution.* Dissolve 6.8 g of *monobasic potassium phosphate* in *water* and dilute to 500 ml with *water*.

*Test solution.* Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.4 g of cefixime, disperse in 100.0 ml of *phosphate buffer pH 7.0,* mix with the aid of ultrasound and centrifuge. Dilute 5.0 ml of the clear supernatant to 100.0 ml with *phosphate buffer pH 7.0.*

*Reference solution (a).* A 0.02 per cent w/v solution of *cefixime RS* in *phosphate buffer pH 7.0.*

*Reference solution (b).* Dissolve 10 mg of *cefixime RS* in 10 ml of *water*. Heat this solution at 95° for 45 minutes. Cool and inject immediately.

Chromatographic system

- a stainless steel column 12.5 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm),
- column temperature 40°,
- mobile phase: a mixture of 30 volumes of *tetrabutylammonium hydroxide solution* prepared by diluting 25 ml of *0.4 M tetrabutylammonium hydroxide solution* to 1000 ml with *water* and adjusting the pH to 6.5 with *1.5 M orthophosphoric acid*, and 10 volumes of *acetonitrile*,
- flow rate adjusted so that the retention time of cefixime is about 10 minutes,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Inject reference solution (b).The relative retention times are about 0.9 for cefixime *E*-isomer and 1.0 for cefixime and the resolution between cefixime and cefixime *E*-isomer is not less than 2.0.

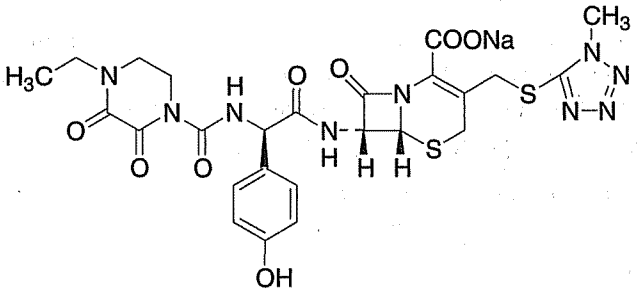
Inject reference solution (a). The column efficiency is not less than 2000 theoretical plates, the tailing factor is not less than 0.9 and not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and reference solution (a).

Calculate the content of $C_{16}H_{15}N_5O_7S_2$ in the tablets.

**Storage.** Store protected from moisture.

# Cefoperazone Sodium



$C_{25}H_{26}N_9NaO_8S_2$ Mol Wt. 667.7

Cefoperazone sodium is sodium salt of 7-D-(-)-α-(4-ethyl-2,3-dioxo-1-piperazinecarboxamido)-α-(4-hydroxyphenyl) acetamido-3-[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl-3-cephem-4-carboxylic acid.

Cefoperazone Sodium contains not less than 95.0 per cent and not more than 102.0 per cent of $C_{25}H_{26}N_9NaO_8S_2$, calculated on the anhydrous and solvent-free basis.

**Category.** Antibacterial.

**Description.** A white or almost white crystalline powder.

## Identification

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

B. Gives the reactions of sodium salts (2.3.1).

## Tests

**pH** (2.4.24). 4.5 to 6.5, determined in a 25.0 per cent w/v solution.

**Related substances.** Determine by liquid chromatography (2.4.14).

*Note —Use freshly prepared solutions.*



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*Test solution (a)*. Dissolve 25 mg of the substance under examination in 250 ml of the mobile phase.

*Test solution (b)*. Dissolve 25 mg of the substance under examination in 50 ml of the mobile phase.

*Reference solution (a)*. A 0.01 per cent w/v solution of *cefoperazone dihydrate RS* in the mobile phase.

*Reference solution (b)*. Dilute 5.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm packed with endcapped octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 884 volumes of *water*, 110 volumes of *acetonitrile*, 3.5 volumes of a 6 per cent w/v solution of *acetic acid*, 2.5 volumes of *triethylammonium acetate* solution prepared by diluting 14 ml of *triethylamine* and 5.7 ml of *glacial acetic acid* to 100 ml with *water*,
- flow rate.1.0 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (a). The test is not valid unless the theoretical plates is not less than 5000 and symmetary factor is not more than 1.6.

Inject test solution (b) and reference solution (b). Run the chromatogram 2.5 times the retention time of the principal peak. In the chromatogram obtained with test solution (b) the area of any secondary peak is not more 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent), the sum of area of all the secondary peaks is not more than 4.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (4.5 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

**Acetone**. Not more than 2.0 per cent.

Determine by gas chromatography (2.4.13).

*Test solution*. Dissolve 0.5 g of the substance under examination in 10.0 ml of *water*.

*Reference solution*. Dissolve 0.35 g of *acetone* in 100.0 ml of *water*. Dilute 10.0 ml of the solution to 100.0 ml with *water*.

Chromatographic system
- a fused-silica capillary or wide-bore column 30 m long and 0.32 mm or 0.53 mm, coated with *macrogol 20 000* (0.25 µm),
- temperature:
  column.165°,
  inlet port. 140°,
- a flame ionisation detector at 250°,
- flow rate. 30 ml per minute of the carrier gas.

Head-space injection conditions:
- equilibration time: 15 minutes,
- transfer-line temperature: 110°,

Calculate the percentage content of acetone.

**Water** (2.3.43). Not more than 5.0 per cent, determined on 0.5 g.

**Assay**. Determine by liquid chromatography (2.4.14).

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

*Reference solution*. A 0.01 per cent w/v solution of *cefoperazone dihydrate RS* in the mobile phase.

Chromatographic system
- a stainless steel column 30 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 884 volumes of *water*, 110 volumes of *acetonitrile*, 3.5 volumes of a 6 per cent w/v solution of *acetic acid* and 2.5 volumes of a solution prepared by dissolving 14 ml of *triethylamine* and 5.7 ml of *glacial acetic acid* in 100 ml of *water*, and mixed,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the theoretical plates is not less than 5000, the symmetry factor is at most 1.6 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_{25}H_{26}N_9NaO_8S_2$ by multiplying the content of cefoperazone by 1.034.

*Cefoperazone Sodium intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.*

**Bacterial endotoxins** (2.2.3). Not more than 0.20 Endotoxin Unit per mg of cefoperazone sodium.

*Cefoperazone Sodium intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.*

**Sterility** (2.2.11). Complies with the test for sterility.

**Storage**. Store protected from moisture.

**Labelling**. The label states whether it is intended for use in the manufacture of parenteral preparations.



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# Cefoperazone Injection

Cefoperazone Sodium Injection

Cefoperazone Injection is a sterile material consisting of Cefoperazone Sodium with or without excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

*The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).*

**Usual strengths**. The equivalent of 250 mg; 500 mg; 1 g and 2 g of cefoperazone.

**Storage**. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Cefoperazone Injection contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of cefoperazone, $C_{25}H_{27}N_9O_8S_2$.

**Description**. A white or almost white powder.

*The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.*

## Identification

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

B. It gives the reactions of sodium salts (2.3.1).

## Tests

**pH** (2.4.24). 4.5 to 6.5, determined in a 25.0 per cent w/v solution.

**Bacterial endotoxins** (2.2.3). Not more than 0.20 Endotoxin Unit per mg of cefoperazone.

**Water** (2.3.43). Not more than 5.0 per cent, except that where it is in the freeze-dried form, the limit is not more than 2.0 per cent.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Determine the weight of the contents of 10 containers. Weigh accurately a quantity of the mixed contents of 10 containers containing about 25 mg of cefoperazone, dissolve in the mobile phase and dilute to 250.0 ml with the mobile phase.

*Reference solution*. A 0.01 per cent w/v solution of *cefoperazone dihydrate RS* in the mobile phase.

Chromatographic system

- a stainless steel column 30 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 884 volumes of *water*, 110 volumes of *acetonitrile*, 3.5 volumes of a 6 per cent w/v solution of *acetic acid* and 2.5 volumes of a solution prepared by dissolving 14 ml of *triethylamine* and 5.7 ml of *glacial acetic acid* in 100 ml of *water*, and mixed,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the theoretical plates is not less than 5000, the symmetry factor is at most 1.6 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

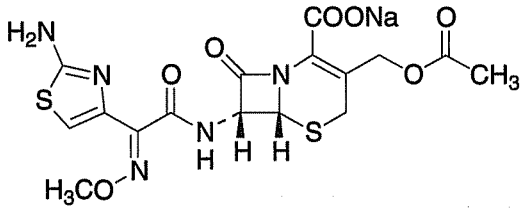
Inject alternately the test solution and the reference solution.

Calculate the content of $C_{25}H_{27}N_9O_8S_2$ in the injection.

**Storage**. Store protected from light at a temperature not exceeding 30°.

**Labelling**. The label states the quantity of Cefoperazone Sodium contained in the sealed container in terms of the equivalent amount of cefoperazone.

# Cefotaxime Sodium



$C_{16}H_{16}N_5NaO_7S_2$ Mol. Wt. 477.4

Cefotaxime Sodium is sodium (7*R*)-3-acetoxymethyl-7-[(*Z*)-2-(2-aminothiazol-yl)-2-(methoxyimino)acetamido]-3-cepham-4-carboxylate.

Cefotaxime Sodium contains the equivalent of not less than 91.6 per cent and not more than 96.4 per cent of cefotaxime, $C_{16}H_{17}N_5O_7S_2$, calculated on the anhydrous basis.

**Category**. Antibacterial.

**Dose**. By intramuscular or intravenous injection, the equivalent of 1 to 2 g of cefotaxime every 8 to 12 hours depending on severity of infection; by intravenous infusion, 1 to 2 g given over 20 to 60 minutes. For children, the equivalent of 100 to 150 mg of cefotaxime per kg of body weight daily in 2 to 4



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divided doses increasing to 200 mg per kg of body weight daily, if necessary. (Each g of cefotaxime sodium is approximately equivalent to 0.95 g of cefotaxime).

**Description.** An off-white to pale yellow, crystalline powder.

### Identification

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

B. Gives the reactions of sodium salts (2.3.1).

### Tests

**pH** (2.4.24). 4.5 to 6.5, determined in a 10.0 per cent w/v solution.

**Water** (2.3.43). Not more than 3.0 per cent, determined on 0.15 g.

**Assay.** Determine by liquid chromatography (2.4.14).

*Test solution.* A 0.01 per cent w/v solution of the substance under examination in *water*.

*Reference solution.* A 0.01 per cent w/v solution of *cefotaxime sodium RS* in *water*.

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a solution prepared by dissolving 60 mg of *potassium dihydrogen phosphate* and 1.2 g of *disodium hydrogen phosphate* in 1000 ml of *water* and mixing with 120 ml of *methanol,*
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_{16}H_{17}N_5O_7S_2$.

*Cefotaxime Sodium intended for use in the manufacture of parenteral preparations without a further appropriate procedure for removal of bacterial endotoxins complies with the following additional requirement.*

**Bacterial endotoxins** (2.2.3). Not more than 0.20 Endotoxin Unit per mg of cefotaxime.

*Cefotaxime Sodium intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.*

**Sterility** (2.2.11). Complies with the test for sterility.

**Storage.** Store protected from moisture in tamper-evident containers.

**Labelling.** The label states whether or not the contents are intended for use in the manufacture of parenteral preparations.

## Cefotaxime Sodium Injection

Cefotaxime Injection

Cefotaxime Sodium Injection is a sterile material consisting of Cefotaxime Sodium with or without excipients. It is filled in sealed containers.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

*The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).*

**Storage.** The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Cefotaxime Sodium Injection contains a quantity of Cefotaxime Sodium equivalent to not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of cefotaxime, $C_{16}H_{17}N_5O_7S_2$.

**Usual strengths**: The equivalent of 250 mg, 1 g and 2 g of cefotaxime.

**Description.** An off-white to pale yellow, crystalline powder.

*The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.*

### Identification

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

B. Gives the reactions of sodium salts (2.3.1).

### Tests

**pH** (2.4.24). 4.5 to 6.5, determined in a 10.0 per cent w/v solution.

**Bacterial endotoxins** (2.2.3). Not more than 0.20 Endotoxin Unit per mg of cefotaxime.

**Water** (2.3.43). Not more than 6.0 per cent, determined on 0.15 g.

**Assay.** Determine by liquid chromatography (2.4.14).



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*Test solution*. Determine the weight of the contents of 10 containers. Weigh accurately a quantity of the mixed contents of the 10 containers dissolve in *water* and dilute to obtain a solution containing 0.01 per cent w/v of cefotaxime.

*Reference solution*. A 0.01 per cent w/v solution of *cefotaxime sodium RS* in *water*.

Chromatographic system
- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a solution prepared by dissolving 60 mg of *potassium dihydrogen phosphate* and 1.2 g of *disodium hydrogen phosphate* in 1000 ml of *water* and mixing with 120 ml of *methanol*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

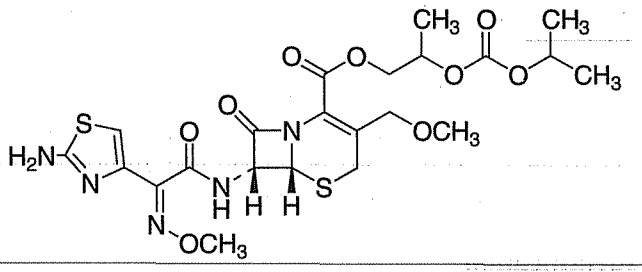
Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_{16}H_{17}N_5O_7S_2$ in the injection.

**Storage**. Store protected from light at a temperature not exceeding 30°.

# Cefpodoxime Proxetil



$C_{21}H_{27}N_5O_9S_2$ Mol. Wt. 557.6

Cefpodoxime Proxetil is 1-(isopropoxycarbonyloxy)ethyl (6*R*, 7*R*)-7-[2-(2-amino-4-thiazolyl)-(*Z*)-2-(methoxyimino) acetamido]-3-methoxymethyl-3-cephem-4-carboxylate.

Cefpodoxime Proxetil contains not less than 690 µg and not more than 804 µg of cefpodoxime, $C_{15}H_{17}N_5O_6S_2$, calculated on the anhydrous basis.

**Category**. Antibacterial.

**Description**. A white to light brownish-white powder.

## Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cefpodoxime proxetil RS* or with the reference spectrum of cefpodoxime proxetil.

## Tests

**Specific optical rotation** (2.4.22). +35° to +48°, determined in a 1.0 per cent w/v solution in *methanol*.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Note — Prepare the solutions immediately before use.*

*Solvent mixture*. 20 volumes of *water* and 10 volumes of *acetonitrile*.

*Test solution*. Dissolve 50 mg of the substance under examination in 5 ml of *methanol* and dilute to 50.0 ml with the solvent mixture. This solution should be injected promptly.

*Reference solution*. Dissolve a quantity of *cefpodoxime proxetil RS* in the solvent mixture to obtain a solution containing about 10 µg per ml.

*Note — A volume of methanol not exceeding 10 per cent of the total volume in the final solution may be used to facilitate dissolution.*

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature 30°,
- mobile phase: A. *0.02 M ammonium acetate*, B. *acetonitrile*,
- flow rate. 2 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 260 nm,
- injection volume. 20 µl.

| Time (in min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0 | 90 | 10 |
| 10 | 68 | 32 |
| 40 | 68 | 32 |
| 80 | 50 | 50 |
| 85 | 50 | 50 |
| 90 | 25 | 75 |
| 95 | 25 | 75 |
| 100 | 90 | 10 |

Inject the reference solution. The retention time for the cefpodoxime proxetil *R*–epimer is between 37 and 42 minutes. The relative retention times for cefpodoxime proxetil *S*-epimer is about 0.9 and for cefpodoxime proxetil *R*-epimer is about 1.0, the resolution between cefpodoxime proxetil *S*-epimer and cefpodoxime proxetil *R*-epimer is not less than 4.0. The test is



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not valid unless the column efficiency for cefpodoxime proxetil *R*-epimer peak is not less than 15,000 theoretical plates.

Inject the test solution and measure the areas of all the peaks. Calculate the percentage of each impurity in the portion of cefpodoxime proxetil taken, from the expression, 100 ($r_i/r_s$) where, $r_i$ is the peak area for each impurity and $r_s$ is the sum of the areas of all the peaks. Any peak at a relative retention time of about 0.86 is not more than 3.0 per cent, any peak at relative retention times of about 1.27, 1.39 is not more than 1.0 per cent, and other individual peaks having relative retention times higher than 2.0 is not more than 0.5 per cent and the sum of the areas of all the secondary peaks is not more than 6.0 per cent. Ignore any peak with an area less than 0.05 per cent.

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

**Isomer ratio**. Using the chromatogram of the test solution obtained in the Assay, calculate the ratio of the cefpodoxime proxetil *R*-epimer peak response to the sum of the peak responses of the cefpodoxime proxetil *S*-epimer peak and the cefpodoxime proxetil *R*-epimer peak: the ratio is between 0.5 and 0.6.

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Water** (2.3.43). Not more than 3.0 per cent, determined on 1.0 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture.* A mixture of 60 volumes of *water* and 40 volumes of *acetonitrile*.

*Test solution*. Dissolve 50 mg of the substance under examination in 10 ml of *methanol*, dilute to 100.0 ml with the solvent mixture. Dilute 5.0 ml of this solution to 100.0 ml with the solvent mixture and filter.

*Reference solution.* Dissolve 25 mg of *cefpodoxime proxetil RS* in 5 ml of *methanol*, dilute to 50.0 ml with the solvent mixture. Dilute 5.0 ml of this solution to 100.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature 30°,
- mobile phase: 60 volumes of *0.02 M ammonium acetate* and 40 volumes of *acetonitrile,*
- flow rate. 2 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative retention time for cefpodoxime proxetil *S*-epimer is about 0.9 and for cefpodoxime proxetil *R*-epimer is about 1.0. The resolution between cefpodoxime proxetil *S*-epimer and cefpodoxime proxetil *R*-epimer is not less than 2.5, the tailing factor for cefpodoxime proxetil *R*-epimer is not more than 1.5 and the relative standard deviation determined from the sum of the areas of the cefpodoxime proxetil *S*-epimer and cefpodoxime proxetil *R*-epimer peaks for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{15}H_{17}N_5O_6S_2$.

**Storage**. Store protected from moisture, at a temperature not exceeding 30°.

# Cefpodoxime Oral Suspension

Cefpodoxime Proxetil Oral Suspension

Cefpodoxime Oral Suspension is a mixture consisting of Cefpodoxime Proxetil with buffering agents and other excipients. It contains a suitable flavouring agent.

*The suspension is constituted by dispersing the contents of the sealed container in the specified volume of water just before issue.*

Cefpodoxime Oral Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cefpodoxime, $C_{15}H_{17}N_5O_6S_2$.

**Storage**. Store protected from moisture, at a temperature not exceeding 30°.

**Usual strength**. 30 ml.

## Identification

In the Assay, the principal peaks of cefpodoxime proxetil *S*-epimer and cefpodoxime proxetil *R*-epimer in the chromatogram obtained with the test solution correspond to the peaks in the chromatogram obtained with the reference solution.

## Tests

**Water** (2.3.43). Not more than 1.5 per cent, determined on 1.0 g.

*The constituted suspension complies with the tests stated under Oral liquids and with the following tests.*

**pH** (2.4.24). 4.0 to 5.5.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture.* A mixture of 60 volumes of *water* and 40 volumes of *acetonitrile*.

*Test solution.* Weigh accurately a quantity of the suspension containing about 50 mg of cefpodoxime, disperse in 10 ml of *water,* add 20 ml of *acetonitrile,* mix with the aid of ultrasound for 15 minutes and dilute to 100.0 ml with the solvent mixture. Dilute 5.0 ml of this solution to 100.0 ml with the solvent mixture and filter.



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*Reference solution*. Dissolve a quantity of *cefpodoxime proxetil RS* in the solvent mixture to obtain a solution containing about 30 µg per ml.

*Note — A volume of methanol not exceeding 10 per cent of the total volume in the final solution may be used to facilitate dissolution.*

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature 30°,
- mobile phase: 60 volumes of *0.02 M ammonium acetate* and 40 volumes of *acetonitrile*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative retention time for cefpodoxime proxetil *S*-epimer is about 0.9 and for cefpodoxime proxetil *R*-epimer is about 1.0. The resolution between cefpodoxime proxetil *S*-epimer and cefpodoxime proxetil *R*-epimer is not less than 2.5, the tailing factor for cefpodoxime proxetil *R*-epimer is not more than 1.5 and the relative standard deviation determined from the sum of the areas of the cefpodoxime proxetil *S*-epimer and cefpodoxime proxetil *R*-epimer peaks for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{15}H_{17}N_5O_6S_2$ in the suspension.

**Labelling**. The label states (1) the quantity of active ingredient in terms of the equivalent amount of cefpodoxime; (2) the temperature of storage and the period during which the constituted suspension may be expected to be satisfactory for use.

# Cefpodoxime Tablets

Cefpodoxime Proxetil Tablets

Cefpodoxime Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cefpodoxime, $C_{15}H_{17}N_5O_6S_2$.

**Usual strengths**. 50 mg; 100 mg; 200 mg.

## Identification

In the Assay, the principal peaks of cefpodoxime proxetil *S*-epimer and cefpodoxime proxetil *R*-epimer in the chromatogram obtained with the test solution correspond to the peaks in the chromatogram obtained with the reference solution.

## Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of a solution prepared by dissolving 3.03 g of *glycine* and 3.37 g of *sodium chloride* in about 500 ml of *water*, adding cautiously with swirling 0.8 ml of *hydrochloric acid*, adjusting the pH to 3.0 and diluting to 1000 ml with *water*,
Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 259 nm (2.4.7). Calculate the content of $C_{15}H_{17}N_5O_6S_2$ in the medium from the absorbance obtained from a solution of known concentration of *cefpodoxime proxetil RS* in the same medium.

D. Not less than 70 per cent of the stated amount of $C_{15}H_{17}N_5O_6S_2$.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. A mixture of 60 volumes of *water* and 40 volumes of *acetonitrile*.

*Test solution*. Weigh and powder 20 tablets. Weigh accurately a quantity of powder containing about 50 mg of cefpodoxime, disperse in 100.0 ml of the solvent mixture. Dilute 5.0 ml of the solution to 100.0 ml with the solvent mixture and filter.

*Reference solution*. Dissolve a quantity of *cefpodoxime proxetil RS* in the solvent mixture to obtain a solution containing about 30 µg per ml.

*Note — A volume of methanol not exceeding 10 per cent of the total volume in the final solution may be used to facilitate dissolution.*

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature 30°,
- mobile phase: 60 volumes of *0.02 M ammonium acetate* and 40 volumes of *acetonitrile*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative retention time for cefpodoxime proxetil *S*-epimer is about 0.9 and for cefpodoxime proxetil *R*-epimer is about 1.0. The resolution between cefpodoxime proxetil *S*-epimer and cefpodoxime proxetil *R*-epimer is not less than 2.5, the tailing factor for cefpodoxime proxetil *R*-epimer is not more than 1.5 and the relative standard deviation determined from the sum of the areas of the cefpodoxime proxetil *S*-epimer and cefpodoxime proxetil *R*-epimer peaks for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.



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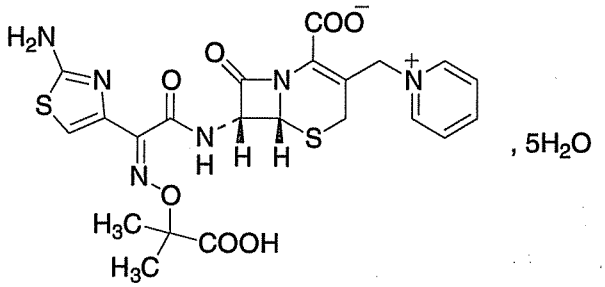


Calculate the content of $C_{15}H_{17}N_5O_6S_2$ in the tablets.

**Storage**. Store protected from moisture, at a temperature not exceeding 30°.

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

## Ceftazidime



$C_{22}H_{22}N_6O_7S_2,5H_2O$ Mol. Wt. 636.6

Ceftazidime is pentahydrate of the inner salt of (7*R*)-7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-(1-carboxy-1-methylethoxyimino)-acetamido]-3-(1-pyridinomethyl)-3-cepham-4-carboxylate pentahydrate.

Ceftazidime contains not less than 95.0 per cent and not more than 102.0 per cent of $C_{22}H_{22}N_6O_7S_2$, calculated on the dried basis.

**Category**. Antibacterial.

**Dose**. By intramuscular or slow intravenous injection or intravenous infusion, 1 to 2 g, every 8 to 12 hours.

**Description**. A white to cream-coloured, crystalline powder.

### Identification

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

### Tests

**pH** (2.4.24). 3.0 to 4.0, determined in a 0.5 per cent w/v solution.

**Pyridine**. Not more than 0.4 per cent.

Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh accurately a quantity containing 0.5 g of ceftazidime and dissolve in sufficient *mixed phosphate buffer pH 7.0* to produce 100.0 ml.

*Reference solution*. Weigh accurately about 200 mg of *pyridine* and dissolve in sufficient *water* to produce 100.0 ml. Immediately prior to chromatography add to 2.0 ml of the resulting solution sufficient *mixed phosphate buffer pH 7.0* to produce 200.0 ml and mix well.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a mixture of 10 volumes of a 2.88 per cent w/v solution of *ammonium dihydrogen* phosphate previously adjusted to *pH 7.0* with *dilute ammonia solution*, 30 volumes of *acetonitrile* and 60 volumes *of water*,
- column temperature. 38° to 42°,
- flow rate. 1.6 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Inject the reference solution. Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained is at least 50 per cent of the full scale deflection of the recorder.

Inject the reference solution six times. The test is not valid unless the relative standard deviation of the area of the principal peak in replicate injections is not more than 3.0 per cent.

Inject alternately the test solution and the reference solution. Record the chromatograms and measure the areas of the pyridine peaks.

Calculate the content of pyridine.

**Loss on drying** (2.4.19). 13.0 to 15.0 per cent, determined on 0.3 g by drying in an oven over *phosphorus pentoxide* at 60° at a pressure not exceeding 0.7 kPa for 3 hours.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve about 30 mg of the substance under examination in 2.5 ml of *phosphate buffer pH 7.0*, dilute to 25.0 ml with *water* and mix. Protect this solution from light. Immediately before chromatography, dilute 5.0 ml of this solution to 50.0 ml with *water*.

*Reference solution*. Treat 30 mg *ceftazidime RS* in a similar manner.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 100 ml of *phosphate buffer pH 7.0* and 20 ml of *acetonitrile* diluted to 1000 ml with *water*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.



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Inject alternately the test solution and the reference solution.

Calculate the content of $C_{22}H_{22}N_6O_7S_2$.

*Ceftazidime intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.*

**Bacterial endotoxins** (2.2.3). Not more than 0.10 Endotoxin Unit per mg.

*Ceftazidime intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.*

**Sterility** (2.2.11). Complies with the test for sterility.

**Storage**. Store protected from light and moisture.

## Ceftazidime for Injection

Ceftazidime for Injection is a sterile mixture of sterile Ceftazidime and Sodium Carbonate.

Ceftazidime for Injection contains not less than 90.0 per cent and not more than 105.0 per cent of ceftazidime, $C_{22}H_{22}N_6O_7S_2$, calculated on the dried and sodium carbonate-free basis.

**Description**. A white or almost white powder.

### Identification

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

B. Gives the reactions of sodium salts and reaction A of carbonates (2.3.1).

### Tests

**pH** (2.4.24). 5.0 to 7.5, determined in a solution containing 100 mg of anhydrous ceftazidime per ml.

**Pyridine**. Not more than 0.4 per cent.

Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh accurately a quantity containing about 0.5 g of ceftazidime and dissolve in sufficient *mixed phosphate buffer pH 7.0* to produce 100.0 ml.

*Reference solution.* Weigh accurately about 200 mg of *pyridine* and dissolve in sufficient *water* to produce 100.0 ml. Immediately prior to chromatography add to 2.0 ml of the resulting solution sufficient *mixed phosphate buffer pH 7.0* to produce 200.0 ml and mix well,

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a mixture of 10 volumes of a 2.88 per cent w/v solution of *ammonium dihydrogen* phosphate previously adjusted to *pH 7.0* with *dilute ammonia solution*, 30 volumes of *acetonitrile* and 60 volumes *of water,*
- flow rate. 1.6 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Inject the reference solution and adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained is at least 50 per cent of the full-scale deflection of the recorder.

Inject the reference solution six times. The test is not valid unless the relative standard deviation of the area of the principal peak in replicate injections is not more than 3.0 per cent.

Inject alternately the test solution and the reference solution. Measure the areas of the pyridine peaks.

Calculate the content of pyridine.

**Sodium carbonate**. Weigh accurately a quantity containing about 50 mg of anhydrous ceftazidime and dissolve in sufficient *water* to produce 100.0 ml. Dilute the resulting solution appropriately with *water* and determine by Method A for flame photometry (2.4.4), measuring at 589 nm or by Method A for atomic absorption spectrophotometry (2.4.2), using *sodium solution FP*, suitably diluted with *water* for the reference solutions.

1 g of Na is equivalent to 2.305 g of $Na_2CO_3$.

**Bacterial endotoxins** (2.2.3). Not more than 0.10 Endotoxin Unit per mg of ceftazidime.

**Sterility** (2.2.11). Complies with the tests for sterility.

**Loss on drying** (2.4.19). Not more than 13.5 per cent, determined on 0.3 g by drying at 25° for 4 hours at a pressure not exceeding 0.7 kPa and continuing the drying by heating in an oven at 100° for 3 hours at a pressure not exceeding 0.7 kPa.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh accurately a quantity containing about 50 mg of anhydrous ceftazidime dissolve in water and dilute to 50.0 ml with the same solvent. Protect this solution from light. Immediately before chromatography, dilute 5.0 ml to 50.0 ml with *water.*

*Reference solution.* Dissolve about 29 mg *ceftazidime RS* in 2.5 ml of *mixed phosphate buffer pH 7.0* and dilute to 25.0 ml with water. Protect this solution from light. Immediately before chromatography, dilute 5.0 ml to 50.0 ml with *water.*



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Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 100 ml of *phosphate buffer pH 7.0* and 20 ml of *acetonitrile* diluted to 1000 ml with *water*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_{22}H_{22}N_6O_7S_2$.

**Storage**. Store in sterile containers, sealed so as to exclude microorganisms, protected from moisture, at a temperature not exceeding 30°.

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

# Ceftazidime Injection

Ceftazidime Injection is a sterile material consisting of Ceftazidime for Injection with or without auxiliary substances. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of Water for Injections immediately before use.

*The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).*

**Storage**. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Ceftazidime Injection contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of ceftazidime, $C_{22}H_{22}N_6O_7S_2$.

**Usual strengths**: The equivalent of 250 mg; 500 mg; 1 g of ceftazidime.

**Description**. A white or almost white crystalline powder.

*The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.*

## Identification

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

B. Gives the reactions of sodium salts and reaction A of carbonates (2.3.1).

## Tests

**pH** (2.4.24). 5.0 to 7.5, determined in a solution containing 100 mg of ceftazidime per ml.

**Pyridine**. Not more than 0.4 per cent.

Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh accurately a quantity containing about 0.5 g of ceftazidime and dissolve in sufficient *mixed phosphate buffer pH 7.0* to produce 100.0 ml.

*Reference solution.* Weigh accurately about 200 mg of *pyridine* and dissolve in sufficient *water* to produce 100.0 ml. Immediately prior to chromatography add to 2.0 ml of the resulting solution sufficient *mixed phosphate buffer pH 7.0* to produce 200.0 ml and mix well.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 10 volumes of a 2.88 per cent w/v solution of *ammonium dihydrogen* phosphate previously adjusted to *pH 7.0* with *dilute ammonia solution*, 30 volumes of *acetonitrile* and 60 volumes *of water*,
- flow rate. 1.6 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Inject the reference solution and adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained is at least 50 per cent of the full-scale deflection of the recorder.

Inject the reference solution six times. The test is not valid unless the relative standard deviation of the area of the principal peak in replicate injections is not more than 3.0 per cent.

Inject alternately the test solution and the reference solution. Measure the areas of the pyridine peaks.

Calculate the content of pyridine.

**Bacterial endotoxins** (2.2.3). Not more than 0.10 Endotoxin Unit per mg of ceftazidime.

**Sterility** (2.2.11). Complies with the test for sterility.

**Loss on drying** (2.4.19). Not more than 13.5 per cent, determined on 0.3 g by drying at 25° for 4 hours at a pressure not exceeding 0.7 kPa and continuing the drying by heating in an oven at 100° for 3 hours at a pressure not exceeding 0.7 kPa.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Determine the weight of the contents of 10 containers. Weigh accurately a quantity of the mixed



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contents of the 10 containers containing about 50 mg of ceftazidime, dissolve in *water* and dilute to 50.0 ml with the same solvent. Protect this solution from light. Immediately before chromatography, dilute 5.0 ml to 50.0 ml with *water*.

*Reference* solution. Dissolve about 29 mg *ceftazidime RS* in 2.5 ml of mixed phosphate buffer pH 7.0 and dilute to 25.0 ml with *water*. Protect this solution from light. Immediately before chromatography, dilute 5.0 ml to 50.0 ml with *water*.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 100 ml of *phosphate buffer pH 7.0* and 20 ml of *acetonitrile* diluted to 1000 ml with *water*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

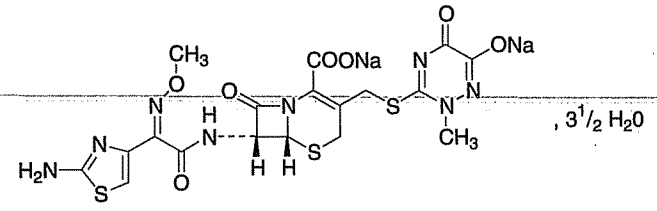
Inject alternately the test solution and the reference solution.

Calculate the content of $C_{22}H_{22}N_6O_7S_2$ in the injection.

**Storage**. Store in sterile containers, sealed so as to exclude microorganisms, protected from moisture, at a temperature not exceeding 30 °.

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

## Ceftriaxone Sodium




$C_{18}H_{16}N_8Na_2O_7S_3,3½H_2O$ Mol. Wt. 662.0

Ceftriaxone sodium is disodium (6*R*,7*R*)-7-[[(*Z*)-(2-aminothiazol-4-yl)(methoxyimino)acetyl]amino]-3-[[(2-methyl-6-oxido-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl) sulphanyl]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate hemiheptahydrate.

Ceftriaxone sodium contains not less than 96.0 per cent and not more than 102.0 per cent of $C_{18}H_{16}N_8Na_2O_7S_3$, calculated on the anhydrous basis.

**Category**. Antibacterial.

**Description**. A white or yellowish, crystalline powder, slightly hygroscopic.

### Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ceftriaxone sodium RS* or with the reference spectrum of ceftriaxone sodium.

B. Gives reaction A of sodium salts (2.3.1).

### Tests

**Appearance of solution**. Dissolve 2.4 g in 20 ml of *carbon dioxide-free water* (Solution A). Dilute 2 ml of solution A to 20 ml with *water*; the resulting solution is clear (2.4.1) and not more intensely coloured than reference solution BYS5 or YS5 (2.4.1).

**pH** (2.4.24). 6.0 to 8.0, determined in solution A.

**Specific optical rotation** (2.4.22). -155.0° to -170.0°, determined in a 1.0 per cent w/v solution in *water*.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 30.0 mg of the substance under examination in the mobile phase and dilute to 100.0 ml with the mobile phase.

*Reference solution (a)*. A 0.03 per cent w/v solution of *ceftriaxone sodium RS* in the mobile phase.

*Reference solution (b)*. A solution containing 0.005 per cent w/v each of *ceftriaxone sodium RS* and *ceftriaxone sodium E- isomer RS* in the mobile phase.

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

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: dissolve 2.0 g of *tetradecylammonium bromide* and 2.0 g of *tetraheptylammonium bromide* in a mixture of 440 ml of *water*, 55 ml of *0.067 M mixed phosphate buffer solution pH 7.0*, 5.0 ml of a buffer solution prepared by dissolving 20.17 g of *citric acid* in 800 ml of *water*, adjusting the pH to 5.0 with *strong sodium hydroxide solution* and diluting to 1000.0 ml with *water*, and 500 ml of *acetonitrile*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject the test solution, reference solution (b) and (c). Continue the chromatography for twice the retention time of the ceftriaxone peak. The test is not valid unless the resolution



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between the peaks due to ceftriaxone and ceftriaxone sodium *E*-isomer is at least 3.0; the area of any peak other than the principal peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1 per cent); the sum of the areas of all such peaks is not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (c) (4 per cent). Ignore any peak with an area 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent).

**Water** (2.3.43). 8.0 per cent to 11.0 per cent, determined on 0.1 g.

**Assay**. Determine by liquid chromatography (2.4.14) as described under Related substances.

Inject alternately the test solution and reference solution (a).

Calculate the content of $C_{18}H_{16}N_8Na_2O_7S_3$.

*Ceftriaxone sodium intended for use in the manufacture of parenteral preparations without a further appropriate procedure for removal of bacterial endotoxins complies with the following additional requirement.*

**Bacterial endotoxins** (2.2.3). Not more than 0.20 Endotoxin Unit per mg of ceftriaxone sodium.

*Ceftriaxone Sodium intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with following requirement.*

**Sterility** (2.2.11). Complies with the test for sterility.

**Storage**. Store protected from light and moisture.

**Labelling**. The label states, where applicable, that the substance is free from bacterial endotoxins.

# Ceftriaxone Injection

Ceftriaxone Injection is a sterile material consisting of Ceftriaxone Sodium with or without excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

*The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).*

**Usual strengths**. The equivalent of 250 mg; 500 mg; 1 g and 2 g of ceftriaxone.

**Storage**. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Ceftriaxone Injection contains not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of ceftriaxone, $C_{18}H_{18}N_8O_7S_3$.

**Description.** A white or almost white powder.

*The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.*

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ceftriaxone sodium RS* or with the reference spectrum of ceftriaxone sodium.

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

C. It gives the reaction A of sodium salts (2.3.1).

## Tests

**Appearance of solution**. A 1.2 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1) and not more intensely coloured than reference solution BYS5 or YS5 (2.4.1).

**pH** (2.4.24). 6.0 to 8.0, determined in a 10.0 per cent w/v solution.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve the substance under examination containing about 30 mg of ceftriaxone in 100 ml of the mobile phase and filter.

*Reference solution (a)*. A 0.03 per cent w/v solution of *ceftriaxone sodium RS* in the mobile phase.

*Reference solution (b)*. A solution containing 0.005 per cent w/v each of *ceftriaxone sodium RS* and *ceftriaxone sodium E-isomer RS* in the mobile phase.

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

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Lichrosphere RP-18),
- mobile phase: dissolve 2 g of *tetradecylammonium bromide* and 2 g of *tetraheptylammonium bromide* in a mixture of 440 ml of *water*, 55 ml of *0.067 M mixed phosphate buffer pH 7.0*, 5 ml of a buffer prepared by dissolving 20.17 g of *citric acid* in 800 ml of *water*, adjusting the pH to 5.0 with *10 M sodium hydroxide* and diluting to 1000 ml with *water*, and 500 ml of *acetonitrile*,



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- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is at least 3.0.

Inject the test solution and reference solution (c). Run the chromatogram at least twice the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any secondary peak is not greater than the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent) and the sum of the areas of all the secondary peaks is not greater than 5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (5.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent).

**Bacterial endotoxins** (2.2.3). Not more than 0.2 Endotoxin Unit per mg of ceftriaxone.

**Water** (2.3.43). Not more than 11.0 per cent, determined on 0.1 g.

**Assay**. Determine by liquid chromatography (2.4.14), as described under Related substances.

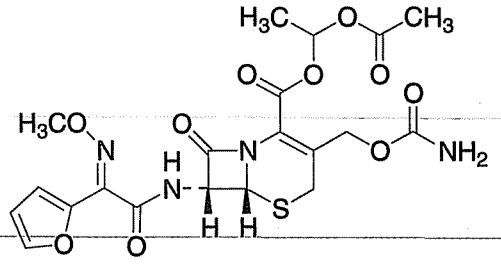
Inject alternately the test solution and reference solution (a).

Calculate the content of $C_{18}H_{18}N_8O_7S_3$ in the injection.

**Storage**. Store protected from light at a temperature not exceeding 30°.

**Labelling**. The label on the sealed container states the quantity of Ceftriaxone Sodium contained in it in terms of the equivalent amount of ceftriaxone.

# Cefuroxime Axetil



$C_{20}H_{22}N_4O_{10}S$ Mol. Wt. 510.5

Cefuroxime Axetil is a mixture of the 2 diastereoisomers of (1*RS*)-1-(acetyloxy)ethyl (6*R*,7*R*)-3-[(carbamoyloxy)methyl]-7-[[(*Z*)-2-(furan-2-yl)-2-(methoxyimino)acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate.

Cefuroxime Axetil contains not less than 79.8 per cent and not more than 84.8 per cent of cefuroxime, $C_{16}H_{16}N_4O_8S$, calculated on the anhydrous and acetone-free basis.

**Category**. Antibacterial.

**Description**. A white or almost white powder.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cefuroxime axetil RS* or with the reference spectrum of cefuroxime axetil.

B. In the Assay, the principal peaks in the chromatogram obtained with the test solution corresponds to the peaks due to diastereomer A and B in the chromatogram obtained with reference solution (d).

## Tests

**Diastereoisomer ratio**. Determine by liquid chromatography (2.4.14).

Use chromatographic system, test solution, reference solution (a), (b), (c) and (d), as described under Assay.

In the chromatogram obtained with the test solution, the ratio of the peak due to cefuroxime axetil diastereoisomer A to the sum of the peaks due to cefuroxime axetil diastereoisomers A and B is between 0.48 and 0.55 by the normalisation procedure.

**Related substances**. Determine by liquid chromatography (2.4.14).

Use chromatographic system, test solution, reference solution (a), (b), (c) and (d), as described under Assay.

The percentage sum of the pair of peaks corresponding to the *E*-isomers located by comparison with the chromatogram obtained with reference solution (c) is not more than 1.0 per cent, the percentage sum of the pair of peaks corresponding to the $D^3$-isomers located by comparison with the chromatogram obtained with reference solution (b) is not greater than 1.5 per cent and the area of any other secondary peak is not more than 0.5 per cent and the sum of all the secondary peaks found is not more than 3.0 per cent.

**Acetone** (5.4). Not more than 1.1 per cent.

**Water** (2.3.43). Not more than 1.5 per cent, determined on 0.4 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*NOTE – Prepare the solutions immediately before use.*

*Test solution*. Dissolve 10.0 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the mobile phase.

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



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*Reference solution (b).* Warm 5 ml of the test solution to 60° for one hour to generate the $D^3$-isomers.

*Reference solution (c)* Expose 5 ml of the test solution to ultraviolet light at 254 nm for 24 hours to generate *E*-isomers.

*Reference solution (d).* A 0.02 per cent w/v solution of *cefuroxime axetil RS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with trimethylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 38 volumes of *methanol* and 62 volumes of a 2.3 per cent solution of *ammonium dihydrogen phosphate*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 278 nm,
- injection volume. 20 µl.

Inject reference solution (d). The test is not valid unless the relative standard deviation for replicate injections for the sum of diastereomer A and B peaks is not more than 2.0 per cent.

Inject the test solution, and reference solutions (a), (b) and (c). The retention times relative to cefuroxime axetil diastereoisomer A (second peak) are approximately 0.9 for cefuroxime axetil diastereoisomer B, 1.2 for the cefuroxime axetil $D^3$-isomers and 1.7 and 2.1 for the *E*-isomers. The test is not valid unless in the chromatogram obtained with reference solution (d), the resolution between the peaks corresponding to cefuroxime axetil diastereoisomers A and B is at least 1.5. In the chromatogram obtained with reference solution (b), the resolution between the peaks corresponding to cefuroxime axetil diastereoisomer A and cefuroxime axetil $D^3$-isomer is at least 1.5.

Calculate the content of $C_{16}H_{16}N_4O_8S$ as the sum of areas of the two diastereoisomer peaks.

1 mg of $C_{20}H_{22}N_4O_{10}S$ is equivalent to 0.8313 mg of $C_{16}H_{16}N_4O_8S$.

**Storage**. Store protected from light and moisture.

# Cefuroxime Axetil Tablets

Cefuroxime Axetil Tablets contain Cefuroxime Axetil. They may be coated.

Cefuroxime Axetil Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cefuroxime, $C_{16}H_{16}N_4O_8S$.

**Usual strengths**. 125 mg; 250 mg; 500 mg.

## Identification

A. Extract a quantity of the powdered tablets containing 0.1 g of cefuroxime with 5 ml of *dichloromethane*, filter and evaporate the filtrate to dryness. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cefuroxime axetil RS* or with the reference spectrum of cefuroxime axetil.

B. In the Assay, the principal peaks in the chromatogram obtained with the test solution corresponds to the peaks due to diastereomer A and B in the chromatogram obtained with reference solution (c).

## Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly. Dilute the filtrate, if necessary, with the dissolution medium. Measure the absorbance of the resulting solution at the maximum at about 278 nm (2.4.7). Calculate the content of $C_{16}H_{16}N_4O_8S$ in the medium from the absorbance obtained from a solution of known concentration of *cefuroxime axetil RS.*

D. Not less than 70 per cent of the stated amount of $C_{16}H_{16}N_4O_8S$.

**Related substances**. Determine by liquid chromatography (2.4.14).

Use chromatographic system, test solution, reference solution (a), (b), and (c), as described under Assay.

In the chromatogram obtained with the test solution the sum of the areas of the pair of peaks corresponding to the *E*-isomers in the chromatogram obtained with reference solution (b) is not more than 1.5 per cent by normalisation, the sum of the areas of any peaks corresponding to the $D^3$-isomers in the chromatogram obtained with reference solution (a) is not more than 2.0 per cent by normalisation and the area of any other secondary peak is not more than 1.0 per cent by normalisation.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*NOTE – Prepare the solutions immediately before use.*

*Test solution.* Disperse 10 tablets in *0.2 M ammonium dihydrogen orthophosphate* with the pH previously adjusted to 2.4 with *orthophosphoric acid,* using 10 ml per g of the stated content of cefuroxime. Immediately add sufficient *methanol* to produce a solution containing the equivalent of 0.5 per cent w/v of cefuroxime and shake vigorously. Filter and dilute a quantity of the filtrate with sufficient of the mobile phase to produce a solution containing 0.025 per cent w/v of cefuroxime.

*Reference solution (a).* Warm a quantity of the test solution at 60° for one hour or until sufficient impurities ($D^3$-isomers) have been generated.



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*Reference solution (b).* Expose a quantity of the test solution to ultraviolet light at 254 nm for 24 hours or until sufficient impurities (*E*-isomers) have been generated.

*Reference solution (c).* A 0.03 per cent w/v solution of *cefuroxime axetil RS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with particles of silica (5 µm) the surface of which has been modified by chemically-bonded trimethylsilyl groups (Such as Hypersil SAS),
- mobile phase: a mixture of 38 volumes of *methanol* and 62 volumes of *0.2 M ammonium dihydrogen orthophosphate,* adjusted, if necessary, so that the resolution between the peaks corresponding to the cefuroxime axetil diastereoisomers A and B in reference solution (c) and between the peaks corresponding to cefuroxime axetil diastereoisomer A and the cefuroxime axetil $D^3$-isomer in reference solution (a) is in each case not less than 1.5,
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 278 nm,
- injection volume. 20 µl.

Inject reference solution (c). The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

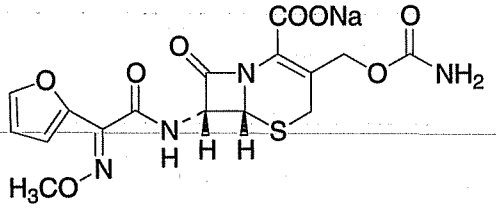
Inject the test solution, reference solution (a), (b) and (c). The retention time relative to cefuroxime axetil diastereoisomer A are approximately 0.9 for cefuroxime axetil diastereoisomer B, 1.2 for the cefuroxime axetil $D^3$-isomers and 1.7 and 2.1 for the *E*-isomers.

Calculate the content of $C_{16}H_{16}N_4O_8S$ as the sum of the areas of the two peaks corresponding to diastereoisomers A and B.

1 mg of $C_{20}H_{22}N_4O_{10}S$ is equivalent to 0.8313 mg of $C_{16}H_{16}N_4O_8S$.

**Labelling.** The quantity of active ingredient is stated in terms of the equivalent amount of cefuroxime.

# Cefuroxime Sodium




$C_{16}H_{15}N_4NaO_8S$ Mol. Wt. 446.4

Cefuroxime Sodium is sodium (7*R*)-3-carbamoyloxymethyl-7-[(*Z*)-furan-2-yl-2-methoxyiminoacetamido]-3-cephem-4-carboxylate.

Cefuroxime Sodium contains not less than 90.0 per cent and not more than 105.0 per cent of cefuroxime, $C_{16}H_{16}N_4O_8S$, calculated on the anhydrous basis.

**Category.** Antibacterial.

**Dose.** Orally, 250 mg twice daily; by intramuscular or intravenous injection or intravenous infusion, 750 mg to 1.5 g, every 6 to 8 hours.

**Description.** A white or faintly yellow powder.

## Identification

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

B. Gives the reactions of sodium salts (2.3.1).

## Tests

**pH** (2.4.24). 6.0 to 8.5, determined in a 10.0 per cent w/v solution.

**Water** (2.3.43). Not more than 3.5 per cent, determined on 0.15 g.

**Assay.** Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh accurately a quantity of the substance under examination containing 25 mg of cefuroxime and dissolve in sufficient *water* to produce 25.0 ml. Immediately transfer 5.0 ml of the resulting solution to a 100-ml volumetric flask, add 20.0 ml of a 0.15 per cent w/v solution of *orcinol* (internal standard) in *water,* dilute to volume with *water* and mix.

*Reference solution.* Treat a quantity of *cefuroxime sodium RS* equivalent to 25 mg of cefuroxime in a similar manner.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with hexylsilane chemically bonded to totally porous silica particles (5 µm),
- mobile phase: a mixture of 100 volumes of *acetate buffer pH 3.4* and 10 volumes of *acetonitrile*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_{16}H_{16}N_4O_8S$.

*Cefuroxime Sodium intended for use in the manufacture of parenteral preparations complies with the following additional requirements.*



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**Bacterial endotoxins** (2.2.3). Not more than 0.10 Endotoxin Unit per mg of cefuroxime.

**Sterility** (2.2.11). Complies with the test for sterility, using the membrane filtration method.

**Storage**. Store protected from moisture. If it is intended for use in the manufacture of parenteral preparations, it should be sterile and sealed so as to exclude micro-organisms.

**Labelling**. The label states whether or not the contents are intended for use in the manufacture of injectable preparations.

## Cefuroxime Injection

Cefuroxime Sodium Injection

Cefuroxime Injection is a sterile material consisting of Cefuroxime Sodium, with or without auxiliary substances. It is filled in a sealed container.

The injection is constituted by dissolving the contents of a sealed container in the requisite amount of Water for Injections immediately before use.

*The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).*

**Storage**. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Cefuroxime Injection contains a quantity of Cefuroxime Sodium equivalent to not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of cefuroxime, $C_{16}H_{16}N_4O_8S$.

**Usual strengths**: The equivalent of 250 mg, 750 mg and 1.5 g of cefuroxime.

**Description**. A white or faintly yellow powder.

*The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.*

### Identification

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

B. Gives the reactions of sodium salts (2.3.1).

### Tests

**pH** (2.4.24). 6.0 to 8.5, determined in a 10.0 per cent w/v solution.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve a quantity of the mixed content of 10 containers containing 0.1 g of cefuroxime in 100 ml of *water*.

*Reference solution (a)*. A 0.1 per cent w/v solution of *cefuroxime sodium RS* in *water*.

*Reference solution (b)*. Heat 20.0 ml of reference solution (a) in water bath at 60° for 10 minutes, cool.

*Reference solution (c)*. Dilute 1.0 ml of reference solution (a) to 100.0 ml with *water*.

Chromatographic system

- a stainless steel column 12.5 cm x 4.6 mm packed with silica chemically bonded to hexylsilane groups (5 µm) (such as Spherisorb S5 C6),
- mobile phase: a mixture of 1 volume of *acetonitrile* and 99 volumes of *acetate buffer pH 3.4*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 273 nm,
- injection volume. 20 µl.

Inject reference solution (b). The chromatogram obtained shows peaks corresponding to cefuroxime and descarbamoyl-cefuroxime. The test is not valid unless the resolution factor between the two principal peaks is not less than 2.0.

Inject the test solution, reference solution (a) and (c). Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of peak due to descarbamoyl-cefuroxime is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent). The sum of areas of all the secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (c) (3.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent).

**Bacterial endotoxins** (2.2.3). Not more than 0.1 Endotoxin Unit per mg of cefuroxime.

**Water** (2.3.43). Not more than 3.5 per cent, determined on 0.15 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Determine the weight of the contents of 10 containers. Weigh accurately a quantity of the mixed contents of the 10 containers containing about 25 mg of cefuroxime and dissolve in sufficient *water* to produce 25.0 ml. Immediately transfer 5.0 ml of the resulting solution to a 100-ml volumetric flask, add 20.0 ml of a 0.15 per cent w/v solution of *orcinol* (internal standard) in *water*, dilute to volume with *water* and mix.



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*Reference solution*. Treat a quantity of *cefuroxime sodium RS* equivalent to 25 mg of cefuroxime in a similar manner.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with hexylsilane chemically bonded to totally porous silica particles (5 µm),
- mobile phase: a mixture of 100 volumes of *acetate buffer pH 3.4* and 10 volumes of *acetonitrile*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_{16}H_{16}N_4O_8S$ in the injection.

**Storage**.Store in tightly-closed containers protected from moisture at a temperature not exceeding 30°.

**Labelling**. The label on the sealed container states the quantity of Cefuroxime Sodium contained in it in terms of the equivalent amount of cefuroxime.

## Cellulose Acetate Phthalate

Cellacephate; Cellacefate

Cellulose Acetate Phthalate is a cellulose, some of the hydroxyl groups of which are esterified by acetyl groups and others by hydrogen phthaloyl groups.

Cellulose Acetate Phthalate contains not less than 17.0 per cent and not more than 26.0 per cent of acetyl groups, $C_2H_3O$ and not less than 30.0 per cent and not more than 40.0 per cent of hydrogen phthaloyl groups, $C_8H_5O_3$ both calculated on the dried, acid-free basis.

**Category**. Pharmaceutical aid (for enteric coating of tablets).

**Description**. A white, free-flowing powder or colourless flakes; odourless or with a faint odour of acetic acid; hygroscopic.

### Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cellulose acetate phthalate RS* or with the reference spectrum of cellulose acetate phthalate.

B. To about 10 mg contained in a small test-tube add 10 mg of *resorcinol*, mix, add 0.5 ml of *sulphuric acid* and heat in a liquid paraffin bath at 160° for 3 minutes. Cool and pour the solution into a mixture of 25 ml of *sodium hydroxide solution* and 200 ml of *water*; the solution shows a vivid green fluorescence.

### Tests

**Viscosity** (2.4.28). 50 $mm^2s^{-1}$ to 90 $mm^2s^{-1}$, determined in the following manner. Weigh accurately about 15 g, previously dried at 105° for 2 hours, and dissolve in 85 g of a mixture of 249 parts of *dry acetone* and 1 part of *water*. Determine at 25° the viscosity of the resulting solution by Method A, using a size D viscometer.

**Appearance of a film**. Dissolve 3.0 g in 17 ml of *acetone* with a water content of 0.35 to 0.45 per cent w/w. Allow 1 ml of this solution to flow over a glass plate and dry; a thin, colourless, transparent and glossy film is produced.

**Free acid**. Not more than 3.0 per cent, calculated as phthalic acid, $C_8H_6O_4$, on the anhydrous basis and determined in the following manner. Weigh accurately 1.0 g, in fine powder, shake for 5 minutes with 100 ml of boiled *water* and filter. Wash the flask and the filter with two quantities, each of 10 ml, of *water*. Combine the filtrate and washings, add 5 drops of *phenolphthalein solution* and titrate with *0.1 M sodium hydroxide* until a faint pink colour is obtained.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.0083 g of phthalic acid.

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

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 5.0 per cent, using 0.5 g dissolved in 20 ml of a mixture of equal volumes of *anhydrous methanol* and *chloroform*.

**Assay**. *For acetyl groups* — Weigh accurately about 0.1 g and heat on a water-bath for 30 minutes with 25.0 ml of *0.1 M sodium hydroxide* under reflux. Cool, add 5 drops of *phenolphthalein solution* and titrate with *0.1 M hydrochloric acid* until the colour is discharged. Carry out a blank titration. Calculate the acetyl groups, $C_2H_3O$, from the expression

$$0.43c/w - (0.578p + 0.518s),$$

where, $c$ = volume, in ml, of *0.1 M hydrochloric acid* consumed

$w$ = weight, in g, of the sample, calculated with reference to anhydrous substance

$p$ = percentage of hydrogen phthaloyl groups as determined in Assay for hydrogen phthaloyl groups

$s$ = percentage of free acid.

*For hydrogen phthaloyl groups* — Weigh accurately about 0.4 g (calculated on the anhydrous basis) and dissolve without



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heating in 20 ml of *2-methoxyethanol*, previously neutralised in the presence of 5 drops of *phenolphthalein solution*. Titrate with *0.1 M sodium hydroxide* until a faint pink colour is produced. Calculate the hydrogen phthaloyl groups, $C_8H_5O_3$, from the expression

$$1.49b/w - 1.795s$$

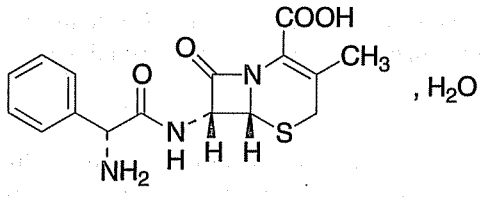
where, $b$ = volume, in ml, of *0.1 M sodium hydroxide* consumed

$w$ = weight, in g, of the sample, calculated with reference to the anhydrous substance

$s$ = percentage of free acid.

**Storage**. Store protected from moisture at a temperature between 8° and 15°.

## Cephalexin




$C_{16}H_{17}N_3O_4S,H_2O$ Mol. Wt. 365.4

Cephalexin is (7*R*)-3-methyl-7-(α-D-phenylglycylamino)-3-cephem-4-carboxylic acid monohydrate.

Cephalexin contains not less than 95.0 per cent and not more than 101.0 per cent of $C_{16}H_{17}N_3O_4S$, calculated on the anhydrous basis.

**Category**. Antibacterial.

**Dose**. 1 to 4 g daily, in divided doses.

**Description**. A white or almost white, crystalline powder; odour, characteristic.

### Identification

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

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

### Tests

**pH** (2.4.24). 4.0 to 5.5, determined in a 0.5 per cent w/v solution.

**Specific optical rotation** (2.4.22). +149° to +158°, determined in a 0.5 per cent w/v solution in *phthalate buffer solution pH 4.4* and in a 2-dm tube.

**Light absorption** (2.4.7). Dissolve 50 mg in sufficient *water* to produce 100.0 ml. Absorbance of the solution at about 330 nm, not greater than 0.05 (2.4.7). Dilute 2 ml to 50.0 ml. When examined between 230 nm and 360 nm the solution shows an absorption maximum at about 262 nm; absorbance at about 262 nm, 0.44 to 0.49.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 50 mg of the substance under examination in mobile phase A and dilute to 50 ml with the same solvent.

*Reference solution (a)*. Dissolve 10 mg of *D-phenylglycine* in mobile phase A and dilute to 10 ml with the same solvent.

*Reference solution (b)*. Dissolve 10 mg of *7-amino-desacetoxycephalosporanic acid RS* in *phosphate buffer solution pH 7.0* and dilute to 10 ml with the same solvent.

*Reference solution (c)*. Dilute 1 ml of reference solution (a) and 1 ml of reference solution (b) to 100 ml with mobile phase A.

*Reference solution (d)*. Dissolve 10 mg of *dimethylformamide* and 10 mg of *dimethylacetamide* in mobile phase A and dilute to 10 ml with the same solvent. Dilute 1 ml to 100 ml with mobile phase A.

*Reference solution (e)*. Dilute 1 ml of reference solution (c) to 20 ml with mobile phase A.

*Reference solution (f)*. Dissolve 10 mg of *cefotaxime sodium RS* in mobile phase A and dilute to 10 ml with the same solvent. To 1 ml of the solution, add 1 ml of the test solution and dilute to 100 ml with mobile phase A.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. *phosphate buffer solution pH 5.0*, B. *methanol*,
- flow rate. 1.5 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 220 nm,
- injection volume. 20 µl.

| Time (min) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0 – 1 | 98 | 2 |
| 1 – 20 | 98 → 70 | 2 → 30 |
| 20 – 23 | 70 → 98 | 30 → 2 |
| 23 – 30 | 98 | 2 |



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Inject reference solution (c) and (f). In the chromatogram obtained with reference solution (c) the resolution between the peaks due to D-phenylglycine and 7-aminodesacetoxycephalosporanic acid is not less than 2.0 and in the chromatogram obtained with reference solution (f) the resolution between the peaks due to cephalexin and cefotaxime is not less than 1.5.

Inject the test solution and reference solution (c) (d) and (e). In the chromatogram obtained with the test solution the area of peak corresponding to D-phenylglycine is not more than the area of the first peak in the chromatogram obtained with reference solution (c) (1.0 per cent). Ignore any peaks due to dimethylformamide and dimethylacetamide The area of any other secondary peak is not more than the area of the first peak in the chromatogram obtained with reference solution (c) (1.0 per cent). The sum of the secondary peaks is not more than the three times the area of the first peak in the chromatogram obtained with reference solution (c). Ignore any peak with an area less than the second peak in the chromatogram obtained with reference solution (e) (0.05 per cent).

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Water** (2.3.43). 4.0 to 8.0 per cent, determined on 0.3 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve 50 mg of the substance under examination in *water* and dilute to 100.0 ml with the same solvent.

*Reference solution (a).* Dissolve 50 mg of *cephalexin monohydrate RS* in *water* and dilute to 100.0 ml with the same solvent.

*Reference solution (b).* Dissolve 10 mg of *cephradine RS* in 20 ml of reference solution (a) and dilute to 100 ml with *water.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 2 volumes of *methanol*, 5 volumes of *acetonitrile*, 10 volumes of a 13.6 g per litre solution of *potassium dihydrogen phosphate* and 83 volumes of *water*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (b). In the chromatogram obtained, the resolution between the peaks due to cephalexin and cephradine is not less than 4.0.

Inject alternately the test solution and reference solution (a).

Calculate the content of $C_{16}H_{17}N_3O_4S$.

**Storage**. Store protected from light at a temperature not exceeding 30°.

## Cephalexin Capsules

Cephalexin Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of anhydrous cephalexin, $C_{16}H_{17}N_3O_4S$.

**Usual strengths**. 250 mg; 500 mg.

### Identification

Shake a quantity of the contents of the capsules containing 0.5 g of anhydrous cephalexin with 1 ml of *water* and 1.4 ml of *1 M hydrochloric acid*, filter and wash the filter with 1 ml of *water.* Add slowly to the filtrate a saturated solution of *sodium acetate* until precipitation occurs. Add 5 ml of *methanol,* filter and wash the precipitate with two quantities, each of 1 ml, of *methanol*. The residue after drying at a pressure not exceeding 0.7 kPa complies with the following tests.

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

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

### Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF* (such as Analtech plates). Impregnate the plate by development with a 5 per cent v/v solution of *n-tetradecane* in hexane. Allow the solvent to evaporate and carry out the chromatography in the same direction as the impregnation.

*Mobile phase*. A mixture of 3 volumes of *acetone*, 80 volumes of a 7.2 per cent w/v solution of *disodium hydrogen orthophosphate* and 120 volumes of a 2.1 per cent w/v solution of *citric acid.*

*Test solution*. Shake a quantity of the contents of the capsules containing about 0.25 g of anhydrous cephalexin with 10 ml of *2 M hydrochloric acid*, filter and use the filtrate.

*Reference solution (a).* Dilute 1 ml of the test solution to 100 ml with *2 M hydrochloric acid.*

*Reference solution (b).* A 0.025 per cent w/v solution of *7-amino-desacetoxycephalosporanic acid RS* in *2 M hydrochloric acid.*

*Reference solution (c).* A 0.025 per cent w/v solution of *DL-phenylglycine* in *2 M hydrochloric acid.*

*Reference solution (d).* A solution containing 2.5 per cent w/v of *cephalexin RS* and 0.025 per cent w/v each of *7-amino-desacetoxycephalosporanic acid RS* and *DL-phenylglycine* in *2 M hydrochloric acid.*



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Apply to the plate 5 µl of each solutions. After development, dry the plate at 90° for 3 minutes, spray the hot plate with a 0.1 per cent w/v solution of *ninhydrin* in the mobile phase, heat the plate at 90° for 15 minutes and allow to cool. In the chromatogram obtained with the test solution any spot corresponding to 7-aminodesacetoxy-cephalosporanic acid is not more intense than the spot in the chromatogram obtained with reference solution (b) (1 per cent), any spot corresponding to DL-phenylglycine is not more intense than the spot in the chromatogram obtained with reference solution (c) (1 per cent) and any other *secondary spot* is not more intense than the spot in the chromatogram obtained with reference solution (a) (1 per cent). The test is not valid unless the chromatogram obtained with reference solution (d) shows three clearly separated spots.

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *water* freshly prepared by distillation,
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc with an average pore diameter not greater than 0.8 µm. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate with *water*. Measure the absorbance of the resulting solution at the maximum at about 261 nm (2.4.7). Calculate the content of $C_{16}H_{17}N_3O_4S$ taking 235 as the specific absorbance at 261 nm.

D. Not less than 75 per cent of the stated amount of $C_{16}H_{17}N_3O_4S$.

**Other tests**. Comply with the tests stated under Capsules.

**Water** (2.3.43). Not more than 10.0 per cent, determined on 0.3 g of the contents of the capsules.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Shake a quantity of the powdered mixed contents of 20 capsules containing about 0.25 g of anhydrous cephalexin with 100.0 ml of *water* for 30 minutes, add sufficient amount of *water* to produce 250.0 ml and filter. Dilute 25.0 ml of the filtrate to 50.0 ml with *water*.

*Reference solution (a).* A 0.05 per cent w/v solution of *cephalexin RS* in *water*.

*Reference solution (b).* A solution containing 0.01 per cent w/v each of *cephalexin RS* and *cephradine RS* in *water*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Nucleosil C 18),
- mobile phase: a mixture of 2 volumes of *methanol*, 5 volumes of *acetonitrile*, 10 volumes of a 13.6 g per litre solution of *potassium dihydrogen phosphate* and 83 volumes of *water*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (b). Adjust the sensitivity so that the height of the peaks in the chromatogram obtained is at least half the full-scale deflection on the recorder. The test is not valid unless the resolution between the peaks corresponding to cephalexin and cephradine is at least 4.0.

Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject alternately the test solution and reference solution (a).

Calculate the content of $C_{16}H_{17}N_3O_4S$ in the capsules.

**Storage**. Store protected from moisture at a temperature not exceeding 30°.

**Labelling**. The label states the strength in terms of the equivalent amount of anhydrous cephalexin.

# Cephalexin Oral Suspension

Cephalexin Dry Syrup; Cephalexin Mixture

Cephalexin Oral Suspension is a mixture of Cephalexin with buffering agents and other excipients. It contains a suitable flavouring agent. It is filled in a sealed container.

The suspension is constituted by dispersing the contents of the sealed container in the specified volume of water just before use.

Cephalexin Oral Suspension contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of $C_{16}H_{17}N_3O_4S$.

When stored at the temperature and for the period stated on the label during which the constituted suspension may be expected to be satisfactory for use, it contains not less than 80.0 per cent of the stated amount of cephalexin, $C_{16}H_{17}N_3O_4S$.

**Usual strengths**. 125 mg; 250 mg of anhydrous cephalexin per 5 ml.

## Identification

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

B. Weigh a quantity containing 0.1 g of anhydrous cephalexin, shake with 20 ml of *methanol*, filter and evaporate the filtrate to dryness using a rotary evaporator. Dissolve the residue in



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the minimum volume of a 1 per cent v/v solution of *glacial acetic acid,* decolorise if necessary by the addition of sufficient *decolorising charcoal*, shake and filter. To 0.25 ml of the resulting solution add 0.1 ml of a 1 per cent w/v solution of *cupric sulphate* and 0.05 ml of *2 M sodium hydroxide*; an olive-green colour is produced.

*The constituted suspension complies with the tests stated under Oral liquids and with the following tests.*

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Shake an accurately weighed quantity of the oral suspension containing about 0.25 g of anhydrous cephalexin with 100.0 ml of *water* for 30 minutes, add sufficient of *water* to produce 250.0 ml and filter. Dilute 25.0 ml of the filtrate to 50.0 ml with *water*.

*Reference solution (a).* A 0.05 per cent w/v solution of *cephalexin RS* in *water.*

*Reference solution (b).* A solution containing 0.01 per cent w/v each of *cephalexin RS* and *cephradine RS* in *water.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Nucleosil C 18),
- mobile phase: a mixture of 2 volumes of *methanol*, 5 volumes of *acetonitrile*, 10 volumes of a 13.6 g per litre solution of *potassium dihydrogen phosphate* and 83 volumes of *water*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (b).Adjust the sensitivity so that the height of the peaks in the chromatogram obtained is at least half the full-scale deflection on the recorder. The test is not valid unless the resolution between the peaks corresponding to cephalexin and cephradine is at least 4.0.

Inject reference solution (a). The relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject alternately the test solution and reference solution (a).

Determine the weight per ml (2.4.29) of the suspension and calculate the content of $C_{16}H_{17}N_3O_4S$, weight in volume.

Repeat the procedure using a portion of the suspension that has been stored at the temperature and for the period stated on the label during which it may be expected to be satisfactory for use.

**Storage**. Store protected from moisture, at a temperature not exceeding 30°.

**Labelling**. The label states the strength in terms of the equivalent amount of anhydrous cephalexin.

## Cephalexin Tablets

Cephalexin Tablets contain not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of anhydrous cephalexin, $C_{16}H_{17}N_3O_4S$.

**Usual strengths**. 250 mg; 500 mg.

### Identification

Remove any coating. Shake a quantity of the powdered tablet cores containing 0.5 g of anhydrous cephalexin with 1 ml of *water* and 1.4 ml of *1 M hydrochloric acid*, add 0.1 g of *decolorising charcoal,* shake, filter and wash the filter with 1 ml of *water.* Add slowly to the filtrate a saturated solution of *sodium acetate* until precipitation occurs. Add 5 ml of *methanol*, filter and wash the precipitate with two quantities, each of 1 ml, of *methanol.* The residue, after drying at a pressure not exceeding 0.7 kPa, complies with the following test.

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

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

### Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF* . Impregnate the plate by development with a 5 per cent v/v solution of *n-tetradecane* in hexane. Allow the solvent to evaporate and carry out the chromatography in the same direction as the impregnation.

*Mobile phase.* A mixture of 3 volumes of *acetone*, 80 volumes of a 7.2 per cent w/v solution of *disodium hydrogen orthophosphate* and 120 volumes of a 2.1 per cent w/v solution of *citric acid.*

*Test solution.* Shake a quantity of the powdered tablets containing 0.25 g of anhydrous cephalexin with 10 ml of *2 M hydrochloric acid*, filter and use the filtrate.

*Reference solution (a).* Dilute 1 ml of the test solution to 100 ml with *2 M hydrochloric acid.*

*Reference solution (b).* A 0.025 per cent w/v solution of *7-aminodesacetoxycephalosporanic acid RS* in *2 M hydrochloric acid.*

*Reference solution (c).* A 0.025 per cent w/v solution of DL-*phenylglycine* in *2 M hydrochloric acid.*

*Reference solution (d).* A solution containing 2.5 per cent w/v of *cephalexin RS* and 0.025 per cent w/v each of *7-amino-*



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*desacetoxycephalosporanic acid RS* and DL-*phenylglycine* in *2 M hydrochloric acid.*

Apply separately to the plate 5 µl of each solution. After development, dry the plate at 90° for 3 minutes, spray the hot plate with a 0.1 per cent w/v solution of *ninhydrin* in the mobile phase, heat the plate at 90° for 15 minutes and allow to cool. In the chromatogram obtained with the test solution any spot corresponding to 7-aminodesacetoxycephalosporanic acid is not more intense than the spot in the chromatogram obtained with reference solution (b) (1.0 per cent), any spot corresponding to DL-phenylglycine is not more intense than the spot in the chromatogram obtained with reference solution (c) (1.0 per cent) and any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (a) (1.0 per cent). The test is not valid unless the chromatogram obtained with reference solution (d) shows three clearly separated spots.

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *water* freshly prepared by distillation,
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc with an average pore diameter not greater than 1.0 µm. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate with *water.* Measure the absorbance of the resulting solution at the maximum at about 261 nm *(*2.4.7). Calculate the content of $C_{16}H_{17}N_3O_4S$ taking 235 as the specific absorbance at 261 nm.

D. Not less than 75 per cent of the stated amount of $C_{16}H_{17}N_3O_4S$.

### Tests

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh and powder 20 tablets. Shake a quantity of the powdered tablets containing about 0.25 g of anhydrous cephalexin with 100.0 ml of *water* for 30 minutes, add sufficient amount of *water* to produce 250.0 ml and filter. Dilute 25.0 ml of the filtrate to 50.0 ml with *water.*

*Reference solution (a).* A 0.05 per cent w/v solution of *cephalexin RS* in *water.*

*Reference solution (b).* A solution containing 0.01 per cent w/v each of *cephalexin RS* and *cephradine RS* in *water.*

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Nucleosil C 18),
- mobile phase: a mixture of 2 volumes of *methanol*, 5 volumes of *acetonitrile*, 10 volumes of a 13.6 g per litre solution of *potassium dihydrogen phosphate* and 83 volumes of *water*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (b). Adjust the sensitivity so that the height of the peaks in the chromatogram obtained is at least half the full-scale deflection on the recorder. The test is not valid unless the resolution between the peaks corresponding to cephalexin and cephradine is at least 4.0.

Inject reference solution (a) six times. The relative standard deviation is not more than 1.0 per cent.

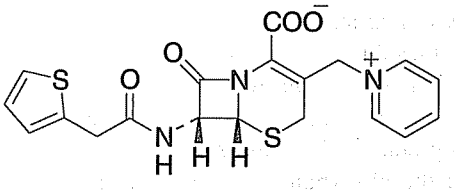
Inject alternately the test solution and reference solution (a).

Calculate the content of $C_{16}H_{17}N_3O_4S$ in the tablets.

**Storage**. Store protected from light and moisture at a temperature not exceeding 30°.

**Labelling**. The label states the strength in terms of the equivalent amount of anhydrous cephalexin. If the tablets are dispersible, the label also states that the tablets should be dispersed in water immediately before use.

## Cephaloridine




$C_{19}H_{17}N_3O_4S_2$ Mol. Wt. 415.5

Cephaloridine is (7*R*)-3-(1-pyridiniomethyl)-7-[(2-thienyl)-acetamido]-3-cephem-4-carboxylate (α-form or δ-form).

Cephaloridine contains not less than 96.0 per cent and not more than 102.0 per cent of $C_{19}H_{17}N_3O_4S_2$, calculated on the anhydrous basis.

**Category**. Antibacterial.

**Dose**. By intravenous, intramuscular or deep subcutaneous injection, 1 to 4 g daily, in divided doses.

**Description**. A white or almost white, crystalline powder; odour, slight and resembling that of pyridine.

### Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cephaloridine (α-form) RS* or *cephaloridine (δ-form) RS* or with the reference spectrum of cephaloridine (α-form) *or* cephaloridine (δ-form).



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B. Mix 20 mg with a few drops of an 80 per cent v/v solution of *sulphuric acid* containing 1 per cent v/v of *nitric acid;* a bluish-green colour is produced.

C. To a 0.5 per cent w/v solution add 1 ml of *chloramine solution* and 2 ml of *0.1 M sodium hydroxide*; a dull red colour is produced which persists for 1 minute.

D. Gives the reactions of penicillins and cephalosporins (2.3.1).

### Tests

**Appearance of solution** (2.4.1). Solution A is clear.

**pH** (2.4.24). 4.0 to 6.0, determined in a 10.0 per cent w/v solution (solution A) prepared by dissolving in *carbon dioxide-free water,* warming to 30° and cooling to 20°.

**Specific optical rotation** (2.4.22). +46.0° to +50.0°, determined at 25° in a 1.0 per cent w/v solution.

**Light absorption**. When examined in the range 230 nm to 360 nm (2.4.7) a 0.0012 per cent w/v solution shows absorption maxima at about 240 nm and 255 nm; absorbance at the maximum at about 240 nm, 0.43 to 0.48. The ratio of the absorbance at the maximum at about 240 nm to that at about 255 nm is not more than 1.10.

**Pyridine**. Dissolve about 25 mg in 10 ml of *water* and add 2.5 ml of a buffer solution prepared by adjusting a 5 per cent w/v solution of *disodium hydrogen phosphate* to pH 6.0 with *phosphoric acid* and adding 1 per cent v/v of *aniline*. Add 1.25 ml of a solution prepared by decolorising a 0.5 per cent v/v solution of *bromine* with *potassium cyanide solution*, shaking and allowing to stand for 2 minutes, and sufficient *water* to produce 25 ml and allow to stand for 25 minutes. Measure the absorbance of the resulting solution at the maximum at about 462 nm, using as the blank a solution prepared in a similar manner but omitting the substance under examination (2.4.7). The absorbance is not more than that of a solution prepared by treating 2.5 ml of a 0.005 per cent w/v solution of *pyridine* in a similar manner.

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Water** (2.3.43). Not more than 0.5 per cent w/w (α-form) and not more than 3.0 per cent w/w (δ-form), determined on 0.25 g. Use as the solvent a mixture of equal volumes of dehydrated methanol and dehydrated pyridine in place of methanol.

**Assay**. Weigh accurately about 60 mg and dissolve in sufficient *water* to produce 50.0 ml. Transfer 10.0 ml to a stoppered flask, add 5 ml of *1 M sodium hydroxide* and allow to stand for 20 minutes. Add 20 ml of a buffer solution containing 35.0 per cent w/v of *sodium acetate* and 42.4 per cent v/v of *glacial acetic acid*, 5 ml of *1 M hydrochloric acid* and 25.0 ml of *0.01 M iodine*, close the flask with a wet stopper and allow to stand for 3 hours in a water-bath at 30°, protected from light. Titrate the excess of iodine with *0.02 M sodium thiosulphate* using *starch solution*, added towards the end of the titration, as indicator. To a further 10.0 ml of the solution add 20 ml of the buffer solution and 25.0 ml of *0.01 M iodine*, allow to stand for 3 hours in a water-bath at 30°, protected from light. Titrate the excess of iodine with *0.02 M sodium thiosulphate* using *starch* solution, added towards the end of the titration, as indicator. The difference between the titrations represents the volume of *0.01 M iodine* equivalent to the cephaloridine present. Calculate the content of $C_{19}H_{17}N_3O_4S_2$ from the difference obtained by simultaneously carrying out the Assay using *cephaloridine (δ-form) RS* instead of the substance under examination.

Cephaloridine intended for use in the manufacture of parenteral preparations complies with the following additional tests.

**Pyrogens**. Complies with the test for pyrogens (2.2.8), using not less than 50 mg per kg of the rabbit's weight, dissolved in 1 ml of Water for injections.

**Sterility** (2.2.11). Complies with the test for sterility.

**Storage**. Store protected from light and moisture in a refrigerator (8° to 15°). If the material is intended for use in the manufacture of parenteral preparations, the container should be sterile, tamper-evident and sealed so as to exclude micro-organisms.

**Labelling**. The label states (1) whether the contents are Cephaloridine (α-form) or Cephaloridine (δ-form); (2) whether or not it is intended for use in the manufacture of injectable preparations.

## Cephaloridine Injection

Cephaloridine Injection is a sterile material consisting of Cephaloridine with or without auxiliary substances. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of Water for Injections immediately before use.

*The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).*

**Storage**. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Cephaloridine Injection contains not less than 90.0 per cent and not more than 105.0 per cent of the stated amount of anhydrous cephaloridine, $C_{19}H_{17}N_3O_4S_2$.

**Usual strengths**. 250 mg; 500 mg; 1 g.

**Description**. A white or almost white, crystalline powder; odour, slight and resembling that of pyridine.



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*The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.*

### Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cephaloridine (α-form) RS* or *cephaloridine (δ-form) RS* or with the reference spectrum of cephaloridine (α-form) *or* cephaloridine (δ-form).

B. Mix 20 mg with a few drops of an 80 per cent v/v solution of *sulphuric acid* containing 1 per cent v/v of *nitric acid;* a bluish-green colour is produced.

C. To a 0.5 per cent w/v solution add 1 ml of *chloramine solution* and 2 ml of *0.1 M sodium hydroxide*; a dull red colour is produced which persists for 1 minute.

D. Gives the reactions of penicillins and cephalosporins (2.3.1).

### Tests

**Appearance of solution** (2.4.1). Solution A is clear.

**pH** (2.4.24). 4.0 to 6.0, determined in a 10.0 per cent w/v solution (solution A) prepared by dissolving in *carbon dioxide-free water,* warming to 30° and cooling to 20°.

**Specific optical rotation** (2.4.22). +46.0° to +50.0°, determined at 25° in a 1.0 per cent w/v solution.

**Light absorption**. When examined in the range 230 nm to 360 nm (2.4.7) a 0.0012 per cent w/v solution shows absorption maxima at about 240 nm and 255 nm; absorbance at the maximum at about 240 nm, 0.43 to 0.48. The ratio of the absorbance at the maximum at about 240 nm to that at about 255 nm is not more than 1.10.

**Pyridine**. Dissolve about 25 mg in 10 ml of *water* and add 2.5 ml of a buffer solution prepared by adjusting a 5 per cent w/v solution of *disodium hydrogen phosphate* to pH 6.0 with *phosphoric acid* and adding 1 per cent v/v of *aniline*. Add 1.25 ml of a solution prepared by decolorising a 0.5 per cent v/v solution of *bromine* with *potassium cyanide solution*, shaking and allowing to stand for 2 minutes, and sufficient *water* to produce 25 ml and allow to stand for 25 minutes. Measure the absorbance of the resulting solution at the maximum at about 462 nm, using as the blank a solution prepared in a similar manner but omitting the substance under examination (2.4.7). The absorbance is not more than that of a solution prepared by treating 2.5 ml of a 0.005 per cent w/v solution of *pyridine* in a similar manner.

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Water** (2.3.43). Not more than 0.5 per cent w/w (α-form) and not more than 3.0 per cent w/w (δ-form), determined on 0.25 g. Use as the solvent a mixture of equal volumes of *dehydrated methanol* and dehydrated pyridine in place of *methanol.*

**Assay**. Determine the weight of the contents of 10 containers Weigh accurately a quantity of the mixed contents of the 10 containers containing about 60 mg of cephaloridine and dissolve in sufficient *water* to produce 50.0 ml. Transfer 10.0 ml to a stoppered flask, add 5 ml of *1 M sodium hydroxide* and allow to stand for 20 minutes. Add 20 ml of a buffer solution containing 35.0 per cent w/v of *sodium acetate* and 42.4 per cent v/v of *glacial acetic acid*, 5 ml of *1 M hydrochloric acid* and 25.0 ml of *0.01 M iodine*, close the flask with a wet stopper and allow to stand for 3 hours in a water-bath at 30°, protected from light. Titrate the excess of iodine with *0.02 M sodium thiosulphate* using *starch solution*, added towards the end of the titration, as indicator. To a further 10.0 ml of the solution add 20 ml of the buffer solution and 25.0 ml of *0.01 M iodine*, allow to stand for 3 hours in a water-bath at 30°, protected from light. Titrate the excess of iodine with *0.02 M sodium thiosulphate* using *starch* solution, added towards the end of the titration, as indicator. The difference between the titrations represents the volume of *0.01 M iodine* equivalent to the cephaloridine present. Calculate the content of $C_{19}H_{17}N_3O_4S_2$ from the difference obtained by simultaneously carrying out the Assay using *cephaloridine (δ–form) RS* instead of the substance under examination.

Cephaloridine intended for use in the manufacture of parenteral preparations complies with the following additional tests.

**Pyrogens**. Complies with the test for pyrogens (2.2.8), using not less than 50 mg per kg of the rabbit's weight, dissolved in 1 ml of Water for Injections.

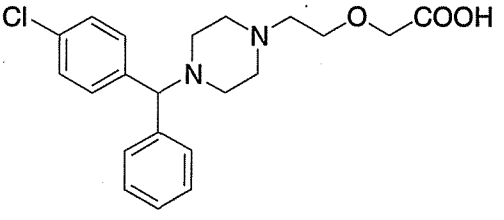
**Sterility** (2.2.11). Complies with the test for sterility.

**Storage**. Store protected from light and moisture at a temperature not exceeding 30°. The constituted solution should be used within 24 hours when stored at a temperature not exceeding 20° or within 4 days when stored in a refrigerator (2° to 8°).

**Labelling**. The label states (1) the weight of Cephaloridine contained in the sealed container; (2) whether the contents are Cephaloridine (α-form) or Cephaloridine (δ-form).

## Cetirizine Hydrochloride

Cetirizine Dihydrochloride




, 2HCl

$C_{21}H_{25}ClN_2O_3,2HCl$ Mol. Wt. 461.8



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Cetirizine Hydrochloride is [2-[4-[(4-chlorophenyl) phenylmethyl]-1-piperazinyl]ethoxy]acetic acid dihydrochloride.

Cetirizine Hydrochloride contains not less than 99.0 per cent and not more than 100.5 per cent of $C_{21}H_{25}ClN_2O_3,2HCl$, calculated on the dried basis.

**Category.** Antihistaminic.

**Description.** A white or almost white powder.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cetirizine hydrochloride RS* or with the reference spectrum of cetirizine hydrochloride.

B. Dissolve 20.0 mg in 50 ml of a 1.03 per cent w/v solution of *hydrochloric acid* and dilute to 100.0 ml with the same acid. Dilute 10.0 ml of this solution to 100.0 ml with the acid.

When examined in the range 210 nm to 350 nm (2.4.7), the resulting solution shows an absorption maximum at about 231 nm. The specific absorbance at 231 nm is 359 to 381.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

*Mobile phase.* A mixture of 1 volume of *ammonia, 10* volumes of *methanol* and 90 volumes of *dichloromethane.*

*Test solution.* Dissolve 10 mg of the substance under examination in *water* and dilute to 5 ml with the same solvent.

*Reference solution (a).* Dissolve 10 mg of *cetirizine hydrochloride RS* in *water* and dilute to 5 ml with the same solvent.

*Reference solution (b).* Dissolve 10 mg of *chlorphenamine maleate RS* in *water* and dilute to 5 ml with the same solvent. To 1 ml of the solution add 1 ml of reference solution (a).

Apply to the plate 5 µl of each solution. After development, dry in a current of cold air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows 2 clearly separated spots.

D. It gives reaction A of chlorides (2.3.1).

## Tests

**Appearance of solution.** A 5.0 per cent w/v solution in *carbon dioxide-free water* (solution A) is clear (2.4.1) and not more intensely coloured than reference solution BYS7 (2.4.1).

**pH** (2.4.24). 1.2 to 1.8, determined in solution A.

**Related substances.** Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve 20 mg of the substance under examination in the mobile phase and dilute to 100 ml with the mobile phase.

*Reference solution (a).* A solution containing 0.02 per cent w/v each of *cetirizine dihydrochloride RS* and *(RS)-1-[(4-chlorophenyl)phenylmethyl]piperazine RS ( cetirizine impurity A)* in the mobile phase. Dilute 1 ml of the solution to 100 ml with the mobile phase.

*Reference solution (b).* Dilute 2 ml of the test solution to 50 ml with the mobile phase. Dilute 5 ml of the solution to 100 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with silica gel (5 µm),
- mobile phase: a mixture of 0.4 volume of *dilute sulphuric acid,* 6.6 volumes of *water* and 93 volumes of *acetonitrile,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 230 nm.
- injection volume. 20 µl.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to cetirizine and impurity A is not less than 2 and the tailing factors are not more than 2.0.

Inject the test solution and reference solution (b). Run the chromatogram for 3 times the retention time of cetirizine. In the chromatogram obtained with the test solution, the area of any impurity peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The sum of the areas of all such peaks is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent). Ignore any peak with an area 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.02 per cent).

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 100° to 105°.

**Assay.** Weigh accurately about 0.1 g, dissolve in 70 ml of a mixture of 30 volumes of *water* and 70 volumes of *acetone.* Titrate with *0.1 M sodium hydroxide* to the second point of inflexion. Determine the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.01539 g of $C_{21}H_{27}Cl_3N_2O_3$.



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**Storage**. Store protected from light.

## Cetirizine Syrup

Cetirizine Oral Liquid

Cetirizine Syrup contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cetirizine hydrochloride $C_{21}H_{25}ClN_2O_3$, 2HCl.

**Usual strength**. 5 mg per ml.

### Identification

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

### Tests

**pH** (2.4.24). 4.5 to 5.5.

**Other tests**. Complies with the tests stated under Oral Liquids.
**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. 60 volumes of *water* and 40 volumes of *acetonitrile*.

*Test solution*. Weigh accurately a quantity of the syrup containing 5 mg of Cetirizine Hydrochloride, dissolve in 100.0 ml of the solvent mixture and filter.

*Reference solution*. A 0.005 per cent w/v solution of *cetirizine dihydrochloride RS* in the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane chemically bonded to porous silica (5 µm),
- mobile phase: a mixture of 40 volumes of *acetonitrile* and 60 volumes of *0.0025 M 1-heptane sulphonic acid* prepared by dissolving 0.55 g of *1-heptane sulphonic acid sodium* in 1000 ml of *water*, adjusting the pH to 3.5 with *0.1 M sulphuric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

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

Inject the test solution and the reference solution.

Determine the weight per ml ( 2.4.29 ) of the syrup and calculate the content of $C_{21}H_{25}ClN_2O_3$,2HCl, weight in volume.

**Storage**. Store protected from light, at a temperature not exceeding 30°.

## Cetirizine Tablets

Cetirizine Hydrochloride Tablets

Cetirizine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cetirizine hydrochloride, $C_{21}H_{25}ClN_2O_3$.2HCl.

**Usual strengths**. 10 mg; 20 mg.

### Identification

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

### Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted with the dissolution medium if necessary, at the maximum at about 230 nm (2.4.7). Calculate the content of $C_{21}H_{25}ClN_2O_3$.2HCl in the medium from the absorbance obtained from a solution of known concentration of *cetirizine hydrochloride RS* in the same medium.

D. Not less than 75 per cent of the stated amount of $C_{21}H_{25}ClN_2O_3$.2HCl.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 20 mg of Cetirizine Hydrochloride, add 50 ml of the mobile phase, mix and dilute to 100 ml with the mobile phase.

*Reference solution (a)*. A solution containing 0.02 per cent w/v each of *cetirizine hydrochloride RS* and *(RS)-1-[(4-chlorophenyl)phenylmethyl]piperazine RS ( cetirizine impurity A)* in the mobile phase. Dilute 1 ml of the solution to 100 ml with the mobile phase.

*Reference solution (b)*. Dilute 1 ml of the test solution to 100 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with silica gel (5 µm),
- mobile phase: a mixture of 0.4 volume of *dilute sulphuric acid,* 6.6 volumes of *water* and 93 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,



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- spectrophotometer set at 230 nm,
- injection volume. 20 µl.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to cetirizine and impurity A is not less than 2 and the tailing factors not more than 2.0.

Inject the test solution and reference solution (b). Run the chromatogram 3 times the retention time of cetirizine. In the chromatogram obtained with the test solution the area of any impurity peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). The sum of the areas of all such peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore any peak with an area 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

**Uniformity of content**. Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay, using the following solution as the test solution.

*Test solution.* Disperse 1 tablet in the mobile phase, mix and dilute to 100.0 ml with the mobile phase, filter. Dilute 5.0 ml of the solution to 10.0 ml with mobile phase.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 25 mg of Cetirizine Hydrochloride, add the mobile phase, mix and dilute to 50.0 ml with the mobile phase, filter. Dilute 1.0 ml of the solution to 10.0 ml with mobile phase.

*Reference solution.* A 0.05 per cent w/v solution of *cetirizine hydrochloride RS* in the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm),
- mobile phase: dissolve 0.19 g of *heptane sulphonic acid sodium salt* in 300 ml *water* add 700 ml *acetonitrile* and mix. Adjust pH to 3.2 with *0.05 M sulphuric acid*, filter,
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, the column efficiency is not less than 2000 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternatively the test solution and the reference solution.

Calculate the content of $C_{21}H_{25}ClN_2O_3.2HCl$ in the tablets.

**Storage**. Store protected from moisture, at a temperature not exceeding 30°.

# Cetostearyl Alcohol

Cetostearyl Alcohol is a mixture of solid aliphatic alcohols consisting chiefly of stearyl and cetyl alcohols.

**Category**. Pharmaceutical aid (ointment base).

**Description**. A white or pale yellow, wax like mass, plates, flakes or granules.

## Identification

In the Assay, the two principal peaks in the chromatogram obtained with the test solution corresponds to the principal spots in the chromatogram obtained with the reference solution.

## Tests

**Melting range** (2.4.21). 47° to 56°, determined by Method II. Introduce the substance under examination into the capillary tubes and allow to stand at 2° to 8° for 12 hours before carrying out the determination.

**Appearance of solution**. Dissolve 0.5 g in 20 ml of boiling *ethanol (95 per cent).* The solution is clear (2.4.1) and not more intensely coloured than reference solution BS6 (2.4.1).

**Acid value** (2.3.23). Not more than 1.0.

**Hydroxyl value** (2.3.27). 208 to 228.

**Saponification value** (2.3.37). Not more than 2.0.

**Iodine value** (2.3.28). Not more than 3.0, determined by Method B in a 8.0 per cent w/v solution in *chloroform.*

**Hydrocarbons.** Dissolve 2.0 g in 100 ml of *light petroleum (40° to 60°)*, warming slightly if necessary, and transfer the solution to a column (25 cm x 10 mm) of *anhydrous alumina* which has been slurried with *light petroleum (40° to 60°).* Elute with two portions, each of 50 ml, of *light petroleum (40° to 60°)* into a flask, remove the light petroleum and dry at 80°; the residue weighs not more than 30 mg.

**Assay**. Determine by gas chromatography (2.4.13).

*Test solution.* Dissolve 0.1 g of the substance under examination in 10 ml of the *ethanol (95 per cent).*

*Reference solution.* A solution containing 0.6 per cent w/v of *cetyl alcohol RS* and 0.4 per cent w/v of *stearyl alcohol RS* in *ethanol (95 per cent).* Dilute 1.0 ml of this solution to 10.0 ml with the same solvent.



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Chromatographic system

- a glass column 30 m x 0.32 mm packed with poly(dimethyl)siloxane (1 µm),
- temperature:

| column | time (min) | temperature (°) |
|---|---|---|
| | 0-20 | 150- 250 |
| | 20-40 | 250 |

- Inlet port and detector at 250°,
- detector. flame ionization,
- flow rate. 1ml per minute using nitrogen as carrier gas,

Inject 1 µl of the reference solution. The test is not valid unless the resolution between the peaks due to cetyl alcohol and stearyl alcohol is not less than 5.0.

Inject the test solution and the reference solution.

Calculate the content of cetyl alcohol, $C_{16}H_{34}O$ and stearyl alcohol, $C_{18}H_{38}O$.

## Cetrimide

Cetrimide consists chiefly of tetradecyltrimethylammonium bromide together with smaller amounts of dodecyl- and hexadecyltrimethylammonium bromides.

Cetrimide contains not less than 96.0 per cent and not more than 101.0 per cent of alkyltrimethylammonium bromides, calculated as $C_{17}H_{38}BrN$ (336.4) on the dried basis.

**Category**. Pharmaceutical aid; bactericide.

**Description**. A white or creamy-white, voluminous, free-flowing powder; odour, faint and characteristic.

### Identification

A. To 10 ml of a 1 per cent w/v solution add 2 ml of *potassium ferricyanide solution*; a yellow precipitate is produced.

B. To 10 ml of a 1 per cent w/v solution add 2 ml of a 10 per cent w/v solution of *sodium silicate*; a white flocculent precipitate is produced.

C. To 10 ml of a 1 per cent w/v solution add 2 ml of *dilute nitric acid*; a yellow precipitate is produced. Filter and to the filtrate add 2 ml of *dilute nitric acid* and 1 ml of *silver nitrate solution*; a yellow precipitate is produced.

D. Dissolve 0.25 g in sufficient *ethanol (95 per cent)* to produce 25 ml. Absorbance of the resulting solution between 260 nm and 280 nm (2.4.7) is not more than 0.05.

### Tests

**Appearance of solution**. A 2.0 per cent w/v solution is clear (2.4.1) and colourless (2.4.1).

**Acidity or alkalinity**. Dissolve 1.0 g in 50 ml of *water* and add 2 drops of *bromocresol purple solution*. Not more than 0.1 ml of either *0.1 M hydrochloric acid* or *0.1 M sodium hydroxide* is required to change the colour of the solution.

**Amine salts**. Carry out the Assay described below using a further 25.0 ml of the original solution and 10 ml of *0.1 M hydrochloric acid* instead of the *0.1 M sodium hydroxide*. The difference between the volume of *0.05 M potassium iodate* required in the titration and that required in the Assay is not more than 1.0 ml for each g of the substance used.

**Sulphated ash** (2.3.18). Not more than 0.5 per cent.

**Loss on drying** (2.4.19). Not more than 2.0 per cent, determined on 1.0 g by drying in an oven at 105° for 2 hours.

**Assay**. Weigh accurately about 2.0 g and dissolve in sufficient *water* to produce 100.0 ml. Transfer 25.0 ml of the solution to a separator, add 25 ml of *chloroform,* 10 ml of *0.1 M sodium hydroxide* and 10.0 ml of a freshly prepared 5.0 per cent w/v solution of *potassium iodide*. Shake well, allow to separate, and discard the chloroform layer. Shake the aqueous solution with three quantities, each of 10 ml, of *chloroform* and discard the chloroform solution. Add 40 ml of *hydrochloric acid*, allow to cool and titrate with *0.05 M potassium iodate* until the deep brown colour is almost discharged. Add 2 ml of *chloroform* and continue the titration, with shaking, until the chloroform layer no longer changes colour. Carry out a blank titration on a mixture of 20 ml of *water,* 10.0 ml of the freshly prepared *potassium iodide solution* and 40 ml of *hydrochloric acid.* The difference between the titrations represents the amount of potassium iodate required.

1 ml of *0.05 M potassium iodate* is equivalent to 0.03364 g of $C_{17}H_{38}BrN$.

## Cetrimide Cream

| | |
|---|---|
| Cetrimide | 5 g |
| Cetostearyl Alcohol | 50 g |
| Liquid Paraffin | 500 g |
| Purified Water | 1000 g |

Melt the Cetostearyl Alcohol and heat with the Liquid Paraffin to about 60°. Dissolve the Cetrimide in sufficient Purified Water to produce about 450 g. Add the aqueous solution to the oily phase when both are at about 60° and mix. Stir gently until cool, add sufficient of the Purified Water to produce 1000 g and mix.

Cetrimide Cream contains not less than 88.0 per cent and not more than 106.0 per cent w/w of the stated amount of cetrimide, $C_{17}H_{38}BrN$.

**Usual strength**. 0.1 per cent w/w.



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### Identification

Mix 1 g with 50 ml of *water*. The diluted cream complies with the following tests:

A. To 10 ml, add 2 ml of *potassium ferricyanide* solution; a yellow precipitate is produced.

B. Shake 3 ml of *water* with 1 ml of *1 M sulphuric acid,* 2 ml of *chloroform* and 0.5 ml of *methyl orange solution.* Add 2 ml of the diluted cream shake and allow to separate; a yellow colour develops in the chloroform layer.

### Tests

**Other tests**. Complies with the tests stated under Creams.

**Assay**. Weigh accurately a quantity of the cream containing 5 mg of Cetrimide add 10 ml of hot *water* and shake gently until dispersed. Add 5 ml of *1 M sulphuric acid,* 20 ml of *chloroform* and 0.25 ml of *dimethyl yellow solution* and titrate with *0.001 M dioctyl sodium sulphosuccinate.*

1 ml of *0.001 M dioctyl sodium sulphosuccinate* is equivalent to 0.0003364 g of $C_{17}H_{38}BrN$.

**Labelling**. The label states the strength as the percentage w/w of Cetrimide.

## Cetyl Alcohol

Palmityl Alcohol; *n*-Hexadecyl Alcohol; 1-Hexadecanol

Cetyl Alcohol is a mixture of solid alcohols consisting mainly of 1-hexadecanol, $C_{16}H_{34}O$.

**Category**. Pharmaceutical aid (stiffening, emulsifying and tablet coating agent).

**Description**. A white, unctuous mass, powder, flakes or granules; odour, slight.

### Identification

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

### Tests

**Melting range** (2.4.21). 46° to 52°, determined by Method II. Introduce the substance under examination into the capillary tubes and allow to stand at 2° to 8° for 12 hours before carrying out the determination.

**Appearance of solution**. Dissolve 0.5 g in boiling *ethanol (95 per cent)*, cool and dilute to 20 ml with the same solvent. The resulting solution is clear (2.4.1) and not more intensely coloured than reference solution BS6 (2.4.1).

**Acid value** (2.3.23). Not more than 1.0.

**Hydroxyl value** (2.3.27). 218 to 238.

**Saponification value** (2.3.37). Not more than 2.0.

**Iodine value** (2.3.28). Not more than 2.0, determined by Method B in a 8.0 per cent w/v solution in *chloroform.*

**Assay.** Determine by gas chromatography (2.4.13).

*Test solution*. Dissolve 0.1 g of the substance under examination in 10 ml of the *ethanol (95 per cent).*

*Reference solution (a).* Dissolve 50 mg of *cetyl alcohol RS* in 5.0 ml of the *ethanol (95 per cent).*

*Reference solution (b).* Dissolve 50 mg of *stearyl alcohol RS* in 10.0 ml of the *ethanol (95 per cent).*

*Reference solution (c).* Mix 1.0 ml each of reference solution (a) and (b) and dilute to 10.0 with the *ethanol (95 per cent).*

Chromatographic system

- a glass column 30 m x 0.32 mm packed with poly(dimethyl)siloxane (1µm),
- temperature:

| column | time (min) | temperature (°) |
|---|---|---|
| | 0-20 | 150-250 |
| | 20-40 | 250 |

- inlet port and detector at 250°,
- flame ionization detector,
- flow rate. 1ml per minute using nitrogen as carrier gas.

Inject 1µl of reference solution (c). The test is not valid unless the resolution between the peaks due to cetyl alcohol and stearyl alcohol is not less than 5.0.

Inject the test solution and reference solution (a).

Calculate the content of cetyl alcohol, $C_{16}H_{34}O$.

## Cetyl Palmitate

$CH_3(CH_2)_{14}COOCH_2(CH_2)_{14}CH_3$

$C_{32}H_{64}O_2$ Mol. Wt. 480.9

Cetyl Palmitate is hexadecyl palmitate.

Cetyl Palmitate is a mixture of $C_{14}$ to $C_{18}$ esters of lauric acid (dodecanoic), myristic acid (tetradecanoic), palmitic acid (hexadecanoic) and stearic acid (octadecanoic) (Cetyl esters wax).

Cetyl Palmitate contains not less than 10.0 per cent and not more than 20.0 per cent for cetyl palmitate 15; not less than 60.0 per cent and more than 70.0 per cent for cetyl palmitate 65 and not less than 90.0 per cent for Cetyl palmitate 95.

**Category**. Pharmaceutical aid.



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**Description**. A white or almost white, waxy plates, flakes or powder.

### Identification

In the Assay, the principal peaks in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with reference solution (a) and (b).

### Tests

**Appearance of solution**. A 20.0 per cent w/v solution in *dichloromethane*, is not more intensely coloured than reference solution YS6 (2.4.1).

**Melting point** (2.4.21). About 45° for Cetyl palmitate 15 and Cetyl palmitate 65 and about 52° for Cetyl palmitate 95.

**Acid value** (2.3.23). Not more than 4.0. Dissolve 10 g in 50 ml of the solvent mixture described by heating under reflux on a water-bath for 5 minutes.

**Hydroxyl value** (2.3.27). Not more than 20.

**Saponification value** (2.3.37). 105 to 120. Heat under reflux for 2 hours.

**Iodine value** (2.3.28). Not more than 2.0.

**Alkaline substances**. Dissolve 2.0 g with gentle heating in a mixture of 1.5 ml of *ethanol (95 per cent)* and 3 ml of *toluene*. Add 0.05 ml of a 4 per cent w/v solution of *bromophenol blue* in *ethanol (95 per cent)*. Not more than 0.4 ml of *0.01 M hydrochloric acid* is required to change the colour of the solution to yellow.

**Nickel**. Dissolve 10.0 g in sufficient *water* to produce 20 ml, add 3 ml of *bromine water* and 2 ml of a 20 per cent w/v solution of *citric acid*, mix and add 10 ml of *6 M ammonia* and 1 ml of a 1 per cent w/v solution of *dimethylglyoxime* in *ethanol (95 per cent)*. Mix, dilute to 50 ml with *water* and allow to stand for 5 minutes; any colour produced is not more intense than that produced by treating in the same manner and at the same time 1.0 ml of *nickel standard solution (10 ppm Ni)* diluted to 20 ml with *water* (1 ppm).

**Total ash** (2.3.19). Not more than 0.2 per cent.

**Water** (2.3.43). Not more than 0.3 per cent w/w, determined on 1.0 g using a mixture of equal volumes of *anhydrous methanol* and *dichloromethane*.

**Assay**. Determine by gas chromatography (2.4.13).

*Test solution*. Dissolve 25 mg of the substance under examination in 25 ml of *hexane*.

*Reference solution (a)*. Dissolve 25 mg of *cetyl palmitate 95 RS* in 25 ml of *hexane*.

*Reference solution (b)*. Dissolve 25 mg of *cetyl palmitate 15 RS* in 25 ml of *hexane*.

Chromatographic system
- a capillary column 10 m x 0.53 mm, packed with poly(dimethyl)siloxane (film thickness 2.65 µm),
- temperature:
  column100° to 300° @10° per minute,
  inlet port and detector 350°,
- a flame ionisation detector,
- flow rate. 6.5 ml per minute using nitrogen as carrier gas.

The relative retention time with reference to cetyl palmitate is about 9 minutes, for cetyl alcohol is about 0.3, for palmitic acid is about 0.4, for lauric ester is about 0.8, for myristic ester is about 0.9 and for stearic ester is about 1.1

Inject 1 µl of reference solution (b). The test is not valid unless the resolution between the peaks due to cetyl palmitate and cetyl stearate is not less than 1.5.

Inject 1 µl each of reference solution (a), (b) and the test solution.

**Storage**. Store protected from moisture, at a temperature not exceeding 30°.

**Labelling**. The label states the type of cetyl palmitate.

## Activated Charcoal

Decolorising Charcoal

Activated Charcoal is obtained from vegetable matter by suitable carbonisation processes intended to confer a high adsorbing power.

**Category**. Adsorbent.

**Dose**. 50 g.

**Description**. A light, black powder, free from grittiness; odourless.

### Identification

A. When heated to redness, burns slowly without flame.

B. Complies with the test for Adsorbing power.

### Tests

**Acidity or alkalinity**. Boil 2.0 g with 40 ml of *water* for 5 minutes. Cool, restore to the original volume with *carbon dioxide-free water* and filter, discarding the first 20 ml of the filtrate. To 10 ml of the filtrate add 0.25 ml of *bromothymol blue solution* and 0.25 ml of *0.02 M sodium hydroxide*. The solution is blue and not more than 0.75 ml of *0.02 M hydrochloric acid* is required to change the colour to yellow.

**Acid-soluble substances**. Boil 1.0 g with a mixture of 20 ml of *water* and 5 ml of *hydrochloric acid* for 5 minutes, filter whilst



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hot and collect the filtrate in a previously weighed porcelain crucible, wash the residue with 10 ml of hot *water*, adding the washing to the filtrate. To the combined filtrate and washing add 1 ml of *hydrochloric acid*, evaporate to dryness and ignite gently to constant weight; the residue weighs not more than 30 mg.

**Ethanol-soluble substances**. Boil 2.0 g with 50 ml of *ethanol (95 per cent)* under a reflux condenser for 10 minutes. Filter immediately, cool and adjust the volume to 50 ml with *ethanol (95 per cent)*. The filtrate is not more intensely coloured than reference solution BYS6 or YS6 (2.4.1). Evaporate 40 ml of the filtrate to dryness; the residue, after drying to constant weight at 105°, weighs not more than 8 mg.

**Alkali-soluble coloured matter**. Boil 0.25 g with 10 ml of *2 M sodium hydroxide* for 1 minute, cool and filter; the filtrate, when diluted to 10 ml with *water*, is not more intensely coloured than reference solution GYS4, (2.4.1).

**Chlorides** (2.3.12). Boil 3.0 g with 75 ml of *water* for 5 minutes, cool. Dilute to 100.0 ml with water and filter; 6.0 ml of the filtrate complies with the limit test for chlorides. (0.14 per cent).

**Sulphates** (2.3.17). 10.0 ml of the filtrate obtained in the test for Chloride complies with the limit test for sulphates (450 ppm).

**Sulphide**. Heat 1.0 g with a mixture of 20 ml of *water* and 5 ml of *7 M hydrochloric acid* to boiling; the fumes evolved do not turn *lead acetate paper* brown.

**Uncarbonised constituents**. Boil 0.25 g with 10 ml of *1 M sodium hydroxide* for few seconds and filter; the filtrate is colourless.

**Copper**. Determine by atomic absorption spectrophotometry (2.4.2), measuring at 325.0 nm using an air-acetylene flame and a solution prepared in the following manner. Boil 2.0 g with 50 ml of *2 M hydrochloric acid* under a reflux condenser for 1 hour. Filter, wash the filter with *2 M hydrochloric acid* and evaporate the combined filtrate to dryness on a water-bath. Dissolve the residue in sufficient *0.1 M hydrochloric acid* to produce 50.0 ml. Use *copper solution AAS*, suitably diluted with *0.1 M hydrochloric acid*, for preparing the standard solutions (25 ppm).

Reserve the solution for the tests for Lead and Zinc.

**Lead**. Determine by atomic absorption spectrophotometry (2.4.2), measuring at 283.3 nm or 217.0 nm using an air-acetylene flame. Use the solution prepared in the test for Copper as the test solution and *lead solution AAS*, suitably diluted with *0.1 M hydrochloric acid*, for preparing the standard solutions (10 ppm).

**Zinc**. Determine by atomic absorption spectrophotometry (2.4.2), measuring at 214.0 nm using an air-acetylene flame. Use the solution prepared in the test for Copper as the test solution and *zinc solution AAS*, suitably diluted with *0.1 M hydrochloric acid*, for preparing the *standard solutions* (25 ppm).

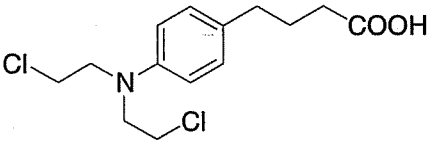
**Sulphated ash** (2.3.18). Not more than 5.0 per cent.

**Loss on drying** (2.4.19). Not more than 15.0 per cent, determined on 1.0 g by drying in an oven at 120° for 4 hours.

**Adsorbing power**. Not less than 40 per cent of its own weight of *phenazone*, calculated on the dried basis, determined by the following method. To 0.3 g add 25 ml of a freshly prepared 1 per cent w/v solution of *phenazone*, shake thoroughly for 15 minutes, filter and discard the first 5 ml of the filtrate. To 10 ml of the filtrate add 1 g of *potassium bromide* and 20 ml of *2 M hydrochloric acid* and titrate with *0.0167 M potassium bromate*, using 0.1 ml of *methyl red solution* as indicator, until the colour changes from reddish pink to yellowish pink and titrate slowly towards the end of the titration (*a* ml). Repeat the titration using 10 ml of the phenazone solution beginning at the words "add 1 g..... titration" (*b* ml). Calculate the percentage of phenazone adsorbed with reference to the dried substance using the expression 2.353 $(a-b)/w$ where $w$ is the weight, in g, of the substance under examination.

**Storage**. Store protected from moisture.

## Chlorambucil




$C_{14}H_{19}Cl_2NO_2$ Mol. Wt. 304.2

Chlorambucil is 4-[4-bis(2-chloroethyl)amino]phenylbutyric acid.

Chlorambucil contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{14}H_{19}Cl_2NO_2$, calculated on the anhydrous basis.

**Category**. Anticancer.

**Dose**. 100 to 200 mg per kg of body weight daily for 4 to 8 weeks.

**Description**. A white, crystalline powder.

*CAUTION — Chlorambucil must be handled with care; contact with the skin and inhalation of airborne particles must be avoided.*

### Identification

*Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.*



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A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *chlorambucil RS*.

B. Shake 0.4 g with 10 ml of *2 M hydrochloric acid* and allow to stand for 30 minutes, shaking occasionally. Filter, wash the residue with two quantities, each of 10 ml, of *water* and add 0.5 ml of *potassium mercuri-iodide solution* to 10 ml of the mixed filtrate and washings; a buff precipitate is produced. To a further 10 ml add 0.5 ml of *potassium permanganate solution*; the purple colour is immediately discharged.

C. Dissolve 50 mg in 5 ml of *acetone* and dilute to 10 ml with *water*. Add 0.05 ml of *2 M nitric acid* and 0.2 ml of *dilute silver nitrate solution*; no opalescence is produced immediately. Heat on a water-bath; an opalescence is produced.

### Tests

**Related substances**. *Carry out all operations as rapidly as possible, protected from light, and prepare the solutions immediately before use.*

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 40 volumes of *toluene*, 25 volumes of *methanol*, 20 volumes of *2-butanone* and 20 volumes of *n-heptane*.

*Test solution*. A 2 per cent w/v solution in *acetone*.

*Reference solution (a)*. Dilute 1 ml of the test solution to 50 ml with *acetone*.

*Reference solution (b)*. Dilute 5 ml of reference solution (a) to 20 ml with *acetone*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 0.5 per cent, determined on 1.0 g.

**Assay**. Weigh accurately about 0.2 g, dissolve in 10 ml of *acetone*, add 10 ml of *water* and titrate with *0.1 M sodium hydroxide* using *dilute phenolphthalein solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.03042 g of $C_{14}H_{19}Cl_2NO_2$.

**Storage**. Store protected from light.

## Chlorambucil Tablets

Chlorambucil Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of chlorambucil, $C_{14}H_{19}Cl_2NO_2$. The tablets are coated.

**Usual strengths**. 2 mg; 5 mg.

### Identification

Shake 0.4 g of the powdered tablets with 10 ml of *2 M hydrochloric acid* and allow to stand for 30 minutes, shaking occasionally. Filter, wash the residue with two quantities, each of 10 ml, of *water* and add 0.5 ml of *potassium mercuri-iodide solution* to 10 ml of the mixed filtrate and washings; a buff precipitate is produced. To a further 10 ml add 0.5 ml of *potassium permanganate solution*; the purple colour is immediately discharged.

### Tests

**Uniformity of content**. Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve one tablet as completely as possible in 10 ml of *0.1 M hydrochloric acid*, add 40 ml of *acetonitrile* and mix in an ultrasonic bath for 5 minutes. Add sufficient *acetonitrile* to produce a solution containing 0.002 per cent w/v of Chlorambucil. Filter the solution, preferably through a glass microfibre filter paper (such as Whatman GF/C), discarding the first 20 ml of the filtrate, and use the filtrate.

*Reference solution*. A 0.002 per cent w/v solution of *chlorambucil RS* in a mixture of 90 volumes of *acetonitrile* and 10 volumes of *0.1 M hydrochloric acid*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of *acetonitrile* and 40 volumes of *0.02 M potassium dihydrogen phosphate*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Calculate the content of $C_{14}H_{19}Cl_2NO_2$ in the tablet.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh and powder 20 tablets. Dissolve as completely as possible a quantity of the powder containing about 10 mg of Chlorambucil in a mixture of 25 ml of *0.1 M hydrochloric acid* and 100 ml of *acetonitrile* by mixing in an ultrasonic bath for at least 10 minutes. Dilute to 250.0 ml with



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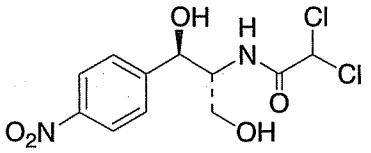
*acetonitrile* and filter the solution, preferably through a glass microfibre filter paper (such as Whatman GF/C), discarding the first 20 ml of the filtrate. Dilute 50.0 ml of the filtrate to 100.0 ml with a mixture of 90 volumes of *acetonitrile* and 10 volumes of *0.1 M hydrochloric acid.*

*Reference solution.* A 0.002 per cent w/v solution of *chlorambucil RS* in a mixture of 90 volumes of *acetonitrile* and 10 volumes of *0.1 M hydrochloric acid.*

Carry out the chromatographic procedure described under Uniformity of content.

Calculate the content of $C_{14}H_{19}Cl_2NO_2$ in the tablets.

# Chloramphenicol




$C_{11}H_{12}Cl_2N_2O_5$ Mol. Wt. 323.1

Chloramphenicol is 2,2-dichloro-*N*-[(1*R*,2*R*)-2-hydroxy-1-hydroxymethyl-2-(4-nitrophenyl)ethyl]acetamide. It is produced by the growth of certain strains of *Streptomyces venezuelae* in a suitable medium, but is normally prepared by synthesis.

Chloramphenicol contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{11}H_{12}Cl_2N_2O_5$, calculated on the dried basis.

**Category.** Antibacterial.

**Dose.** For an adult, 1.5 to 3 g daily, in divided doses; for a child, 25 to 50 mg per kg of body weight daily, in divided doses.

**Description.** A white to greyish-white or yellowish-white, fine crystalline powder or fine-crystals, needles or elongated plates; odourless.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

A. Determine by infrared absorption spectr*ophotometry* (2.4.6). Compare the spectrum with that obtained with *chloramphenicol RS* or with the reference spectrum of chloramphenicol.

B. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

C. Dissolve 10 mg in 1 ml of *ethanol (50 per cent)*, add 3 ml of a 1 per cent w/v solution of *calcium chloride* and 50 mg of *zinc powder* and heat on a water-bath for 10 minutes. Decant the clear supernatant liquid into a test-tube, add 0.1 g of *anhydrous sodium acetate* and 0.1 ml of *benzoyl chloride,* shake for 1 minute and add 0.5 ml of a 10.5 per cent w/v solution of *ferric chloride hexahydrate* and, if necessary, add sufficient *dilute hydrochloric acid* to produce a clear solution; a red-violet to purple colour is produced. Repeat the test omitting the zinc powder; no red colour is produced.

D. Heat 50 mg with 2 ml of *ethanolic potassium hydroxide solution* in a covered test-tube on a water-bath for 15 minutes; the resulting solution gives the reactions of chlorides (2.3.1).

## Tests

**pH** (2.4.24). 4.5 to 7.5, determined in a suspension prepared by shaking 50 mg with 10 ml of *carbon dioxide-free water.*

**Specific optical rotation** (2.4.22). +17.0° to +20.0°, determined in a 5.0 per cent w/v solution in *ethanol.*

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

*Mobile phase.* A mixture of 90 volumes of *chloroform,* 10 volumes of *methanol* and 1 volume of *water.*

*Test solution.* Dissolve 1 g of the substance under examination in 100 ml of *acetone.*

*Reference solution (a).* A 1 per cent w/v solution of *chloramphenicol RS* in *acetone.*

*Reference solution (b).* Dilute 0.5 ml of reference solution (a) to 100 ml with *acetone.*

Apply to the plate 1 µl and 20 µl of the test solution, 1 µl of reference solution (a) and 20 µl of reference solution (b). After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with 20 µl of the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b).

**Chlorides** (2.3.12). To 2.0 g add 20 ml of *water* and 10 ml of *nitric acid* and shake for 5 minutes. Filter through a filter paper previously washed by filtering 5-ml quantities of *water* until 5 ml of the filtrate is no longer opalescent on addition of 0.1 ml of *nitric acid* and 0.1 ml of a 4.25 per cent w/v solution of *silver nitrate.* The resulting filtrate complies with the limit test for chlorides (125 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay.** Weigh accurately about 0.125 g and dissolve in sufficient *water* to produce 250.0 ml. Dilute 10.0 ml with



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sufficient *water* to produce 250.0 ml. Measure the absorbance of the resulting solution at the maximum at about 278 nm (2.4.7). Calculate the content of $C_{11}H_{12}Cl_2N_2O_5$ taking 297 as the specific absorbance at 278 nm.

*Chloramphenicol intended for use in the manufacture of parenteral preparations without a further process for the removal of bacterial endotoxins complies with the following additional requirement.*

**Bacterial endotoxins** (2.2.3). Not more than 0.2 Endotoxin Unit per mg of chloramphenicol.

*Chloramphenicol intended for use in the manufacture of parenteral or ophthalmic preparations without a further sterilisation procedure complies with the following additional requirement.*

**Sterility** (2.2.11). Complies with the test for sterility.

**Storage**. Store protected from light and moisture. If the material is intended for use in the manufacture of parenteral or ophthalmic preparations without a further appropriate procedure of sterilisation, the container should be sterile, tamper-evident and sealed so as to exclude micro-organisms.

**Labelling**. The label states whether or not the contents are intended for use in the manufacture of parenteral or ophthalmic preparations.

## Chloramphenicol Capsules

Chloramphenicol Capsules contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of chloramphenicol, $C_{11}H_{12}Cl_2N_2O_5$.

**Usual strengths**. 250 mg; 500 mg.

### Identification

Suspend a quantity of the contents of the capsules containing about 1.25 g of Chloramphenicol in 60 ml of *water* and extract with two quantities, each of 20 ml, of *light petroleum (60° to 80°)* or *light petroleum (100° to 120°)*. Wash the combined extracts with two quantities, each of 15 ml, of *water*, add the washings to the aqueous layer, extract with four quantities, each of 50 ml, of *ether* and remove the ether from the combined extracts. The residue, after drying to constant weight at 105°, complies with the following tests.

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

B. Heat 50 mg with 2 ml of *ethanolic potassium hydroxide solution* in a covered test-tube on a water-bath for 15 minutes; the resulting solution gives the reactions of chlorides (2.3.1).

### Tests

**Specific optical rotation** (2.4.22). +17.0° to +20.0°, determined in a 5.0 per cent w/v solution in *ethanol* of the residue obtained in the test for Identification.

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 100 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first 1 ml of the filtrate. Dilute 5.0 ml of the filtrate to 100.0 ml with the same solvent. Measure the absorbance of the resulting solution at the maximum at about 278 nm (2.4.7). Calculate the content of $C_{11}H_{12}Cl_2N_2O_5$ taking 297 as the specific absorbance at 278 nm.

D. Not less than 85 per cent of the stated amount of $C_{11}H_{12}Cl_2N_2O_5$.

**Other tests**. Comply with the tests stated under Capsules.

**Assay**. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 0.2 g of Chloramphenicol, dissolve in 800 ml of *water*, warming if necessary to effect solution and add sufficient *water* to produce 1000.0 ml. Dilute 10.0 ml of this solution to 100.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 278 nm (2.4.7). Calculate the content of $C_{11}H_{12}Cl_2N_2O_5$, taking 297 as the specific absorbance at 278 nm.

**Storage**. Store protected from moisture.

## Chloramphenicol Eye Drops

Chloramphenicol Eye Drops are a sterile solution of Chloramphenicol in Purified water.

Chloramphenicol Eye Drops contain not less than 90.0 per cent and not more than 130.0 per cent of the stated amount of chloramphenicol, $C_{11}H_{12}Cl_2N_2O_5$.

**Usual strength**. 0.5 per cent w/v.

### Identification

To a volume containing 50 mg of Chloramphenicol add 15 ml of *water* and extract with four quantities, each of 25 ml, of *ether*. Combine the extracts and evaporate to dryness. The residue complies with the following tests.

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G F254*.

*Mobile phase*. A mixture of 90 volumes of *chloroform*, 10 volumes of *methanol* and 1 volume of *water*.



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*Test solution*. Dissolve 0.1 g of the residue in sufficient *ethanol (95 per cent)* to produce 10 ml.

*Reference solution*. Dissolve 0.1 g of *chloramphenicol RS* in sufficient *ethanol (95 per cent)* to produce 10 ml.

Apply to the plate 1µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in a current of warm air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. Dissolve 10 mg in 2 ml of *ethanol (50 per cent)*, add 4.5 ml of *1 M sulphuric acid* and 50 mg of *zinc powder* and allow to stand for 10 minutes. Decant the supernatant liquid or filter, if necessary. Cool the resulting solution in ice and add 0.5 ml of *sodium nitrite solution* and, after 2 minutes, 1 g of *urea* followed by 1 ml of *2-naphthol solution* and 2 ml of *10 M sodium hydroxide*; a red colour is produced. Repeat the test omitting the zinc powder; no red colour is produced.

### Tests

**pH** (2.4.24). 7.0 to 7.5.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dilute a suitable volume of the eye drops containing about 50 mg of chloramphenicol to 100.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 25.0 ml with the mobile phase and filter through a 0.5 µm or finer porosity filter and use the clear filtrate.

*Reference solution*. A 0.01 per cent w/v solution of *chloramphenicol RS* in the mobile phase. Filter this solution through a 0.5 µm or finer porousity filter and use the clear filtrate.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 55 volumes of *water*, 45 volumes of *methanol* and 0.1 volume of *glacial acetic acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume. 20 µl.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_{11}H_{12}Cl_2N_2O_5$ in the drops.

**Storage**. Store in light resistant containers at a temperature not exceeding 30°.

## Chloramphenicol Eye Ointment

Chloramphenicol Eye Ointment contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of chloramphenicol, $C_{11}H_{12}Cl_2N_2O_5$.

**Usual strength**. 1 per cent w/w.

### Identification

Mix a quantity of the ointment containing 30 mg of Chloramphenicol with 10 ml of *light petroleum (40° to 60°)*, centrifuge and discard the supernatant liquid. Repeat this procedure using three quantities, each of 10 ml, of the same solvent. Combine the extracts and evaporate to dryness. The residue complies with the following tests.

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

B. Heat 50 mg with 2 ml of *ethanolic potassium hydroxide solution* in a covered test-tube on a water-bath for 15 minutes; the resulting solution gives the reactions of chlorides (2.3.1).

### Tests

**Other tests**. Complies with the tests stated under Eye Ointments.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Transfer an accurately weighed quantity of the ointment, containing about 25 mg of Chloramphenicol, to a suitable conical flask, add 20 ml of *cyclohexane*, mix with the aid of ultrasound for about 2 minutes add 60 ml of *methanol*, and mix. Filter this mixture, collecting the filtrate in a 100- ml volumetric flask. Wash the filter with *methanol*, collecting the washings in the volumetric flask. Dilute with *methanol* to volume, and mix. Transfer 50.0 ml of the resulting solution to a suitable round-bottom flask, and evaporate to dryness by rotating the flask under vacuum in a water-bath at 35°. Dissolve the residue in 50.0 ml of *methanol*. Transfer 10.0 ml of the resulting solution to a 25-ml volumetric flask, dilute with the mobile phase to volume, and mix. Filter a portion of this solution through a 0.5 µm or finer porosity filter, and use the clear filtrate.

*Reference solution*. A 0.01 per cent w/v solution of *chloramphenicol RS* in the mobile phase. Filter this solution through a 0.5 µm or finer porosity filter and use the clear filtrate.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 55 volumes of *water*, 45 volumes of *methanol* and 0.1 volume of *glacial acetic acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume. 20 µl.



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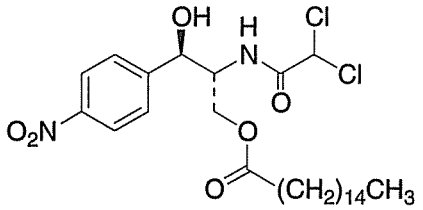


Inject alternately the test solution and the reference solution.

Calculate the content of $C_{11}H_{12}Cl_2N_2O_5$ in the ointment.

**Storage**. Store at a temperature not exceeding 30°.

## Chloramphenicol Palmitate



$C_{27}H_{42}Cl_2N_2O_6$ Mol. Wt. 561.6

Chloramphenicol Palmitate is (2*R*,3*R*)-2-(2,2-dichloroacetamido)-3-hydroxy-3-(4-nitrophenyl)propyl hexadecanoate.

Chloramphenicol Palmitate contains not less than 97.0 per cent and not more than 103.0 per cent of $C_{27}H_{42}Cl_2N_2O_6$, calculated on the dried basis.

**Category**. Antibacterial.

**Dose**. For an adult, the equivalent of 1.5 to 3 g of chloramphenicol daily, in divided doses; for a child, the equivalent of 25 to 50 mg of chloramphenicol per kg of body weight daily, in divided doses. (175 mg of chloramphenicol palmitate is approximately equivalent to 100 mg of chloramphenicol).

**Description**. A fine, white or almost white, unctuous powder; odour, faint.

### Identification

A. When examined in the range 230 nm to 360 nm (2.4.7), a 0.003 per cent w/v solution in *ethanol (95 per cent)* shows an absorption maximum only at about 271 nm; absorbance at about 271 nm, about 0.53.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silanised silica gel H*.

*Mobile phase*. A mixture of 70 volumes of *ethanol (95 per cent)* and 30 volumes of a 10 per cent w/v solution of *ammonium acetate*.

*Test solution*. Dissolve 50 mg of the substance under examination in a mixture of 1 ml of *1 M sodium hydroxide* and 5 ml of *acetone*, allow to stand for 30 minutes and add 1.1 ml of *1 M hydrochloric acid* and 3 ml of *acetone*.

*Reference solution (a)*. A 0.2 per cent w/v solution of *chloramphenicol RS* in *acetone*.

*Reference solution (b)*. A 0.2 per cent w/v solution of *palmitic acid* in *acetone*.

*Reference solution (c)*. A 0.2 per cent w/v solution of the substance under examination in *acetone*.

Apply to the plate 4 µl of each solution. After development, dry the plate in air and spray with a solution containing 0.02 per cent w/v of *2,7-dichlorofluorescein* and 0.01 per cent w/v of *rhodamine B* in *ethanol (95 per cent)*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. The chromatogram obtained with the test solution shows three spots corresponding in position to the principal spots in chromatograms obtained with reference solutions (a), (b) and (c).

C. Dissolve 10 mg in 4 ml of *ethanol (95 per cent)* add 1 ml of *1 M sulphuric acid* and 50 mg of *zinc powder* and allow to stand for 10 minutes. Filter, cool the filtrate in ice and add 0.5 ml of *sodium nitrite solution* and, after 2 minutes, 1 g of *urea* followed by 1 ml of *2-naphthol solution* and 2 ml of *10 M sodium hydroxide*; a red colour develops. Repeat the test omitting the zinc powder; no red colour is produced.

D. Heat 50 mg with 2 ml of *ethanolic potassium hydroxide solution* in a covered test-tube on a water-bath for 15 minutes; the resulting solution gives the reactions of chlorides (2.3.1).

### Tests

**Free acid**. Dissolve 1.0 g by warming to 35° in 5 ml of a mixture of a equal volumes of *ethanol (95 per cent)* and *ether* and add 0.2 ml of *phenolphthalein solution*; not more than 0.4 ml of *0.1 M sodium hydroxide* is required to produce a pink colour persisting for 30 seconds.

**Specific optical rotation** (2.4.22). +21.0° to +25.0°, determined in a 5.0 per cent w/v solution in *ethanol*.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 50 volumes of *cyclohexane*, 40 volumes of *chloroform* and 10 volumes of *methanol*.

*Test solution*. Dissolve 1 g of the substance under examination in 100 ml of *acetone*.

*Reference solution*. Dilute 2 ml of the test solution to 100 ml with *acetone*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Free chloramphenicol**. Not more than 450 ppm, determined by the following method. Dissolve, with the aid of gentle heat, 1.0 g in 80 ml of *xylene*, cool and extract with three successive



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quantities, each of 15 ml, of *water*; discard the xylene and dilute the combined aqueous extracts to 50 ml with *water*. Extract the solution with 10 ml of *carbon tetrachloride*, allow to separate, discard the carbon tetrachloride and centrifuge a portion of the aqueous solution. Measure the absorbance of the clear aqueous solution at the maximum at about 278 nm, using as the blank a solution obtained by repeating the procedure without the substance under examination; the absorbance of this blank solution must not be greater than 0.05 (2.4.7). Calculate the content of free chloramphenicol, in ppm, from the expression $(A \times 10^4)/5.96$, where A is the absorbance of the clear aqueous solution of the substance under examination.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven over *phosphorus pentoxide* at 80° at a pressure not exceeding 0.1 kPa for 3 hours.

**Assay**. Weigh accurately about 60 mg and dissolve in sufficient *ethanol (95 per cent)* to produce 100.0 ml. Dilute 10.0 ml of this solution to 200.0 ml with *ethanol (95 per cent)* and measure the absorbance of the resulting solution at the maximum at about 271 nm (2.4.7). Calculate the content of $C_{27}H_{42}Cl_2N_2O_6$ taking 178 as the specific absorbance at 271 nm.

**Storage**. Store protected from light and moisture.

## Chloramphenicol Oral Suspension

Chloramphenicol Palmitate Oral Suspension; Chloramphenicol Palmitate Mixture

Chloramphenicol Oral Suspension is a suspension of Chloramphenicol Palmitate in a suitable flavoured vehicle.

Chloramphenicol Oral Suspension contains not less than 95.0 per cent and not more than 115.0 per cent of the stated amount of chloramphenicol, $C_{11}H_{12}Cl_2N_2O_5$.

**Usual strength**. The equivalent of 125 mg of chloramphenicol per 5 ml. (175 mg of chloramphenicol palmitate is approximately equivalent to 100 mg of chloramphenicol).

### Identification

Extract a quantity of the suspension containing about 7.5 mg of chloramphenicol with 10 ml of *chloroform* and carefully evaporate the clear chloroform solution on a water-bath to dryness. Dissolve the residue in 250 ml of *ethanol (95 per cent.* When examined in the range 230 nm to 360 nm (2.4.7) the resulting solution shows an absorption maximum only at about 271 nm.

### Tests

**pH** (2.4.24). 4.5 to 7.0.

**Polymorph A**. To a volume of the suspension containing 125 mg of chloramphenicol add 35 ml of *water*, mix, centrifuge for 40 minutes at not less than 18,000 rpm and discard the supernatant liquid. Wash the residue by adding 2 ml of *water*, triturating to form a paste, adding 18 ml of *water*, mixing thoroughly centrifuging and discarding the supernatant liquid. Wash the residue twice more in a similar manner, dry at 20° for 16 hours at a pressure not exceeding 0.7 kPa and grind to a *fine powder*. Prepare a mull of the residue by triturating a small quantity with about twice its weight of *liquid paraffin* until a smooth creamy paste is obtained. Determine by infrared absorption spectrophotometry (2.4.6) over the range 770 $cm^{-1}$ to 910 $cm^{-1}$ using conditions such that between 20 per cent and 30 per cent transmittance occurs at 810 $cm^{-1}$ to 910 $cm^{-1}$. Repeat the operation using a mull prepared with a standard mixture obtained by mixing together thoroughly 1 part by weight of *chloramphenicol palmitate (polymorph A) RS* and 9 parts by weight of *chloramphenicol palmitate nonpolymorph A RS*. On each of the spectra, draw a straight base line between the minima occurring at about 880 $cm^{-1}$ and 790 $cm^{-1}$ and using these base lines measure the heights of the peaks occuring at the maxima at about 858 $cm^{-1}$ and 840 $cm^{-1}$. In the spectrum obtained with preparation under examination, the ratio of the peak height at about 858 $cm^{-1}$ to that at the maximum at about 840 $cm^{-1}$ is greater than the corresponding ratio in the spectrum obtained with the standard mixture.

**Other tests**. Complies with the tests stated under Oral Liquids.

**Assay**. Weigh accurately a quantity of the suspension containing about 125 mg of chloramphenicol, add 10 ml of *water* and shake with four quantities, each of 20 ml, of *chloroform*, filtering each extract through cotton wool, previously washed with *chloroform*, into a 100-ml volumetric flask. Dilute to volume with *chloroform* and mix well. Dilute 2.0 ml of this solution to 100.0 with *ethanol (95 per cent)* and measure the absorbance of the resulting solution at the maximum at about 271 nm using 1 ml of *chloroform* diluted to 50 ml with *ethanol (95 per cent)* as the blank (2.4.7). Calculate the content of chloramphenicol palmitate, $C_{27}H_{42}Cl_2N_2O_6$, taking 178 as the specific absorbance at 271 nm.

Determine the weight per ml of the suspension (2.4.29) and calculate the content of chloramphenicol, $C_{11}H_{12}Cl_2N_2O_5$, weight in volume using a factor of 0.575 for the conversion of the content of chloramphenicol palmitate to chloramphenicol.

**Storage**. Store protected from light.

**Labelling**. The label states (1) the strength in terms of the equivalent amount of chloramphenicol; (2) that if the preparation is diluted, it must be used immediately after dilution.

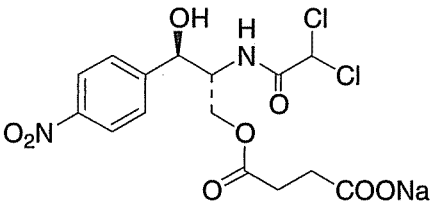


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# Chloramphenicol Sodium Succinate



$C_{15}H_{15}Cl_2N_2NaO_8$ Mol. Wt. 445.2

Chloramphenicol Sodium Succinate is a mixture of variable proportions of sodium (2*R*,3*R*)-2-(2,2-dichloroacetamido)-3-hydroxy-3-(4-nitrophenyl)propyl succinate (3-isomer) and of sodium (1*R*,2*R*)-2-(2,2-dichloroacetamido)-3-hydroxy-1-(4-nitrophenyl)propyl succinate (1-isomer).

Chloramphenicol Sodium Succinate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{15}H_{15}Cl_2N_2NaO_8$, calculated on the anhydrous basis.

**Category**. Antibacterial.

**Dose**. By intravenous injection, the equivalent of 3 to 4 g of chloramphenicol daily, in divided doses. (140 mg of chloramphenicol sodium succinate is approximately equivalent to 100 mg of chloramphenicol).

**Description**. A white or yellowish-white powder; hygroscopic.

## Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 85 volumes of *chloroform*, 14 volumes of *methanol* and 1 volume of *2 M acetic acid*.

*Test solution*. Dissolve 0.1 g of the substance under examination in 10 ml of *acetone*.

*Reference solution (a)*. A 1 per cent w/v solution of *chloramphenicol sodium succinate RS* in *acetone*.

*Reference solution (b)*. A 1 per cent w/v solution of *chloramphenicol RS* in *acetone*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The two principal spots in the chromatogram obtained with the test solution are similar in position and size to those in the chromatogram obtained with reference solution (a) and their positions are different from that of the principal spot in the chromatogram obtained with reference solution (b).

B. Dissolve 10 mg in 2 ml of *ethanol (95 per cent)* add 4.5 ml of *1 M sulphuric acid* and 50 mg of *zinc powder*, allow to stand for 10 minutes and decant the supernatant liquid or filter, if necessary. Cool the resulting solution in ice and add 0.5 ml of *sodium nitrite solution* and, after 2 minutes, 1 g of *urea* followed by 1 ml of *2-naphthol solution* and 2 ml of *10 M sodium hydroxide*; a red colour develops. Repeat the test omitting the zinc powder; no red colour is produced.

C. To 5 ml of a 0.1 per cent w/v solution add a few drops of *silver nitrate solution*; no precipitate is produced. Heat 50 mg with 2 ml of *ethanolic potassium hydroxide solution* on a water-bath for 15 minutes, add 50 mg of *decolorising charcoal*, shake and filter. The filtrate when treated with *silver nitrate solution*, yields a curdy precipitate which is insoluble in *nitric acid* but soluble, after being well washed with *water*, in *dilute ammonia solution* from which it is reprecipitated on addition of *nitric acid*.

D. A 5 per cent w/v solution gives the reactions of sodium salts (2.3.1).

## Tests

**pH** (2.4.24). 6.4 to 7.0, determined in a 25.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). +5.0° to +8.0°, determined in a 5.0 per cent w/v solution.

**Free chloramphenicol**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 90 volumes of *chloroform*, 10 volumes of *methanol* and 1 volume of *water*.

*Test solution*. Dissolve 0.1 g of the substance under examination in 10 ml of *acetone*.

*Reference solution*. A 0.02 per cent w/v solution of *chloramphenicol RS* in *acetone*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any spot corresponding to chloramphenicol in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Water** (2.3.43). Not more than 2.0 per cent, determined on 0.3 g.

**Assay**. Weigh accurately about 0.2 g and dissolve in sufficient *water* to produce 500.0 ml; dilute 5.0 ml of this solution to 100.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 276 nm (2.4.7). Calculate the content of $C_{15}H_{15}Cl_2N_2NaO_8$ taking 220 as the specific absorbance at 276 nm.

*Chloramphenicol Sodium Succinate intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.*

**Bacterial endotoxins** (2.2.3). Not more than 0.2 Endotoxin Unit per mg of chloramphenicol.



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*Chloramphenicol Sodium Succinate intended for use in the manufacture of parenteral preparations without a further sterilisation procedure complies with the following additional requirement.*

**Sterility** (2.2.11). Complies with the test for sterility.

**Storage**. Store protected from light and moisture. If the material is intended for use in the manufacture of parenteral preparations, the container should be sterile, tamper-evident and sealed so as to exclude micro-organisms.

**Labelling**. The label states whether or not the material is intended for use in the manufacture of parenteral preparations.

## Chloramphenicol Sodium Succinate Injection

Chloramphenicol Sodium Succinate Injection is a sterile material consisting of Chloramphenicol Sodium Succinate with or without excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

*The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).*

**Storage**. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Chloramphenicol Sodium Succinate Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of chloramphenicol, $C_{11}H_{12}Cl_2N_2O_5$.

**Usual strengths**. The equivalent of 300 mg and 1 g of chloramphenicol. (140 mg of chloramphenicol sodium succinate is approximately equivalent to 100 mg of chloramphenicol).

**Description**. A white or yellowish-white powder; hygroscopic.

*The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.*

### Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 85 volumes of *chloroform*, 14 volumes of *methanol* and 1 volume of *2 M acetic acid.*

*Test solution*. Dissolve 0.1 g of the substance under examination in 10 ml of *acetone.*

*Reference Solution (a)*. A 1 per cent w/v solution of *chloramphenicol sodium succinate RS* in *acetone.*

*Reference solution (b)*. A 1 per cent w/v solution of *chloramphenicol RS* in *acetone.*

Apply to the plate 2 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The two principal spots in the chromatogram obtained with the test solution are similar in position and size to those in the chromatogram obtained with reference solution (a) and their positions are different from that of the principal spot in the chromatogram obtained with reference solution (b).

B. Dissolve 10 mg in 2 ml of *ethanol (95 per cent)* add 4.5 ml of *1 M sulphuric acid* and 50 mg of *zinc powder,* allow to stand for 10 minutes and decant the supernatant liquid or filter, if necessary. Cool the resulting solution in ice and add 0.5 ml of *sodium nitrite solution* and, after 2 minutes, 1 g of *urea* followed by 1 ml of *2-naphthol solution* and 2 ml of *10 M sodium hydroxide*; a red colour develops. Repeat the test omitting the zinc powder; no red colour is produced.

C. To 5 ml of a 0.1 per cent w/v solution add a few drops of *silver nitrate solution*; no precipitate is produced. Heat 50 mg with 2 ml of *ethanolic potassium hydroxide solution* on a water-bath for 15 minutes, add 50 mg of *decolorising charcoal,* shake and filter. The filtrate when treated with *silver nitrate solution*, yields a curdy precipitate which is insoluble in *nitric acid* but soluble, after being well washed with *water,* in *dilute ammonia solution* from which it is reprecipitated on addition of *nitric acid.*

D. A 5 per cent w/v solution gives the reactions of sodium salts (2.3.1).

### Tests

**pH** (2.4.24). 6.4 to 7.0, determined in a 25.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). +5.0° to +8.0°, determined in a 5.0 per cent w/v solution.

**Free chloramphenicol**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

*Mobile phase.* A mixture of 90 volumes of *chloroform,* 10 volumes of *methanol* and 1 volume of *water.*

*Test solution.* Dissolve 0.1 g of the substance under examination and dissolve in 10 ml of *acetone.*

*Reference solution.* A 0.02 per cent w/v solution of *chloramphenicol RS* in *acetone.*

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any spot corresponding to chloramphenicol in the chromatogram obtained with the test solution is not more



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intense than the spot in the chromatogram obtained with the reference solution.

**Water** (2.3.43). Not more than 2.0 per cent, determined on 0.3 g.

**Bacterial endotoxins** (2.2.3). Not more than 0.2 Endotoxin Unit per mg of chloramphenicol.

**Sterility** (2.2.11). Complies with the test for sterility.

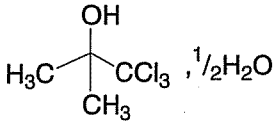
**Assay**. Determine the weight of the contents of 10 containers. Weigh accurately about 0.2 g of the mixed contents of the 10 containers and dissolve in sufficient *water* to produce 500.0 ml; dilute 5.0 ml of this solution to 100.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 276 nm (2.4.7). Calculate the content of $C_{15}H_{15}Cl_2N_2NaO_8$ taking 220 as the specific absorbance at 276 nm.

**Storage**. Store protected from light and moisture.

**Labelling**. The label states the quantity of Chloramphenicol Sodium Succinate in the sealed container in terms of the equivalent amount of chloramphenicol.

## Chlorbutol

Chlorobutanol




$C_4H_7Cl_3O$, ½ $H_2O$ Mol.Wt. 186.5

Chlorbutol is 1,1,1-trichloro-2-methylpropan-2-ol hemihydrate.

Chlorbutol contains not less than 98.0 per cent and not more than 101.0 per cent of $C_4H_7Cl_3O$, calculated on the anhydrous basis.

**Category**. Pharmaceutical aid (antimicrobial preservative), analgesic; local anaesthetic.

**Description**. Colourless crystals or a white, crystalline powder; odour, characteristic and somewhat camphoraceous; sublimes readily.

### Identification

A. To 5 ml of a freshly prepared 0.5 per cent w/v solution add 1 ml of *1 M sodium hydroxide* and then, slowly, 2 ml of *iodine solution*; a yellow precipitate of iodoform is produced.

B. Heat about 20 mg with 2 ml of *10 M sodium hydroxide* and 1 ml of *pyridine* on a water-bath and shake; the separated pyridine layer becomes red.

C. Warm gently about 20 mg with 5 ml of *ammoniacal silver nitrate solution*; a black precipitate is produced.

### Tests

**Appearance of solution**. A 50.0 per cent w/v solution in *ethanol (95 per cent)* is not more opalescent than opalescence standard OS2 (2.4.1), and not more intensely coloured than reference solution BYS5 (2.4.1).

**Acidity**. Dissolve 2.0 g in 20 ml of *ethanol (95 per cent)*, add 0.1 ml of *bromothymol blue solution* and titrate with *0.1 M sodium hydroxide*; not more than 0.1 ml of *0.1 M sodium hydroxide* is required to change the colour of the solution.

**Chlorides** (2.3.12). 0.5 g dissolved in 10 ml of *ethanol (95 per cent)* complies with the limit test for chlorides (500 ppm). Use 5 ml of *ethanol (95 per cent)* in place of 5 ml of *water* to prepare the standard.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

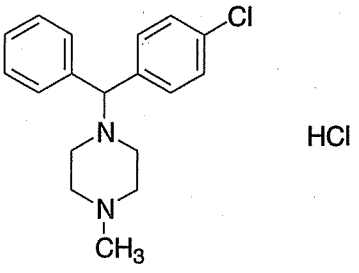
**Water** (2.3.43). 4.5 per cent to 6.0 per cent, determined on 0.3 g.

**Assay**. Weigh accurately about 0.2 g and dissolve in 5 ml of *ethanol (95 per cent)*. Add 5 ml of *sodium hydroxide solution* and boil under a reflux condenser for 15 minutes. Cool, dilute with 20 ml of *water*, add 5 ml of *nitric acid*, 1 ml of *nitrobenzene* and 50.0 ml of *0.1 M silver nitrate* and shake vigorously for 1 minute. Add 4 ml of *ferric ammonium sulphate solution* and titrate the excess of silver nitrate with *0.1 M ammonium thiocyanate*.

1 ml of *0.1 M silver nitrate* is equivalent to 0.005917 g of $C_4H_7Cl_3O$.

**Storage**. Store protected from moisture at a temperature not exceeding 30°.

## Chlorcyclizine Hydrochloride




$C_{18}H_{21}ClN_2$,HCl Mol. Wt. 337.3

Chlorcyclizine Hydrochloride is 1-(4-chlorobenzhydryl)-4-methylpiperazine hydrochloride.



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Chlorcyclizine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of the stated amount of $C_{18}H_{21}ClN_2,HCl$, calculated on the dried basis.

**Category**. Antihistaminic.

**Dose**. 50 mg thrice daily.

**Description**. A white crystalline powder.

### Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *chlorcyclizine hydrochloride RS* or with the reference spectrum of chlorcyclizine hydrochloride.

B. Weigh accurately about 10 mg, dissolve in 100 ml of 0.5 per cent w/v of *suphuric acid*. Dilute 10 ml of the solution to 100 ml with 0.5 per cent w/v *sulphuric acid*. When examined in the range 215 to 300 nm (2.4.7), exhibits maximum only at about 231 nm; absorbance at about 231 nm, about 0.475 to 0.525.

C. In the test for Related substances, the principle spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

D. Gives the reactions of chlorides (2.3.1).

### Tests

**Appearance of solution**. A 5.0 per cent w/v solution is clear (2.4.1) and colourless (2.4.1).

**pH** (2.4.24). 5.0 to 6.0, determined in a 1.0 per cent w/v solution.

**Related substance**. Determine by thin layer chromatography (2.4.17). coating the plate with *silica gel*.

*Mobile phase*. A mixture of 85 volumes of *dichloromethane*, 13 volumes of *methanol* and 2 volumes of *strong ammonia solution*.

*Test solution (a)*. A 2.0 per cent w/v solution of the substance under examination in *methanol*.

*Test solution (b)*. A 0.1 per cent w/v solution of the substance under examination in *methanol*.

*Reference solution (a)*. A 0.10 per cent w/v solution of *chlorcyclizine hydrochloride RS* in *methanol*.

*Reference solution (b)*. A 0.01 per cent w/v solution of *methylpiperzine RS* in *methanol*.

*Reference solution (c)*. A 0.004 per cent w/v solution of the substance under examination in *methane*.

*Reference solution (d)*. 0.10 per cent w/v each of *hydroxyzine hydrochloride RS* and *chlorcyclizine hydrochloride RS* in *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and expose to iodine vapours for 10 minutes. Any secondary spot in the chromatogram obtained with the test solution (a) is not more intense than the corresponding spot in the chromatogram obtained with the test solution (b). Any spot other than the principle spot but corresponding to the spot obtained with reference solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.50 per cent). The test is not valid unless the chromatogram obtained with reference solution (d) shows to clearly separated spot.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

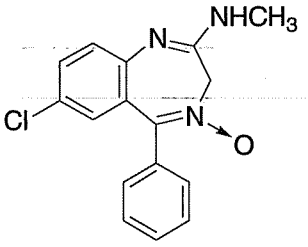
**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 130°.

**Assay**. Weigh accurately about 0.2 g, dissolve in 1 ml of *0.1 M hydrochloric acid* and add 50 ml of *methanol*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.03373 g of $C_{18}H_{21}ClN_2,HCl$.

**Storage**. Store protected from light and moisture.

## Chlordiazepoxide




$C_{16}H_{14}ClN_3O$ Mol. Wt. 299.8

Chlordiazepoxide is 7-chloro-2-methylamino-5-phenyl-3*H*-1,4-benzodiazepine 4-oxide.

Chlordiazepoxide contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{16}H_{14}ClN_3O$, calculated on the dried basis.

**Category**. Anxiolytic.

**Dose**. 10 to 100 mg daily, in divided doses.

**Description**. An almost white to light yellow, crystalline powder; practically odourless.

### Identification

*Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.*



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A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *chlordiazepoxide RS.*

B. When examined in the range 230 nm to 360 nm (2.4.7) a 0.0005 per cent w/v solution prepared immediately before use in subdued light in *0.1 M hydrochloric acid* shows absorption maxima at about 246 nm and 308 nm. Absorbance at the maximum at about 246 nm, 0.56 to 0.60 and at the maximum at about 308 nm, 0.16 to 0.17.

C. Dissolve 0.2 g in 4 ml of hot *dilute hydrochloric acid*, heat at 100° for 10 minutes, cool and filter. 2 ml of the filtrate gives the reactions of primary aromatic amines (2.3.1).

### Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Note—Use freshly prepared solutions and protected from light.*

*Test solution*. Dissolve 20 mg of the substance under examination in 100 ml of the mobile phase.

*Reference solution (a)*. Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 2.0 ml of this solution to 10.0 ml with the mobile phase.

*Reference solution (b)*. Dissolve 5 mg of *chlordiazepoxide impurity A RS* in the mobile phase, add 25.0 ml of the test solution and dilute to 100 ml with the mobile phase. Dilute 2.0 ml of this solution to 50.0 ml with the mobile phase.

*Reference solution (c)*. Dissolve 4 mg of *aminochlorobenzophenone* in 100 ml of the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of *acetonitrile* and 50 volumes of *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to chlordiazepoxide impurity A and chlordiazepoxide is not less than 5.0.

Inject the test solution and reference solution (a). Run the chromatogram 6 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent) and sum of areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent). Ignore any peak with a retention time less than 1.4 minutes.

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

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.25 g and dissolve by heating, if necessary, in 80 ml of *anhydrous glacial acetic acid.* Titrate with *0.1 M perchloric acid,* determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02998 g of $C_{16}H_{14}ClN_3O$.

**Storage**. Store protected from light and moisture.

## Chlordiazepoxide Tablets

Chlordiazepoxide Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of chlordiazepoxide, $C_{16}H_{14}ClN_3O$.

**Usual strengths**. 5 mg; 10 mg; 25 mg.

### Identification

A. Dilute 1 ml of the final solution obtained in the Assay to 2 ml with *0.1 M hydrochloric acid.* When examined in the range 230 nm to 360 nm (2.4.7) the resulting solution shows absorption maxima at about 246 nm and 308 nm.

B. To a quantity of the powdered tablets containing 0.2 g of Chlordiazepoxide add 4 ml of hot *2 M hydrochloric acid*, heat at 100° for 10 minutes, cool and filter; 2 ml of the filtrate gives the reactions of primary aromatic amines (2.3.1).

### Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

*Mobile phase.* A mixture of 85 volumes of *chloroform,* 14 volumes of *methanol* and 1 volume of *strong ammonia solution.*

*Test solution.* Shake a quantity of the powdered tablets containing 0.1 g of Chlordiazepoxide with 10 ml of a mixture of *acetone* containing 2 per cent v/v of *strong ammonia solution*



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and 8 per cent v/v of *water*, allow to settle and use the clear supernatant liquid.

*Reference solution (a)*. Dilute 5 volumes of the test solution to 100 volumes with the same solvent mixture.

*Reference solution (b)*. Dilute 1 volume of the test solution to 100 volumes with the same solvent mixture.

*Reference solution (c)*. A 0.001 per cent w/v solution of *2-amino-5-chlorobenzophenone*.

Apply to the plate 2 µl and 20 µl quantities of the test solution, 2 µl of each of reference solutions (a) and (b) and 20 µl of reference solution (c). After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with 2 µl of the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b). Spray the plate with a freshly prepared 1 per cent w/v solution of *sodium nitrite* in *1 M hydrochloric acid*, dry it in a current of air and spray with a 0.4 per cent w/v solution of *N-(1-naphthyl)ethylenediamine dihydrochloride* in *ethanol (95 per cent)*. Any violet spot corresponding to 2-amino-5-chlorobenzophenone in the chromatogram obtained with 20 µl of the test solution is not more intense than the spot in the chromatogram obtained with reference solution (c).

**Uniformity of content**. Comply with the test stated under Tablets.

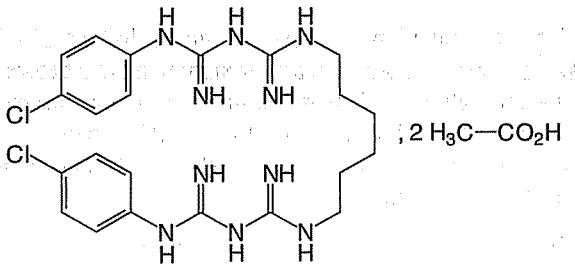
Powder one tablet, shake with 50 ml of *0.1 M hydrochloric acid* for 20 minutes and add sufficient *0.1 M hydrochloric acid* to produce 100.0 ml. Filter and dilute a suitable volume of the filtrate containing 0.8 mg of Chlordiazepoxide with sufficient *0.1 M hydrochloric acid* to produce 50.0 ml. Measure the absorbance of the resulting solution at the maximum at about 308 nm (2.4.7). Calculate the content of $C_{16}H_{14}ClN_3O$ in the tablet taking 327 as the specific absorbance at 308 nm.

**Other tests**. Complies with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 20 mg of Chlordiazepoxide and shake with 150 ml of *0.1 M hydrochloric acid* for 20 minutes. Add sufficient *0.1 M hydrochloric acid* to produce 250.0 ml and filter. Dilute 10.0 ml of the filtrate to 50.0 ml with *0.1 M hydrochloric acid* and measure the absorbance of the resulting solution at the maximum at about 308 nm (2.4.7). Calculate the content of $C_{16}H_{14}ClN_3O$ taking 327 as the specific absorbance at 308 nm.

**Storage**. Store protected from light at a temperature not exceeding 30°.

# Chlorhexidine Acetate




$C_{22}H_{30}Cl_2N_{10},2C_2H_4O_2$ Mol. Wt. 625.6

Chlorhexidine Acetate is 1,1'-(hexane-1,6-diyl)bis[5-(4-chlorophenyl)biguanide] diacetate.

Chlorhexidine Acetate contains not less than 98.0 per cent and not more than 101.0 per cent of chlorhexidine diacetate, $C_{22}H_{30}Cl_2N_{10},2C_2H_4O_2$, calculated on the dried basis.

**Category**. Antiseptic.

**Description**. A white or almost white, microcrystalline powder.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *chlorhexidine acetate RS*.

B. Dissolve about 5 mg in 5 ml of a warm 1.0 per cent w/v solution of *cetrimide* and add 1 ml of *strong sodium hydroxide solution* and 1 ml of *bromine water*. A deep red colour is produced.

C. Dissolve 0.3 g in 10 ml of a mixture of equal volumes of *hydrochloric acid* and *water*. Add 40 ml of *water*, filter if necessary and cool in ice *water*. Make alkaline to *titan yellow paper* by adding dropwise and with stirring *strong sodium hydroxide solution* and add 1 ml in excess. Filter, wash the precipitate with *water* until the washings are free from alkali and recrystallise from *alcohol (70 per cent v/v)*. Dry at 100° to 105°. Melting point (2.4.21). 132° to 136°.

D. It gives reaction (a) of acetates (2.3.1).

## Tests

**Chloroaniline**. Dissolve 0.2 g of the substance under examination in 25 ml of *water* with shaking if necessary. Add 1 ml of *hydrochloric acid* and dilute to 30 ml with *water*. Add rapidly and with thorough mixing after each addition, 2.5 ml of *dilute hydrochloric acid*, 0.35 ml of *sodium nitrite solution*, 2 ml of a 5.0 per cent w/v solution of *ammonium sulphamate*,



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5 ml of a 0.1 per cent w/v solution of *naphthylethylenediamine dihydrochloride* and 1 ml of *alcohol*, dilute to 50.0 ml with *water* and allow to stand for 30 minutes. Any reddish-blue colour in the solution is not more intense than that in a standard prepared at the same time in the same manner using a mixture of 10.0 ml of 0.001 per cent w/v solution of *chloroaniline* in *dilute hydrochloric acid* and 20 ml of *dilute hydrochloric acid* instead of the solution of the substance under examination (500 ppm).

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 0.2 g of the substance under examination in 100 ml of the mobile phase.

*Reference solution (a)*. A 0.15 per cent w/v solution of *chlorhexidine acetate RS* in the mobile phase.

*Reference solution (b)*. Dilute 2.5 ml of the test solution to 100 ml with the mobile phase.

*Reference solution (c)* Dilute 2 ml of reference solution (b) to 10 ml with the mobile phase. Further dilute 1 ml of this solution to 10 ml with the mobile phase.

Chromatographic system

- a stainless steel column 20 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: 2.0 g of *sodium octanesulphonate* in a mixture of 120 ml of *glacial acetic acid*, 270 ml of *water* and 730 ml of *methanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Equilibrate the column with the mobile phase for at least 1 hour. Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (b) is at least 50 per cent of the full scale of the recorder.

Inject the test solution and reference solutions (a), (b) and (c). Record the chromatograms of reference solutions (b) and (c) until the peak due to chlorhexidine has been eluted and record the chromatogram of the test solution for six times the retention time of the peak due to chlorhexidine. In the chromatogram obtained with the test solution, the sum of the areas of all the peaks, other than the principal peak is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.5 per cent). Ignore any peak with a relative retention time of 0.25 or less with respect to the principal peak and any peak whose area is less than that of the principal peak in the chromatogram obtained with reference solution (c).

**Loss on drying** (2.4.19). Not more than 3.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Sulphated ash** (2.3.18). Not more than 0.15 per cent.

**Assay**. Dissolve 0.14 g in 100 ml of *anhydrous acetic acid* and titrate with *0.1 M perchloric acid* determining the end-point potentiometrically (2.4.25).

1 ml of *0.1 M perchloric acid* is equivalent to 0.01564 g of $C_{26}H_{38}Cl_2N_{10}O_4$.

## Chlorhexidine Gluconate Solution

Chlorhexidine Gluconate Solution is an aqueous solution of 1,1'-hexamethylenebis [5-(4-chlorophenyl)biguanide] digluconate.

Chlorhexidine Gluconate Solution contains not less than 19.0 per cent w/v and not more than 21.0 per cent w/v of $C_{22}H_{30}Cl_2N_{10},2C_6H_{12}O_7$.

**Category**. Antiseptic.

**Description**. An almost colourless or pale yellowish, clear or slightly opalescent liquid; almost odourless.

### Identification

*Test A may be omitted if tests B, C and D are carried out. Tests C and D may be omitted if tests A and B are carried out*

A. To 2 ml add 80 ml of *water*, cool in ice, add *5 M sodium hydroxide* dropwise with stirring until the solution is slightly alkaline to titan yellow paper and add 2 ml in excess. Filter, wash the precipitate with *water* until the washings are free from alkali, dissolve it in about 25 ml of *ethanol* on a boiling water-bath and heat until the volume is reduced to about 5 ml. Cool in ice, induce crystallisation, if necessary, by scratching the side of the vessel with a glass rod, filter and dry the crystals at 105°. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *chlorhexidine RS* or with the reference spectrum of chlorhexidine. Examine the substance as a dispersion in *potassium bromide IR* without excessive grinding.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 50 volumes of *ethanol (95 per cent)*, 30 volumes of *water*, 10 volumes of *strong ammonia solution* and 10 volumes of *ethyl acetate*.

*Test solution*. Dilute 10 ml of the substance under examination to 50 ml with *water*.

*Reference solution*. A 2.5 per cent w/v solution of *calcium gluconate RS* in *water*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate at 100° for 20 minutes, allow



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to cool, spray with a 5 per cent w/v solution of *potassium dichromate* in a 40 per cent w/w solution of *sulphuric acid* and allow to stand for 5 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

C. To 0.5 ml add 10 ml of *water* and 0.5 ml of *cupric sulphate solution*; a white precipitate is produced which on boiling flocculates and changes to a pale purple colour.

D. To 0.05 ml add 5 ml of a 1 per cent w/v solution of *cetrimide*, 1 ml of *10 M sodium hydroxide* and 1 ml of *bromine water*; a deep red colour is produced.

### Tests

**pH** (2.4.24). 5.5 to 7.0, determined in a solution obtained by diluting 5 ml to 100 ml.

**Weight per ml** (2.4.29). 1.06 g to 1.07 g, determined at 20°.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating a 0.5-mm thick plate with a slurry consisting of 8 g of *silica gel GF254* and 16 ml of *water* containing 1 g of *sodium formate*.

*Mobile phase*. A mixture of 50 volumes of *chloroform*, 50 volumes of *ethanol (95 per cent)* and 7 volumes of *formic acid*.

*Test solution*. Dilute 1 ml of the substance under examination to 20 ml with *1.5 M acetic acid*.

Apply to the plate, in the form of a band 4 cm wide, 20 µl of the test solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Mark the area around each group of bands above and below the principal band, transfer quantitatively the enclosed areas of silica gel to a glass-stoppered tube, add 5.0 ml of *methanol*, shake for 15 minutes, centrifuge and measure the absorbance of the clear, supernatant liquid at the maximum at about 256 nm (2.4.7), using as the blank a solution prepared by heating in a similar manner equivalent-sized areas of silica gel removed from the coating adjacent to the areas previously removed. The absorbance is not more than that obtained with a solution prepared by diluting 2 ml of the substance under examination with sufficient *1.5 M acetic acid* to produce 10 ml and diluting 0.2 ml of this solution to 50 ml with *methanol*.

**4-Chloroaniline**. Not more than 0.25 per cent, calculated with reference to chlorhexidine solution at a nominal concentration of 20 per cent w/v, determined by the following method. Dilute 2.0 ml to 100.0 with *water*. To 10.0 ml of this solution add 2.5 ml of *2 M hydrochloric acid* and dilute to 20 ml with *water*. Add rapidly, with continuous mixing after each addition, 0.35 ml of *sodium nitrite* solution, 2 ml of a 5 per cent w/v solution of *ammonium sulphamate* and 5 ml of a 0.01 per cent w/v solution of *N-(1-naphthyl) ethylenediamine dihydrochloride*. Add 1 ml of *ethanol (95 per cent)* and sufficient *water* to produce 50 ml, mix and set aside for 30 minutes. Any reddish blue colour produced is not more intense than that produced by treating at the same time in the same manner a mixture of 10.0 ml of 0.001 per cent w/v solution of *4-chloroaniline* in *2 M hydrochloric acid* and 10 ml of *water* in place of the dilution of the substance under examination.

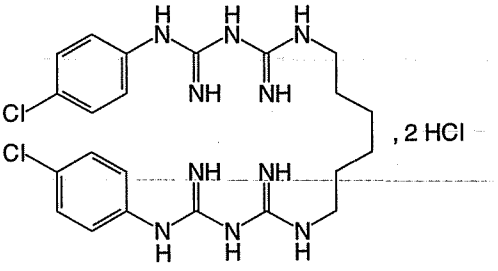
**Assay**. Weigh accurately about 1.0 g and evaporate to a low bulk. Dissolve in 50 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02244 g of $C_{22}H_{30}Cl_2N_{10},2C_6H_{12}O_7$.

Determine the weight per ml (2.4.29) and calculate the percentage content of $C_{22}H_{30}Cl_2N_{10},2C_6H_{12}O_7$, weight in volume.

**Storage**. Store protected from light.

## Chlorhexidine Hydrochloride




$C_{22}H_{30}Cl_2N_{10},2HCl$ Mol. Wt. 578.4

Chlorhexidine Hydrochloride is 1,1'-(hexane-1,6-diyl)bis[5-(4-chlorophenyl)biguanide] dihydrochloride.

Chlorhexidine Hydrochloride contains not less than 98.0 per cent and not more than 101.0 per cent of chlorhexidine dihydrochloride, $C_{22}H_{30}Cl_2N_{10}$, 2HCl calculated on the dried basis.

**Category**. Antiseptic.

**Description**. A white or almost white, crystalline powder.

### Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *chlorhexidine hydrochloride RS*.



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B. Dissolve about 5 mg in 5 ml of a warm 1.0 per cent w/v solution of *cetrimide* and add 1 ml of *strong sodium hydroxide solution* and 1 ml of *bromine water*. A deep red colour is produced.

C. Dissolve 0.3 g in 10 ml of a mixture of equal volumes of *hydrochloric acid* and *water*. Add 40 ml of *water*, filter if necessary and cool in ice *water*. Make alkaline to titan yellow paper by adding dropwise and with stirring *strong sodium hydroxide solution* and add 1 ml in excess. Filter, wash the precipitate with *water* until the washings are free from alkali and recrystallise from *alcohol (70 per cent v/v)*. Dry at 100° to 105°. Melting point (2.4.21). 132° to 136°.

D. It gives reaction (a) of chlorides (2.3.1).

### Tests

**Chloroaniline**. To 0.2 g of the substance under examination, add 1 ml of *hydrochloric acid*, dilute to 30 ml with *water* and shake until a clear solution is obtained. Add rapidly and with thorough mixing after each addition, 2.5 ml of *dilute hydrochloric acid*, 0.35 ml of *sodium nitrite solution*, 2 ml of a 5.0 per cent w/v solution of *ammonium sulphamate*, 5 ml of a 0.1 per cent w/v solution of *naphthylethylenediamine dihydrochloride* and 1 ml of *alcohol*, dilute to 50.0 ml with *water* and allow to stand for 30 minutes. Any reddish-blue colour in the solution is not more intense than that in a standard prepared at the same time and in the same manner using a mixture of 10.0 ml of a 0.001 per cent solution of *chloroaniline* in *dilute hydrochloric acid* and 20 ml of *dilute hydrochloric acid* instead of the solution of the substance under examination (500 ppm).

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 0.2 g of the substance under examination in 100 ml of the mobile phase.

*Reference solution (a)*. A 0.15 per cent w/v solution of *chlorhexidine hydrochloride RS* in the mobile phase.

*Reference solution (b)*. Dilute 2.5 ml of the test solution to 100 ml with the mobile phase.

*Reference solution (c)*. Dilute 2 ml of reference solution (b) to 10 ml with the mobile phase. Dilute 1 ml of the solution to 10 ml with the mobile phase.

Chromatographic system

- a stainless steel column 20 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: 2.0 g of *sodium octanesulphonate* in a mixture of 120 ml of *glacial acetic acid*, 270 ml of *water* and 730 ml of *methanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Equilibrate the column with the mobile phase for at least 1 hour. Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (b) is at least 50 per cent of the full scale of the recorder.

Inject the test solution and reference solutions (a), (b) and (c). Record the chromatograms until the peak due to chlorhexidine has been eluted and record the chromatogram of the test solution for six times the retention time of the peak due to chlorhexidine. In the chromatogram obtained with the test solution, the sum of the areas of all the peaks, other than the principal peak is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.5 per cent). Ignore any peak with a relative retention time of 0.25 or less with respect to the principal peak and any peak whose area is less than that of the principal peak in the chromatogram obtained with reference solution (c).

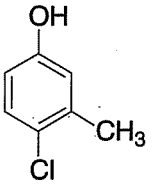
**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Assay**. Dissolve 0.1 g in 5 ml of *anhydrous formic acid* and add 70 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically (2.4.25).

1 ml of *0.1 M perchloric acid* is equivalent to 0.01446 g of $C_{22}H_{32}Cl_4N_{10}$.

## Chlorocresol




$C_7H_7ClO$ Mol. Wt. 142.6

Chlorocresol is 4-chloro-3-methylphenol.

Chlorocresol contains not less than 98.0 per cent and not more than 101.0 per cent of $C_7H_7ClO$.

**Category**. Antiseptic; pharmaceutical aid (antimicrobial preservative).

**Description**. Colourless or almost colourless crystals or a white, crystalline powder; odour, characteristic and not tarry; volatile in steam.

### Identification

A.To a saturated solution in *water* add one drop of *ferric chloride test solution*; a bluish colour is produced.



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B.To 0.1 g add 0.2 ml of *benzoyl chloride* and 0.5 ml of *2 M sodium hydroxide.* Shake vigorously until a white precipitate is produced, add 5 ml of *water* and filter. The melting range of the residue, after crystallisation from *methanol* and drying at 70°, is 85° to 88° (2.4.21).

### Tests

**Appearance of solution.** A 5.0 per cent w/v solution in *ethanol (95 per cent)* is clear (2.4.1) and not more intensely coloured than reference solution BYS6 (2.4.1).

**Acidity or alkalinity.** To 10 ml of a 5.0 per cent w/v solution add 0.1 ml of *methyl red solution.* The solution is orange or red and not more than 0.2 ml of *0.01 M sodium hydroxide* is required to change the colour of the solution to yellow.

**Related substances.** Determine by gas chromatography (2.4.13).

*Test solution.* A 1 per cent w/v solution of the substance under examination in *acetone.*

Chromatographic system

- a glass column 1.8m x 3.5 mm, packed with silanised diatomaceous support (80 to 120 mesh) impregnated with 3 to 5 per cent w/w of phenyl methyl silicone fluid (50 per cent phenyl) (such as OV-17),
- temperature:
  column.125°,
  inlet port. 210°,
  detector. 230°,
- flow rate. 30 ml per minute of the carrier gas (nitrogen).

Allow the chromatography to proceed for three times the retention time of chlorocresol (about 8 minutes).

The sum of the areas of any secondary peaks in the chromatogram is not greater than 1.0 per cent of the total area of the peaks.

**Non-volatile matter.** Not more than 0.1 per cent, determined on 2.0 g by volatilising on a water-bath and drying at 105°.

**Assay.** Weigh accurately about 70 mg, dissolve in 30 ml of *glacial acetic acid*, add 25.0 ml of *0.0167 M potassium bromate*, 20.0 ml of a 15 per cent w/v solution of *potassium bromide* and 10 ml of *hydrochloric acid.* Stopper the flask and allow to stand in the dark for 15 minutes, shaking occasionally. Add 1 g of *potassium iodide* and 100 ml of *water.* Titrate with *0.1 M sodium thiosulphate*, shaking vigorously and using *starch solution*, added towards the end of the titration, as indicator. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of potassium bromate required.

1 ml of *0.0167 M potassium bromate* is equivalent to 0.003565 g of $C_7H_7ClO$.

**Storage.** Store protected from light and moisture.

## Chloroform

$CHCl_3$ Mol. Wt. 119.4

Chloroform is trichloromethane to which either 1.0 per cent to 2.0 per cent v/v of ethanol or 50 mg per litre of amylene has been added.

**Category.** General anaesthetic; pharmaceutical aid (solvent and antimicrobial preservative).

**Description.** A colourless, volatile liquid; odour, characteristic.

*NOTE - Care should be taken not to vaporise chloroform in the presence of a flame because of the production of harmful gases.*

### Identification

*Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.*

A. Shake with an equal volume of *water* and dry with *anhydrous sodium sulphate.* Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with the reference spectrum of chloroform.

B. Non-flammable. The vapour introduced into a Bunsen flame produces a green colour and gives rise to noxious vapours having a characteristic odour.

C. Warm 0.5 ml with 0.05 ml of *aniline* and 1 ml of *5 M sodium hydroxide.* The characteristic odour of phenyl isocyanide is produced.

### Tests

**Weight per ml** (2.4.29). 1.474 g to 1.478 g.

**Boiling range** (2.4.8). Not more than 5.0 per cent v/v distils below 60° and the remainder distils between 60° and 62°.

**Acidity or alkalinity.** Shake 10 ml with 20 ml of freshly boiled and cooled *water* for 3 minutes and allow to separate. To 5 ml of the aqueous layer (solution A) add 0.1 ml of *litmus solution;* the colour produced is similar to that produced on adding 0.1 ml of *litmus solution* to 5 ml of freshly boiled and cooled *water.*

**Chlorides.** To 5 ml of solution A add 5 ml of *water* and 0.2 ml of *silver nitrate solution*; the solution is *clear.*

**Free chlorine.** To 10 ml of solution A add 1 ml of *cadmium iodide solution* and 2 drops of *starch solution*; no blue colour is produced.

**Aldehyde.** Shake 5 ml with 5 ml of *water* and 0.2 ml of *alkaline potassium mercuri-iodide solution* in a stoppered bottle and set aside in the dark for 15 minutes; not more than a pale yellow colour is produced.

**Foreign chlorine compounds.** Shake 20 ml with 10 ml of *sulphuric acid* in a stoppered flask for 5 minutes, allow to



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stand in the dark for 30 minutes and discard the acid layer. Shake 15 ml of the chloroform layer with 30 ml of *water* in a stoppered flask for 3 minutes and allow to separate. To the aqueous layer add 0.2 ml of *silver nitrate solution* and set aside in the dark for 5 minutes; no opalescence is produced.

**Related substances**. Determine by gas chromatography (2.4.13).

*Test solution (a).* A solution containing 0.2 per cent v/v of *carbon tetrachloride,* 0.2 per cent v/v of *1,1,1-trichloroethane* (internal standard), 0.2 per cent v/v of *dichloromethane,* 0.2 per cent v/v of *ethanol,* 0.5 per cent v/v of *bromochloromethane* and 0.2 per cent v/v of the substance under examination in *1-propanol.*

*Test solution (b).* The substance under examination.

*Reference solution (a).* A solution containing 0.2 per cent v/v of the internal standard in the substance under examination.

*Reference solution (b). 1-propanol.*

Chromatographic system

- a glass column 4m x 3 mm, packed with acid-washed kieselguhr (60 to 100 mesh) coated with 15 per cent w/w of *di-2-cyanoethyl ether,*
- temperature:
  column.40°,
  inlet port and detector. 100°,
- flow rate. 30 ml per minute of the carrier gas.
- Inject 0.1 µl of each solution.

The test is not valid unless the column efficiency, determined using the chloroform peak in the chromatogram obtained with test solution (a), is greater than 700 plates per metre and the total number of plates is greater than 2,500.

In the chromatogram obtained with test solution (a) the peaks, in the order of emergence, are due to carbon tetrachloride, 1,1,1-trichloroethane, dichloromethane, chloroform, ethanol, bromochloromethane and 1-propanol (solvent).

Using the chromatogram obtained with reference solution (b) make any corrections due to the contribution of secondary peaks from the solvent to the peaks in the chromatogram obtained with test solution (a).

In the chromatogram obtained with reference solution (a), the ratio of the areas of any peaks due to carbon tetrachloride, dichloromethane and bromochloromethane to the area of the peak due to the internal standard is not greater than the corresponding ratios in the chromatogram obtained with test solution (a) and the ratio of the area of any other secondary peak that elutes prior to the solvent peak, except for the peak corresponding to ethanol, to the area of the peak due to the internal standard is not greater than the ratio of the area of the peak due to chloroform to the area of the peak due to the internal standard in the chromatogram obtained with test solution (a).

Calculate the percentage content of each of the specified impurities and also calculate the percentage content of each of any other impurities assuming the same response per unit volume as with chloroform. The total content of all impurities is not more than 1.0 per cent v/v.

**Ethanol** *(if present).* Determine by gas chromatography (2.4.13).

*Test solution (a).* The substance under examination.

*Test solution (b).* A solution containing 1.0 per cent v/v of *1-propanol* (internal standard) in the substance under examination.

*Reference solution.* A solution containing 1.0 per cent v/v of *ethanol* and 1.0 per cent v/v of the internal standard in *water.*

Inject 0.1 µl of each solution.

Follow the chromatographic procedure described under Related substances.

The test is not valid unless the height of the trough separating the ethanol peak from the chloroform peak in the chromatogram obtained with test solution (a) is less than 15 per cent of the height of the ethanol peak.

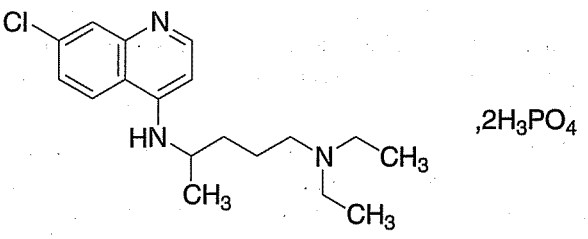
Calculate the percentage content of ethanol from the areas of the peaks due to ethanol and the internal standard in the chromatograms obtained with reference solution and test solution (b).

**Non-volatile matter**. Not more than 0.004 per cent w/v, determined on 25 ml by evaporation to dryness and drying at 105°.

**Storage**. Store protected from light in tightly-closed, glass-stoppered containers.

**Labelling**. The label states whether it contains ethanol or amylene.

## Chloroquine Phosphate




$C_{18}H_{26}ClN_3,2H_3PO_4$ Mol. Wt. 515.9

Chloroquine Phosphate is (*RS*)-7-chloro-4-(4-diethylamino-1-methylbutylamino)quinoline diphosphate.



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Chloroquine Phosphate contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{18}H_{26}ClN_3,2H_3PO_4$, calculated on the anhydrous basis.

**Category**. Antimalarial; antiamoebic.

**Dose**. Chloroquine. Prophylactic, 300 mg once weekly; therapeutic, initial dose, 600 mg followed by a single dose of 300 mg after 6 to 8 hours and then by a single dose of 300 mg daily for the next 2 to 4 days; by slow intravenous infusion, 200 to 300 mg (antimalarial); by intramuscular injection, 160 to 200 mg repeated at intervals of 12 hours until oral therapy is possible (antimalarial and in hepatic amoebiasis) 600 mg daily for 5 to 7 days followed by 300 to 450 mg daily or twice a week for 2 weeks or longer; by intramuscular injection, same dose as for malaria therapy (in hepatic amoebiasis).

(250 mg of chloroquine phosphate is approximately equivalent to 155 mg of chloroquine).

**Description**. A white or almost white, crystalline powder; odourless. It slowly gets discoloured on exposure to light. It may exist in two polymorphic forms differing in their behaviour, one of which melts at about 195° and the other at about 218°.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.*

A. Dissolve 0.1 g in 10 ml of *water*, add 2 ml of *2 M sodium hydroxide* and extract with two quantities, each of 20 ml, of *chloroform*. Wash the combined chloroform extracts with *water*, dry over *anhydrous sodium sulphate*, evaporate to dryness and dissolve the residue in 2 ml of *chloroform*. The resulting solution complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with 80 mg of *chloroquine phosphate RS* treated in the same manner.

B. When examined in the range 210 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution shows absorption maxima at about 220 nm, 235 nm, 256 nm, 329 nm and 342 nm; absorbance at about 220 nm, 0.60 to 0.66, at about 235 nm, 0.35 to 0.39, at about 256 nm, 0.30 to 0.33, at about 329 nm, 0.325 to 0.355 and at about 342 nm, 0.36 to 0.39.

C. Dissolve 25 mg in 20 ml of *water* and add 8 ml of *picric acid solution*; the precipitate, after washing successively with *water*, *ethanol (95 per cent)* and *ether*, melts at 205° to 210° (2.4.21).

D. Neutralise with *dilute nitric acid* the aqueous layer obtained in test A. Add an equal volume of *ammonium molybdate solution* and warm; a yellow precipitate is produced.

## Tests

**Appearance of solution**. A 10.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and not more intensely coloured than reference solution BYS5 *or* GYS5 (2.4.1).

**pH** (2.4.24). 3.5 to 4.5, determined in a 10.0 per cent w/v solution.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 50 volumes of *chloroform*, 40 volumes of *cyclohexane* and 10 volumes of *diethylamine*.

*Test solution*. A 5 per cent w/v solution of the substance under examination in *water*.

*Reference solution (a)*. Dilute 1 ml of the test solution to 100 ml with *water*.

*Reference solution (b)*. Dilute 25 ml of reference solution (a) to 50 ml with *water*.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals, Method A (10 ppm).

**Water** (2.3.43). Not more than 2.0 per cent, determined on 1.0 g.

**Assay**. Weigh accurately about 0.2 g and dissolve in 50 ml of *anhydrous glacial acetic acid* with the aid of heat (if necessary, heat under a reflux condenser). Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02579 g of $C_{18}H_{26}ClN_3,2H_3PO_4$.

**Storage**. Store protected from light.

# Chloroquine Phosphate Injection

Chloroquine Phosphate Injection is a sterile solution of Chloroquine Phosphate in Water for Injections.

Chloroquine Phosphate Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of chloroquine, $C_{18}H_{26}ClN_3$.

**Usual strength**. The equivalent of 40 mg of chloroquine per ml. (250 mg of chloroquine phosphate is approximately equivalent to 155 mg of chloroquine).

**Description**. A clear, colourless or almost colourless solution.

## Identification

A. To a volume of the injection containing 60 mg of chloroquine add 2 ml of *2 M sodium hydroxide* and extract with two



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quantities, each of 20 ml, of *chloroform.* Wash the combined chloroform extracts with *water,* dry over *anhydrous sodium sulphate*, evaporate to dryness and dissolve the residue in 2 ml of *chloroform.* The resulting solution complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with 80 mg of *chloroquine phosphate RS* treated in the same manner.

B. Dilute a volume of the injection containing 15 mg of chloroquine to 20 ml with *water* and add 8 ml of *picric acid solution*; the precipitate, after washing successively with *water*, *ethanol (95 per cent)* and *ether,* melts at about 207° (2.4.21).

C. Neutralise the aqueous layer obtained in test A with *dilute nitric acid*, add an equal volume of *ammonium molybdate solution* and warm; a yellow precipitate is produced.

### Tests

**pH** (2.4.24). 3.5 to 4.5.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. To an accurately measured volume of the injection containing 0.4 g of chloroquine add 20 ml of *1 M sodium hydroxide* and extract with four quantities, each of 25 ml, of *chloroform.* Combine the chloroform extracts and evaporate to a volume of about 10 ml. Add 40 ml of *anhydrous glacial acetic acid* and mix. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01599 g of chloroquine, $C_{18}H_{26}ClN_3$.

**Storage**. Store protected from light.

**Labelling**. The label states the strength in terms of the equivalent amount of chloroquine in a suitable dose-volume.

## Chloroquine Phosphate Suspension

Chloroquine Phosphate Suspension is a suspension of Chloroquine Phosphate in a suitable flavoured vehicle.

Chloroquine Phosphate Suspension contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of chloroquine, $C_{18}H_{26}ClN_3$.

**Usual strength**. The equivalent of 50 mg of chloroquine in 5 ml.

(80 mg of chloroquine phosphate is approximately equivalent to 50 mg of chloroquine).

### Identification

To a volume of the suspension containing 50 mg of chloroquine add 2 ml of *2 M sodium hydroxide* and extract with two quantities, each of 20 ml, of *chloroform.* Wash the combined chloroform extracts with *water,* dry over *anhydrous sodium sulphate*, evaporate to dryness and dissolve the residue in 2 ml of *chloroform.* The resulting solution complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with 80 mg of *chloroquine phosphate RS* treated in the same manner.

### Tests

**pH** (2.4.24). 5.5 to 6.5.

**Other tests**. Complies with the tests stated under Oral Liquids.

**Assay**. Weigh accurately a quantity of the suspension containing about 100 mg of chloroquine, add 50 ml of *1 M hydrochloric acid*, shake well and dilute to 100.0 ml with *1 M hydrochloric acid.* Filter and discard the first few ml of the filtrate. Dilute 10.0 ml of the filtrate to 100.0 ml with *1 M hydrochloric acid* and mix. Further dilute 10.0 ml to 100.0 ml with the same solvent and mix. Measure the absorbance of the resulting solution at the maximum at about 342 nm (2.4.7). Calculate the content of $C_{18}H_{26}ClN_3$ from the absorbance obtained by repeating the operation using *chloroquine phosphate RS* in place of the substance under examination.

## Chloroquine Phosphate Tablets

Chloroquine Phosphate Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of chloroquine phosphate, $C_{18}H_{26}ClN_3,2H_3PO_4$ The tablets are coated.

**Usual strength**. 250 mg. (250 mg of chloroquine phosphate is approximately equivalent to 155 mg of chloroquine).

### Identification

A. To a quantity of the powdered tablets containing 0.1 g of Chloroquine Phosphate add 10 ml of *water* and 2 ml of *2 M sodium hydroxide* and extract with two quantities, each of 20 ml, of *chloroform.* Wash the combined chloroform extracts with *water,* dry over *anhydrous sodium sulphate*, evaporate to dryness and dissolve the residue in 2 ml of *chloroform.* The resulting solution complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with 80 mg of *chloroquine phosphate RS* treated in the same manner.



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B. Extract a quantity of the powdered tablets containing 25 mg of Chloroquine Phosphate with 20 ml of *water*, filter and to the filtrate add 8 ml of *picric acid solution*; the precipitate, after washing successively with *water*, *ethanol (95 per cent)* and *ether*, melts at about 207° (2.4.21).

C. Extract a quantity of the powdered tablets containing 0.5 g of Chloroquine Phosphate with 25 ml of *water* and filter. To the filtrate add 2.5 ml of *5 M sodium hydroxide* and extract with three quantities, each of 10 ml, of *ether*. The aqueous layer, after neutralisation with *2 M nitric acid*, gives the reactions of phosphates (2.3.1).

### Tests

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 50 volumes of *chloroform*, 40 volumes of *cyclohexane* and 10 volumes of *diethylamine*.

*Test solution*. Shake a quantity of the powdered tablets containing 1 g of Chloroquine Phosphate with 20 ml of *water* for 30 minutes, centrifuge and use the clear, supernatant liquid.

*Reference solution (a)*. Dilute 1 ml of the test solution to 100 ml with *water*.

*Reference solution (b)*. Dilute 25 ml of reference solution (a) to 50 ml with *water*.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 75 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc with an average pore diameter not greater than 1.0 µm. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate with *0.1 M hydrochloric acid*. Measure the absorbance of the resulting solution at the maximum at about 344 nm (2.4.7). Calculate the content of $C_{18}H_{26}ClN_3,2H_3PO_4$ per tablet taking 371 as the specific absorbance at 344 nm.

D. Not less than 70 per cent of the stated amount of $C_{18}H_{26}ClN_3,2H_3PO_4$.

**Other tests**. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.5 g of Chloroquine Phosphate, add 20 ml of *1 M sodium hydroxide* and extract with four quantities, each of 25 ml, of *chloroform*. Combine the chloroform extracts and evaporate to a volume of about 10 ml. Add 40 ml of *anhydrous glacial acetic acid* and mix. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02579 g of $C_{18}H_{26}ClN_3,2H_3PO_4$.

**Storage**. Store protected from light.

## Chloroquine Sulphate

$C_{18}H_{26}ClN_3,H_2SO_4,H_2O$ Mol. Wt. 435.9

Chloroquine Sulphate is *(RS)*-4-(7-chloro-4- quinolyl-amino) pentyldiethylamine sulphate monohydrate.

Chloroquine Sulphate contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{18}H_{26}ClN_3,H_2SO_4$, calculated on the anhydrous basis.

**Category**. Antimalarial; antiamoebic.

**Dose**. Chloroquine. Prophylactic, 300 mg once weekly; therapeutic, initial dose, 600 mg followed by a single dose of 300 mg after 6 to 8 hours and then by a single dose of 300 mg daily for the next 2 to 4 days; by slow intravenous infusion, 200 to 300 mg (antimalarial); by intramuscular injection, 160 to 200 mg repeated at intervals of 12 hours until oral therapy is possible (antimalarial and in hepatic amoebiasis) 600 mg daily for 5 to 7 days followed by 300 to 400 mg daily or twice a week for 2 weeks or longer (in hepatic amoebiasis).

(200 mg of chloroquine sulphate is approximately equivalent to 147 mg of chloroquine).

**Description**. A white or almost white, crystalline powder; odourless.

### Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.*

A. Dissolve 0.1 g in 10 ml of *water*, add 2 ml of *2 M sodium hydroxide* and extract with two quantities, each of 20 ml, of *chloroform*. Wash the combined chloroform extracts with *water*, dry with *anhydrous sodium sulphate*, evaporate to dryness and dissolve the residue in 2 ml of *chloroform*. The resulting solution complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained by treating 0.1 g of *chloroquine sulphate RS* in the same manner.



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B. When examined in the range 210 nm to 360 nm, a 0.001 per cent w/v solution shows absorption maxima at about 220 nm, 235 nm, 256 nm, 329 nm and 342 nm; absorbance at about 220 nm, 0.73 to 0.81, at about 235 nm, 0.43 to 0.47, at about 256 nm, 0.37 to 0.41, at about 329 nm, 0.40 to 0.44 and at about 342 nm, 0.43 to 0.47 (2.4.7).

C. Dissolve 25 mg in 20 ml of *water* and add 8 ml of *picric acid solution*; the precipitate, after washing successively with *water*, *ethanol (95 per cent)* and *ether*, melts at 205° to 210° (2.4.21).

D. Gives reaction A of sulphates (2.3.1).

### Tests

**Appearance of solution**. An 8.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and not more intensely coloured than reference solution BYS5 or GYS5 (2.4.1).

**pH** (2.4.24). 4.0 to 5.0, determined in an 8.0 per cent w/v solution.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 50 volumes of *chloroform*, 40 volumes of *cyclohexane* and 10 volumes of *diethylamine*.

*Test solution*. A 5 per cent w/v solution of the substance under examination in *water*.

*Reference solution (a)*. Dilute 1 ml of the test solution to 100 ml with *water*.

*Reference solution (b)*. Dilute 25 ml of reference solution (a) to 50 ml with *water*.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

**Heavy metals** (2.3.13). 1.0 g dissolved in 25 ml of *water* complies with the limit test for heavy metals, Method A (20 ppm).

**Chlorides** (2.3.12). 1.25 g complies with the limit test for chlorides (200 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). 3.0 to 5.0 per cent, determined on 0.5 g.

**Assay**. Weigh accurately about 0.5 g and dissolve in 50 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.0418 g of $C_{18}H_{26}ClN_3,H_2SO_4$.

**Storage**. Store protected from light.

## Chloroquine Sulphate Injection

Chloroquine Sulphate Injection is a sterile solution of Chloroquine Sulphate in Water for Injections.

Chloroquine Sulphate Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of chloroquine, $C_{18}H_{26}ClN_3$.

**Usual strength**. The equivalent of 40 mg of chloroquine per ml. (200 mg of chloroquine sulphate is approximately equivalent to 147 mg of chloroquine).

**Description**. A clear, colourless or almost colourless solution.

### Identification

A. To a volume of the injection containing 70 mg of chloroquine add sufficient *water* to produce 10 ml, add 2 ml of *2 M sodium hydroxide* and extract with two quantities, each of 20 ml, of *chloroform*. Wash the combined chloroform extracts with *water*, dry with *anhydrous sodium sulphate*, evaporate to dryness and dissolve the residue in 2 ml of *chloroform*. The resulting solution complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained by treating 0.1 g of *chloroquine sulphate RS* in the same manner.

B. When examined in the range 210 nm to 360 nm, a 0.001 per cent w/v solution shows absorption maxima at about 220 nm, 235 nm, 256 nm, 329 nm and 342 nm; absorbance at about 220 nm, 0.73 to 0.81, at about 235 nm, 0.43 to 0.47, at about 256 nm, 0.37 to 0.41, at about 329 nm, 0.40 to 0.44 and at about 342 nm, 0.43 to 0.47 (2.4.7).

C. Gives reaction A of sulphates (2.3.1).

### Tests

**pH** (2.4.24). 4.0 to 5.5.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. To an accurately measured volume of the injection containing 0.4 g of chloroquine add 20 ml of *1 M sodium hydroxide* and extract with four quantities, each of 25 ml, of *chloroform*. Combine the chloroform extracts and evaporate to a volume of about 10 ml. Add 40 ml of *anhydrous glacial acetic acid* and mix. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml *0.1 M perchloric acid* is equivalent to 0.01599 g of $C_{18}H_{26}ClN_3$.

**Storage**. Store protected from light.

**Labelling**. The label states the strength in terms of the equivalent amount of chloroquine in a suitable dose-volume.



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# Chloroquine Sulphate Tablets

Chloroquine Sulphate Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of chloroquine sulphate, $C_{18}H_{26}ClN_3,H_2SO_4$. The tablets are coated.

**Usual strength**. 200 mg. (200 mg of chloroquine sulphate is approximately equivalent to 147 mg of chloroquine).

## Identification

A. To a quantity of the powdered tablets equivalent to 0.1 g of Chloroquine Sulphate add 10 ml of *water* and 2 ml of *2 M sodium hydroxide* and extract with two quantities, each of 20 ml, of *chloroform*. Wash the combined chloroform extracts with *water*, dry with *anhydrous sodium sulphate*, evaporate to dryness and dissolve the residue in 2 ml of *chloroform*. The resulting solution complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained by treating 0.1 g of *chloroquine sulphate RS* in the same manner.

B. Extract a quantity of the powdered tablets containing 25 mg of Chloroquine Sulphate with 20 ml of *water*, filter and to the filtrate add 8 ml of *picric acid solution*; the precipitate, after washing successively with *water*, *ethanol (95 per cent)* and *ether*, melts at about 207° (2.4.21).

C. Extract a quantity of the powdered tablets containing about 0.1 g of Chloroquine Sulphate with 10 ml of *water* and 1 ml of *dilute hydrochloric acid* and filter. To the filtrate add 1 ml of *barium chloride solution*; a white precipitate is produced.

## Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 75 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc with an average pore diameter not greater than 1.0 µm. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate with *0.1 M hydrochloric acid*. Measure the absorbance of the resulting solution at the maximum at about 344 nm (2.4.7). Calculate the content of $C_{18}H_{26}ClN_3,H_2SO_4$ per tablet taking 450 as the specific absorbance at 344 nm.

D. Not less than 70 per cent of the stated amount of $C_{18}H_{26}ClN_3,H_2SO_4$.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.5 g of Chloroquine Sulphate, add 20 ml of *1 M sodium hydroxide* and extract with four quantities, each of 25 ml, of *chloroform*. Combine the chloroform extracts and evaporate to a volume of about 10 ml. Add 40 ml of *anhydrous glacial acetic acid* and mix. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.0436 g of $C_{18}H_{26}ClN_3,H_2SO_4$.

**Storage**. Store protected from light.

# Chloroquine Syrup

Chloroquine Syrup is a solution of Chloroquine Phosphate or Chloroquine Sulphate in a suitable flavoured vehicle.

Chloroquine Syrup contains Chloroquine Phosphate or Chloroquine Sulphate equivalent to not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of chloroquine, $C_{18}H_{26}ClN_3$.

**Usual strength**. The equivalent of 50 mg of chloroquine in 5 ml. (80 mg of Chloroquine Phosphate or 67 mg of Chloroquine Sulphate is approximately equivalent to 50 mg of chloroquine).

## Identification

To a volume of the syrup containing 50 mg of chloroquine add 2 ml of *2 M sodium hydroxide* and extract with two quantities, each of 20 ml, of *chloroform*. Wash the combined chloroform extracts with water, dry with *anhydrous sodium sulphate*, evaporate to dryness and dissolve the residue in 2 ml of *chloroform*. The resulting solution complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained by treating 0.1 g of *chloroquine sulphate RS* in the same manner.

## Tests

**pH** (2.4.24). 4.0 to 6.5.

**Other tests**. Complies with the tests stated under Oral Liquids.

**Assay**. To an accurately measured volume of the syrup containing about 0.4 g of chloroquine add 20 ml of *1 M sodium hydroxide* and extract with four quantities, each of 25 ml, of *chloroform*. Combine the chloroform extracts and evaporate to a volume of about 10 ml. Add 40 ml of *anhydrous glacial acetic acid* and mix. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01599 g of $C_{18}H_{26}ClN_3$.



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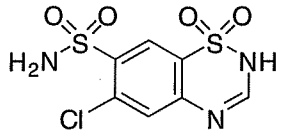
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**Storage**. Store protected from light.

**Labelling**. The label states (1) whether the syrup contains Chloroquine Phosphate or Chloroquine Sulphate; (2) the strength in terms of equivalent amount of chloroquine in each 5 ml.

## Chlorothiazide



$C_7H_6ClN_3O_4S_2$ Mol. Wt. 295.7

Chlorothiazide is 6-chloro-2*H*-1,2,4-benzothiadiazine-7-sulphonamide 1,1-dioxide

Chlorothiazide contains not less than 98.0 per cent and not more than 102.0 per cent of $C_7H_6ClN_3O_4S_2$, calculated on the dried basis.

**Category**. Diuretic; antihypertensive.

**Description**. A white or almost white, crystalline powder.

### Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

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

B. Dissolve 80 mg in 100 ml of *0.1 M sodium hydroxide* and dilute to 1000.0 ml with *water*. Dilute 10.0 ml of the solution to 100.0 ml with *0.01 M sodium hydroxide*. When examined in the range 220 nm to 320 nm (2.4.7), shows two absorption maxima at about 225 nm and 292 nm. The specific absorbance at the maxima are 725 to 800 and 425 to 450 respectively.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. *Ethyl acetate*.

*Test solution*. Dissolve 25 mg of the substance under examination in 5 ml of *acetone*.

*Reference solution*. A 0.5 per cent w/v solution of *chlorothiazide RS* in *acetone*.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution.

D. To 0.1 g, add a pellet of *sodium hydroxide* and heat strongly. Gas is evolved which turns *red litmus paper* to blue. After cooling, take up the residue with 10 ml of *dilute hydrochloric acid*. Gas is evolved which turns *lead acetate paper* to black.

### Tests

*Solution A*. Dissolve 1.0 g of the substance under examination in 50 ml of *water*.

**Acidity or alkalinity**. To 10 ml of solution A, add 0.2 ml of *0.01 M sodium hydroxide* and 0.15 ml of *methyl red solution*. The solution is yellow. Not more than 0.4 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator to red.

**Related substances**. Determine by thin-layer chromatography (2.4.17) coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 15 volumes of *2-propanol* and 85 volumes of *ethyl acetate*.

*Test solution*. Dissolve 25 mg of the substance under examination in 5.0 ml of *acetone*.

*Reference solution*. Dilute 1.0 ml of the test solution to 100.0 ml with *acetone*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and spray with a mixture of equal volumes of *alcoholic solution of sulphuric acid* and *alcohol*. Heat the plate at 105° for 30 minutes and immediately place the plate in the tank having 10 ml of a saturated solution of *sodium nitrite* in a beaker. Carefully add 0.5 ml of *sulphuric acid* to the *sodium nitrite solution*, close the tank, and allow to stand for 15 minutes. Remove the plate, heat in a ventilated oven at 40° for 15 minutes and spray with three quantities, each of 5 ml, of a freshly prepared 0.5 per cent w/v solution of *naphthylethylenediamine dihydrochloride* in *alcohol*. Examine the plate in day light. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (1.0 per cent).

**Chlorides** (2.3.12). 15 ml of Solution A complies with the limit test for chlorides (160 ppm).

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

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Dissolve 0.25 g in 50 ml of *dimethylformamide*. Titrate with *0.1 M tetrabutylammonium hydroxide* in *2-propanol* to the first point of inflexion. Determine the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.02957 g of $C_7H_6ClN_3O_4S_2$.



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# Chlorothiazide Oral Suspension

Chlorothiazide Oral Suspension is a dry mixture of Chlorothiazide with buffering agent and other excipients. It contains a suitable flavouring agent. It is filled in a sealed container.

The suspension is constituted by dispersing the content of the sealed container in the specified volume of water just before use.

Chlorothiazide Oral Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of chlorothiazide, $C_7H_6ClN_3O_4S_2$.

When stored at the temperature and for the period stated on the label during which the constituted suspension may be expected to be satisfactory for use, it contains not less than 80.0 per cent of the stated amount of chlorothiazide, $C_7H_6ClN_3O_4S_2$.

**Usual strength**. 50 mg per ml.

**Storage**. Store protected from moisture, at a temperature not exceeding 30°.

*The constituted suspension complies with the tests stated under Oral Liquids and with the following tests.*

## Identification

When examined in the range 230 nm to 360 nm (2.4.7), the solution obtained in the assay shows an absorption maxima at the same wavelength as that of solution of *chlorothiazide RS* prepared in the same manner.

## Tests

**pH** (2.4.24). 3.2 to 4.0, determined on constituted solution.

**Other tests**. Complies with tests stated under Oral Suspension.

**Assay**. Weigh accurately a quantity of the suspension containing about 250 mg of chlorothiazide, diluted to 250 ml with *sodium hydroxide solution* (1 in 250) and mix. Dilute 10.0 ml of this solution to 100 ml with *diluted hydrochloric acid* (1 in 100) and mix. Transfer 50.0 ml of the resulting solution to a 125 ml separator, and wash with two, 25 ml portions of *chloroform*, discarding the washing. Dilute 10.0 ml of the washed solution to 100 ml with *sodium hydroxide solution* (1 in 250) and mix. Dissolve an accurately weighed quantity of *chlorothizide RS* in *sodium hydroxide solution* (1 in 250) to obtain a concentration of about 10 µg per ml and measure the absorbance of the both solutions at the maxima (2.4.7) at about 292 nm.

Determine the weight per ml of the suspension (2.4.29) and calculate the content of chlorothiazide $C_7H_6ClN_3O_4S_2$ weight in oral suspension.

# Chlorothiazide Tablets

Chlorothiazide Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of chlorothiazide, $C_7H_6ClN_3O_4S_2$.

**Usual strengths**. 250 mg; 500 mg.

## Identification

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

B. Gives the reaction of sulphite (2.3.1).

## Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *phosphate buffer pH 8.0*,
Speed and time. 75 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter. Dilute the filtrate, if necessary, with the dissolution medium. Measure the absorbance of the resulting solution at the maximum at about 294 nm (2.4.7). Calculate the content of $C_7H_6ClN_3O_4S_2$ in the medium from the absorbance obtained from a solution of known concentration of *chlorothiazide RS*.

D. Not less than 75 per cent of the stated amount of $C_7H_6ClN_3O_4S_2$.

**Other tests**. Comply with tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*NOTE—Prepare the solutions immediately before use.*

*Test solution.* Weigh and powder 20 Tablets. Disperse a quantity of the powder containing about 250 mg of Chlorothiazide with 50.0 ml of *0.05 M monobasic sodium phosphate buffer*, shake for 15 minutes and add 100 ml of *acetonitrile*, dilute to 500 ml with *water*, filter.

*Reference solution.* Dissolve 25 mg of *chlorothiazide RS* in 5.0 ml of *0.05 M monobasic sodium phosphate buffer*, add 10.0 ml of *acetonitrile* and dilute to 50.0 ml with *water.*

Chromatographic system
- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 95 volumes of *0.08 M monobasic sodium phosphate*, adjusted to pH 2.9 with *orthophosphoric acid* and 5 volumes of *methanol*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.



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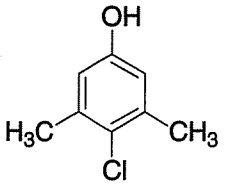
Inject the reference solution. The test is not valid unless the theoritical plates is not less than 1300, the capacity factor is not less than 4.3. The tailing factor is not less than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_7H_6ClN_3O_4S_2$ in the tablets.

**Storage**. Store protected from moisture.

## Chloroxylenol




$C_8H_9ClO$ Mol. Wt. 156.6

Chloroxylenol is 4-chloro-3,5-dimethylphenol.

Chloroxylenol contains not less than 98.0 per cent and not more than 103.0 per cent of $C_8H_9ClO$.

**Category**. Antiseptic; disinfectant.

**Description**. A white or creamy-white crystals or crystalline powder; odour characteristic. It is volatile in steam.

### Identification

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

B. Dissolve 0.1 g in 5 ml of *chloroform* and add 0.5 ml of a filtered 1 per cent w/v solution of *ferric chloride* in *chloroform* and 0.1 ml of *pyridine*; a blue colour is produced.

C. To 5 ml of a saturated solution in *water* add 0.5 ml of *ferric chloride test solution*; no blue colour is produced.

D. Mix 50 mg with 0.5 g of *anhydrous sodium carbonate* and ignite strongly, cool, boil the residue with 5 ml of *water*, acidify with *nitric acid*, filter and add 2 ml of *silver nitrate solution*; a white precipitate is produced.

### Tests

**Related substances**. Determine by gas chromatography (2.4.13).

*Test solution*. A 2 per cent w/v solution of the substance under examination in *chloroform*.

*Reference solution*. A solution containing 2 per cent w/v of the substance under examination and 0.04 per cent w/v of *4-chloro-o-cresol* (internal standard) in chloroform.

Chromatographic system
- a glass column 1.5m × 4 mm, packed with acid-washed diatomaceous support (80 to 100 mesh) coated with 3 per cent w/w of polyethylene glycol (such as Carbowax 20M),
- temperature:
  column.160°,
  inlet port and detector. 220°,
- a flame ionisation detector,
- flow rate. 30 ml per minute of the carrier gas.

In the chromatogram obtained with the reference solution the sum of the areas of any secondary peaks is not greater than the area of the peak due to internal standard.

**Assay**. Weigh accurately about 70 mg, dissolve in 30 ml of *glacial acetic acid*, add 25.0 ml of *0.0167 M potassium bromate*, 20 ml of a 15 per cent w/v solution of *potassium bromide* and 10 ml of *hydrochloric acid*, stopper the flask and allow to stand protected from light for 15 minutes. Add 1 g of *potassium iodide* and 100 ml of *water* and titrate with *0.1 M sodium thiosulphate*, shaking vigorously and using 1 ml of *starch solution* as indicator. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of *potassium bromate* required.

1 ml of *0.0167 M potassium bromate* is equivalent to 0.003915 g of $C_8H_9ClO$.

## Chloroxylenol Solution

Chloroxylenol solution is a solution of Chloroxylenol solubilised in a saponaceous base containing Ethanol (95 per cent) and essential oils. Ethanol (95 per cent) may be replaced by Industrial Methylated Spirit in making Chloroxylenol Solution.

Chloroxylenol Solution contains not less than 4.75 per cent and not more than 5.25 per cent of $C_8H_9ClO$.

**Usual strength**. 5 per cent w/v.

### Tests

**pH** (2.4.24). 7.0 to 11.0.

**Ethanol content** (2.3.45). 16 to 21 per cent v/v.

**Assay**. Determine by gas chromatography (2.4.13).

*Test solution*. Extract 4 ml of the solution under examination with 20.0 ml of *chloroform* after adding 4 ml of *2 M hydrochloric acid*. Extract with two further quantities, each



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of 10.0 ml, of *chloroform*. Combine the chloroform extracts, shake with *anhydrous sodium sulphate* and filter.

*Reference solution (a)*. Dissolve 0.1 g of *chloroxylenol RS* in 10.0 ml of a 0.8 per cent w/v solution of *4-chloro-o-cresol* (internal standard) in *chloroform* (solution A) and dilute to 20.0 ml with *chloroform*.

*Reference solution (b)*. Prepare in the same manner as the test solution but use 20.0 ml of solution A instead of 20 ml of *chloroform*.

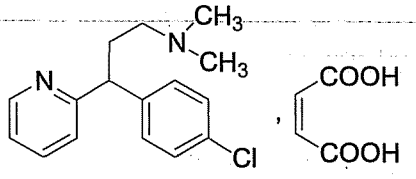
Chromatographic system

- a glass column 1.5m ξ 4 mm, packed with acid-washed, silanised diatomaceous support (80 to 100 mesh) coated with 3 per cent w/w of polyethylene glycol (such as Carbowax 20M),
- temperature:
  column.160°,
  inlet port and detector. 220°,
- a flame ionisation detector,
- flow rate. 30 ml per minute of the carrier gas.

Calculate the content of $C_8H_9ClO$ in the solution.

**Labelling**. The label states that the preparation is meant for external use only.

## Chlorpheniramine Maleate



$C_{16}H_{19}ClN_2,C_4H_4O_4$ Mol. Wt. 390.9

Chlorpheniramine Maleate is (*RS*)-3-(4-chlorophenyl)-3-(pyrid-2-yl)propyldimethylamine hydrogen maleate.

Chlorpheniramine Maleate contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{16}H_{19}ClN_2,C_4H_4O_4$, calculated on the dried basis.

**Category**. Antihistaminic.

**Dose**. Orally, 4 to 16 mg daily, in divided doses. By subcutaneous or intramuscular injection, 10 to 20 mg, repeated if required; maximum 40 mg in 24 hours. By slow intravenous injection over 1 minute, 10 to 20 mg diluted in the syringe with 5 to 10 ml of blood.

**Description**. A white, crystalline powder; odourless.

### Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *chlorpheniramine maleate RS* or with the reference spectrum of chlorpheniramine maleate.

B. When examined in the range 230 nm to 360 nm, a 0.002 per cent w/v solution in *0.05 M sulphuric acid* shows an absorption maximum only at about 265 nm; absorbance at about 265 nm, about 0.42 (2.4.7).

C. To 0.2 g add 3 ml of *water* and 1 ml of *10 M sodium hydroxide* and extract with three quantities, each of 5 ml, of *ether*. To 0.1 ml of the aqueous layer add a solution of 10 mg of *resorcinol* in 3 ml of *sulphuric acid* and heat in a water-bath for 15 minutes; the solution is colourless. To the remainder of the aqueous layer add 2 ml of *bromine solution*, heat in a water-bath for 15 minutes, heat to boiling and cool. To 0.2 ml of the resulting solution add a solution of 10 mg of *resorcinol* in 3 ml of *sulphuric acid* and heat in a water-bath for 15 minutes; a blue colour is produced.

D. Dissolve 0.1 g in 10 ml of *water* and add dropwise with shaking 25 ml of a 1 per cent w/v solution of *picric acid*. Collect the precipitate on a sintered-glass filter, wash with 3 ml of *ethanol (95 per cent)*, recrystallise from *ethanol (50 per cent)* and dry at 100° to 105°. The crystals melt between 196° and 200° (2.4.21).

### Tests

**Appearance of solution**. A 10.0 per cent w/v solution is clear (2.4.1), and not more intensely coloured than reference solution BYS6 (2.4.1).

**pH** (2.4.24). 4.0 to 5.0, determined in a 1.0 per cent w/v solution.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 50 volumes of *cyclohexane*, 40 volumes of *chloroform* and 10 volumes of *diethylamine*.

*Test solution*. A 5 per cent w/v solution of the substance under examination in *chloroform*.

*Reference solution*. Dilute 1 ml of the test solution to 100 ml with *chloroform* and mix. Dilute 5 ml of the resulting solution to 25 ml with *chloroform*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution. Ignore any spot remaining on the line of application.



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**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 4 hours.

**Assay**. Weigh accurately about 0.2 g and dissolve in 20 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01954 g of $C_{16}H_{19}ClN_2,C_4H_4O_4$.

**Storage**. Store protected from light and moisture.

## Chlorpheniramine Injection

Chlorpheniramine Maleate Injection

Chlorpheniramine Injection is a sterile solution of Chlorpheniramine Maleate in Water for Injections free from dissolved air and containing suitable buffering and stabilising agents.

Chlorpheniramine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of chlorpheniramine maleate, $C_{16}H_{19}ClN_2,C_4H_4O_4$.

**Usual strength**. 10 mg in 1 ml.

**Description**. A colourless solution.

### Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*. Heat the plate at 105° for 30 minutes before use.

*Mobile phase*. A mixture of 50 volumes of *ethyl acetate*, 30 volumes of *methanol* and 20 volumes of *1 M acetic acid*.

*Test solution*. Evaporate an appropriate volume of the injection to dryness in a current of nitrogen using the minimum amount of heat, dissolve the residue as completely as possible in sufficient *chloroform* to produce a solution containing 0.5 per cent w/v of Chlorpheniramine Maleate and centrifuge.

*Reference solution*. A 0.5 per cent w/v solution of *chlorpheniramine maleate RS* in *chloroform*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The two principal spots in the chromatogram obtained with the test solution correspond to those in the chromatogram obtained with the reference solution. Spray the plate with *dilute potassium iodobismuthate solution*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

### Tests

**pH** (2.4.24). 4.0 to 5.2.

**Related substances**. Carry out the method described under the Identification test using as the test solution a solution prepared in the following manner. Evaporate an appropriate volume of the injection to dryness in a current of nitrogen using the minimum amount of heat. Dissolve the residue in sufficient *chloroform* to produce a solution containing 5.0 per cent w/v of Chlorpheniramine Maleate and centrifuge. For the reference solution, dilute 1 volume of the test solution to 500 volumes with *chloroform*. After development, dry the plate in air and spray with *dilute potassium iodobismuthate solution*. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. Dilute an accurately measured volume of the injection containing 10 mg of Chlorpheniramine Maleate to 500.0 ml with *0.25 M sulphuric acid*. Measure the absorbance of the resulting solution at the maximum at about 265 nm (2.4.7). Calculate the content of $C_{16}H_{19}ClN_2,C_4H_4O_4$ taking 212 as the specific absorbance at 265 nm.

**Storage**. Store protected from light.

## Chlorpheniramine Tablets

Chlorpheniramine Maleate Tablets

Chlorpheniramine Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of chlorpheniramine maleate, $C_{16}H_{19}ClN_2,C_4H_4O_4$.

**Usual strengths**. 4 mg; 8 mg.

### Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*. Heat the plate at 105° for 30 minutes before use.

*Mobile phase*. A mixture of 50 volumes of *ethyl acetate*, 30 volumes of *methanol* and 20 volumes of *1 M acetic acid*.

*Test solution*. Extract a quantity of the powdered tablets containing 5 mg of Chlorpheniramine Maleate with *chloroform*, filter, evaporate the filtrate to dryness and dissolve the residue in 1 ml of *chloroform*.

*Reference solution*. A 0.5 per cent w/v solution of *chlorpheniramine maleate RS* in *chloroform*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm.



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The two principal spots obtained in the chromatogram obtained with the test solution correspond to those in the chromatogram obtained with the reference solution. Spray the plate with *dilute potassium iodobismuthate solution*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

### Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 50 volumes of *cyclohexane*, 40 volumes of *chloroform* and 10 volumes of *diethylamine*.

*Test solution*. Extract a quantity of the powdered tablets containing 100 mg of Chlorpheniramine Maleate with *chloroform*, filter, evaporate to dryness and dissolve the residue in 2 ml of *chloroform*.

*Reference solution*. Dilute 1 ml of the test solution to 50 ml with *chloroform* and dilute 1.0 ml of the resulting solution to 10.0 ml with the same solvent.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution. Ignore any spot remaining on the line of application.

**Uniformity of content**. Comply with test stated under Tablets.

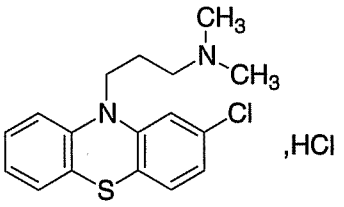
Powder one tablet and carry out the Assay beginning at the words "shake with 20 ml of *0.05 M sulphuric acid*....". Calculate the content of $C_{16}H_{19}ClN_2,C_4H_4O_4$ in the tablet.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 4 mg of Chlorpheniramine Maleate, shake with 20 ml of *0.05 M sulphuric acid* for 5 minutes, add 20 ml of *ether*, shake carefully and filter the acid layer into a second separator. Extract the ether layer with two quantities, each of 10 ml, of *0.05 M sulphuric acid*, filter each acid layer into the second separator and wash the filter with *0.05 M sulphuric acid*. Make the combined acid extracts and washing just alkaline to *litmus paper* with *1 M sodium hydroxide*, add 2 ml in excess, and extract with two quantities, each of 50 ml, of ether. Wash each ether extract with the same 20 ml of *water* and extract in succession with 20, 20 and 5 ml of *0.25 M sulphuric acid*, dilute the combined acid extracts to 50.0 ml with *0.25 M sulphuric acid*; dilute 10.0 ml to 50.0 ml with *0.25 M sulphuric acid* and measure the absorbance of the resulting solution at the maximum at about 265 nm (2.4.7). Calculate the content of $C_{16}H_{19}ClN_2,C_4H_4O_4$. taking 212 as the specific absorbance at 265 nm.

**Storage**. Store protected from light and moisture.

## Chlorpromazine Hydrochloride




$C_{17}H_{19}ClN_2S,HCl$ Mol. Wt. 355.3

Chlorpromazine Hydrochloride is 2-chloro-10-(3-dimethylaminopropyl)phenothiazine hydrochloride.

Chlorpromazine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{17}H_{19}ClN_2S,HCl$, calculated on the dried basis.

**Category**. Antipsychotic; antiemetic.

**Dose**. As antipsychotic, orally, 75 to 300 mg daily, in divided doses; by intramuscular injection, 25 to 50 mg. As antiemetic, orally, 10 to 25 mg every 4 to 6 hours; by deep intramuscular injection, 25 mg initially followed by 25 to 50 mg every 3 to 4 hours until necessary.

**Description**. A white or creamy-white, crystalline powder; odourless. It decomposes on exposure to air and light becoming yellow, pink and finally violet.

### Identification

*Test A may be omitted if tests B, C and D are carried out.*
*Test B may be omitted if tests A, C and D are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *chlorpromazine hydrochloride RS* or with the reference spectrum of chlorpromazine hydrochloride.

B. When examined in the range 230 nm to 360 nm, a 0.0005 per cent w/v solution in *0.1 M hydrochloric acid* shows absorption maxima at about 254 nm and 306 nm; absorbance at about 254 nm, 0.45 to 0.48 (2.4.7).

C. Complies with the test for identification of phenothiazines (2.3.3)

D. A 5 per cent w/v solution gives reaction B of chlorides (2.3.1).



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### Tests

**pH** (2.4.24). 3.5 to 4.5, determined in a 10.0 per cent solution.

**Related substances**. Complies with the test for Related substances in Phenothiazines (2.3.5), using mobile phase (a).

**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.6 g, dissolve in 200 ml of *acetone* and add 15 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, using a saturated solution of methyl orange in acetone as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03553 g of $C_{17}H_{19}ClN_2S,HCl$.

**Storage**. Store protected from light and moisture.

## Chlorpromazine Injection

Chlorpromazine Hydrochloride Injection

Chlorpromazine Injection is a sterile solution of Chlorpromazine hydrochloride in Water for Injections free from air and containing buffering and stabilizing agents.

Chlorpromazine Hydrochloride contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of chlorpromazine hydrochloride, $C_{17}H_{19}ClN_2S,HCl$.

*NOTE — Protect the solutions from light throughout the tests.*

**Usual strength**. 25 mg per ml.

**Description**. A colourless or almost colourless solution.

### Identification

A. To a volume containing 0.1 g of Chlorpromazine Hydrochloride, add 20 ml *of water* and 2 ml *of 10 M sodium hydroxide*. Extract with 25 ml *of ether*, wash the ether extract with two quantities, each of 5 ml, of *water*, dry the ether extract with *anhydrous sodium sulphate*, evaporate the ether and dissolve the residue in 1 ml of *chloroform*. The resulting solution complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *chlorpromazine hydrochloride RS* treated in the same manner or with the reference spectrum of chlorpromazine hydrochloride.

B. Dilute a volume of the injection with sufficient *0.1 M hydrochloric acid* to produce a solution containing 0.0005 per cent w/v of Chlorpromazine Hydrochloride. The resulting solution, when examined in the range 230 nm to 360 nm shows absorption maxima at about 254 nm and 306 nm; absorbance at about 254 nm, 0.45 to 0.48 (2.4.7).

C. Gives reaction B of chlorides (2.3.1).

### Tests

**Related substances**. Complies with the test for Related substances in Phenothiazines (2.3.5), using mobile phase (a) and the following solution.

*Test solution*. Dilute a volume of the injection with sufficient of a mixture of 95 volumes of *methanol* and 5 volumes of *diethylamine* to produce a solution containing 2.0 per cent of Chlorpromazine Hydrochloride.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. Dilute an accurately measured volume of the injection with sufficient *0.1 M hydrochloric acid* to produce a solution containing 0.0005 per cent w/v of Chlorpromazine Hydrochloride and measure the absorbance of the resulting solution at the maximum at about 254 nm (2.4.7). Calculate the content of $C_{17}H_{19}ClN_2S,HCl$, taking 915 as the specific absorbance at 254 nm.

**Storage**. Store protected from light.

## Chlorpromazine Tablets

Chlorpromazine Hydrochloride Tablets

Chlorpromazine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of chlorpromazine hydrochloride, $C_{17}H_{19}ClN_2S,HCl$. The tablets are coated.

*NOTE — Protect the solutions from light throughout the tests.*

**Usual strengths**. 10 mg; 25 mg; 50 mg; 100 mg; 200 mg.

### Identification

A. To a quantity of the powdered tablets containing 40 mg of Chlorpromazine Hydrochloride add 10 ml of *water* and 2 ml of *10 M sodium hydroxide*. Extract with 15 ml of *ether* and wash the ether extract with two quantities each of 5 ml, *of water*, dry with *anhydrous sodium sulphate*. Evaporate the ether and dissolve the residue in 0.4 ml *of chloroform*. The resulting solution complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *chlorpromazine*



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*hydrochloride RS* treated in the same manner or with the reference spectrum of chlorpromazine hydrochloride.

B. Digest a quantity of the powdered tablets containing 25 mg of Chlorpromazine Hydrochloride with 25 ml of *water* and filter. Reserve a portion of the filtrate for Identification C. Dilute a volume of the filtrate with sufficient *0.1 M hydrochloric acid* to produce a solution containing 0.0005 per cent w/v of Chlorpromazine Hydrochloride. The resulting solution, when examined in the range 230 nm to 360 nm shows absorption maxima at about 254 nm and 306 nm; absorbance at about 254 nm, 0.45 to 0.48 (2.4.7).

C. The filtrate reserved in test B gives reaction B of chlorides (2.3.1).

### Tests

**Related substances**. Complies with the test for Related substances in Phenothiazines (2.3.5), using mobile phase (a) and the following solutions.

*Test solution*. Extract a quantity of the powdered tablets containing 0.2 g of Chlorpromazine Hydrochloride with 10 ml of a mixture of 95 volumes of *melhanol* and 5 volumes of *dielhylamine* and filter.

*Reference solution*. Dilute 1 volume of the test solution to 200 volumes with the same solvent mixture.

**Uniformity of content**. Comply with the test stated under Tablets.

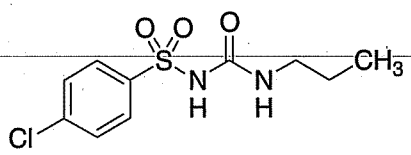
Powder one tablet, shake with 1 ml *of dilute hydrochloric acid* and 40 ml of *water* for 15 minutes, add sufficient *water* to produce 100.0 ml and mix. Centrifuge about 15 ml and to 10.0 ml of the clear, supernatant liquid add 2 ml of *1 M hydrochloric acid* and sufficient *water* to produce a solution containing about 0.0005 per cent w/v of Chlorpromazine Hydrochloride. Measure the absorbance of the resulting solution at the maximum at about 254 nm (2.4.7). Calculate the content of $C_{17}H_{19}ClN_2S,HCl$ in the tablet taking 915 as the specific absorbance at 254 nm.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.1 g of Chlorpromazine Hydrochloride, add 5 ml of *dilute hydrochloric acid* and 200 ml of *water*. Shake for 15 minutes and add sufficient *water* to produce 500.0 ml. Centrifuge about 15 ml and to 5.0 ml of the clear, supernatant liquid add 10 ml of *dilute hydrochloric acid* and sufficient *water* to produce 200.0 ml. Measure the absorbance of the resulting solution at the maximum at about 254 nm (2.4.7). Calculate the content of $C_{17}H_{19}ClN_2S,HCl$, taking 915 as the specific absorbance at 254 nm.

**Storage**. Store protected from light.

## Chlorpropamide



$C_{10}H_{13}ClN_2O_3S$ Mol. Wt. 276.7

Chlorpropamide is 1-(4-chlorobenzenesulphonyl)-3-propylurea.

Chlorpropamide contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{10}H_{13}ClN_2O_3S$, calculated on the dried basis.

**Category**. Hypoglycaemic.

**Dose**. 100 to 500 mg daily.

**Description**. A white, crystalline powder; odourless or almost odourless.

### Identification

*Test A may be omitted if tests B, C, D and E are carried out. Tests B, C, D and E may be omitted if test A is carried out.*

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

B. Dissolve 0.16 g in 50 ml of *methanol*, dilute 5 ml to 100 ml with *0.01 M hydrochloric acid* and dilute 5 ml of this solution to 100 ml with *0.01 M hydrochloric acid*. When examined in the range 220 nm to 360 nm, the resulting solution shows an absorption maximum only at about 232 nm; absorbance at about 232 nm, about 0.48 (2.4.7).

C. Boil 0.1 g with 8 ml of a 50 per cent w/w solution of *sulphuric acid* under a reflux condenser for 30 minutes, cool and filter, reserving the filtrate for test D. The precipitate, after recrystallisation from *water* and drying, melts at about 143° (2.4.21).

D. Make the filtrate reserved in test C alkaline with *sodium hydroxide solution* and heat; an ammoniacal odour is produced.

E. Heat 0.1 g with 1 g of *anhydrous sodium carbonate* at a dull red heat for 10 minutes. Cool, extract the residue with *water* and filter. Acidify the filtrate with *dilute nitric acid* and add *silver nitrate solution*; a white precipitate is produced.

### Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.



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*Mobile phase*. A mixture of 100 volumes of *chloroform*, 50 volumes of *methanol*, 30 volumes of *cyclohexane* and 11.5 volumes of *strong ammonia solution*.

*Test solution*. Dissolve 0.6 g of the substance under examination in 10 ml of *acetone*.

*Reference solution (a)*. A 0.02 per cent w/v solution of *4-chlorobenzenesulphonamide* in *acetone*.

*Reference solution (b)*. A 0.02 per cent w/v solution of *1,3-dipropylurea RS* in *acetone*.

*Reference solution (c)*. A 0.02 per cent w/v solution of the substance under examination in *acetone*.

Apply to the plate 5 µl of each solution. After development, dry the plate in a current of cold air, heat at 110° for 10 minutes, place the plate, while hot, in a tank of chlorine gas prepared by adding *hydrochloric acid* to a 5 per cent w/v solution of *potassium permanganate* contained in a beaker placed in the tank and allow to stand for 2 minutes. Dry it in a current of cold air until an area of the plate below the line of application gives at most a very faint blue colour with a 0.5 per cent w/v solution of *potassium iodide* in *starch solution;* avoid prolonged exposure to cold air. Any spots corresponding to 4-chlorobenzenesulphonamide and 1,3-dipropylurea in the chromatogram obtained with the test solution are not more intense than the spots in the chromatogram obtained with reference solutions (a) and (b) respectively. Any other secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (c).

**Heavy metals** (2.3.13). 0.66 g complies with the limit test for heavy metals, Method B (30 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent, determined on 2.0 g.

**Loss on drying** (2.4.19). Not more than 1.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.5 g and dissolve in 50 ml of *ethanol (95 per cent)* previously neutralised to *phenolphthalein solution*. Add 25 ml of *water* and titrate with *0.1 M sodium hydroxide* using *phenolphthalein solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.02767 g of $C_{10}H_{13}ClN_2O_3S$.

# Chlorpropamide Tablets

Chlorpropamide Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of chlorpropamide, $C_{10}H_{13}ClN_2O_3S$.

**Usual strengths**. 100 mg; 250 mg.

## Identification

Extract a quantity of the powdered tablets containing 1 g of Chlorpropamide with five quantities, each of 4 ml, of *acetone*, filter and carefully evaporate the filtrate to dryness on a water-bath. The residue complies with the following tests.

A. Boil 0.1 g with 8 ml of a 50 per cent w/w solution of *sulphuric acid* under a reflux condenser for 30 minutes, cool and filter, reserving the filtrate for test B. The precipitate, after recrystallisation from *water* and drying, melts at about 143° (2.4.21).

B. Make the filtrate reserved in test A alkaline with *sodium hydroxide solution* and heat; an ammonical odour is produced.

C. Heat 0.1 g with 1 g of *anhydrous sodium carbonate* at a dull red heat for 10 minutes. Cool, extract the residue with *water* and filter. Acidify the filtrate with *dilute nitric acid* and add *silver nitrate solution*; a white precipitate is produced.

## Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 100 volumes of *chloroform*, 50 volumes of *methanol*, 30 volumes of *cyclohexane* and 11.5 volumes of *strong ammonia solution*.

*Test solution*. Shake a quantity of the powdered tablets containing 0.6 g of Chlorpropamide with 10 ml of *acetone* and filter.

*Reference solution (a)*. A 0.02 per cent w/v solution of *4-chlorobenzenesulphonamide* in *acetone*.

*Reference solution (b)*. A 0.02 per cent w/v solution of *1,3-dipropylurea RS* in *acetone*.

*Reference solution (c)*. A 0.02 per cent w/v solution of the substance under examination in *acetone*.

Apply to the plate 5 µl of each solution. After development, dry the plate in a current of cold air, heat at 110° for 10 minutes, place the plate, while hot, in a tank of chlorine gas prepared by adding *hydrochloric acid* to a 5 per cent w/v solution of *potassium permanganate* contained in a beaker placed in the tank and allow to stand for 2 minutes. Dry it in a current of cold air until an area of the plate below the line of application gives at most a very faint blue colour with a 0.5 per cent w/v solution of *potassium iodide* in *starch solution;* avoid prolonged exposure to cold air. Any spots corresponding to 4-chlorobenzenesulphonamide and 1,3-dipropylurea in the chromatogram obtained with the test solution are not more intense than the spots in the chromatogram obtained with reference solutions (a) and (b) respectively. Any other secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (c).



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**Dissolution** (2.5.2).

Apparatus. No 1

Medium. 900 ml of a 0.68 per cent w/v solution of *potassium dihydrogen phosphate* adjusted to pH 7.4 by the addition of *1 M sodium hydroxide*

Speed and time. 100 rpm and 60 minutes.

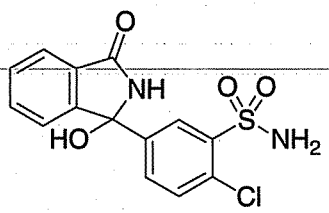
Withdraw a suitable volume of the medium and filter through a membrane filter with an average pore diameter not greater than 1.0 µm. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate with *0.1 M hydrochloric acid* to obtain a solution containing about 10 µg of chlorpropamide per ml. Measure the absorbance of the resulting solution at the maximum at about 232 nm (2.4.7). Calculate the content of $C_{10}H_{13}ClN_2O_3S$ taking 598 as the specific absorbance at 232 nm.

D. Not less than 75 per cent of the stated amount of $C_{10}H_{13}ClN_2O_3S$.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.25 g of Chlorpropamide and shake with 40 ml of *methanol* for 20 minutes, add sufficient *methanol* to produce 50.0 ml, mix, filter and dilute 5.0 ml of the filtrate to 100.0 ml with *0.1 M hydrochloric acid*. Mix, dilute 10.0 ml of this solution to 250.0 ml with *0.1 M hydrochloric acid* and measure the absorbance of the resulting solution at the maximum at about 232 nm (2.4.7). Calculate the content of $C_{10}H_{13}ClN_2O_3S$ taking 598 as the specific absorbance at 232 nm.

# Chlorthalidone




$C_{14}H_{11}ClN_2O_4S$ Mol. Wt 338.8

Chlorthalidone is (*RS*)-2-chloro-5-(1-hydroxy-3-oxoisoindolin-1-yl)benzenesulphonamide.

Chlorthalidone contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{14}H_{11}ClN_2O_4S$, calculated on the dried basis.

**Category**. Diuretic.

**Dose**. 50 to 200 mg daily.

**Description**. A white to yellowish-white, crystalline powder; almost odourless.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

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

B. When examined in the range 230 nm to 360 nm, a 0.01 per cent w/v solution in *ethanol (95 per cent)* shows absorption maxima at about 275 nm and at about 284 nm; absorbance at about 275 nm, about 0.6 and at about 284 nm, about 0.45 (2.4.7).

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 197 volumes of *ethyl acetate* and 3 volumes of *water*.

*Test solution*. Dissolve 0.1 g of the substance under examination in 100 ml of *acetone*.

*Reference solution*. A 0.1 per cent w/v solution of *chlorthalidone RS* in *acetone*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

D. Dissolve 10 mg in 1 ml of *sulphuric acid;* an intense yellow colour is produced.

## Tests

**Appearance of solution**. Dissolve 1.0 g in sufficient *2 M sodium hydroxide* to produce 10 ml. The solution is clear (2.4.1), and not more intensely coloured than degree 6 of the appropriate range of reference solutions (2.4.1).

**Acidity**. Dissolve 1 g in a mixture of 25 ml of *acetone* and 25 ml of *carbon dioxide-free water* with the aid of heat, cool and titrate with *0.1 M sodium hydroxide* using *methyl red solution* as indicator. Repeat the operation without the substance under examination. The difference between the titrations is not more than 0.75 ml.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 30 volumes of *dioxan*, 30 volumes of *2-propanol*, 30 volumes of *toluene* and 20 volumes of *strong ammonia solution*.

*Test solution*. A 2 per cent w/v solution of the substance under examination in *acetone*.



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*Reference solution (a)*. Dilute 10 ml of the test solution to 20 ml with *acetone* and mix. Dilute 1 ml of the resulting solution to 100 ml with *acetone*.

*Reference solution* (b). A 0.02 per cent w/v solution of *2-(4-chloro-3-sulphamoylbenzoyl)benzoic acid RS* in *acetone*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any spot corresponding to 2-(4-chloro-3-sulphamoylbenzoyl)benzoic acid in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b) and any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (a).

**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

**Chlorides** (2.3.12). Triturate 0.5 g with 30 ml of *water*, shake for 5 minutes and filter. 15 ml of the filtrate complies with the limit test for chlorides. Use 5.0 ml of *chloride standard solution (25 ppm Cl)* to prepare the standard (500 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.2 g and dissolve in 50 ml of *acetone*. Titrate with *0.1 M tetrabutylammonium hydroxide* in an atmosphere of nitrogen, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.03388 g of $C_{14}H_{11}ClN_2O_4S$.

## Chlorthalidone Tablets

Chlorthalidone Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of chlorthalidone, $C_{14}H_{11}ClN_2O_4S$.

**Usual strength**. 50 mg.

### Identification

Heat a quantity of the powdered tablets containing 0.2 g of Chlorthalidone with 20 ml of *acetone* on a water-bath for 10 minutes, cool and filter. Add 40 ml of *water* to the filtrate and heat on a water-bath for 20 minutes using a gentle current of air to remove the solvent. Cool to room temperature and allow to stand, filter and dry the crystals at 105° for 4 hours. The crystals comply with the following tests.

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

B. When examined in the range 230 nm to 360 nm, a 0.01 per cent w/v solution in *ethanol (95 per cent)* shows absorption maxima at about 275 nm and at about 284 nm; absorbance at about 275 nm, about 0.6 and at about 284 nm, about 0.45 (2.4.7).

C. Wash with *water* a quantity of the crystals obtained in test A and dissolve 50 mg in 3 ml of *sulphuric acid*; an intense yellow colour is produced.

### Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Test solution*. Shake a quantity of the powdered tablets containing 50 mg of Chlorthalidone with 5 ml of *acetone*, centrifuge and use the supernatant liquid.

*Reference solution*. A solution containing 0.01 per cent w/v of *2-(4-chloro-3-sulphamoylbenzoyl)benzoic acid RS* in *acetone*.

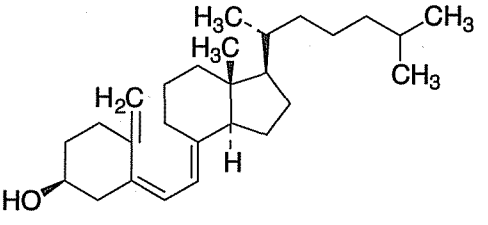
Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.1 g of Chlorthalidone, boil with 30 ml of *methanol* under a reflux condenser for 5 minutes, shake vigorously for 15 minutes, cool and filter; wash the residue with *methanol* and filter. Dilute the combined filtrate and washings to 100.0 ml with *methanol*. To 5.0 ml add 2 ml of *1 M hydrochloric acid* and sufficient *methanol* to produce 50.0 ml. Measure the absorbance of the resulting solution at the maximum at about 275 nm (2.4.7). Calculate the content of $C_{14}H_{11}ClN_2O_4S$ taking 57.4 as the specific absorbance at 275 nm.

## Cholecalciferol

Vitamin $D_3$




$C_{27}H_{44}O$ Mol Wt. 384.6

Cholecalciferol is (5*Z*,7*E*)-(3*S*)-9,10-secocholesta-5,7,10(19)-triene-3-ol.



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Cholecalciferol contains not less than 97.0 per cent and not more than 103.0 per cent of $C_{27}H_{44}O$.

**Category**. Vitamin D (antirachitic).

**Dose**. Orally, in prevention of rickets, not more than 20 µg (800 Units) daily, allowance being made for vitamin D obtained from other sources; in the treatment of rickets and osteomalacia, 125 µg to 1.25 mg (5,000 to 50,000 Units) daily; in the treatment of hypocalcaemia of hypoparathyroidism, 1.25 to 5 mg (50,000 to 200,000 Units) daily. By intramuscular injection, 5 to 10 mg.

[Cholecalciferol contains 40,000 Units of antirachitic activity (vitamin D) in each mg].

**Description**. White or almost white crystals; odourless or almost odourless. It is sensitive to air, heat and light. A reversible isomerisation to precholecalciferol may occur in solution, depending on temperature and time.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cholecalciferol RS*.

B. Dissolve 1 mg in 1 ml of *1,2-dichloroethane* and 4 ml of *antimony trichloride solution*; a yellowish-orange colour is produced.

C. In the test for 7-Dehydrocholesterol, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (b).

D. To a solution of about 0.5 mg in 5 ml of *chloroform* add 0.3 ml of *acetic anhydride* and 0.1 ml *sulphuric acid* and shake vigorously; a bright red colour is produced which rapidly changes through violet and blue to green.

## Tests

**Specific optical rotation** (2.4.22). +105° to +112°, determined, within 30 minutes of preparation, in a solution prepared by dissolving 0.2 g rapidly and without heating in sufficient *aldehyde-free ethanol (95 per cent)* to produce 25.0 ml.

**Light absorption**. Dissolve 10 mg, rapidly and without heating, in sufficient *aldehyde-free ethanol (95 per cent)* to produce 100.0 ml. Dilute 5.0 ml of this solution to 50.0 ml with *aldehyde-free ethanol (95 per cent)*. Absorbance of the resulting solution at the maximum at about 265 nm, measured within 30 minutes of preparation, 0.46 to 0.50 (2.4.7).

**7-Dehydrocholesterol**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A 0.01 per cent w/v solution of *butylated hydroxytoluene* in a mixture of equal volumes of *cyclohexane* and *peroxide-free ether*.

*NOTE—Prepare the following solutions immediately before use.*

*Test solution*. Dissolve 0.25 g of the substance under examination in sufficient of *1,2-dichloroethane* containing 1 per cent w/v of *squalane* and 0.01 per cent w/v of *butylated hydroxytoluene* (solvent A) to produce 5 ml.

*Reference solution (a)*. A solution containing 0.005 per cent w/v of *7-dehydrocholesterol RS* in solvent A.

*Reference solution (b)*. A solution containing 2.5 per cent w/v of *cholecalciferol RS* in solvent A.

*Reference solution (c)*. Mix equal volumes of reference solutions (a) and (b).

Apply to the plate 10 µl of each solution. Develop the chromatograms immediately, protected from light. After development, dry the plate in air and spray three times with *antimony trichloride reagent*. Examine the chromatograms for not more than 4 minutes after spraying. The principal spot in the chromatogram obtained with the test solution is initially orange-yellow but becomes brown later. In the chromatogram obtained with the test solution any violet spot with an Rf value slightly lower than that of the principal spot (due to 7-dehydrocholesterol and appearing slowly) is not more intense than the spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated principal spots.

**Assay**. Determine by liquid chromatography (2.4.14).

*NOTE—Carry out the following procedure as rapidly as possible in subdued light and protected from air.*

*Test solution*. Weigh accurately about 50.0 mg of the substance under examination, dissolve in 10 ml of *toluene* without heating and dilute to 100.0 ml with the mobile phase; dilute 5.0 ml of this solution to 50.0 ml with the mobile phase; further dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

*Reference solution (a)*. Dissolve 50.0 mg of *cholecalciferol RS* in 10 ml of toluene without heating and dilute to 100.0 ml with the mobile phase; dilute 5.0 ml of this solution to 50.0 ml with the mobile phase (Solution A); further dilute 5.0 ml of solution A to 50.0 ml with the mobile phase.

*Reference solution (b)*. Reflux 5.0 ml of solution A, under nitrogen, on a water-bath for 60 minutes to obtain a solution of cholecalciferol, precholecalciferol and *trans*-cholecalciferol.

Chromatographic system

– a stainless steel column 25 cm × 4.6 mm, packed with porous silica particles (5 µm) (such as Nucleosil 50-S),



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- mobile phase: a mixture of 997 volumes of *hexane* and 3 volumes of *1-pentanol*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (b) and record the chromatogram adjusting the sensitivity so that the height of the peak due to cholecalciferol is more than 50 per cent of full-scale deflection. The approximate relative retention times calculated with reference to cholecalciferol are 0.4 for precholecalciferol and 0.5 for *trans*-cholecalciferol. The resolution between precholecalciferol and *trans*-cholecalciferol should be not less than 1.0; if necessary adjust the proportions of the constituents and flow rate of the mobile phase to obtain the required resolution.

Inject reference solution (a) and record the chromatogram adjusting the sensitivity so that the height of the peak due to cholecalciferol is more than 50 per cent of full-scale deflection.

Inject the test solution. Measure the areas for the major peaks.

Calculate the content of $C_{27}H_{44}O$.

**Storage**. Store protected from light in hermetically sealed containers under nitrogen in a refrigerator. The contents of an opened container should be used immediately.

# Chorionic Gonadotrophin

Human Chorionic Gonadotrophin

Chorionic Gonadotrophin is a dry, sterile preparation of placental glycoproteins that has luteinising activity. It is extracted from the urine of pregnant women. The material is sterilised by filtration and dried under reduced pressure or freeze-dried.

Chorionic Gonadotrophin contains not less than 2500 Units per mg.

**Category**. Gonadotrophic hormone.

**Dose**. By intramuscular injection, 500 to 5000 Units twice weekly or in accordance with the needs and response of the patient.

**Description**. A white or almost white, amorphous powder.

## Identification

It causes an increase in the weight of the seminal vesicles or of the prostate glands of immature male rats when administered as directed in the Assay.

## Tests

**Appearance of solution**. A 1.0 per cent w/v solution is clear (2.4.1), and colourless (2.4.1).

**Water**. Not more than 5 per cent, determined by the following method.

Determine by gas chromatography (2.4.13).

*NOTE—Use throughout dry glassware that may be siliconised.*

*Internal standard solution.* Dilute 15 µl of *anhydrous methanol* with sufficient *anhydrous 2-propanol* to produce 100 ml.

*Test solution (a).* Dissolve 4 mg of the substance under examination in 0.5 ml of *anhydrous 2-propanol.*

*Test solution (b).* Dissolve 4 mg of the substance under examination in 0.5 ml of internal standard solution.

*Reference solution.* Add 10 µl of *water* to 50 ml of internal standard solution.

Chromatographic system

- a stainless steel column 1m × 2 mm, packed with porous polymer beads (60 to 80 mesh) (such as Chromosorb 102),
- temperature:
  column.120°,
  inlet port and detector. 150°,
- thermal conductivity detector,
- flow rate. 30 ml per minute of the carrier gas (helium).

From the chromatograms obtained, and taking into account any water detectable in internal standard solution, calculate the percentage of water taking 0.9960 g as the weight per ml at 25°.

**Assay**. Carry out the biological assay of chorionic gonadotrophin described below.

*Standard preparation.* The 3rd International Standard for Chorionic Gonadotrophin, human, established in 1986, consisting of a freeze-dried extract of human chorionic gonadotrophin with human albumin (supplied in ampoules containing 650 Units), or another suitable preparation the potency of which has been determined in relation to the International Standard.

Dissolve a sufficient quantity corresponding to the daily doses to be used in sufficient *albumin-phosphate buffer pH 7.2* so that the daily dose is about 0.2 ml. Add a suitable antimicrobial preservative such as 0.4 per cent w/v of *phenol* or 0.002 per cent w/v of *thiomersal.* Store the solution at a temperature of 2° to 8°.

*Test preparation.* Dissolve a sufficient quantity of the preparation under examination corresponding to the daily doses to be used in sufficient *albumin-phosphate buffer pH 7.2* so that the daily dose is about 0.2 ml. Add a suitable antimicrobial preservative such as 0.4 per cent w/v of *phenol* or 0.002 per cent w/v of *thiomersal.* Store the solution at a temperature of 2° to 8°.



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Use immature male rats of the same strain, approximately 21 days old and of approximately equal weight within the range 25 to 35 g. Assign the rats at random to four equal groups of at least eight animals. If sets of four littermates are available, allot one littermate from each set at random to each group and mark according to the litter.

Choose two doses of the standard preparation and two of the test solution such that the smaller dose is sufficient to produce a positive response in some of the rats and the larger dose does not produce a maximum response in all of the rats. As an initial approximation, doses of 7.5 and 15 Units may be tried although the dose will depend on the sensitivity of the animals used, which may vary widely.

Inject subcutaneously into each rat the daily dose allocated to its group on 4 consecutive days at the same time each day. On the fifth day, about 24 hours after the last injection, kill the rats and remove the seminal vesicles or the prostate glands from each animal. Remove any extraneous fluid and tissue from the vesicles or glands and weigh them immediately. Calculate the result of the assay by standard statistical methods using the weight of the vesicles or prostate glands as the response.

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The fiducial limits of error are not less than 64 per cent and not more than 156 per cent of the stated potency.

*Chorionic Gonadotrophin intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.*

**Bacterial endotoxins** (2.2.3). Not more than 15 Endotoxin Units per ml of a solution prepared in the following manner. Dissolve a quantity in *water BET* to obtain a solution containing 500 units of chorionic gonadotrophin per ml. Carry out the test using Maximum Valid dilution of this solution calculated from the declared sensitivity of the lysate used in the test.

*Chorionic Gonadotrophin intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirements.*

**Sterility** (2.2.11). Complies with the test for sterility.

**Abnormal toxicity** (2.2.1). Complies with the test for abnormal toxicity (2.2.1) using a quantity equivalent to 1000 Units dissolved in 0.5 ml of *sodium chloride injection* and observing the animals for 48 hours.

**Storage**. Store protected from light in a tamper-evident container, which is sealed so as to exclude micro-organisms, in a refrigerator (2° to 8°).

**Labelling**. The label states (1) the number of Units contained in the container; (2) the number of Units per mg; (3) whether or not it is intended for use in the manufacture of parenteral preparations.

## Chorionic Gonadotrophin Injection

Chorionic Gonadotrophin Injection is a sterile material consisting of Chorionic Gonadotrophin with or without excipients such as buffers, diluents or other inert substances such as Lactose or Sodium Chloride. It may also contain an antimicrobial agent. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

*The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).*

**Storage**. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Chorionic Gonadotrophin Injection contains not less than 80.0 per cent and not more than 125.0 per cent of the stated potency.

**Usual strengths**. 500, 1000, 2000, 5000 and 10,000 Units in each sealed container.

**Description**. A white or almost white powder.

*The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.*

### Identification

It causes an increase in the weight of the seminal vesicles or of the prostate glands of immature male rats when administered as directed in the Assay.

### Tests

**pH** (2.4.24). 6.0 to 8.0, determined in a 1.0 per cent w/v solution.

**Assay**. Carry out the biological assay of chorionic gonadotrophin described below.

*Standard preparation*. The 3rd International Standard for Chorionic Gonadotrophin, human, established in 1986, consisting of a freeze-dried extract of human chorionic gonadotrophin with human albumin (supplied in ampoules



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containing 650 Units), or another suitable preparation the potency of which has been determined in relation to the International Standard.

Dissolve a sufficient quantity corresponding to the daily doses to be used in sufficient *albumin-phosphate buffer pH 7.2* so that the daily dose is about 0.2 ml. Add a suitable antimicrobial preservative such as 0.4 per cent w/v of *phenol* or 0.002 per cent w/v of *thiomersal*. Store the solution at a temperature of 2° to 8°.

*Test preparation*. Dissolve a sufficient quantity of the injection under examination corresponding to the daily doses to be used in sufficient *albumin-phosphate buffer pH 7.2* so that the daily dose is about 0.2 ml. Add a suitable antimicrobial preservative such as 0.4 per cent w/v of *phenol* or 0.002 per cent w/v of *thiomersal*. Store the solution at a temperature of 2° to 8°.

Use immature male rats of the same strain, approximately 21 days old and of approximately equal weight within the range 25 to 35 g. Assign the rats at random to four equal groups of at least eight animals. If sets of four littermates are available, allot one littermate from each set at random to each group and mark according to the litter.

Choose two doses of the standard preparation and two of the test solution such that the smaller dose is sufficient to produce a positive response in some of the rats and the larger dose does not produce a maximum response in all of the rats. As an initial approximation, doses of 7.5 and 15 Units may be tried although the dose will depend on the sensitivity of the animals used that may vary widely.

Inject subcutaneously into each rat the daily dose allocated to its group on 4 consecutive days at the same time each day. On the fifth day, about 24 hours after the last injection, kill the rats and remove the seminal vesicles or the prostate glands from each animal. Remove any extraneous fluid and tissue from the vesicles or glands and weigh them immediately. Calculate the result of the assay by standard statistical methods using the weight of the vesicles or prostate glands as the response.

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The fiducial limits of error are not less than 64 per cent and not more than 156 per cent of the stated potency.

**Bacterial endotoxins** (2.2.3). Not more than 15 Endotoxin Units per ml of a solution prepared in the following manner. Dissolve the contents of a sealed container in *water BET* to obtain a solution containing 500 units of chorionic gonadotrophin per ml. Carry out the test using Maximum Valid Dilution of this solution calculated from the declared sensitivity of the lysate used in the test.

**Abnormal toxicity** (2.2.1). Use a quantity equivalent to 1000 Units dissolved in 0.5 ml of *sodium chloride injection* and observing the animals for 48 hours.

**Storage**. Store protected from light in containers, which are sealed so as to exclude micro-organisms, at a temperature not exceeding 20°.

**Labelling**. The label states (1) the number of Units contained in the sealed container; (2) the name(s) of any added substance(s).

# Chymotrypsin

Chymotrypsin is a proteolytic enzyme obtained by the activation of chymotrypsinogen extracted from the pancreas of beef *(Bos taurus* L.*)*. It has an activity of not less than 5.0 microkatals per milligram. In solution it has maximal enzymic activity at about pH 8; the activity is reversibly inhibited at pH 3, the pH at which it is most stable.

**Category**. Proteolytic enzyme.

## Production

The animals from which chymotrypsin is derived must fulfil the requirements for the health of animals suitable for human consumption. Furthermore, the tissues used shall not include any specified risk material as defined by any relevant international or, where appropriate, national legislation.

The method of manufacture is validated to demonstrate that the product, if tested, would comply with the following test.

**Histamine** (2.2.7). Not more than 1 µg (calculated as histamine base) per 5 microkatals of chymotrypsin activity. Before carrying out the test, heat the solution of the substance under examination on a water-bath for 30 minutes.

**Description**. A white or almost white, crystalline or amorphous powder.

## Identification

*Substrate solution*. To 24 mg of *acetyltyrosine ethyl ester* add 0.2 ml of *ethanol (95 per cent)*, and swirl until solution is effected. Add 2.0 ml of *0.067 M phosphate buffer solution pH 7.0* and 1 ml of *methyl red mixed solution* and dilute to 10.0 ml with *water*.

A. A 1.0 per cent w/v solution in *carbon dioxide-free water* (Solution A). Dilute 1 ml of solution A to 10 ml with *water*. In a depression in a white spot plate, mix 0.05 ml of this solution with 0.2 ml of substrate solution; a purple colour develops.



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B. Dilute 0.5 ml of solution A to 5 ml with *water*. Add 0.1 ml of a 2 per cent w/v solution of *tosylphenylalanylchloromethane* in *ethanol (95 per cent)*. Adjust to pH 7.0 and shake for 2 hours. In a depression in a white spot plate, mix 0.05 ml of this solution with 0.2 ml of the substrate solution; no colour develops within 3 minutes of mixing.

### Tests

**Appearance of solution**. Solution A is not more opalescent than Opalescence standard OS2 (2.4.1).

**pH** (2.4.24). 3.0 to 5.0, determined in solution A.

**Light absorption**. Dissolve 30 mg in 100.0 ml of *0.001 M hydrochloric acid*. The solution shows an absorption maximum at 281 nm and a minimum at 250 nm (2.4.7). The specific absorbance at the absorption maximum is 18.5 to 22.5 and at the absorption minimum is not more than 8.

**Trypsin**. Transfer to a depression in a white spot plate 0.05 ml of *tris (hydroxymethyl)aminomethane buffer solution pH 8.1* and 0.1 ml of solution A. Add 0.2 ml of substrate solution. At the same time and in the same manner, prepare a reference solution using the substance under examination to which not more than 1 per cent w/w of *trypsin* has been added. Start a timer. No colour appears in the test solution within 3-5 minutes after the addition of the substrate solution. A purple colour is produced in the control solution.

*Substrate solution*. To 98.5 mg of *tosylarginine methyl ester hydrochloride*, suitable for assaying trypsin, add 5 ml of *tris(hydroxymethyl)aminomethane buffer solution pH 8.1* and swirl to dissolve. Add 2.5 ml of *methyl red mixed solution* and dilute to 25.0 ml with *water*.

**Loss on drying** (2.4.19). Not more than 5.0 per cent, determined on 1.0 g by drying at 60° at a pressure not exceeding 0.7 kPa for 2 hours.

**Assay**. The activity of chymotrypsin is determined by comparing the rate at which it hydrolyses *acetyltyrosine ethyl ester* with the rate at which *chymotrypsin RS* hydrolyses the same substrate under the same conditions.

### Apparatus

Use a reaction vessel of about 30 ml capacity provided with:

- a device that will maintain a temperature of 25.0 ± 0.1°;
- a stirring device, for example a magnetic stirrer;
- a lid with holes for the insertion of electrodes, the tip of a burette, a tube for the admission of nitrogen and the introduction of reagents.

An automatic or manual titration apparatus may be used. For the latter the burette is graduated in 0.005 ml and the pH meter is provided with a wide scale and glass- calomel or glass-silver-silver chloride electrodes.

*Test solution*. Dissolve 25 mg of the substance under examination in 250.0 ml of *0.001 M hydrochloric acid*.

*Reference solution*. A 0.01 per cent w/v solution of *chymotrypsin RS* in *0.001 M hydrochloric acid*.

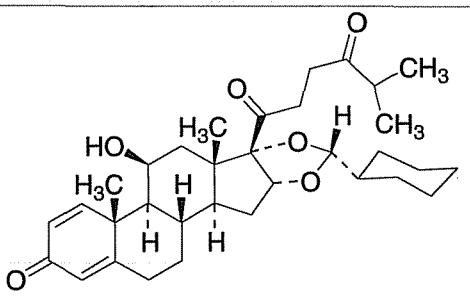
Store the solutions at below 5°. Warm 1 ml of each solution to about 25° over 15 minutes and use 50 µl of each solution (corresponding to about 25 nanokatals) for each titration. Carry out the titration in an atmosphere of nitrogen. Transfer 10.0 ml of *0.01 M calcium chloride solution* to the reaction vessel and, while stirring, add 0.35 ml of *0.2 M acetyltyrosine ethyl ester solution*. When the temperature is steady at 25.0 ± 0.1° (after about 5 minutes) adjust the pH to exactly 8.0 with *0.02 M sodium hydroxide*. Add 50 µl of the test solution (equivalent to about 5 µg of the substance under examination) and start a timer. Maintain the pH at 8.0 by the addition of *0.02 M sodium hydroxide*, noting the volume added every 30 seconds. Calculate the volume of *0.02 M sodium hydroxide* used per second between 30 seconds and 210 seconds. Carry out a titration in the same manner using the reference solution and calculate the volume of *0.02 M sodium hydroxide* used per second.

Calculate the activity in microkatals per milligram.

**Storage**. Store protected from light, in a refrigerator (2° to 8°).

**Labelling**. The quantity of chymotrypsin and the total activity in microkatals per container; for the amorphous substance, that it is hygroscopic.

## Ciclesonide




$C_{32}H_{44}O_7$ Mol. Wt. 540.7

Ciclesonide is (11β,16α)-16,17-[(*R*)-cyclohexylmethylene] bis(oxy)-11-hydroxy-21-(2-methyl-1-oxopropoxy)pregna-1,4-diene-3,20-dione.



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Ciclesonide contains not less than 98.0 per cent and not more than 102.0 per cent of ciclesonide, $C_{32}H_{44}O_7$, calculated on the anhydrous basis.

**Category**. Glucocorticoid.

**Description**. A white to-off white powder.

### Identification

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

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

### Tets

**Specific optical rotation** (2.4.22). +90.0° to +98.0°, determined in a 0.5 per cent w/v solution in *methanol.*

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 50 mg of the substance under examination in 50 ml of *methanol.*

*Reference solution*. A 0.001 per cent w/v solution of *ciclesonide RS* in *methanol.*

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: A. dilute 1 volume of *orthophosphoric acid* to 1000 ml with *water*,
  B. *acetonitrile,*
- flow rate. 1.5 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 240 nm,
- injection volume. 20 µl.

| Time (min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0 | 65 | 35 |
| 20 | 25 | 75 |
| 40 | 25 | 75 |
| 45 | 65 | 35 |
| 55 | 65 | 35 |

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the column efficiency is not less than 30000 theoretical plates.

Inject the reference solution and the test solution. Any individual impurity is not more than 0.5 per cent and the sum of all the impurities found is not more than 1.0 per cent.

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

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 0.5 per cent, determined on 1 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 50 mg of the substance under examination in 50.0 ml of *methanol*. Dilute 5 ml of the resulting solution to 50.0 ml with *methanol.*

*Reference solution*. A 0.01 per cent w/v solution of *ciclesonide RS* in *methanol.*

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 30 volumes of 0.1 per cent *orthophosphoric acid* and 70 volumes of *acetonitrile,*
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 245 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{32}H_{44}O_7$.

**Storage**. Store protected from light, at a temperature not exceeding 30°.

## Ciclesonide Inhalation

Ciclesonide Inhalation is a suspension of microfine Ciclesonide in a suitable liquid filled in a suitable pressurized container. It may contain suitable pharmaceutical aids such as surfactants, stabilizing agents.

Ciclesonide Inhalation delivers not less than 80.0 per cent and not more than 120.0 per cent of the stated amount of ciclesonide, $C_{32}H_{44}O_7$, per inhalation by actuation of the valve.

**Usual strengths**. 80 µg per metered dose; 160 µg per metered dose.

### Identification

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



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### Tests

**Other tests**. Complies with the tests stated under Inhalation Preparations (Pressurised Metered-dose Preparations).

Follow the procedure described under Assay with suitable dilution of the reference solution wherever the amount of active substance is to be determined in any test.

**Assay**. Carry out the test for Content of active ingredient delivered per actuation stated under Inhalation Preparations (Pressurised Metered-dose Preparations).

Determine by liquid chromatography (2.4.14).

*Solvent mixture*. A mixture of equal volumes of *water* and *acetonitrile*.

*Test solution*. Prepare using the solvent mixture as described under the test for Content of active ingredient delivered per actuation stated under Inhalation Preparations (Pressurised Metered-dose Preparations).

*Reference solution (a)*. A 0.04 per cent w/v solution of *ciclesonide RS* in *acetonitrile*.

*Reference solution (b)*. Dilute reference solution (a) with the solvent mixture to obtain a solution containing 32 µg of Ciclesonide per ml.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 30 volumes of a buffer solution prepared by diluting 1 ml of *orthophosphoric acid* to 1000 ml with *water* and 70 volumes of *acetonitrile*,
- flow rate. 3 ml per minute,
- spectrophotometer set at 245 nm,
- injection volume. 200 µl.

Inject reference solution (b). The test is not valid unless the column efficiency is not less than 3500 theoretical plates and the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and reference solution (b).

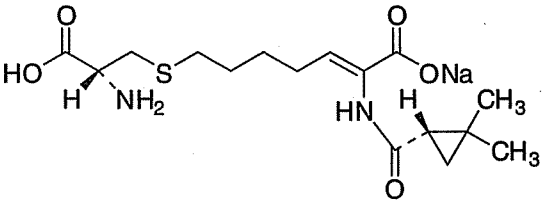
Calculate the content of $C_{32}H_{44}O_7$ in the solution and the amount of $C_{32}H_{44}O_7$ delivered per actuation of the valve.

Determine the content of active ingredient a second and third time by repeating the procedure on the middle ten and on the last ten successive combined actuations of the valve. For each of the three determinations the average content of $C_{32}H_{44}O_7$ delivered per actuation of the valve meets the requirements.

**Storage**. Store protected from moisture at a temperature not exceeding 30°.

**Labelling**. The label states the amount of active ingredient delivered per inhalation.

## Cilastatin Sodium



$C_{16}H_{25}N_2NaO_5S$ Mol. Wt. 380.4

Cilastatin sodium is sodium (*Z*)-7-[[(*R*)-2-amino-2-carboxyethyl]sulphanyl]-2-[[[(1*S*)-2,2-dimethylcyclopropyl]carbonyl]amino]hept-2-enoate.

Cilastatin Sodium contains not less than 98.0 per cent and not more than 101.5 per cent of $C_{16}H_{25}N_2NaO_5S$, calculated on the anhydrous and solvent free basis.

**Category**. Antibacterial.

**Description**. A white or light yellow amorphous powder, hygroscopic.

### Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cilastatin sodium RS* or with the reference spectrum of cilastatin sodium.

B. It gives reaction A of sodium (2.3.1).

### Tests

**Appearance of solution**. A 1.0 per cent w/v solution in *carbon-dioxide free water* (Solution A) is clear (2.4.1) and not more intensely coloured than reference solution YS6 (2.4.1).

**pH** (2.4.24). 6.5 to 7.5, determined in solution A.

**Specific optical rotation** (2.4.22). + 41.5° to + 44.5°, determined in 1.0 per cent w/v solution in a mixture of 1 volume of *hydrochloric acid* and 120 volumes of *methanol*.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 32 mg of the substance under examination in 20.0 ml of *water*.



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*Reference solution (a).* Dilute 2.0 ml of the test solution to 100.0 ml with *water.* Dilute 5.0 ml of the solution to 100.0 ml with *water.*

*Reference solution (b).* Dilute 5.0 ml the test solution to 100.0 ml with *water.* Dilute 5.0 ml of this solution to 50.0 ml with *water.*

*Reference solution (c).* Dissolve 16 mg of the substance under examination in *dilute hydrogen peroxide solution* and dilute to 10.0 ml with the same solvent. Allow to stand for 30 minutes. Dilute 1 ml of this solution to 100 ml with *water.*

*Reference solution (d).* Dissolve 32 mg of *mesityl oxide* in 100 ml of *water.* Dilute 1 ml of the solution to 50 ml with *water.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 50°,
- mobile phase: A. a mixture of 30 volumes of *acetonitrile* and 70 volumes of a 0.1 per cent v/v solution of *orthophosphoric acid* in *water*,
  B. a 0.1 per cent v/v solution of *orthophosphoric acid* in *water*,
- a linear gradient programme using the conditions given below,
- flow rate. 2.0 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

| Time (min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0-30 | 15 → 100 | 85 → 0 |
| 30-46 | 100 | 0 |
| 46-56 | 100 → 15 | 0 → 85 |

Equilibrate the column with a mixture of 15 per cent v/v of mobile phase A and 85 per cent v/v of mobile phase B. Inject separately each solution. Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (b) is at least 15 per cent of the full scale of the recorder.

Inject reference solution (a) and (c). The test is not valid unless, the chromatogram obtained with reference solution (c) shows three principal peaks: the first two peaks (cilastatin impurity A) may elute without being completely resolved and the capacity factor of the third peak (cilastatin) is not less than 10; in the chromatogram obtained with reference solution (a), the principal peak has a signal-to-noise ratio is not less than 5.0.

Inject reference solution (a), (b), (d) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent); the sum of the areas of all secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore any peak with an area less than the area of the principal peak in the chromatogram obtained with reference solution (a) and any peak corresponding to the principal peak in the chromatogram obtained with reference solution (d).

**Mesityl oxide, acetone and methanol.** Not more than 1.0 per cent w/w of *acetone*, 0.5 per cent w/w of *methanol* and 0.4 per cent w/w of *mesityl oxide.*

Determine by gas chromatography (2.4.13).

*Internal standard solution.* Dissolve 0.5 ml of *propanol* in *water* and dilute to 1000 ml with the same solvent.

*Test solution.* Dissolve 0.2 g of the substance under examination in *water*, add 2.0 ml of the internal standard solution and dilute to 10.0 ml with *water.*

*Reference solution.* Dissolve 2.0 ml of *acetone*, 0.5 ml of *methanol* and 0.5 ml of *mesityl oxide* in *water* and dilute to 1000 ml with the same solvent. To 2.0 ml of this solution add 2.0 ml of the internal standard solution and dilute to 10.0 ml with *water.* This solution contains 316 µg of acetone, 79 µg of methanol and 86 µg of mesityl oxide per milliliter.

Chromatographic system

- a fused-silica column 30 m x 0.53 mm, packed with macrogol 20000 (film thickness 1.0 µm),
- temperature:
  column 50° from 0 to 2.5 minutes, 50° - 70° from 2.5 to 5 minutes and hold at 70° from 5 to 5.5 minutes,
  inlet port at 160°and detector at 220°,
- a flame-ionisation detector,
- flow rate. 9 ml per minute using nitrogen as carrier gas.

Inject 1 µl of the reference solution and the test solution. Calculate the contents of *acetone*, *methanol* and mesityl oxide.

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

**Water** (2.3.43). Not more than 2.0 per cent, determined on 0.5 g.

*Cilastatin Sodium intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.*

**Bacterial endotoxins** (2.2.3). Not more than 0.17 Endotoxin Units per mg of cilastatin sodium.

**Assay.** Dissolve 0.3 g in 30 ml of *methanol* and add 5 ml of *water.* Add *0.1 M hydrochloric acid* to a pH of about 3.0. Titrate with *0.1 M sodium hydroxide,* determining the end point potentiometrically (2.4.25). Three jumps of potential are observed, titrate to the third equivalence point.



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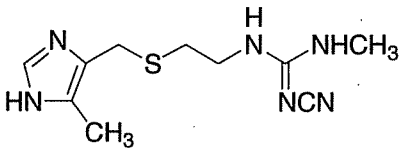


1 ml of *0.1 M sodium hydroxide* is equivalent to 0.01902 g of $C_{16}H_{25}N_2NaO_5S$.

**Storage**. Store protected from moisture, at a temperature not exceeding 8°. If the substance is sterile, store in a sterile, airtight, tamper-proof container .

**Labelling**. The label states, where applicable, that the substance is free from bacterial endotoxins.

# Cimetidine



$C_{10}H_{16}N_6S$. Mol Wt. 252.3

Cimetidine is 2-cyano-1-methyl-3-[2-(5-methylimidazol-4-ylmethylthio)ethyl]guanidine.

Cimetidine contains not less than 98.5 per cent and not more than 101.5 per cent of $C_{10}H_{16}N_6S$, calculated on the dried basis.

**Category**. Antiulcer.

**Dose**. Orally, 400 mg twice daily (with breakfast and at night) or 800 mg as a single daily dose at night; by intramuscular or slow intravenous injection, 200 mg every 4 to 6 hours. The oral or parenteral dose should not exceed 2.4 g daily.

**Description**. A white or almost white powder.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6), using a *potassium bromide* dispersion obtained from the solid state without prior solvent treatment. Compare the spectrum with that obtained with *cimetidine RS* or with the reference spectrum of cimetidine. No shoulder or peak should be discernible at 1190 $cm^{-1}$.

B. When examined in the range 210 nm to 360 nm, a 0.0008 per cent w/v solution in *1 M sulphuric acid* shows an absorption maximum at about 218 nm and a minimum at about 260 nm (2.4.7).

C. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (d).

D. Dissolve about 1 mg in a mixture of 1 ml of *ethanol* and 5 ml of a freshly prepared 2 per cent w/v solution of *citric acid* in *acetic anhydride*. Heat in a water-bath for 10 to 15 minutes; a reddish violet colour is produced.

## Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase (a)*. A mixture of 65 volumes of *ethyl acetate*, 20 volumes of *methanol* and 15 volumes of *strong ammonia solution*.

*Mobile phase (b)*. A mixture of 84 volumes of *ethyl acetate*, 8 volumes of *methanol* and 8 volumes of *strong ammonia solution*.

*Test solution (a)*. Dissolve 0.5 g of the substance under examination in sufficient *methanol* to produce 10 ml.

*Test solution (b)*. Dilute 1 ml of test solution (a) to 10 ml with *methanol*.

*Reference solution (a)*. Dilute 1 ml of solution (a) to 100 ml with *methanol* and dilute 20 ml of this solution to 100 ml with *methanol*.

*Reference solution (b)*. Dilute 5 ml of reference solution (a) to 10 ml with *methanol*.

*Reference solution (c)*. Dilute 5 ml of reference solution (b) to 10 ml with *methanol*.

*Reference solution (d)*. Dissolve 10 mg of *cimetidine RS* in 2 ml of *methanol*.

Apply separately to two plates 4 µl of each solution. Allow the first plate to stand for 15 minutes in the tank saturated with vapour from mobile phase (a). Develop the second plate using mobile phase (b). After development, dry the plates in a current of air, expose to iodine vapour until maximum contrast of the spots has been obtained and examine in ultraviolet light at 254 nm. The following limits apply to both methods. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than two such spots are more intense than the spot in the chromatogram obtained with reference solution (b). The test is not valid unless the chromatogram obtained with reference solution (c) shows a clearly visible spot.

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

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.



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**Assay**. Weigh accurately about 0.25 g and dissolve in 75 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02523 g of $C_{10}H_{16}N_6S$.

**Storage**. Store protected from light.

## Cimetidine Tablets

Cimetidine Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of cimetidine, $C_{10}H_{16}N_6S$.

**Usual strengths**. 200 mg; 400 mg; 800 mg.

### Identification

A. Shake a quantity of the powdered tablets containing 0.1 g of Cimetidine with 10 ml of *methanol*, filter, evaporate the filtrate to dryness using gentle heat and dry the residue at 60° at a pressure not exceeding 0.7 kPa. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cimetidine RS* or with the reference spectrum of cimetidine.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (d).

### Tests

**Dissolution** (2.5.2).

Apparatus No. 2,
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 100 rpm and 15 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter. Measure the absorbance (2.4.7) of the filtrate, suitably diluted if necessary with dissolution medium at 218 nm. Calculate the content of cimetidine, $C_{10}H_{16}N_6S$ in the medium from the absorbance obtained from a solution of known concentration of *cimetidiene RS* in the same medium.

D. Not less than 80 per cent of the stated amount of $C_{10}H_{16}N_6S$.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase (a)*. A mixture of 65 volumes of *ethyl acetate*, 20 volumes of *methanol* and 15 volumes of *strong ammonia solution*.

*Mobile phase (b)*. A mixture of 84 volumes of *ethyl acetate*, 8 volumes of *methanol* and 8 volumes of *strong ammonia solution*.

*Test solution (a)*. Add 20 ml of *methanol* to a quantity of the powdered tablets containing 1 g of Cimetidine, mix with the aid of ultrasound for 2 minutes, shake for 3 minutes and filter using a suitable 0.2 µm filter.

*Test solution (b)*. Dilute 1 ml of test solution (a) to 10 ml with *methanol*.

*Reference solution (a)*. Dilute 1.0 ml of test solution (b) to 20.0 ml with *methanol*.

*Reference solution (b)*. Dilute 1.0 ml of test solution (a) to 100.0 ml with *methanol*. Dilute 20 ml of this solution to 100.0 ml with *methanol*.

*Reference solution (c)*. Dilute 5 ml of reference solution (b) to 10 ml with *methanol*.

*Reference solution (d)*. Dissolve 10 mg of *cimetidine RS* in 2 ml of *methanol*.

Apply separately to two plates 4 µl of each solution. Allow the first plate to stand for 15 minutes in the tank saturated with vapour from mobile phase (a). Develop the second plate using mobile phase (b). After development, dry the plates in a current of air, expose to iodine vapour until maximum contrast of the spots has been obtained and examine in ultraviolet light at 254 nm. The following limits apply to both methods. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than two such spots are more intense than the spot in the chromatogram obtained with reference solution (b). The test is not valid unless the chromatogram obtained with reference solution (c) shows a clearly visible spot.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.25 g of Cimetidine and stir with 20 ml of warm *methanol*. Filter and repeat the extraction with three quantities, each of 20 ml, of warm *methanol*. Evaporate the combined filtrate and washings to dryness and dissolve the residue in 75 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02523 g of $C_{10}H_{16}N_6S$.

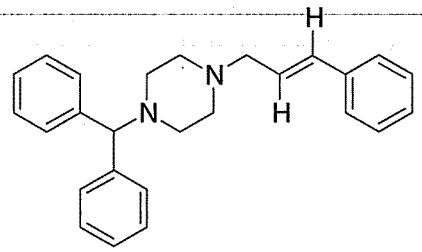


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# Cinnarizine



$C_{26}H_{28}N_2$ Mol. Wt. 368.5

Cinnarizine is (*E*)-1-(diphenylmethyl)-4-(3-phenylprop-2-enyl)piperazine.

Cinnarizine contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{26}H_{28}N_2$, calculated on the dried basis.

**Category**. Antihistaminic.

**Dose**. 25 to 50 mg, thrice daily.

**Description**. A white or almost white powder.

## Identification

*Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cinnarizine RS*.

B. In the test for Related substances the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

C. Dissolve 0.2 g of *anhydrous citric acid* in 10 ml of *acetic anhydride* in a water-bath at 80° and maintain the temperature of the water-bath at 80° for 10 minutes. Add about 20 mg of the substance under examination; a purple colour is produced.

## Tests

**Appearance of solution**. A 2.5 per cent w/v solution in *dichloromethane* is clear (2.4.1) and not more intensely coloured than reference solution BYS6 (2.4.1).

**Acidity or Alkalinity**. Suspend 0.5 g in 15 ml of *water*. Boil for 2 minutes, cool and filter. Dilute the filtrate to 20 ml with *carbon dioxide-free water*. To 10 ml add 0.1 ml of *phenolphthalein solution* and 0.25 ml of *0.01 M sodium hydroxide*; the solution is pink. To 10 ml add 0.1 ml of *methyl red solution* and 0.25 ml of *0.01 M hydrochloric acid*; the solution is red.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 25 mg of the substance under examination in 10.0 ml of the *methanol*.

*Reference solution (a)*. Dissolve 12.5 mg of *cinnarizine RS* and 15.0 mg of *flunarizine dihydrochloride RS* in 100.0 ml of the *methanol*. Dilute 1.0 ml of this solution to 20.0 ml with the same solvent.

*Reference solution (b)*. Dilute 1.0 ml of the test solution to 100.0 ml with *methanol*. Dilute 5.0 ml of this solution to 20.0 ml with the same solvent.

Chromatographic system

- a stainless steel column 10 cm x 4 mm packed with base-deactivated octadecylsilane bonded to porous silica (3 µm),
- mobile phase: A. 1 per cent w/v solution of *ammonium acetate*,
  B. 0.2 per cent v/v solution of *glacial acetic acid* in *acetonitrile*,
- a linear gradient programme using the conditions given below,
- flow rate.1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 10 µl.

| Time (min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0 - 20 | 75→10 | 25→ 90 |
| 20 - 25 | 10 | 90 |

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to cinnarizine and flunarizine is not less than 5.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent), the sum of area of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

**Heavy metals** (2.3.13). Dissolve 1.0 g in a mixture of 85 volumes of *acetone* and 15 volumes of *water* and add *dilute hydrochloric acid* until dissolution is complete. Dilute to 20 ml with the same mixture of acetone and water. 12 ml of the resulting solution complies with the limit test for heavy metals, Method D (20 ppm). Prepare the standard using 10 ml of *lead standard solution (1 ppm Pb)* obtained by diluting *lead*



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*standard solution (100 ppm Pb)* with the mixture of acetone and water.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa for 4 hours.

**Assay**. Weigh accurately about 0.15 g and dissolve in a mixture of 70 volumes of *2-butanone* and 10 volumes of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, using *α-naphtholbenzein solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01843 g of $C_{26}H_{28}N_2$.

## Cinnarizine Tablets

Cinnarizine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cinnarizine, $C_{26}H_{28}N_2$.

**Usual strength**. 25 mg.

### Identification

A. Extract a quantity of the powdered tablets containing 0.1 g of Cinnarizine with 20 ml of *dichloromethane*, filter and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cinnarizine RS*.

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

### Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Shake a suitable quantity of the powdered tablets containing 25 mg of Cinnarizine with *methanol*, dilute to 10 ml with the same solvent and filter.

*Reference solution (a)*. Dissolve 12.5 mg of *cinnarizine RS* and 15 mg of *flunarizine dihydrochloride RS* in *methanol* and dilute to 100 ml with the same solvent. Dilute 1 ml of this solution to 20 ml with *methanol*.

*Reference solution (b)*. Dilute 1 ml of the test solution to 100 ml with *methanol*. Dilute 5 ml of this solution to 20 ml with *methanol*.

Chromatographic system

- a stainless steel column 10 cm × 4 mm, packed with base-deactivated octadecylsilane bonded to porous silica (3 µm),
- mobile phase: A. a 1.0 per cent w/v solution of *ammonium acetate*,
  B. a 0.2 per cent v/v solution of *glacial acetic acid* in *acetonitrile*,
- flow rate. 1.5 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 230 nm,
- injection volume. 10 µl.

| Time (min) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) | Comment |
|---|---|---|---|
| 0 – 20 | 75 →10 | 25 → 90 | linear gradient |
| 20 – 25 | 10 | 90 | isocratic elution |
| 25 – 30 | 75 | 25 | switch to initial eluent composition |
| 30 = 0 | 75 | 25 | restart gradient |

Equilibrate the column for at least 30 minutes at the initial eluent composition.

Inject reference solution (b). Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained is at least 50 per cent of the full scale of the recorder. If necessary, adjust the concentration of *glacial acetic acid* in mobile phase B to obtain a horizontal base-line in the chromatogram.

Inject reference solution (a). When the chromatogram is recorded in the prescribed conditions, the retention times are: cinnarizine about 11 min and flunarizine about 11.5 min. The test is not valid unless the resolution between the peaks corresponding to cinnarizine and flunarizine is at least 5.0. If necessary, adjust the time programme for the gradient elution.

Inject the blank, the test solution and reference solution (b). In the chromatogram obtained with the test solution: the area of any peak, other than the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent); the sum of the areas of the peaks, other than the principal peak, is not greater than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak due to the blank and any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b).

**Other tests**. Comply with the tests stated under tablets.



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**Assay**. Determine by liquid chromatography (2.4.14) as given under the test for Related substances using the following solutions.

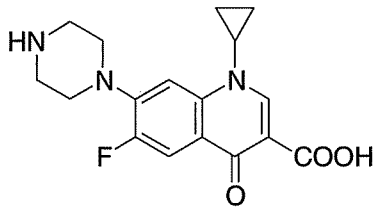
*Test solution*. Weigh and powder 20 tablets. Shake a quantity of the powdered tablets containing about 25 mg of Cinnarazine with *methanol*, dilute to 50.0 ml with the same solvent and filter. Dilute 5.0 ml of this solution to 50.0 ml with *methanol*.

*Reference solution*. A 0.005 per cent w/v solution of *cinnarzine RS* in *methanol*.

Calculate the content of $C_{26}H_{28}N_2$ in the tablets.

**Storage**. Store protected from light.

# Ciprofloxacin




$C_{17}H_{18}FN_3O_3$ Mol. Wt. 331.4

Ciprofloxacin is 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(piperazin-1-yl)quinoline-3-carboxylic acid.

Ciprofloxacin contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{17}H_{18}FN_3O_3$, calculated on the dried basis.

**Category**. Antibacterial.

**Dose**. Orally, 250 to 750 mg twice daily; by intravenous infusion, 100 mg to 200 mg twice daily.

**Description**. A white to pale yellow, crystalline powder.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained from *ciprofloxacin RS* or with the reference spectrum of ciprofloxacin.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 40 volumes of *dichloromethane*, 40 volumes of *methanol*, 20 volumes of *strong ammonia solution* and 10 volumes of *acetonitrile*.

*Test solution*. Dissolve 0.1 g of the substance under examination in 10 ml of *6 M ammonia*.

*Reference solution*. A 1 per cent w/v solution of *ciprofloxacin RS* in *6 M ammonia*.

Apply to the plate, as 1-cm bands, 5 µl of each solution. Place the plate in an atmosphere of ammonia for about 15 minutes and transfer it to an unsaturated chamber containing the mobile phase. Allow the mobile phase to rise 12 cm. Dry the plate in air for 15 minutes and examine in ultraviolet light at 254 nm and at 365 nm. The principal band in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

## Tests

**Appearance of solution**. A 2.5 per cent w/v solution in *0.1 M hydrochloric acid* is clear (2.4.1).

**Related substances**. Carry out the method described in the Assay and calculate the percentage of each impurity from the chromatogram obtained with the test solution. The content of ciprofloxacin ethylenediamine analog or of any other individual impurity peak found is not more than 0.2 per cent and the sum of all the impurity peaks is not more than 0.5 per cent.

**Fluoroquinolonic acid**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 40 volumes of *dichloromethane*, 40 volumes of *methanol*, 20 volumes of *strong ammonia* solution and 10 volumes of *acetonitrile*.

*Test solution*. Dissolve 0.1 g of the substance under examination in 10 ml of *0.1 M acetic acid*.

*Reference solution*. Weigh 10 mg of *fluoroquinolonic acid RS*, add 0.1 ml of *6 M ammonia* and dilute to 100.0 ml with *water*. Dilute 2.0 ml of this solution to 10.0 ml with *water*.

Apply to the plate 5 µl of each solution. Place the plate in an atmosphere of ammonia for about 15 minutes. Remove the plate and place it in a chamber containing the mobile phase. After development, dry the plate in air for 15 minutes and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution corresponding to the spot of fluoroquinolonic acid is not more intense than the spot in the chromatogram obtained with the reference solution.

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

**Chlorides** (2.3.12). To 2.0 g add 30 ml of *water*, shake for 5 minutes and filter through a chloride-free filter paper. 15 ml of the filtrate complies with the limit test for chlorides (250 ppm).

**Sulphates** (2.3.17). Dissolve 0.75 g in 5.0 ml of *2 M acetic acid* and 20.0 ml of *water*. 10 ml of the resulting solution complies with the limit test for sulphates (400 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.



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**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 120° for 6 hours at a pressure not exceeding 0.7 kPa.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh accurately about 25 mg, add 0.2 ml of a solution containing 7 per cent v/v of *phosphoric acid* and add sufficient of the mobile phase to produce 50.0 ml.

*Reference solution (a).*Prepare in the same manner as the test solution using an accurately weighed quantity of *ciprofloxacin RS* in place of the substance under examination.

*Reference solution (b).* A 0.05 per cent w/v solution of *ciprofloxacin ethylenediamine analog RS* in reference solution (a).

Chromatographic system

- a stainless steel column 25 cm × 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 87 volumes of *0.025 M phosphoric acid*, previously adjusted with *triethylamine* to a pH of 3.0 ± 0.1, and 13 volumes of *acetonitrile,*
- flow rate. 1.5 ml per minute,
- column temperaure. 30° ± 1°,
- spectrophotometer set at 278 nm,
- injection volume. 10 µl.

Inject reference solution (b) and record the chromatogram adjusting the sensitivity and flow rate suitably so that the retention time for ciprofloxacin is between 6.4 and 10.8 minutes, the relative retention times are about 0.7 for ciprofloxacin ethylenediamine analog and 1.0 for ciprofloxacin and the resolution between ciprofloxacin ethylenediamine analog peak and ciprofloxacin peak is not less than 6.

Inject reference solution (a). The column efficiency, determined from ciprofloxacin peak, is not less than 2500 theoretical plates, the tailing factor for the ciprofloxacin peak is not more than 2.0 and the relative standard deviation for replicate injections is not more than 1.5 per cent.

Inject alternately the test solution and reference solution (a).

Calculate the content of $C_{17}H_{18}FN_3O_3$.

**Storage**. Store protected from light.

# Ciprofloxacin Injection

Ciprofloxacin Injection is a sterile solution of Ciprofloxacin in 5 per cent Dextrose Injection or in Sodium Chloride Injection prepared with the aid of Lactic Acid.

Ciprofloxacin Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ciprofloxacin, $C_{17}H_{18}FN_3O_3$.

**Usual strength**. 2 mg per ml.

## Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*. Place the plate in an atmosphere of ammonia for about 15 minutes and transfer it to an unsaturated chamber.

*Mobile phase*. A mixture of 40 volumes of *dichloromethane*, 40 volumes of *methanol*, 20 volumes of *strong ammonia solution* and 10 volumes of *acetonitrile*.

*Test solution.* Dilute sufficient of the injection with water to obtain a solution containing the equivalent of 0.05 per cent w/v of Ciprofloxacin.

*Reference solution.* A 0.05 per cent w/v solution of *ciprofloxacin RS* in *6 M ammonia.*

Apply to the plate, as 1-cm bands, 5 µl of each solution. Place the plate in an atmosphere of ammonia for about 15 minutes and transfer it to an unsaturated chamber containing the mobile phase. Allow the mobile phase to rise 12 cm. Dry the plate in air for 15 minutes and examine in ultraviolet light at 254 nm and at 365 nm. The principal band in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

## Tests

**pH** (2.4.24). 3.5 to 4.6.

**Ciprofloxacin ethylenediamine analog**. Not more than 0.5 per cent, determined by the method described in the Assay. Calculate the percentage of ciprofloxacin ethylenediamine analog from the chromatogram obtained with the test solution from the following expression.

Per cent of the analog = $100[0.7 \times r_a /(0.7 \times r_a + r_c)]$, where 0.7 is the response factor for ciprofloxacin ethylenediamine analog relative to that of ciprofloxacin, $r_a$ and $r_c$ are the responses of ciprofloxacin ethylenediamine analog peak and the ciprofloxacin peak respectively.

**Lactic acid**. 0.288 mg to 0.352 mg for each mg of Ciprofloxacin stated on the label.

Determine by liquid chromatography (2.4.14).

*Test solution.* The substance under examination.

*Reference solution.* A 0.08 per cent w/v solution of *sodium lactate RS* in *water.*

Chromatographic system

- a stainless steel column 30 cm × 7.8 mm, packed with a strong cation-exchange resin consisting of sulphonated cross-linked styrene-divinylbenzene copolymer in the hydrogen form (7 to 11 µm),



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- mobile phase: a mixture of 85 volumes of *0.0025 M sulphuric acid* and 15 volumes of *acetonitrile*,
- column temperature. 40° ± 1°,
- flow rate. 0.6 ml per minute,
- spectrophotometer set at 208 nm,
- injection volume. 20 µl.

Inject the reference solution and record the chromatograms adjusting the sensitivity and flow rate suitably so that the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and reference solution, record the chromatograms and measure the peak responses for the major peaks. Calculate the content of lactic acid, $C_3H_6O_3$, in the substance under examination.

*NOTE - After each analysis, the column should be rinsed with a mixture of 85 volumes of 0.005 M sulphuric acid and 15 volumes of acetonitrile to elute the ciprofloxacin from the column. The column may be regenerated with 0.005 M sulphuric acid and may be reused or stored.*

**Dextrose** *(if present)*. 4.75 per cent to 5.25 per cent w/v of $C_6H_{12}O_6,H_2O$, determined by the following method. To 50.0 ml add 0.2 ml of *6 M ammonia* and dilute to 100.0 ml. Mix well and determine the optical rotation at 25° in a 2-dm tube (2.4.22). The observed rotation in degrees multiplied by 2.085 represents the percentage of dextrose monohydrate, $C_6H_{12}O_6,H_2O$, in the preparation under examination.

**Sodium chloride** *(if present)*. 0.855 per cent to 0.945 per cent w/v of NaCl, determined by the following method. To 10.0 ml add 150 ml of water and titrate with *0.1 M silver nitrate* using *potassium chromate solution* as indicator.

1 ml of *0.1 M silver nitrate* is equivalent to 0.005844 g of NaCl.

**Bacterial endotoxins** (2.2.3). Not more than 0.25 Endotoxin Unit per mg of ciprofloxacin.

**Sterility** (2.2.11). Complies with the test for sterility, using Method A.

**Particulate contamination** (2.5.9). Complies with the limit test for particulate contamination.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. Deteremine by liquid chromatography (2.4.14).

*Test solution*. Dilute a volume of the injection containing 25 mg of Ciprofloxacin to 100.0 ml with the mobile phase and mix.

*Reference solution (a)*. A 0.03 per cent w/v solution of *ciprofloxacin hydrochloride RS* in the mobile phase.

*Reference solution (b)*. Dissolve a sufficient quantity of *ciprofloxacin ethylenediamine analog RS* in reference solution (a) so as to obtain a solution containing 0.025 per cent w/v of the reference substance.

Chromatographic system
- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 87 volumes of *0.025 M phosphoric acid*, previously adjusted with *triethylamine* to a pH of 3.0 ± 0.1, and 13 volumes of *acetonitrile*,
- flow rate. 1.5 ml per minute,
- column temperaure. 30° ± 1°,
- spectrophotometer set at 278 nm,
- injection volume. 10 µl.

Inject reference solution (b) and record the chromatogram adjusting the sensitivity and flow rate suitably so that the retention time for ciprofloxacin is between 6.4 and 10.8 minutes, the relative retention times are about 0.7 for ciprofloxacin ethylenediamine analog and 1.0 for ciprofloxacin and the resolution between ciprofloxacin ethylenediamine analog peak and ciprofloxacin peak is not less than 6.

Inject reference solution (a). The column efficiency, determined from ciprofloxacin peak, is not less than 2500 theoretical plates, the tailing factor for the ciprofloxacin peak is not more than 2.0 and the relative standard deviation for replicate injections is not more than 1.5 per cent.

Inject alternately the test solution and reference solution (a).

Calculate the content of $C_{17}H_{18}FN_3O_3$ in the injection.

**Storage**. Store protected from light at a temperature not exceeding 30°. The contents should not be allowed to freeze.

**Labelling**. The label states whether Dextrose or Sodium Chloride has been used for preparing the injection.

## Ciprofloxacin Hydrochloride

$C_{17}H_{18}FN_3O_3,HCl,H_2O$ Mol. Wt. 385.8

Ciprofloxacin Hydrochloride is 1-cyclopropyl-6-fluoro-1, 4- dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid hydrochloride monohydrate.

Ciprofloxacin Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{17}H_{18}FN_3O_3,HCl$, calculated on the anhydrous basis.

**Category**. Antibacterial.

**Dose**. The equivalent of 250 to 750 mg of ciprofloxacin twice daily (116 mg of ciprofloxacin hydrochloride is approximately equivalent to 100 mg of ciprofloxacin).

**Description**. A pale yellow, crystalline powder.



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## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ciprofloxacin hydrochloride RS*.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*. Place the plate in an atmosphere of ammonia for about 15 minutes and transfer it to an unsaturated chamber.

*Mobile phase.* A mixture of 40 volumes of *dichloromethane*, 40 volumes of *methanol*, 20 volumes of *strong ammonia* solution and 10 volumes of *acetonitrile*.

*Test solution.* Dissolve 0.1 g of the substance under examination in 10 ml of *water*.

*Reference solution.* A 1 per cent w/v solution of *ciprofloxacin hydrochloride RS* in *water*.

Apply to the plate, as 1-cm bands, 5 µl of each solution. Place the plate in an atmosphere of ammonia for about 15 minutes and transfer it to an unsaturated chamber containing the mobile phase. Allow the mobile phase to rise 12 cm. Dry the plate in air for 15 minutes and examine in ultraviolet light at 254 nm and at 365 nm. The principal band in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

C. Gives the reactions of chlorides (2.3.1).

## Tests

**pH** (2.4.24). 3.0 to 4.5, determined in a 2.5 per cent w/v solution.

**Related substances**. Carry out the method described in the Assay and calculate the percentage of each impurity peak in the chromatogram obtained with the test solution. The content of ciprofloxacin ethylenediamine analog or of any other individual impurity peak found is not more than 0.2 per cent and the sum of all the impurity peaks is not more than 0.5 per cent.

**Fluoroquinolonic acid**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase.* A mixture of 40 volumes of *dichloromethane*, 40 volumes of *methanol*, 20 volumes of *strong ammonia* solution and 10 volumes of *acetonitrile*.

*Test solution.* Dissolve 0.1 g of the substance under examination in 10 ml of *water*.

*Reference solution.* Weigh 10 mg of *fluoroquinolonic acid RS*, add 0.1 ml of *6 M ammonia* and dilute to 100.0 ml with *water*. Dilute 2.0 ml of this solution to 10.0 ml with *water*.

Apply to the plate 5 µl of each solution. Place the plate in an atmosphere of ammonia for about 15 minutes. Remove the plate and place it in a chamber containing the mobile phase. After development, dry the plate in air for 15 minutes and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution corresponding to the spot of fluoroquinolonic acid is not more intense than the spot in the chromatogram obtained with the reference solution.

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

**Sulphates** (2.3.17). 0.375 g complies with the limit test for sulphates (400 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.4.19). 4.7 to 6.7 per cent, determined on 0.2 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh accurately about 50 mg of the substance under examination and dissolve in 100.0 ml of *water*.

*Reference solution (a).* A 0.05 per cent w/v solution of *ciprofloxacin hydrochloride RS* in *water*.

*Reference solution (b).* A 0.05 per cent w/v solution of *ciprofloxacin ethylenediamine analog RS* in *reference solution (a)*.

Chromatographic system

- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 87 volumes of *0.025 M phosphoric acid*, previously adjusted with *triethylamine* to a pH of 3.0 ± 0.1, and 13 volumes of *acetonitrile*,
- flow rate. 1.5 ml per minute,
- column temperature. 30° ± 1°,
- spectrophotometer set at 278 nm,
- injection volume. 10 µl.

Inject reference solution (b) and record the chromatogram adjusting the sensitivity and flow rate suitably so that the retention time for ciprofloxacin is between 6.4 and 10.8 minutes, the relative retention times are about 0.7 for ciprofloxacin ethylenediamine analog and 1.0 for ciprofloxacin and the resolution between ciprofloxacin ethylenediamine analog peak and ciprofloxacin peak is not less than 6.

Inject reference solution (a). The column efficiency, determined from ciprofloxacin peak, is not less than 2500 theoretical plates, the tailing factor for the ciprofloxacin peak is not more than 2.0 and the relative standard deviation for replicate injections is not more than 1.5 per cent.

Inject alternately the test solution and reference solution (a).

Calculate the content of $C_{17}H_{18}FN_3O_3$,HCl.

**Storage**. Store protected from light.



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# Ciprofloxacin Eye Drops

Ciprofloxacin Eye Drops are a sterile solution of Ciprofloxacin Hydrochloride in Purified water.

Ciprofloxacin Eye Drops contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ciprofloxacin, $C_{17}H_{18}FN_3O_3$.

**Usual strength**. 0.3 per cent w/v.

## Identification

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

B. Give reaction A of chlorides (2.3.1).

## Tests

**pH** (2.4.24). 3.5 to 5.5.

**Other tests**. Comply with the tests stated under Eye Drops.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Transfer an accurately measured volume of Eye drops containing 6 mg of ciprofloxacin, to a 50-ml volumetric flask, dilute with *water* to volume, and mix.

*Reference solution (a).* A 0.014 per cent w/v solution of *ciprofloxacin hydrochloride RS* in *water.*

*Reference solution (b).* A 0.001 per cent w/v solution of *ciprofloxacin ethylenediamine analog RS* in *reference solution (a).*

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 30°,
- mobile phase: a mixture of 75 volumes of *0.005 M tetrabutylammonium phosphate*, adjusted to pH 2.0 with *orthophosphoric acid* and 25 volumes of *methanol,*
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume. 20 µl.

Inject reference solution (b). The relative retention time are about 0.8 for the ciprofloxacin ethylenediamine analog and 1.0 for ciprofloxacin and the resolution between the ciprofloxacin ethylenediamine analog peak and the ciprofloxacin peak is not less than 1.5.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 500 theoretical plates, the tailing factor not more than 2.0, and the relative standard deviation for replicate injections is not more than 2 per cent.

Inject alternatively the test solution and reference solution (a). Calculate the content of $C_{17}H_{18}FN_3O_3$ in the eye drops.

**Storage**. Store protected from light.

# Ciprofloxacin Tablets

Ciprofloxacin Hydrochloride Tablets

Ciprofloxacin Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ciprofloxacin, $C_{17}H_{18}FN_3O_3$.

**Usual strengths**. The equivalent of 250 mg; 500 mg; 750 mg of ciprofloxacin.

## Identification

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

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*. Place the plate in an atmosphere of ammonia for about 15 minutes and transfer it to an unsaturated chamber.

*Mobile phase*. A mixture of 40 volumes of *dichloromethane*, 40 volumes of *methanol*, 20 volumes of *strong ammonia solution* and 10 volumes of *acetonitrile*.

*Test solution*. Shake a quantity of the powdered tablets containing about 0.15 g of ciprofloxacin with 75 ml of *water* for 20 minutes, dilute to 100.0 ml with *water*, mix, centrifuge and use the clear supernatant liquid.

*Reference solution*. A 0.15 per cent w/v solution of *ciprofloxacin hydrochloride RS* in *water*.

Apply to the plate, as 1-cm bands, 5 µl of each solution. Place the plate in an atmosphere of ammonia for about 15 minutes and transfer it to an unsaturated chamber containing the mobile phase. Allow the mobile phase to rise 12 cm. Dry the plate in air for 15 minutes and examine in ultraviolet light at 254 nm and at 365 nm. The principal band in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

## Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *water,*
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted with *water* if necessary, at the maximum at about 276 nm (2.4.7). Calculate



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the content of ciprofloxacin, $C_{17}H_{18}FN_3O_3$, in the medium from the absorbance obtained by repeating the determination using a solution of known concentration of *ciprofloxacin hydrochloride RS*.

D. Not less than 80 per cent of the stated amount of $C_{17}H_{18}FN_3O_3$.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 1.25 g of ciprofloxacin, add about 400 ml of *0.01 M hydrochloric acid*, shake for 20 minutes, dilute to 500.0 ml with *0.01 M hydrochloric acid*. and filter. Dilute 10.0 ml of the filtrate to 100.0 ml with *0.01 M hydrochloric acid*.

*Reference solution (a)*. A 0.03 per cent w/v solution of *ciprofloxacin hydrochloride RS* in *0.01 M hydrochloric acid*.

*Reference solution (b)*. A 0.05 per cent w/v solution of *ciprofloxacin ethylenediamine analog RS* in *reference solution (a)*.

Chromatographic system
- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 30° ± 1°,
- mobile phase: a mixture of 87 volumes of *0.025 M phosphoric acid*, previously adjusted with *triethylamine* to a pH of 3.0 ± 0.1, and 13 volumes of *acetonitrile*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 278 nm,
- injection volume. 10 µl.

Inject reference solution (b) and record the chromatogram adjusting the sensitivity and flow rate suitably so that the retention time for ciprofloxacin is between 6.4 and 10.8 minutes, the relative retention times are about 0.7 for ciprofloxacin ethylenediamine analog and 1.0 for ciprofloxacin and the resolution between ciprofloxacin ethylenediamine analog peak and ciprofloxacin peak is not less than 6.

Inject reference solution (a). The column efficiency determined from ciprofloxacin peak, is not less than 2500 theoretical plates, the tailing factor for the ciprofloxacin peak is not more than 2.0 and the relative standard deviation for replicate injections is not more than 1.5 per cent.

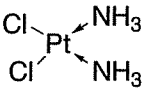
Inject alternately the test solution and reference solution (a).

Calculate the content of $C_{17}H_{18}FN_3O_3$ in the tablets.

**Storage**. Store protected from light.

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

# Cisplatin




$H_6Cl_2N_2Pt$ Mol. Wt. 300.0

Cisplatin is *cis*-diamminedichloroplatinum(II).

Cisplatin contains not less than 97.0 per cent and not more than 102.0 per cent of $H_6Cl_2N_2Pt$.

**Description**. A yellow powder or orange yellow crystals.

*CAUTION — Cisplatin is potentially cytotoxic. Great care should be taken in handling the powder and preparing solutions.*

*NOTE — Carry out all the tests and the Assay, except Identification tests A and C and the test for Silver, protected from light.*

**Category**. Anticancer.

**Dose**. By intravenous infusion, 15 to 20 mg per sq. m. of body surface daily for 5 days.

## Identification

*Test A may be omitted if tests B and C are carried out. Test C may be omitted if tests A and B are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cisplatin RS*.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

C. Add 50 mg to 2 ml of *2 M sodium hydroxide*, evaporate to dryness, dissolve the residue in a mixture of 0.5 ml of *nitric acid* and 1.5 ml of *hydrochloric acid* and evaporate to dryness again; the residue is orange. Dissolve the residue in 0.5 ml of *water* and add 0.5 ml of *ammonium chloride solution*; a yellow crystalline precipitate is produced.

## Tests

**Appearance of solution**. Solution A is clear (2.4.1) and not more intensely coloured than reference solution GYS5 or BYS5 (2.4.1). A 2.0 per cent w/v solution in *dimethylformamide* is clear (2.4.1).

**pH** (2.4.24). 4.5 to 6.0, determined in a 0.1 per cent w/v solution in *normal saline* prepared in *carbon dioxide-free water* (solution A), measured immediately after preparation

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *microcrystalline cellulose* and activating the plate by heating at 150° for 1 hour.



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*Mobile phase*. A mixture of 90 volumes of *dimethylformamide* and 10 volumes of *acetone*.

*Test solution (a)*. A 2 per cent w/v solution of the substance under examination in *dimethylformamide*.

*Test solution (b)*. Dilute 1 ml of test solution (a) to 10 ml with *dimethylformamide*.

*Reference solution (a)*. Dilute 5 ml of test solution (b) to 25 ml with *dimethylformamide*.

*Reference solution (b)*. A 0.2 per cent w/v solution of *cisplatin RS* in *dimethylformamide*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and spray with a 5 per cent w/v solution of *stannous chloride* in *1 M hydrochloric acid*. After 1 hour, the chromatogram obtained with the test solution shows no secondary spot with an Rf value lower than that of the principal spot and any secondary spot with an Rf value higher than that of the principal spot is not more intense than the spot in the chromatogram obtained with reference solution (b).

**Silver**. Determine by atomic absorption spectrophotometry (2.4.2), measuring at 328 nm using a silver hollow-cathode light as a radiation source, a fuel-lean air-acetylene flame, preferably a spectral width of 0.5 nm.

*Test solution*. Dissolve 0.1 g of the substance under examination in 15 ml of *nitric acid* by heating up to 80°. Cool and dilute to 25.0 ml with *water*.

*Reference solutions*. Add 50 ml of *nitric acid* to suitable volumes (10 to 30 ml) of *silver solution AAS* and dilute to 100.0 ml with *water*.

Carry out a blank determination (250 ppm).

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Prepare immediately before use a 0.05 per cent w/v solution of the substance under examination in *normal saline*.

*Reference solution*. A 0.05 per cent w/v solution of *cisplatin RS* in *normal saline*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with strong anion-exchange silica gel (10 µm),
- mobile phase: a mixture of 90 volumes of *methanol* and 10 volumes of *normal saline*
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 20 µl.

Calculate the content of $H_6Cl_2N_2Pt$.

**Storage**. Store protected from light.

# Cisplatin Injection

Cisplatin Injection is a sterile, freeze-dried mixture of Cisplatin, Mannitol and Sodium Chloride. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

*The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).*

**Storage**. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Cisplatin Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cisplatin, $H_6Cl_2N_2Pt$.

**Usual strength**. 50 mg.

**Description**. A yellow powder.

*The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.*

*CAUTION — Cisplatin is potentially cytotoxic. Great care should be taken in handling the powder and preparing solutions.*

## Identification

A. When examined in the range 230 nm to 360 nm, a 0.1 per cent w/v solution in *0.1 M hydrochloric acid* shows an absorption maximum only at about 300 nm (2.4.7).

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

## Tests

**pH** (2.4.24). 3.5 to 6.5, determined in a solution constituted as directed in the label, in *water for injections*.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *microcrystalline cellulose* and activating the plate by heating at 150° for 1 hour.

*Mobile phase*. A mixture of 90 volumes of *dimethylformamide* and 10 volumes of *acetone*.

*Test solution*. Shake the contents of one vial with *dimethylformamide* to produce a solution containing 0.5 per cent w/v of Cisplatin, mix with the aid of ultrasound for 10 minutes and filter.

*Reference solution (a)*. Dilute 5 ml of the test solution to 50 ml with *dimethylformamide*.



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*Reference solution (b).* Dilute 1 ml of the test solution to 50 ml with *dimethylformamide.*

*Reference solution (c).* A solution containing 0.05 per cent w/v of *cisplatin RS* in *dimethylformamide.*

Apply to the plate 20 µl of each solution. After development, dry the plate in air and spray with a 5 per cent w/v solution of *stannous chloride* in *1 M hydrochloric acid.* After 1 hour, the chromatogram obtained with the test solution shows no secondary spot with an Rf value lower than that of the principal spot and any secondary spot with an Rf value higher than that of the principal spot is not more intense than the spot in the chromatogram obtained with reference solution (b).

**Bacterial endotoxins** (2.2.3). Not more than 2.0 Endotoxin Units per mg of cisplatin.

**Sterility** (2.2.11). Comply with the test for sterility, Method A.

**Assay.** Determine by liquid chromatography (2.4.14).

*Test solution.* Determine the weight of the contents of 10 containers. Dissolve the mixed contents of 10 containers in *dimethylformamide* to obtain a solution containing about 0.1 per cent w/v of cisplatin.

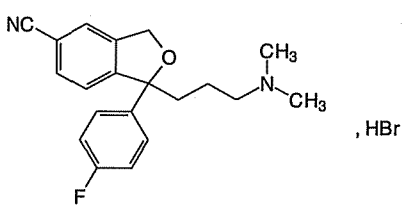
*Reference solution.* A 0.1 per cent w/v solution of *cisplatin RS* in *dimethylformamide* (use within one hour).

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with strong anion-exchange silica gel (10 µm),
- mobile phase: a mixture of 90 volumes of *methanol* and 10 volumes of *normal saline,*
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 20 µl.

Calculate the content of $H_6Cl_2N_2Pt$ in the injection.

**Storage.** Store protected from light.

# Citalopram Hydrobromide




$C_{20}H_{21}FN_2O,HBr$ Mol.Wt. 405.3

Citalopram Hydrobromide is (*RS*)-1-(3-dimethylaminopropyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile hydrobromide.

Citalopram Hydrobromide contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{20}H_{21}FN_2O,HBr$, calculated on the anhydrous basis.

**Category.** Antidepressent.

**Description.** A white to off-white crystalline powder.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *citalopram hydrobromide RS* or with the reference spectrum of citalopram hydrobromide.

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

C. It gives the reactions of bromides (2.3.1).

## Tests

**pH** (2.4.24). 5.5 to 6.5, determined in a 0.5 per cent w/v solution in *water.*

**Specific optical rotation** (2.4.22). –0.2° to +0.2°, determined at 20°, in a 2.5 per cent w/v solution in *methanol.*

**Related substances.** Determine by liquid chromatography (2.4.14).

*Solvent mixture.* 50 volumes of *methanol* and 50 volumes of *water.*

*Test solution.* Dissolve 62.5 mg of the substance under examination in 100.0 ml of the solvent mixture and filter.

*Reference solution (a).* A 0.625 µg per ml solution of *citalopram hydrobromide RS* in the solvent mixture.

*Reference solution (b).* A solution containing 0.0001 per cent w/v of each of *citalopram hydrobromide RS* and *[1-(4-Fluorophenyl)-1-(-3-(methylamino[propyl-1,dihydroisobenzofuran-5-carbonitrile hydrochloride] RS* (*citalopram impurity A RS*) in the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- column temperature 50°,
- mobile phase: a mixture of 80 volumes of a buffer solution prepared by dissolving 1.0 g of *sodium acetate* in 800 ml of *water*, adding 6 ml of *triethylamine*, adjusting the pH to 4.6 with *acetic acid,* and diluting to 1000 ml with *water*, and 20 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 239 nm,
- injection volume. 20 µl.

Inject reference solution (b). The relative retention time for impurity A with respect to citalopram is about 0.9. The test is



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not valid unless the resolution between impurity A and citalopram is not less than 1.8, the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the test solution and reference solution (a). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with the reference solution (a) (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than 5 times the area of the peak in the chromatogram obtained with the reference solution (a) (0.5 per cent).

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

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 0.5 per cent, determined on 0.25 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. 50 volumes of *methanol* and 50 volumes of *water*.

*Test solution*. Weigh accurately about 62.5 mg of the substance under examination, dissolve in 100.0 ml of the solvent mixture and filter.

*Reference solution*. A 0.0625 per cent w/v solution of *citalopram hydrobromide RS* in the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- column temperature 50°,
- mobile phase: a mixture of 80 volumes of a buffer solution prepared by dissolving 1.0 g of *sodium acetate* in 800 ml of *water*, adding 6 ml of *triethylamine*, adjusting the pH to 4.6 with *acetic acid* and diluting to 1000 ml with *water*, and 20 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 239 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 3.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{20}H_{21}FN_2O$, HBr.

**Storage**. Store protected from moisture, at a temperature not exceeding 30°.

# Citalopram Tablets

Citalopram Hydrobromide Tablets

Citalopram Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of citalopram, $C_{20}H_{21}FN_2O$.

**Usual strengths**. 10 mg; 20 mg; 40 mg.

## Identification

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

## Tests

**Dissolution** (2.5.2).

Apparatus No. 2,

Medium. 900 ml of a buffer solution prepared by mixing 118 ml of *1 M hydrochloric acid* and 82 ml of *1 M sodium hydroxide* and diluting to 1000 ml with *water*. Adjust the pH to 1.5 with *1 M sodium hydroxide*,

Speed and time. 100 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 239 nm (2.4.7). Calculate the content of $C_{20}H_{21}FN_2O$ in the medium from the absorbance obtained from a solution of known concentration of *citalopram hydrobromide RS* in the same medium.

D. Not less than 80 per cent of the stated amount of $C_{20}H_{21}FN_2O$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh a quantity of the powdered tablets containing 50.0 mg of citalopram, disperse in 100.0 ml of the mobile phase and filter.

*Reference solution (a)*. A 0.625 µg per ml solution of *citalopram hydrobromide RS* in the mobile phase.

*Reference solution (b)*. A solution containing 0.0001 per cent w/v of *[3-(3N,N-dimethylamino)-1-(4-fluorophenyl)-6-cvano-1(3H)-isobenzofuranone]RS* (*citalopram impurity B RS*) and 0.025 per cent w/v of *citalopram hydrobromide RS* in the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 45°,
- mobile phase: a mixture of 55 volumes of a buffer solution prepared by dissolving 3.15 g of *potassium dihydrogen phosphate* and 3.6 g of *disodium hydrogen phosphate*



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in 1000 ml of *water*, 38 volumes of *methanol* and 7 volumes of *acetonitrile*, with the pH adjusted to 6.5 with *orthophosphoric acid*,
- flow rate. 0.8 ml per minute,
- spectrophotometer set at 239 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between impurity B and citalopram is not less than 3.0.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 5000 theoretical plates, the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the test solution and reference solution (a). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 2.5 times the area of the peak in the chromatogram obtained with the reference solution (a) (0.25 per cent) and the sum of the areas of all the secondary peaks is not more than 8 times the area of the peak in the chromatogram obtained with the reference solution (a) (0.8 per cent).

**Uniformity of content** *(For tablets containing 10 mg or less)*. Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14).

Use the chromatographic system described in the Assay using the following test solution.

*Test solution*. Powder one tablet, disperse in 10 ml of a 0.142 per cent w/v solution of *anhydrous dibasic sodium phosphate*, add 40 ml of *methanol* and mix with the aid of ultrasound for 5 minutes. Add sufficient volume of the internal standard solution and dilute stepwise, if necessary with the solvent mixture to obtain a solution containing 0.01 per cent w/v of citalopram and 0.0025 per cent w/v of internal standard solution and filter.

Calculate the content of $C_{20}H_{21}FN_2O$ in the tablet.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. 80 volumes of *methanol* and 20 volumes of a 0.142 per cent w/v solution of *anhydrous dibasic sodium phosphate*.

*Test solution*. Weigh and powder 20 tablets. Weigh accurately a quantity of powder containing about 100 mg of citalopram, disperse in 100.0 ml of the solvent mixture and filter. To 5.0 ml of this solution, add 5.0 ml of the internal standard solution and dilute to 50.0 ml with the solvent mixture.

*Reference solution*. A 0.125 per cent w/v solution of *citalopram hydrobromide RS* (equivalent to 0.1 per cent w/v of citalopram) in the solvent mixture. To 5.0 ml of this solution, add 5.0 ml of the internal standard solution and dilute to 50.0 ml with the solvent mixture.

*Internal standard solution*. A 0.025 per cent w/v solution of *dimethyl-(1-methyl-3,3-diphenylallyl)amine hydrochloride RS* (*citalopram impurity C RS*) in the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature 45°,
- mobile phase: a 0.077 per cent w/v solution of *dodecyltrimethylammonium bromide*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Inject the reference solution. The relative retention time for citalopram impurity C is about 1.36 and the resolution between citalopram and citalopram impurity C is not less than 1.5. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the relative standard deviation for replicate injections is not more than 1.5 per cent.

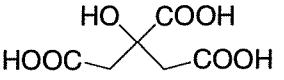
Inject the test solution and the reference solution.

Calculate the content of $C_{20}H_{21}FN_2O$ in the tablets.

**Storage**. Store protected from moisture, at a temperature not exceeding 30°.

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

# Citric Acid




$C_6H_8O_7$ Mol. Wt. 192.1

Citric Acid is 2-hydroxypropane-1,2,3-tricarboxylic acid.

Citric Acid contains not less than 99.0 per cent and not more than 101.0 per cent of $C_6H_8O_7$, calculated on the anhydrous basis.

**Category**. Pharmaceutical aid; anticoagulant for storage of whole blood (along with sodium citrate).

**Description**. Colourless crystals or a white powder; slightly hygroscopic in moist air.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *citric acid RS* or with the reference spectrum of citric acid. Dry the substance under examination and the reference substance at 105° for 2 hour.



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B. Gives reaction A of citrates (2.3.1).

C. A 10 per cent w/v solution is strongly acidic.

### Tests

**Appearance of solution**. Dissolve 2.0 g in sufficient *water* to produce 10 ml. The solution is clear (2.4.1), and not more intensely coloured than reference solution YS7, BYS7 or GYS7 (2.4.1).

**Arsenic** (2.3.10). Dissolve 10.0 g in 50 ml of *water* and add 10 ml of *stannated hydrochloric acid*; the resulting solution complies with the limit test for arsenic (1 ppm).

**Barium**. Dissolve 5.0 g in several portions in 39 ml of *2 M sodium hydroxide* and dilute to 50 ml with *distilled water* (solution A). To 5 ml of solution A add 5 ml of *1 M sulphuric acid* and allow to stand for 1 hour. Any opalescence produced is not more intense than that of a mixture of 5 ml of solution A and 5 ml of *distilled water*.

**Calcium**. To 0.2 ml of *ethanolic calcium standard solution (100 ppm Ca)* add 1 ml of a 4 per cent w/v solution of *ammonium oxalate*. After 1 minute add 1 ml of *2 M acetic acid* and 5 ml of solution A diluted to 10 ml with *distilled water* and shake. After 15 minutes any opalescence produced is not more intense than that of a standard prepared in the same manner using a mixture of 10 ml of *calcium standard solution (10 ppm Ca)* and 5 ml of *water* in place of solution A (200 ppm).

**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals, Method A (10 ppm).

**Iron** (2.3.14). 8 ml of solution A diluted to 10 ml with *water* complies with the limit test for iron (50 ppm).

**Chlorides** (2.3.12). Dissolve 5.0 gm in 10 ml of *water*, add 1 ml of *2 M nitric acid* and dilute to 15 ml with *water*. The resulting solution complies with the limit test for chlorides (50 ppm).

**Sulphates** (2.3.17). Dissolve 1.0 g in sufficient *distilled water* to produce 15 ml. The resulting solution complies with the limit test for sulphates (150 ppm).

**Oxalic acid**. Dissolve 0.8 g in 4 ml of *water*, add 2 ml of *hydrochloric acid* and 1 g of *granulated zinc* and heat in a water-bath for 1 minute. Allow to stand for 2 minutes, decant the liquid into a test-tube containing 0.25 ml of a 1 per cent w/v solution of *phenylhydrazine hydrochloride* and heat to boiling. Cool rapidly, transfer to a graduated measuring cylinder, add an equal volume of *hydrochloric acid* and 0.25 ml of a 5 per cent solution of *potassium ferricyanide*, shake and allow to stand for 30 minutes. Any pink colour produced is not more intense than that produced by carrying out the test using 0.2 mg of *oxalic acid* dissolved in 4 ml of *water*.

**Readily carbonisable substances**. Heat 0.75 g in powder, with 10 ml of *sulphuric acid* (containing 94.5 to 95.5 per cent w/w of $H_2SO_4$) in a water-bath at 90° ± 1°. Shake after one minute, continue the heating for a total of 1 hour and cool rapidly and immediately. Any colour produced is not more intense than that of a mixture of 1.0 ml of CCS and 9.0 ml of FCS (2.4.1).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 1.0 per cent, determined on 2.0 g.

**Assay**. Weigh accurately about 2 g and dissolve in 100 ml of *water*. Titrate with *1 M sodium hydroxide* using 0.5 ml of *phenolphthalein solution* as indicator.

1 ml of *1 M sodium hydroxide* is equivalent to 0.06403 g of $C_6H_8O_7$.

**Storage**. Store protected from moisture.

## Citric Acid Monohydrate

$C_6H_8O_7,H_2O$ Mol. Wt. 210.1

Citric Acid Monohydrate is 2-hydroxypropane-1,2,3-tricarboxylic acid monohydrate.

Citric Acid Monohydrate contains not less than 99.0 per cent and not more than 101.0 per cent of $C_6H_8O_7$, calculated on the anhydrous basis.

**Category**. Pharmaceutical aid; anticoagulant for storage of whole blood (along with sodium citrate).

**Description**. Colourless crystals or a white, crystalline powder; slightly efflorescent in warm, dry air.

### Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *citric acid monohydrate RS* or with the reference spectrum of citric acid monohydrate. Dry the substance under examination and the reference substance at 105° for 2 hour.

B. Gives reaction A of citrates (2.3.1).

C. A 10 per cent w/v solution is strongly acidic.

### Tests

**Appearance of solution**. Dissolve 2.0 g in sufficient *water* to produce 10 ml. The solution is clear (2.4.1), and not more intensely coloured than reference solution YS7, BYS7 or GYS7 (2.4.1).

**Arsenic** (2.3.10). Dissolve 10 g in 50 ml of *water* and add 10 ml of *stannated hydrochloric acid*; the resulting solution complies with the limit test for arsenic (1 ppm).



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**Barium**. Dissolve 5.0 g in several portions in 39 ml of *2 M sodium hydroxide* and dilute to 50 ml with *distilled water* (solution A). To 5 ml of solution A, add 5 ml of *1 M sulphuric acid* and allow to stand for 1 hour. Any opalescence produced is not more intense than that of a mixture of 5 ml of solution A and 5 ml of *distilled water*.

**Calcium**. To 0.2 ml of *ethanolic calcium standard solution (100 ppm Ca)* add 1 ml of a 4 per cent w/v solution of *ammonium oxalate*. After 1 minute add 1 ml of *2 M acetic acid* and 5 ml of solution A diluted to 10 ml with *distilled water* and shake. After 15 minutes any opalescence produced is not more intense than that of a standard prepared in the same manner using a mixture of 10 ml of *calcium standard solution (10 ppm Ca)* and 5 ml of *water* in place of solution A (200 ppm).

**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals, Method A (10 ppm).

**Iron** (2.3.14). 8 ml of solution A diluted to 10 ml with *water* complies with the limit test for iron (50 ppm).

**Chlorides** (2.3.12). Dissolve 5.0 g in 10 ml of *water*, add 1 ml of *2 M nitric acid* and dilute to 15 ml with *water*. The resulting solution complies with the limit test for chlorides (50 ppm).

**Sulphates** (2.3.17). Dissolve 1.0 g in sufficient *distilled water* to produce 15 ml. The resulting solution complies with the limit test for sulphates (150 ppm).

**Oxalic acid**. Dissolve 0.8 g in 4 ml of *water*, add 2 ml of *hydrochloric acid* and 1 g of *granulated zinc* and heat in a water-bath for 1 minute. Allow to stand for 2 minutes, decant the liquid into a test-tube containing 0.25 ml of a 1 per cent w/v solution of *phenylhydrazine hydrochloride* and heat to boiling. Cool rapidly, transfer to a graduated measuring cylinder, add an equal volume of *hydrochloric acid* and 0.25 ml of a 5 per cent solution of *potassium ferricyanide*, shake and allow to stand for 30 minutes. Any pink colour produced is not more intense than that produced by carrying out the test using 0.2 mg of *oxalic acid* dissolved in 4 ml of *water*.

**Readily carbonisable substances**. Heat 0.75 g with 10 ml of *sulphuric acid* (containing between 94.5 and 95.5 per cent w/w of $H_2SO_4$) in a water-bath maintained at 90° ± 1°. Shake after 1 minute, continue the heating for a total of one hour and cool rapidly and immediately. Any colour produced is not more intense than that of a mixture of 1.0 ml of CCS and 9.0 ml of FCS (2.4.1).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

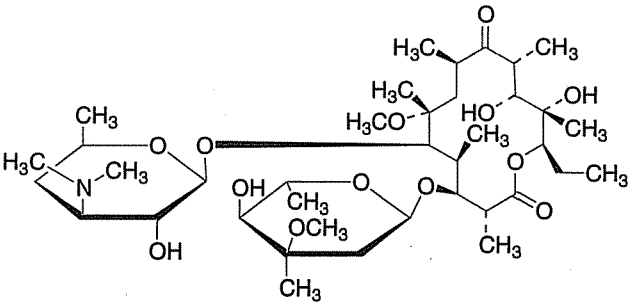
**Water** (2.3.43). 7.5 to 9.0 per cent, determined on 0.5 g.

**Assay**. Weigh accurately about 2 g and dissolve in 50 ml of *water*. Titrate with *1 M sodium hydroxide* using 0.5 ml of *phenolphthalein solution* as indicator.

1 ml of *1 M sodium hydroxide* is equivalent to 0.06403 g of $C_6H_8O_7$.

**Storage**. Store protected from moisture.

# Clarithromycin



$C_{38}H_{69}NO_{13}$ Mol. Wt. 748.0

Clarithromycin is (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-4-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl)oxy]-14-ethyl-12,13-dihydroxy-7-methoxy-3,5,7,9,11,13-hexamethyl-6-[[3,4,6-trideoxy-3-(dimethylamino)-α-D-xylo-hexopyranosyl]oxy]oxacyclotetradecane-2,10-dione (6-O-methylerythromycin A).

Clarithromycin contains not less than 96.0 per cent and not more than 102.0 per cent of $C_{38}H_{69}NO_{13}$, calculated on the anhydrous basis.

**Category**. Antibacterial.

**Description**. A white or almost white, crystalline powder.

## Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *clarithromycin RS* or with the reference spectrum of clarithromycin.

## Tests

**Specific optical rotation** (2.4.22). -94° to -102°, determined on a 1 per cent w/v solution in *methylene chloride* at 20°.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 75 mg of the substance under examination in 25 ml of *acetonitrile* and dilute to 50 ml with *water*.

*Reference solution (a)*. Dissolve 7.5 mg of *clarithromycin RS* in 2.5 ml of *acetonitrile* and dilute to 5.0 ml with *water*.



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*Reference solution (b).* Dilute 1 ml of reference solution (a) to 100 ml with equal volumes of *acetonitrile* and *water.*

Chromatographic system as described under Assay.

Inject reference solution (b). Test is not valid unless the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 2.5 times the area of the peak in the chromatogram obtained with reference solution (b) (2.5 per cent) and the sum of areas of all the secondary peaks is not more than 5 times the area of the peak in the chromatogram obtained with the reference solution (b) (5.0 per cent).

**Heavy metals** (2.3.13). Dissolve 1 g in a mixture of 15 volumes of *water* and 85 volumes of *dioxan* and dilute to 20 ml with the same mixture of solvents. 12 ml of the solution complies with limit test for heavy metals, Method D (20 ppm). Prepare reference solution using 1 ml of lead standard solution (100 ppm) using the same mixture of solvents.

**Water** (2.3.43). Not more than 2.0 per cent w/w, determined on 0.5 g using pyridine as solvent.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve 75 mg of the substance under examination in 25 ml of *acetonitrile* and dilute to 50.0 ml with *water.*

*Reference solution.* Dissolve 15 mg of the *clarithromycin RS* in 5 ml of *acetonitrile* and dilute to 10.0 ml with *water.*

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (3.5µm),
- column temperature 40°,
- mobile phase: A. 0.476 per cent w/v solution of *potassium dihydrogen phosphate* adjusted to pH 4.4 with *dilute orthophosphoric acid* or a 4.5 per cent solution of *potassium hydroxide*, filter,
  B. *acetonitrile*,
- a linear gradient programme using the conditions given below,
- flow rate. 1.1 ml per minute,
- spectrophotometer set at 205 nm,
- injection volume. 10 µl.

| Time (in mins.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0 | 75 | 25 |
| 32 | 40 | 60 |
| 34 | 40 | 60 |
| 36 | 75 | 25 |
| 42 | 75 | 25 |

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{38}H_{69}NO_{13}$.

**Storage**. Store protected from moisture.

## Clarithromycin Tablets

Clarithromycin Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of clarithromycin, $C_{38}H_{69}NO_{13}$.

**Usual strengths.** 250 mg; 500 mg.

### Identification

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

### Tests

**Dissolution** (2.5.2).

Apparatus. No. 1,
Medium. 900 ml of *acetate buffer pH 5.0,*
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

*Test solution.* Use the filtrate.

*Reference solution.* Weigh accurately a suitable quantity of *clarithromycin RS,* dissolve in *methanol,* dilute with dissolution medium to obtain a solution having a known concentration of about 0.125 mg per ml.

Chromatographic system as described under Assay.

Calculate the content of $C_{38}H_{69}NO_{13}$ in the tablet.

D. Not less than 75 per cent of the stated amount of $C_{38}H_{69}NO_{13}$.

**Loss on drying** (2.4.19). Not more than 6.0 per cent, determined on 1 g by drying in an oven at 110°, under vacuum, for 3 hours.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Disperse a quantity of powdered tablet containing 125 mg of Clarithromycin in 200.0 ml with *methanol* and filter. Dilute 5.0 ml of the solution to 25.0 ml with mobile phase.

*Reference solution.* A 0.0625 per cent w/v solution of *clarithromycin RS* in *methanol.* Dilute 5.0 ml of the solution to 25.0 ml with mobile phase.



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Chromatographic system
- a stainless steel column 15 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature 50°,
- mobile phase: a mixture of 65 volumes of *methanol* and 35 volumes of *0.067 M monobasic potassium phosphate* adjusted to pH 4.0 with *orthophosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 50 µl.

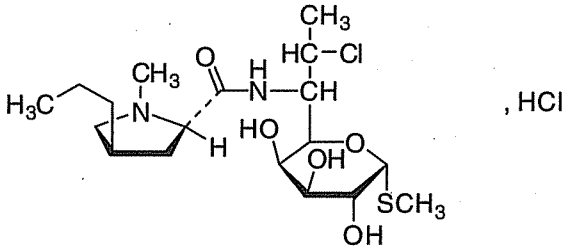
Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, the column efficiency in not less than 750 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{38}H_{69}NO_{13}$.

**Storage**. Store protected from moisture.

# Clindamycin Hydrochloride




$C_{18}H_{33}ClN_2O_5S,HCl$ Mol. Wt. 461.5

Clindamycin Hydrochloride is methyl 7-chloro-6,7,8-trideoxy-6-[[[(2*S*,4*R*)-1-methyl-4-propyl-2-pyrrolidinyl]carbonyl]amino]-1-thio-L-*threo*-α-D-*galacto*-octopyranoside hydrochloride.

Clindamycin Hydrochloride contains not less than 91.0 per cent and not more than 102.0 per cent of $C_{18}H_{33}ClN_2O_5S,HCl$ calculated on the anhydrous basis.

**Category**. Antibacterial.

**Description**. A white or almost white, crystalline powder.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6).Compare the spectrum with that obtained with *clindamycin hydrochloride RS* or with the reference spectrum of clindamycin hydrochloride.

B. Determine by thin- layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 19 volumes of *2-propanol*, 38 volumes of a 15 per cent w/v solution of *ammonium acetate*, adjusted to pH 9.6 with *ammonia* and 43 volumes of *ethyl acetate*.

*Test solution*. Dissolve 10 mg of the substance under examination in 10 ml of *methanol*.

*Reference solution (a)*. A 0.1 per cent w/v solution of *clindamycin hydrochloride RS* in the *methanol*.

*Reference solution (b)*. A solution containing 0.1 per cent w/v each of *clindamycin hydrochloride RS* and *lincomycin hydrochloride RS* in the *methanol*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the pate in air, spray with a 0.1 per cent w/v solution of *potassium permanganate*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows 2 clearly separated spots.

C. Dissolve about 10 mg of the substance under examination in 2 ml of *dilute hydrochloric acid* and heat on a water-bath for 3 minutes, add 3 ml of *sodium carbonate solution* and 1 ml of a 2 per cent w/v solution of *sodium nitroprusside*, a violet-red colour is produced.

D. A 1 per cent w/v solution gives reaction A of chlorides (2.3.1).

## Tests

**pH** (2.4.24). 3.0 to 5.0, determined in a 10 per cent w/v solution in *carbon dioxide-free water*.

**Specific optical rotation** (2.4.22). +135 ° to +150°, determined in a 4.0 per cent w/v solution.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 50 mg of the substance under examination in 50.0 ml of the mobile phase.

*Reference solution (a)*. A 0.1 per cent w/v solution of *clindamycin hydrochloride RS* in the mobile phase.

*Reference solution (b)*. Dilute 2.0 ml of the test solution to 100.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 45 volumes of *acetonitrile* and 55 volumes of a 0.68 per cent w/v solution of



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*potassium dihydrogen phosphate*, adjusted to pH 7.5 with a 25 per cent w/v solution of *potassium hydroxide*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

The relative retention time with reference to clindamycin for methyl 6,8-dideoxy-6-[[[(2*S*,4*R*)-1-methyl-4-propylpyrrolidin-2-yl]carbonyl]amino]-1-thio-D-*erythro*-a-D-galacto-octopyranoside (clindamycin impurity A) is about 0.4; for methyl 7-chloro-6,7,8-trideoxy-6-[[[(2*S*,4*R*)-4-ethyl-1-methylpyrrolidin-2-yl]carbonyl]amino]-1-thio-L-*threo*-a-D-galacto- octopyranoside (clindamycin impurity B) is about 0.65 and for methyl 7-chloro-6,7,8-trideoxy-6-[[[(2*S*,4*R*)-1-methyl-4-propylpyrrolidin-2-yl]carbonyl]amino]-1-thio-D-*erythro*-a-D-galacto- octopyranoside (clindamycin impurity C) is about 0.8.

Inject reference solution (b) and the test solution. In the chromatogram obtained with test solution the area of the peak due to clindamycin impurity B is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent). The area of peak corresponding to clindamycin impurity C is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (4.0 per cent). The area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). The sum of the areas of all the secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (6.0 per cent). Ignore any peak with an area less than 0.025 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

**Sulphated ash** (2.3.18). Not more than 0.5 per cent.

**Water** (2.3.43). 3.0 per cent to 6.0 per cent, determined on 0.5 g.

**Assay**. Determine by liquid chromatography (2.4.14), as described under Related substances.

Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{18}H_{33}ClN_2O_5S,HCl$.

**Storage**. Store protected from moisture.

# Clindamycin Capsules

Clindamycin Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of clindamycin, $C_{18}H_{33}ClN_2O_5S$.

**Usual strength**. 150 mg.

## Identification

A. Shake a quantity of the content of capsules containing about 30 mg of clindamycin with 15 ml of *chloroform*, filter and evaporate the filtrate to dryness. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *clindamycin hydrochloride RS* or with the reference spectrum of clindamycin hydrochloride.

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

## Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Shake a quantity of the content of capsules containing about 50 mg of clindamycin with 50 ml of the mobile phase for 15 minutes and filter.

*Reference solution (a)*. Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase.

*Reference solution (b)*. A 0.1 per cent w/v solution of *clindamycin hydrochloride RS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Hypersil BDS),
- mobile phase: a mixture of 45 volumes of *acetonitrile* and 55 volumes of 0.68 per cent w/v solution of *potassium dihydrogen orthophosphate*, adjusted to pH 7.5 with 25 per cent w/v solution of *potassium hydroxide*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

Inject reference solution (b). The relative retention time with reference to clindamycin for methyl 6,8-dideoxy-6-[[[(2*S*,4*R*)-1- methyl-4-propylpyrrolidin-2-yl]carbonyl]amino]-1-thio-D-erythro-α-D-galacto- octopyranoside (lincomycin) (clindamycin impurity A) is about 0.4, for methyl 7-chloro-6,7,8-trideoxy-6-[[[(2*S*,4*R*)-4-ethyl- 1-methylpyrrolidin-2-yl]carbonyl]amino]-1-thio-L-*threo*-α-D-*galacto*-octopyranoside (clindamycin B) (clindamycin impurity B) is about 0.65 and for methyl 7-chloro-6,7,8-trideoxy-6-[[[(2*S*,4*R*)-1-methyl-4-propylpyrrolidin-2-yl]carbonyl]amino]-1-thio-D-*erythro*-α-D-*galacto*- octopyranoside (7-epiclindamycin) (clindamycin impurity C) is about 0.8.

Inject reference solution (a) and the test solution. Run the chromatogram twice the retention time of the principal peak.



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In the chromatogram obtained with the test solution the area of peak corresponding to clindamycin impurity B is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent), the area of peak corresponding to clindamycin impurity C is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (4.0 per cent), the area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent) and the sum of the areas of all the secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (6.0 per cent). Ignore any peak with an area less than 0.025 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

**Other tests**. Comply with the tests stated under Capsules.

**Water** (2.3.43). Not more than 7.0 per cent, determined on 1 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Shake a quantity of the content of 20 capsules containing about 50 mg of clindamycin with 50 ml of the mobile phase for 15 minutes and filter.

*Reference solution*. A 0.11 per cent w/v solution of *clindamycin hydrochloride RS* in the mobile phase.

Use chromatographic system as described under Related substances.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0.

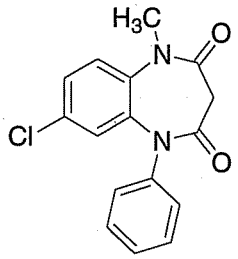
Inject the reference solution and the test solution.

Calculate the content of $C_{18}H_{33}ClN_2O_5S$ in the capsules.

1 mg of $C_{18}H_{33}ClN_2O_5S$, HCl is equivalent to 0.9209 mg of $C_{18}H_{33}ClN_2O_5S$.

**Labelling**. The quantity of active ingredient is stated in terms of the equivalent amount of clindamycin.

# Clobazam




$C_{16}H_{13}ClN_2O_2$ Mol. Wt. 300.7

Clobazam is 7-chloro-1-methyl-5-phenyl-1,5-dihydro-3*H*-1,5-benzodiazepine-2,4-dione.

Clobazam contains not less than 97.0 per cent and not more than 103.0 per cent of $C_{16}H_{13}ClN_2O_2$, calculated on the dried basis.

**Category**. Anticonvulsant.

**Description**. A white or almost white, crystalline powder.

## Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *clobazam RS* or with the reference spectrum of clobazam.

## Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 10 mg of the substance under examination in the mobile phase and dilute to 50 ml with the mobile phase.

*Reference solution (a)*. Dissolve 5.0 mg of *7-chloro-5-phenyl-1,5-dihydro-3H-1,5-benzodiazepine-2,4-dione RS ( clobazam impurity A)* in the mobile phase and dilute to 50 ml with the mobile phase. Dilute 1 ml of this solution to 100 ml with the mobile phase.

*Reference solution (b)*. Dissolve 5 mg of *chlordiazepoxide RS* and 5 mg of *clobazam RS* in the mobile phase and dilute to 50 ml with the mobile phase. Dilute 1 ml of the solution to 100 ml with the mobile phase.

*Reference solution (c)*. Dilute 1 ml of the test solution to 200 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 40 volumes of *acetonitrile* and 60 volumes of *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 20 µl.

Inject reference solution (b). The resolution between the peaks due to chlordiazepoxide and clobazam is not less than 1.3.

Inject the test solution and reference solutions (a) and (c). Continue the chromatography for 5 times the retention time of clobazam (about 15 minutes). In the chromatogram obtained with the test solution the area of the peak obtained due to impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). The area of any other impurity peak is not more than 0.4 times the area of the principal peak in the chromatogram



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obtained with reference solution (c) (0.2 per cent). The sum of the areas of all other impurity peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent, determined on the residue obtained in the test for Loss on drying.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 100°-105°.

**Assay**. Weigh accurately about 50 mg and dissolve in 100.0 ml of *ethanol (95 per cent)*. Dilute 2.0 ml of the solution to 250.0 ml with the same solvent. Measure the absorbance of the resulting solution at the maximum at about 232 nm (2.4.7), taking 1380 as the specific absorbance at 232 nm.

Calculate the content of $C_{16}H_{13}ClN_2O_2$.

**Storage**. Store protected from moisture.

## Clobazam Capsules

Clobazam Capsules contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of clobazam, $C_{16}H_{13}ClN_2O_2$.

**Usual strength**. 10 mg.

### Identification

Shake a quantity of the capsules containing 20 mg of Clobazam with 10 ml of *dichloromethane*, filter and evaporate the filtrate to dryness. Dissolve the residue in the minimum amount of *methanol*, evaporate to dryness and dry the residue at 105° for 10 minutes. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with the spectrum obtained with *clobazam RS* treated in the same manner or with the reference spectrum of clobazam.

### Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Extract a quantity of the contents of the capsules containing 40 mg of Clobazam with three quantities, each of 10 ml, of *dichloromethane*, combine the filtered extracts, evaporate to dryness and dissolve the residue in 2 ml of *methanol*.

*Reference solution (a)*. Dilute 1 volume of the test solution to 200 volumes with *methanol*.

*Reference* solution *(b)*. Dilute 1 volume of reference solution (a) to 2.5 volumes with *methanol*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 40 volumes of *acetonitrile* and 60 volumes of *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 20 µl.

Inject the test solution and reference solutions (a) and (b). Continue the chromatography for 5 times the retention time of clobazam (about 15 minutes). In the chromatogram obtained with the test solution the area of any impurity peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b). The sum of the areas of all the impurity peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a).

**Dissolution** (2.5.2).

Apparatus. No 1
Medium. 900 ml of *0.1 M hydrochloric acid*.
Speed and time. 75 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

*Test solution*. The filtrate obtained as given above.

*Reference solution*. Weigh accurately a suitable quantity of *clobazam RS*, dissolve in *methanol*, and dilute with the dissolution medium to obtain a solution having the same concentration as that of the test solution.

Chromatographic system
- a stainless steel column 12.5 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 ì m)(such as Superspher 100RP-18),
- mobile phase: a mixture of 470 volumes of *acetonitrile* and 530 volumes of *water*,
- flow rate. 0.7 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 50 µl.

Inject alternatively the test solution and the reference solution.

Calculate the content of $C_{16}H_{13}ClN_2O_2$ in the medium.

D. Not less than 75 per cent of the stated amount of $C_{16}H_{13}ClN_2O_2$.

**Other tests**. Comply with the tests stated under Capsules.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh 20 capsules. Open the capsules without losing any part of the shells and transfer the contents as



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completely as possible to a flask. Wash the shells with three quantities, each of 30 ml, of *methanol,* add the washings to the flask and dilute to 200.0 ml with *methanol.* Allow the shells to dry at room temperature and weigh. The difference between the weights represents the weight of the total contents. Mix the contents of the flask with the aid of ultrasound for 10 minutes and stir magnetically for 20 minutes. Centrifuge a portion of the suspension and dilute a volume of the resulting supernatant liquid containing 5 mg of Clobazam to 100.0 ml with *methanol.*

*Reference solution (a).* A 0.005 per cent w/v solution of *clobazam RS* in *methanol.*

*Reference solution (b).* A solution containing 0.006 per cent w/v of *7-chloro-1,5-dihydro-5-phenyl-1,5-benzodiazepine-2,4(3H)-dione RS* (desmethylclobazam) and 0.0125 per cent w/v of *clobazam RS* in *methanol.*

Chromatographic system

- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 470 volumes of *acetonitrile* and 530 volumes of *water,*
- flow rate 0.7 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 20 µl.

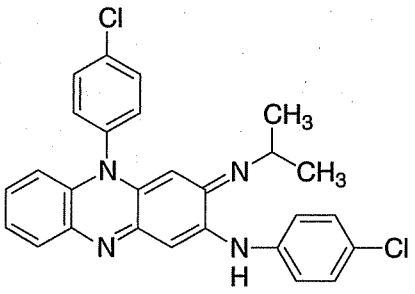
Inject reference solution (b). The resolution between the peaks corresponding to desmethylclobazam and clobazam is not less than 3.

Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternatively the test solution and reference solution (a).

Calculate the content of $C_{16}H_{13}ClN_2O_2$ in the capsules.

# Clofazimine




$C_{27}H_{22}Cl_2N_4$ Mol. Wt. 473.4

Clofazimine is 3-(4-chloroanilino)-10-(4-chlorophenyl)- 2,10-dihydro-2-(isopropylimino)phenazine.

Clofazimine contains not less than 98.5 per cent and not more than 101.5 per cent of $C_{27}H_{22}Cl_2N_4$, calculated on the dried basis.

**Category**. Antibacterial (antileprotic).

**Dose**. For leprosy, for previously untreated patients, 100 mg three times weekly; for sulphone-resistant patients, 100 mg six times weekly. For the suppression of lepra reactions, 200 mg daily.

**Description**. Dark red crystals or a reddish-brown, fine powder; almost odourless.

## Identification

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

B. When examined in the range 230 nm to 600 nm, a 0.0005 per cent w/v solution in *0.01 M methanolic hydrochloric acid* shows absorption maxima, at about 283 nm and 487 nm; absorbance at about 283 nm, about 0.65 and at about 487 nm, about 0.32 (2.4.7).

C. Dissolve 2 mg in 3 ml of *acetone* and add 0.1 ml of *hydrochloric acid;* an intense violet colour is produced. Add 0.5 ml of *5 M sodium hydroxide*; the colour changes to orange-red.

## Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel F254* and exposing the plate to ammonia vapour immediately before use by suspending the plate for 30 minutes in a tank containing a shallow layer of *0.2 M ammonia.*

*Mobile phase.* A mixture of 85 volumes of *dichloromethane* and 4 volumes of *1-propanol.*

*Test solution.* Dissolve 0.2 g of the substance under examination in 10 ml of *choroform.*

*Reference solution (a).* A 0.016 per cent w/v solution of the substance under examination in *chloroform.*

*Reference solution (b).* A 0.01 per cent w/v solution of the substance under examination in *chloroform.*

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in air for 5 minutes and replace it in the tank. When the mobile phase has again risen 12 cm dry the plate in air for 5 minutes and examine in daylight and then in ultraviolet light at 254 nm. Spray the plate with *sulphuric acid (50 per cent)* and examine again in daylight. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not



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more than two such spots are more intense than the spot in the chromatogram obtained with reference solution (b).

**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.5 g and dissolve in 20 ml of *chloroform*. Add 50 ml of *acetone* and titrate with *0.1 M perchloric acid* in *dioxan*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04734 g of $C_{27}H_{22}Cl_2N_4$.

## Clofazimine Capsules

Clofazimine Capsules contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of clofazimine, $C_{27}H_{22}Cl_2N_4$.

**Usual strength**. 100 mg.

### Identification

A. When examined in the range 260 nm to 600 nm (2.4.7), a 0.0005 per cent w/v solution in a mixture prepared by dissolving 2.25 g of *sodium dodecyl sulphate*, 0.85 g of *tertbutylammonium hydrogen sulphate* and 0.885 g of *disodium hydrogen orthophosphate* in 500 ml of *water* adjust the pH to 3.0 with *orthophophoric acid* and 65 volumes of *acetonitrile*, shows two absorbance maximum at 289 nm and 491 nm.

B. To 5 mg of the contents of a capsule add 3 ml of *chloroform* and 1 ml of *2 M hydrochloric acid*; the colour of the chloroform layer changes to violet. Add 2 ml of *2 M sodium hydroxide*; the colour changes to brownish-yellow.

### Tests

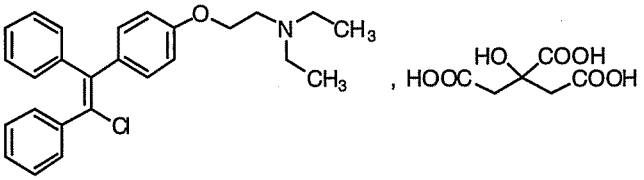
**Other tests**. Comply with the tests stated under Capsules.

**Assay**. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 0.15 g of Clofazimine and dissolve in sufficient *chloroform* to produce 100.0 ml. Filter through a chloroform-washed plug of cotton wool. Dilute 5.0 ml of the clear filtrate to 100.0 ml with *chloroform*. To 5.0 ml add 5.0 ml of *0.1 M methanolic hydrochloric acid* and sufficient *chloroform* to produce 50.0 ml. Measure the *absorbance* of the resulting solution at the maximum at about 491 nm (2.4.7), using as the blank a mixture of 5.0 ml of *0.1 M methanolic hydrochloric acid* and sufficient *chloroform* to produce 50.0 ml. Calculate the content of $C_{27}H_{22}Cl_2N_4$. taking 650 as the specific absorbance at 491 nm.

**Storage**. Store protected from moisture.

## Clomifene Citrate

Clomiphene Citrate



$C_{26}H_{28}ClNO,C_6H_8O_7$ Mol . Wt. 598.1

Clomifene citrate is a mixture of *E*- and *Z*-isomers of 2-[4-(2-chloro-1,2-diphenylvinyl)phenoxy]triethylamine dihydrogen citrate.

Clomifene Citrate contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{26}H_{28}ClNO,C_6H_8O_7$, calculated on the anhydrous basis.

**Category**. Ovulation inducer.

**Dose**. 50 mg daily for 5 consecutive days starting on fifth day of menstruation.

**Description**. A white or pale yellow, crystalline powder.

### Identification

*Tests B and C may be omitted if tests A and D are carried out. Test A may be omitted if Tests B, C and D are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *clomifene citrate RS*.

B. Dissolve about 5 mg in 5 ml of a mixture of 10 volumes of *acetic anhydride* and 50 volumes of *pyridine* and heat in a water-bath; a deep red colour is produced.

C. In the test for Related substances, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution (a).

D. A 5 per cent w/v solution gives the reactions of citrates (2.3.1).

### Tests

*NOTE — In the following tests, the solutions should be protected from light in amber-coloured glassware. Ensure*



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*minimum exposure of the solutions to daylight until they are required for chromatography.*

**Related substances**. Determine by liquid chromatography (2.4.14)

*Test solution*. A 0.125 per cent w/v solution of the substance under examination in the mobile phase.

*Reference solution (a)*. A solution containing 0.125 per cent w/v of *clomifene citrate for performance test RS* in the mobile phase.

*Reference solution (b)*. Dilute 1 ml of the test solution to 50 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with butylsilane bonded to porous silica (Such as Vydac C4),
- mobile phase: mix 400 volumes of acetonitrile with 600 volumes of *water* and add 8 ml of *diethylamine*, adjust the pH of the mixture to 6.2 by the addition of about 1 to 2 ml of *phosphoric acid* taking care to reduce progressively the volume of each addition as the required pH is approached,
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 233 nm,
- injection volume. 10 µl.

Equilibrate the column with the mobile phase at a flow rate of 1.2 ml per minute for about one hour.

Inject reference solution (a). Continue the chromatography for twice the retention time of the principal peak. Measure the height (a) above the baseline of the peak due to clomifene impurity A and the height (B) above the baseline of the lowest point of the curve separating this peak from the peak due to clomifene. The test is not valid unless A is greater than 15 times B and the chromatogram obtained resembles the reference chromatogram. If necessary, adjust the concentration of acetonitrile in the mobile phase.

Inject separately the test solution and reference solution (b). Continue the chromatography for four times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any peak due to 2-[4-(1,2-diphenylvinyl)phenoxy]triethylamine is not greater than that of the principal peak in the chromatogram obtained with reference solution (b) (2 per cent) and the area of any other secondary peak is not greater than half the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent); the sum of the areas of any secondary peaks is not greater than 1.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.5 per cent). Ignore any peak with a retention time relative to the clomifene peak of 0.2 or less and any peak with an area less than 0.025 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

**Z-isomer**. 30 to 50 per cent.

Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 25 mg of the substance under examination in 25 ml of *0.1 M hydrochloric acid*, add 5 ml of *1 M sodium hydroxide* and shake with three quantities, each of 25 ml, of *ethanol-free chloroform*. Wash the combined extracts with 10 ml of *water*, dry over *anhydrous sodium sulphate* and dilute to 100.0 ml with *ethanol-free chloroform* To 20.0 ml of the solution add 0.1 ml of *triethylamine* and dilute to 100.0 ml with *hexane*.

*Reference solution*. Dissolve 25 mg of *clomifene citrate RS* in 25 ml of *0.1 M hydrochloric acid*, add 5 ml of *1 M sodium hydroxide* and shake with three quantities, each of 25 ml, of *ethanol-free chloroform*. Wash the combined extracts with 10 ml of *water*, dry over *anhydrous sodium sulphate* and dilute to 100.0 ml with *ethanol-free chloroform*. To 20.0 ml of the solution add 0.1 ml of *triethylamine* and dilute to 100.0 ml with *hexane*.

Chromatographic system
- a stainless steel column 30 cm x 4 mm, packed with porous silica particles (10 µm) (Such as Parasol),
- mobile phase: a mixture of 1 volume of *triethylamine*, 200 volumes of *ethanol-free chloroform* and 800 volumes of *hexane*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 302 nm,
- injection volume. 50 µl.

Equilibrate the column with the mobile phase for about 2 hours.

Inject the reference solution. The chromatogram obtained shows a peak due to *E*-isomer just before a peak due to *Z*-isomer. The test is not valid unless the resolution between the peaks corresponding to *E*- and *Z*-isomers is at least 1.0. If necessary, adjust the relative proportions of ethanol-free chloroform and hexane in the mobile phase. Measure the area of the peak due to the *Z*-isomer in the chromatogram obtained with the test solution and the reference solution.

Calculate the content of *Z*-isomer as a percentage of the total clomifene citrate present.

**Water** (2.3.43). Not more than 1.0 per cent, determined on 1.0 g.

**Assay**. Weigh accurately about 0.5 g and dissolve in 50 ml of *anhydrous acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.05981 g of $C_{26}H_{28}ClNO,C_6H_8O_7$.



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# Clomifene Tablets

Clomifene Citrate Tablets; Clomiphene Tablets; Clomophene Tablets

Clomifene Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of clomifene citrate, $C_{26}H_{28}ClNO,C_6H_8O_7$.

**Usual strengths**. 25 mg; 50 mg.

## Identification

A. When examined in the range 230 nm to 360 nm (2.4.7), the solution obtained in the Assay shows absorption maxima at about 235 nm and 292 nm.

B. Dissolve a quantity of the powdered tablets containing 5 mg of Clomifene Citrate in 5 ml of a mixture of 10 volumes of *acetic anhydride* and 50 volumes of *pyridine* and heat in a water-bath; a deep red colour is produced.

## Tests

**Z-isomer**. 30 to 50 per cent of the content of clomifene citrate as determined in the Assay.

Determine by liquid chromatography (2.4.14)

*Test solution*. Shake a quantity of the powdered tablets containing about 50 mg of Clomifene Citrate with 50 ml of *0.1 M hydrochloric acid* for 10 minutes and filter. To 25 ml of the filtrate add 5 ml of *1 M sodium hydroxide* and extract with three quantities, each of 25 ml, of *ethanol-free chloroform*. Wash the combined extracts with 10 ml of *water*, dry over *anhydrous sodium sulphate* and add sufficient *ethanol-free chloroform* to produce 100.0 ml. To 20.0 ml of the resulting solution add 0.1 ml of *triethylamine* and sufficient *hexane* to produce 100 ml.

Chromatographic system

- a stainless steel column 30 cm x 4 mm, packed with porous silica particles (10 µm) (Such as Parasol),
- mobile phase: a mixture of *ethanol-free chloroform* and *hexane*, each containing 0.10 per cent v/v of *triethylamine*, adjusted so that the baseline separation is obtained between *E*- and *Z*-isomers of clomifene (a mixture of 20 volumes of *ethanol-free chloroform* and 80 volumes of *hexane* is suitable),
- flow rate. 2 ml per minute,
- spectrophotometer set at 302 nm,
- injection volume. 50 µl.

Stabilise the system by passing about 250 ml of the mobile phase.

Inject the test solution. In the chromatogram a peak due to the *E*-isomer precedes that due to the *Z*-isomer of clomifene. The test is not valid unless baseline separation is achieved between *E*- and *Z*-clomifene and the column efficiency is greater than 10,000 theoretical plates per metre determine using the peak due to E-isomer.

Calculate the percentage of *Z*-isomer from the expression $100A_Z/(1.08A_E+A_Z)$ where $A_Z$ and $A_E$ are the areas of the peaks due to the *Z*- and *E*-isomers respectively.

**Dissolution** (2.5.2).

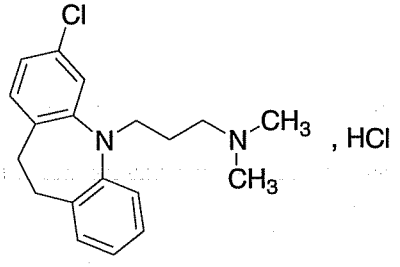
Apparatus No. 1,
Medium. 900 ml of *water*,
Speed and time. 100 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first 1 ml of the filtrate. Dilute a suitable volume of the filtrate with *0.1 M hydrochloric acid*. Measure the absorbance of the resulting solution at the maximum at about 232 nm (2.4.7). Calculate the content of $C_{26}H_{28}ClNO,C_6H_8O_7$ in the medium taking 317 as the specific absorbance at 232 nm.

D. Not less than 80 per cent of the stated amount of $C_{26}H_{28}ClNO,C_6H_8O_7$.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 50 mg of Clomifene Citrate, shake for 30 minutes with 70 ml of *0.1 M hydrochloric acid* prepared in a 30 per cent v/v solution of *2-propanol* (instead of water normally used for the purpose as solvent), dilute to 100.0 ml with the propanolic hydrochloric acid and filter. Dissolve 5.0 ml of the filtrate to 100.0 ml with *0.1 M hydrochloric acid* and measure the absorbance of the resulting solution at the maximum at about 292 nm (2.4.7), using a solution prepared by diluting 5 ml of the propanolic hydrochloric acid to 100 ml with *0.1 M hydrochloric acid* as the blank. Calculate the content of $C_{26}H_{28}ClNO,C_6H_8O_7$ taking 175 as the specific absorbance at 292 nm.

# Clomipramine Hydrochloride




$C_{19}H_{23}ClN_2,HCl$ Mol. Wt. 351.3

Clomipramine is 3-(3-chloro-10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepin-5-yl)-*N*,*N*-dimethylpropan-1-amine hydrochloride.



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Clomipramine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{19}H_{23}ClN_2,HCl$, calculated on the dried basis.

**Category**. Antidepressant.

**Description.** A white or slightly yellow, crystalline powder, slightly hygroscopic.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *clomipramine hydrochloride RS*.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 5 volumes of *ammonia*, 25 volumes of *acetone* and 75 volumes of *ethyl acetate*.

*Test solution*. Dissolve 20 mg of the substance under examination in 10 ml of *methanol*.

*Reference solution*. A 0.2 per cent w/v solution of *clomipramine hydrochloride RS* in *methanol*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air, spray with 0.5 per cent solution of *potassium dichromate* in a 20 per cent solution of *sulphuric acid*. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with the reference solution.

C. Dissolve about 5 mg in 2 ml of *nitric acid*. An intense blue colour develops.

D. Dissolve about 50 mg in 5 ml of *water* and add 1 ml of *dilute ammonia*. Allow to stand for 5 minutes and filter. Acidify the filtrate with *dilute nitric acid*. The solution gives reaction A of chlorides (2.3.1).

## Tests

**Appearance of solution**. A 10 per cent w/v solution in *carbon dioxide-free water* (Solution A) is clear (2.4.1) and not more intensely coloured than reference solution Y5 (2.4.1).

**pH** (2.4.24). 3.5 to 5.0, determined in solution A.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. 25 volumes of mobile phase B and 75 volumes of mobile phase A.

*Test solution*. Dissolve 20 mg of the substance under examination in 10 ml of solvent mixture.

*Reference solution (a)*. A 0.2 per cent w/v solution of *clomipramine hydrochloride RS* in solvent mixture.

*Reference solution (b)*. Dilute 1 ml of reference solution (a) to 100 ml with solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with cyanopropylsilyl silica (5 µm),
- mobile phase: A. 1.2 g of *sodium dihydrogen phosphate* in *water*, add 1.1 ml of *nonylamine*, adjust to pH 3.0 with *phosphoric acid* and dilute to 1000 ml with *water*,
  B. *acetonitrile*,
- a linear gradient programme using the conditions given below,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

| Time (in min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0 | 75 | 25 |
| 10 | 65 | 35 |
| 32 | 65 | 35 |
| 44 | 75 | 25 |

Inject reference solution (a). Test is not valid unless the column effciency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent).

**Heavy metals** (2.3.13). 2 g complies with limit test for heavy metals, Method B (10 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.25 g, dissolve in 50.0 ml of *ethanol* and add 5.0 ml of *0.01 M hydrochloric acid*. Titrate with *0.1 M sodium hydroxide*. Determine the end-point potentiometrically (2.4.25).

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.03513 g of $C_{19}H_{24}Cl_2N_2$.

**Storage**. Store protected from light and moisture.

# Clomipramine Capsules

## Clomipramine Hydrochloride Capsules

Clomipramine Capsules contain Clomipramine Hydrochloride.

Clomipramine Capsules contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of clomipramine hydrochloride, $C_{19}H_{23}ClN_2,HCl$.



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**Usual strengths**. 25 mg; 75 mg; 100 mg.

## Identification

Triturate a quantity of the contents of the capsules containing 0.15 g of Clomipramine Hydrochloride with 10 ml of *chloroform*, filter and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6 ). Compare the spectrum with that obtained with *clomipramine hydrochloride RS*.

## Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Disperse a quantity of the mixed contents of 20 capsules containing 20 mg of Clomipramine Hydrochloride with 5 ml of mobile phase A with the aid of ultrasound for 15 minutes, dilute to 10 ml with the same solvent and filter.

*Reference solution (a)*. A 0.2 per cent w/v solution of *clomipramine hydrochloride RS* in mobile phase A.

*Reference solution (b)*. Dilute 1 ml of reference solution (a) to 100 ml with mobile phase A.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with cyanopropylsilyl silica (5 µm) (Such as Hypersil BDS CN),
- mobile phase: A. a mixture of 75 volumes of solution prepared by dissolving 1.2 g of *sodium dihydrogen orthophosphate* in 950 ml of *water*, add 1.1 ml of *nonylamine*, adjust to pH 3.0 with *orthophosphoric acid* and add sufficient *water* to produce 1000 ml (solution A) and 25 volumes of *acetonitrile*.
  B. a mixture of 65 volumes of solution A and 35 volumes of *acetonitrile*,
- a linear gradient programme using the conditions given below,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

| Time (in min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0 | 75 | 25 |
| 10 | 65 | 35 |
| 32 | 65 | 35 |
| 44 | 75 | 25 |

Inject reference solution (a). Test is not valid unless the column effciency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent).

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 50 mg of Clomipramine, disperse in 100.0 ml of *methanol*. Dilute 5.0 ml of the solution to 50.0 ml with *methanol*.

*Reference solution*. A 0.0125 per cent w/v solution of *clobazam RS* in *methanol*.

Chromatographic system

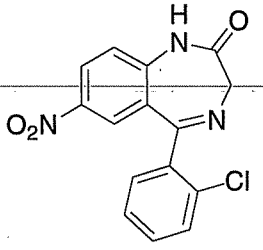
- a stainless steel column 20 cm × 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 47 volumes of *acetonitrile* and 53 volumes of *water*,
- flow rate. 0.7 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{16}H_{13}ClN_2O_2$.

# Clonazepam




$C_{15}H_{10}ClN_3O_3$ Mol. Wt. 315.7

Clonazepam is 5-(2-chlorophenyl)-7-nitro-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one.

Clonazepam contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{15}H_{10}ClN_3O_3$, calculated on the dried basis.

**Category**. Anticonvulsant.

**Description**. A slightly yellowish, crystalline powder.



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### Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *clonazepam RS*.

### Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. 10 volumes of *tetrahydrofuran*, 42 volumes of *methanol* and 48 volumes of *water*.

*Test solution*. Dissolve 0.05 g of the substance under examination in 10 ml of *methanol* and dilute to 100 ml with the solvent mixture.

*Reference solution (a)*. Dissolve 25 mg of *clonazepam RS* in 5 ml of *methanol* and dilute to 50 ml with the solvent mixture.

*Reference solution (b)*. Dilute 1 ml of reference solution (a) to 100 ml with solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 10 volumes of *tetrahydrofuran*, 42 volumes of *methanol* and 48 volumes of a 0.66 per cent solution of *ammonium phosphate* previously adjusted to pH 8.0 with a 4 per cent w/v of *sodium hydroxide* or *orthophosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Inject reference solution (a). Test is not valid unless the column effciency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1 g by drying in an oven at 105° for 4 hours.

**Assay**. Weigh accurately about 0.275 g, dissolve in 50 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid*. Determine the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03157g of $C_{15}H_{10}ClN_3O_3$.

**Storage**. Store protected from light.

## Clonazepam Injection

Clonazepam Injection is a sterile material consisting of Clonazepam with or without excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

*The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).*

**Usual strength**. 1 mg per ml.

**Storage**. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Clonazepam Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of clonazepam, $C_{15}H_{10}ClN_3O_3$.

**Description**. A slightly yellowish, crystalline powder.

*The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.*

### Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 2 volumes of *strong ammonia solution*, 15 volumes of *n-heptane*, 30 volumes of *nitromethane* and 60 volumes of *ether*.

*Test solution*. Dilute 3 ml of a solution containing 3 mg of Clonazepam in a stoppered tube with an equal volume of *water*, shake with 1 ml of *chloroform*, allow to separate and use the chloroform layer.

*Reference solution*. Dissolve 3 mg of *clonazepam RS* in 1 ml of *chloroform*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 10 cm. After development, dry the plate in a current of cold air, spray with 2 *M sodium hydroxide* and heat at 120° for 15 minutes. The yellow spot in the chromatogram obtained with test solution corresponds to that in the chromatogram obtained with reference solution.

### Tests

**pH** (2.4.24). 3.4 to 4.3.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 20 volumes of *chloroform* and 80 volumes of *ether*.



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*Test solution.* Dilute, if necessary, a volume of the solution containing 10 mg of Clonazepam to 20 ml with *water* and extract with three 3 ml quantities of *chloroform.* Wash each *chloroform* extract separately with the same 10 ml volume of *water*, combine the extracts and add sufficient *chloroform* to produce 10 ml.

*Reference solution (a).* A 0.0005 per cent w/v solution of *2-amino-2'-chloro-5-nitrobenzophenone RS* ('nitrobenzophenone') in *chloroform.*

*Reference solution (b).* A 0.0002 per cent w/v solution of *2-amino-2'-chloro-5-nitrobenzophenone RS* ('nitrobenzophenone') in *chloroform.*

*Reference solution (c).* A 0.0002 per cent w/v of *3-amino-4-(2-chlorophenyl)-6-nitroquinolin-2-one RS* ('carbostyril') in *chloroform.*

Apply to the plate 50 µl of each solution. After development, dry the plate in a current of cold air. For the second development use a mixture of 10 volumes of *ether* and 90 volumes of *nitromethane.* After development, dry the plate, heat at a pressure of 2 kPa at 120° for 3 hours, allow to cool and spray with a 10 per cent w/v solution of *zinc chloride* in *0.1 M hydrochloric acid.* Dry the plate in air and examine in visual light. Any spots in the chromatogram obtained with test solution corresponding to the nitrobenzophenone and carbostyril impurities are not more intense than the spots in the chromatograms obtained with reference solutions (b) and (c) respectively (0.2 per cent). Any other secondary spot in the chromatogram obtained with test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent).

**Assay**. *Protect the solutions from light throughout the assay.*

To a volume of the solution containing 20 mg of Clonazepam, dilute to 100.0 ml with *propan-2-ol.* Dilute 10.0 ml of the solution to 100.0 ml with *propan-2-ol* . Measure the absorbance of the resulting solution at the maximum at 310 nm (2.4.7). Calculate the content of $C_{15}H_{10}ClN_3O_3$ taking 364 as the specific absorbance at 310 nm.

**Storage**. Store protected from light.

**Labelling**. The label states (1) 'Sterile Clonazepam Concentrate'; (2) that the diluted injection is to be given by intravenous injection.

# Clonazepam Tablets

Clonazepam Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of clonazepam, $C_{15}H_{10}ClN_3O_3$.

**Usual strengths**. 0.5 mg; 1 mg; 2 mg.

## Identification

A. To an amount of the finely powdered tablets containing about 10 mg of Clonazepam in a small separator add 25 ml of *water*, shake for 2 minutes, and extract with two quantities, each of 40 ml, of *chloroform.* Pass the extracts through *anhydrous sodium sulphate*, combine them, and evaporate to dryness at room temperature with the aid of a stream of nitrogen; the residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *clonazepam RS* or with the reference spectrum of clonazepam.

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

## Tests

**Dissolution** (2.5.2).

Apparatus No.1
Medium. 900 ml of degassed *water.*
Speed and time. 75 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter, discarding about 10 ml of the filtrate.

Determine by liquid chromatography (2.4.14).

*Test solution.* The filtrate obtained as given above.

*Reference solution.* A 0.005 per cent w/v solution of *clonazepam RS* in *methanol.* Dilute suitably with the dissolution medium to produce a solution with a known concentration similar to the expected concentration of the test solution.

Chromatographic system
- a stainless steel column 30 cm × 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 40 volumes of *water*, 30 volumes of *methanol* and 30 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 100 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

D. Not less than 70 per cent of the stated amount of $C_{15}H_{10}ClN_3O_3$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture.* A mixture of 10 volumes of *tetrahydrofuran,* 42 volumes of *methanol* and 48 volumes of *water.*



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*Test solution.* Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 10 mg of Clonazepam, dissolve in 75 ml of the solvent mixture and dilute to 100.0 ml with the solvent mixture and filter.

*Reference solution (a).* Dissolve 25 mg of *clonazepam RS* in 150 ml of the solvent mixture and dilute to 250.0 ml with the solvent mixture.

*Reference solution (b).* Dilute 1 ml of the reference solution (a) to 100.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 10 volumes of *tetrahydrofuran*, 42 volumes of *methanol* and 48 volumes of a 0.66 per cent w/v solution of *ammonium phosphate,* with the pH previously adjusted to 8.0 with a 4 per cent w/v solution of *sodium hydroxide* or *orthophosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 50 µl.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of areas of all secondary peaks is not more than twice the area of the peak in the chromatogram obtained with the reference solution (b) (2.0 per cent). Ignore any peaks with an area 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

**Uniformity of content**. Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay.

*Test solution.* To one tablet add 5 ml of the solvent mixture, disperse with the aid of ultrasound for 10 minutes, dilute, if necessary, with sufficient of the solvent mixture to produce a solution containing 0.01 percent of Clonazepam and filter.

Calculate the content of $C_{15}H_{10}ClN_3O_3$ in the tablet.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture.* A mixture of 10 volumes of *tetrahydrofuran,* 42 volumes of *methanol* and 48 volumes of *water.*

*Test solution.* Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 10 mg of Clonazepam, dissolve in 75 ml of the solvent mixture and dilute to 100.0 ml with the solvent mixture and filter.

*Reference solution.* Dissolve 25 mg of *clonazepam RS* in 150 ml of the solvent mixture and dilute to 250.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 10 volumes of *tetrahydrofuran*, 42 volumes of *methanol* and 48 volumes of a 0.66 per cent w/v solution of *ammonium phosphate,* with the pH previously adjusted to 8.0 with a 4 per cent w/v solution of *sodium hydroxide* or *orthophosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 50 µl.

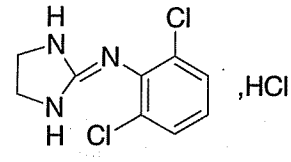
Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{15}H_{10}ClN_3O_3$ in the tablets.

**Storage**. Store protected from moisture.

# Clonidine Hydrochloride




$C_9H_9Cl_2N_3,HCl$ Mol. Wt. 266.6

Clonidine Hydrochloride is 2-[(2,6-dichlorophenyl)imino]-imidazolidine hydrochloride.

Clonidine Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of $C_9H_9Cl_2N_3,HCl$, calculated on the dried basis.

**Category**. Antihypertensive.

**Dose**. Orally, 50 to 100 µg thrice daily increased gradually according to the needs and response of the patient; maximum daily dose 1.2 mg. By slow intravenous injection, 150 to 300 µg; maximum daily dose 750 µg.

**Description**. A white or almost white, crystalline powder.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.*



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A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *clonidine hydrochloride RS* or with the reference spectrum of clonidine hydrochloride.

B. When examined in the range 230 nm to 600 nm, a 0.03 per cent w/v solution in *0.01 M hydrochloric acid* shows absorption maxima, at about 272 nm and 279 nm and an inflection at about 265 nm (2.4.7)

C. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

D. Gives reaction A of chlorides (2.3.1).

### Tests

**Appearance of solution**. A 5.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and not more intensely coloured than reference solution YS7 (2.4.1).

**pH** (2.4.24). 4.0 to 5.0, determined in a 5.0 per cent w/v solution.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. Shake together 50 volumes of *water*, 40 volumes of *1-butanol* and 10 volumes of *glacial acetic acid* and allow the layers to separate. Use the filtered upper layer.

*Test solution (a)*. Dissolve 0.1 g of the substance under examination in 10 ml of *methanol*.

*Test solution (b)*. Dilute 1.0 ml of test solution (a) to 10 ml with *methanol*.

*Reference solution (a)*. Dilute 5 ml of test solution (b) to 100 ml with *methanol*.

*Reference solution (b)*. A 0.1 per cent w/v solution of *clonidine hydrochloride RS* in *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with *modified potassium iodobismuthate solution*. Allow to dry in air for 1 hour, spray again with the same reagent and immediately spray with a 5 per cent w/v solution of *sodium nitrite*. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a).

**Sulphated ash** (2.3.18). Not more than 0.2 per cent w/w.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.2 g and dissolve in 70 ml of *ethanol (95 per cent)*. Titrate with *0.1 M ethanolic sodium hydroxide*, determining the end-point potentiometrically (2.4.25).

1 ml of *0.1 M ethanolic sodium hydroxide* is equivalent to 0.02666 g of $C_9H_9Cl_2N_3$,HCl.

## Clonidine Injection

Clonidine Hydrochloride Injection

Clonidine Injection is a sterile solution of Clonidine Hydrochloride in Water for Injections.

Clonidine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of clonidine hydrochloride, $C_9H_9Cl_2N_3$,HCl.

**Usual strength**. 150 µg in 1 ml.

### Identification

A. Dilute a volume containing 300 µg of Clonidine Hydrochloride to 5 ml with *0.01 M hydrochloric acid*. When examined in the range 230 nm to 360 nm, the resulting solution shows absorption maxima at about 272 nm and 279 nm and an inflection at about 265 nm (2.4.7).

B. To a volume containing 150 µg of Clonidine Hydrochloride add 1 ml of a 10 per cent w/v solution of *ammonium reineckate* and keep aside for 5 minutes; a pink precipitate is obtained.

### Tests

**pH** (2.4.24). 4.0 to 7.0.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. Shake together 50 volumes of *water*, 40 volumes of *1-butanol* and 10 volumes of *glacial acetic acid* and allow the layers to separate. Use the filtered upper layer.

*Test solution*. Add 10 ml of *methanol* to a volume containing 750 µg of Clonidine Hydrochloride, evaporate to dryness and dissolve the residue in 0.5 ml of *methanol*.

*Reference solution*. Dilute 1 volume of the test solution to 100 volumes with *methanol*.

Apply to the plate 20 µl of each solution. After development, dry the plate in air and spray with *modified potassium iodobismuthate solution*. Allow to dry in air for 1 hour, spray again with the same reagent and immediately spray with a 5 per cent w/v solution of *sodium nitrite*. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. To an accurately measured volume of the injection containing 150 µg of Clonidine Hydrochloride add 25 ml of *citrophosphate buffer pH 7.6*, 5 ml of *water*, and 1 ml of a solution containing 0.15 per cent w/v of *bromothymol blue* and 0.15 per cent w/v of *anhydrous sodium carbonate*. Add 30 ml of *chloroform*, shake for 1 minute and centrifuge. To



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15.0 ml of the chloroform layer add 10 ml of *boric acid solution* and measure the absorbance of the resulting solution at the maximum at about 420 nm (2.4.7), using as the blank a solution prepared by diluting 10 ml of *boric acid solution* to 25.0 ml with *chloroform*. Repeat the operation by adding to 5.0 ml of a 0.003 per cent w/v solution of *clonidine hydrochloride RS*, previously dried to constant weight at 105°, 20 ml of *citrophosphate buffer pH 7.6* and completing the procedure described above beginning at the words "5 ml of *water*". Calculate the content of $C_9H_9Cl_2N_3$,HCl from the absorbance obtained using *clonidine hydrochloride RS* in place of the substance under examination.

**Storage**. Store in single dose containers.

## Clonidine Tablets

Clonidine Hydrochloride Tablets

Clonidine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of clonidine hydrochloride, $C_9H_9Cl_2N_3$,HCl.

**Usual strengths**. 25 µg; 100 µg; 300 µg

### Identification

To a quantity of the powdered tablets containing 500 µg of Clonidine Hydrochloride add 30 ml of *water* and 5 ml of *1 M sodium hydroxide*. Swirl gently and extract with 20 ml of *chloroform*. Remove the chloroform layer, dry with *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness. Dissolve the residue in 8 ml of *0.01 M hydrochloric acid*. The resulting solution complies with the following tests.

A. When examined in the range 230 nm to 360 nm, it shows absorption maxima at about 272 nm and 279 nm and an inflection at about 265 nm (2.4.7).

B. To 2 ml add 1 ml of a 10 per cent w/v solution of *ammonium reineckate* and allow to stand for 5 minutes; a pink precipitate is produced.

### Tests

**Uniformity of content**. Comply with the test stated under Tablets.

*For tablets containing 300 µg or more of Clonidine Hydrochloride* – To one tablet add 200 ml of *citrophosphate buffer pH 7.6*, shake until disintegrated and dilute with *citrophosphate buffer pH 7.6*, if necessary, to give a solution containing about 0.0015 per cent w/v of Clonidine Hydrochloride. To 5 ml of the supernatant liquid add 1 ml of a solution containing 0.15 per cent w/v of *bromothymol blue* and 0.15 per cent w/v of *anhydrous sodium carbonate*. Add 10 ml of *chloroform*, shake for 1 minute and centrifuge. To 5 ml of the supernatant liquid add 5 ml of *boric acid solution* and measure the absorbance of a 2-cm layer of the resulting solution at the maximum at about 420 nm (2.4.7), using as the blank a mixture of 5 ml of *boric acid solution* and 5 ml of *chloroform*. Repeat the operation by using a solution prepared by diluting 5 ml of a 0.03 per cent w/v solution of *clonidine hydrochloride RS* to 100 ml with *citrophosphate buffer pH 7.6*, transferring 5 ml to a separator and completing the procedure described above beginning at the words "add 1 ml of a solution...".

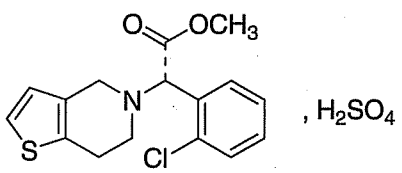
Calculate the content of $C_9H_9Cl_2N_3$,HCl in the tablet from the absorbance obtained using *clonidine hydrochloride RS* in place of the tablet.

*For tablets containing less than 300 µg of Clonidine Hydrochloride* - Use the same procedure but with a concentration of 0.001 per cent w/v or 0.0005 per cent w/v of Clonidine Hydrochloride as appropriate and with corresponding smaller concentrations of *clonidine hydrochloride RS*.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. To an accurately weighed quantity of the powder containing about 100 µg of Clonidine Hydrochloride add 25 ml of *citrophosphate buffer pH 7.6* and shake for 15 minutes. Add 5 ml of *water* and 1 ml of a solution containing 0.15 per cent w/v of *bromothymol blue* and 0.15 per cent w/v of *anhydrous sodium carbonate* and shake to disperse. Add 30 ml of *chloroform*, shake for 1 minute and centrifuge. To 15.0 ml of the chloroform layer add 10 ml of *boric acid solution* and measure the absorbance of the resulting solution at the maximum at about 420 nm (2.4.7), using as the blank a solution prepared by diluting 10 ml of *boric acid solution* to 25.0 ml with *chloroform*. Repeat the operation by adding to 5.0 ml of a 0.003 per cent w/v solution of *clonidine hydrochloride RS*, previously dried to constant weight at 105°, 20 ml of *citrophosphate buffer pH 7.6* and completing the procedure described above beginning at the words "5 ml of *water*". Calculate the content of $C_9H_9Cl_2N_3$,HCl from the absorbance obtained using *clonidine hydrochloride RS* in place of the substance under examination.

## Clopidogrel Bisulphate



, $H_2SO_4$

$C_{16}H_{16}ClNO_2S$, $H_2SO_4$ Mol. Wt. 419.9

Clopidogrel Bisulphate is methyl (*S*)-α-(*o*-chlorophenyl)-6,7-dihydrothieno[3,2-*c*]pyridine-5-(4*H*)-acetate sulphate.



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Clopidogrel Bisulphate contains not less than 97.0 per cent and not more than 101.5 per cent of $C_{16}H_{16}ClNO_2S,H_2SO_4$, calculated on the dried basis.

**Category**. Antidepressant.

**Description**. A white to off-white powder.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *clopidogrel bisulphate RS* or with the reference spectrum of clopidogrel bisulphate.

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

C. Gives reaction A of sulphates (2.3.1).

## Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*NOTE — For all clopidogrel related compounds, the concentrations are expressed as bi-sulphate salts. Use bi-sulphate salt equivalents stated on reference substances labels to calculate the concentrations as appropriate.*

*Test solution*. Dissolve 100 mg of the substance under examination in 5.0 ml of *methanol* and dilute to 200.0 ml with the mobile phase.

*Reference solution*. A solution containing 20 µg per ml of *clopidogrel bisulphate RS*, 40 µg per ml of *(+)-(S)-(o-chlorophenyl)-6,7-dihydrothieno(3,2-c]pyridine-5(4H)-acetic acid RS (clopidogrel impurity A RS)*, 120 µg per ml of *methyl(±)-(o)-chlorophenyl)-4,5 dihydrothieno(2,3-c)pyridine-6(7H)-acetate, hydrochloride RS (clopidogrel impurity B RS)*, and 200 µg per ml of *methyl(-)-(R)-o-chlorophenyl)-6,7 dihydrothieno(3,2-c)pyridine-5(4H)-acetate,hydrogen sulphate RS (clopidogrel impurity C RS)*, in *methanol*. Dilute 5.0 ml of the solution to 200.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with chiral recognition protein, ovomucoid, chemically bonded to porous silica (5 µm),
- mobile phase: a mixture of 75 volumes of a buffer solution prepared by dissolving 1.36 g of *potassium phosphate* in 1000 ml of *water* and 25 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 10 µl.

Inject the reference solution. The relative retention time with respect to clopidogrel, for impurity A is about 0.5, for enantiomers of clopidogrel impurity B, about 0.8 and 1.2 and for clopidogrel impurity C, about 2.0. The test is not valid unless the resolution between clopidogrel and the first enantiomer of clopidogrel impurity B is not less than 2.5.

Inject the test solution and the reference solution. In the chromatogram obtained with the test solution the area of the peak due to clopidogrel impurity A is not more than the area of the corresponding peak in the chromatogram obtained with the reference solution ( 0.2 per cent ), the area of the peak due to the first enantiomer of clopidogrel impurity B is not more than the area of the corresponding peak in the chromatogram obtained with the reference solution ( 0.3 per cent ) and the area of the peak due to clopidogrel impurity C is not more than the area of the corresponding peak in the chromatogram obtained with the reference solution ( 1.0 per cent ). The area of any peak due to other impurities is not more than the area of the peak due to *clopidogrel bisulphate RS* in the chromatogram obtained with the reference solution (0.1 per cent). The sum of all the impurities is not more than 1.5 per cent.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 2 hours.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh accurately about 100 mg of the substance under examination and dissolve in 100.0 ml of *methanol*. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

*Reference solution (a)*. A 0.1 per cent w/v solution of *clopidogrel bisulphate RS* in *methanol*. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

*Reference solution (b)*. A solution containing 100 µg per ml of *clopidogrel bisulphate RS* and 200 µg per ml of *clopidogrel impurity B RS* in *methanol*. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with chiral recognition protein, ovomucoid, chemically bonded to porous silica (5 µm),
- mobile phase : a mixture of 75 volumes of a buffer solution prepared by dissolving 1.36 g of *monobasic potassium phosphate* in 1000 ml of *water* and 25 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 10 µl.

Inject reference solution (b). The relative retention times with respect to clopidogrel, for enantiomers of clopidogrel impurity B are about 0.8 and 1.2 and the resolution between clopidogrel and the first enantiomer of clopidogrel impurity B is not less than 2.5.

Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.



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Inject the test solution and reference solution (a).

Calculate the content of $C_{16}H_{16}ClNO_2S,H_2SO_4$.

**Storage**. Store protected from moisture, at a temperature not exceeding 30°.

## Clopidogrel Tablets

Clopidogrel Bisulphate Tablets

Clopidogrel Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of clopidogrel, $C_{16}H_{16}ClNO_2S$.

**Usual strength**. 75 mg.

### Identification

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

### Tests

**Dissolution** (2.5.2).

Apparatus No.1,

Medium. 900 ml of *0.1 M hydrochloric acid,*

Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 240 nm (2.4.7). Calculate the content of $C_{16}H_{16}ClNO_2S$ in the medium from the absorbance obtained from a solution of known concentration of clopidogrel prepared by dissolving a suitable quantity of *clopidogrel bisulphate RS* in 20 ml of *methanol* and further diluting with the medium.

D. Not less than 80 per cent of the stated amount of $C_{16}H_{16}ClNO_2S$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 75 mg of Clopidogrel, add 5 ml of *methanol* and dilute to 200.0 ml with the mobile phase. Allow to stand for 10 minutes and mix and filter.

*Reference solution (a).* A solution containing *clopidogrel bisulphate RS* equivalent to 40 µg per ml of clopidogrel, 250 µg per ml of *[(+)-(S)-(o-chlorophenyl)-6,7-dihydrothieno(3,2-c]pyridine-5(4H)-acetic acid] RS (clopidogrel impurity A RS)* and 300 µg per ml of *[methyl(-)-(R)-o-chlorophenyl)-6,7 dihydrothieno(3,2-c)pyridine-5(4H)-acetate,hydrogen sulphate]RS (clopidogrel impurity C RS)* in *methanol.* Dilute 5.0 ml of the solution to 200.0 ml with the mobile phase.

*Reference solution (b).* A solution containing *clopidogrel bisulphate RS* equivalent to 100 µg per ml of clopidogrel, and 200 µg per ml of *[methyl(±)-(o)-chlorophenyl)-4,5 dihydrothieno(2,3-c)pyridine-6(7H)-acetate, hydrogen sulphate] RS (clopidogrel impurity B RS)* in *methanol.* Dilute 5.0 ml of the solution to 200.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with chiral recognition protein, ovomucoid, chemically bonded to porous silica (5 µm),
- mobile phase : a mixture of 75 volumes of a buffer solution prepared by dissolving 1.36 g of *potassium phosphate* in 1000 ml of *water* and 25 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 10 µl.

Inject reference solution (b). The relative retention times with respect to clopidogrel for enantiomers of clopidogrel impurity B are about 0.8 and 1.2. The resolution between clopidogrel and the first enantiomer of clopidogrel impurity B is not less than 2.5. Inject reference solution (a). The relative retention time with respect to clopidogrel for clopidogrel impurity A is about 0.5 and for clopidogrel impurity C is about 2.0. The relative standard deviation for replicate injections is not more than 15.0 per cent for each peak.

In the chromatogram obtained with the test solution the area of the peak due to clopidogrel impurity A is not more than 0.7 times the area of the corresponding peak in the chromatogram obtained with reference solution (a) (1.2 per cent ), and the area of the peak due to clopidogrel impurity C is not more than 0.75 times the area of the corresponding peak in the chromatogram obtained with reference solution (a) (1.5 per cent ). The area of any peak due to other impurities is not more than twice the area of the peak due to *clopidogrel bisulphate RS* in the chromatogram obtained with reference solution (a) (0.2 per cent, excluding impurity B). The sum of all the impurities is not more than 2.5 per cent, excluding impurity B.

**Uniformity of content** *(For tablets containing 10 mg or less).* Comply with the test stated under Tablets.

Disperse one tablet in 50 ml of *0.1 M hydrochloric acid.* Dilute 5.0 ml of the solution to 50.0 ml with *0.1 M hydrochloric acid* and filter. Measure the absorbance of the filtered solution at the maximum at about 270 nm (2.4.7). Calculate the content of $C_{16}H_{16}ClNO_2S$ from the absorbance obtained from a solution of known concentration of *clopidogrel bisulphate RS* in *0.1 M hydrochloric acid.*

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh and powder 20 tablets. Weigh accurately a quantity of the powder equivalent to 75 mg of clopidogrel, disperse in 100.0 ml of the *methanol.* Dilute 5.0 ml of the solution to 50.0 ml with the *methanol* and filter.



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*Reference solution (a)*. A 0.01 per cent w/v solution of *clopidogrel bisulphate RS* in *methanol*.

*Reference solution (b)*. A solution containing *clopidogrel bisulphate RS* equivalent to 100 µg per ml of clopidogrel and 200 µg per ml of *[methyl(±)-(o)-chlorophenyl)-4,5 dihydrothieno(2,3-c)pyridine-6(7H)-acetate, hydrogen sulphate] RS (clopidogrel impurity B RS)* in *methanol*. Dilute 5.0 ml of the solution to 200.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with chiral recognition protein, ovomucoid, chemically bonded to porous silica (5 µm),
- mobile phase: a mixture of 75 volumes of a buffer solution prepared by dissolving 1.36 g of *monobasic potassium phosphate* in 1000 ml of *water* and 25 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 10 µl.

Inject reference solution (b). The relative retention times with respect to clopidogrel, for enantiomers of clopidogrel impurity B are about 0.8 and 1.2 and the resolution between clopidogrel and the first enantiomer of clopidogrel impurity B is not less than 2.5.

Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

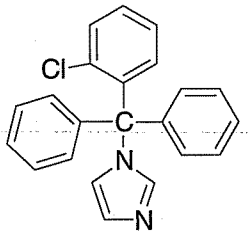
Inject the test solution and reference solution (a).

Calculate the content of $C_{16}H_{16}ClNO_2S$ in the tablets.

**Storage**. Store protected from moisture, at a temperature not exceeding 30°.

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

# Clotrimazole




$C_{22}H_{17}ClN_2$ Mol. Wt. 344.8

Clotrimazole is 1-[(2-chlorophenyl)diphenylmethyl]-1*H*-imidazole.

Clotrimazole contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{22}H_{17}ClN_2$, calculated on the dried basis.

**Category**. Antifungal.

**Description**. A white or pale yellow, crystalline powder.

## Identification

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

B. When examined in the range 230 nm to 360 nm, a 0.04 per cent w/v solution in a mixture of 1 volume of *0.1 M hydrochloric acid* and 9 volumes of *methanol* shows absorption maxima at about 262 and 265 nm; absorbance at about 262 nm, about 0.9 and at about 265 nm, about 0.92 (2.4.7).

## Tests

**Appearance of solution**. A 5.0 per cent w/v solution in *ethanol (95 per cent)* is clear (2.4.1), and not more intensely coloured than reference solution BYS6 (2.4.1).

**2-Chlorotritanol**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. A mixture of 70 volumes of *methanol* and 30 volumes of *0.02 M phosphoric acid*.

*Test solution*. Dissolve 0.1 g of the substance under examination in 100 ml with solvent mixture.

*Reference solution (a)*. A 0.0002 per cent w/v solution of *2-chlorotritanol RS* in the same solvent mixture.

*Reference solution (b)*. Dilute 1 volume of the test solution to 50 volumes with the same solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 70 volumes of *methanol* and 30 volumes of *0.02 M phosphoric acid* adjusted to pH 7.5 with a 10 per cent w/v solution of *triethylamine* in *methanol*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume. 20 µl.

Inject the test solution and reference solution (a). Allow the chromatography to proceed for 1.5 times the retention time of the principal peak in the chromatogram obtained with the test solution. The area of any peak corresponding to 2-chlorotritanol in the chromatogram obtained with the test solution is not greater then the area of the peak in the chromatogram obtained with reference solution (a). The test is not valid unless the column efficiency, determined using the principal peak in the chromatogram obtained with reference solution (b) is not less than 9000 theoretical plates.



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**Imidazole**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 180 volumes of *toluene*, 20 volumes of *1-propanol* and 1 volume of *strong ammonia solution*.

*Test solution*. Dissolve 0.5 g of the substance under examination in 10 ml of *chloroform*.

*Reference solution*. A 0.01 per cent w/v solution of imidazole in *chloroform*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, place in a tank of chlorine prepared by the addition of *hydrochloric acid* to *potassium permanganate* and allow to stand for 2 minutes. Remove any excess chlorine from the plate with a current of air and spray with *potassium iodide and starch solution*. Any spot corresponding to imidazole in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.3 g and dissolve in 50 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, using *1-naphtholbenzein solution* as indicator to a green end-point. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03448 g of $C_{22}H_{17}ClN_2$.

**Storage**. Store protected from light.

# Clotrimazole Cream

Clotrimazole Cream contains Clotrimazole in a suitable base.

Clotrimazole Cream contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of clotrimazole, $C_{22}H_{17}ClN_2$.

**Usual strengths**. 1 per cent w/w; 2 per cent w/w.

## Identification

A. Mix a quantity of the cream containing 40 mg of Clotrimazole with 20 ml of a mixture of 1 volume of *1 M sulphuric acid* and 4 volumes of *methanol* and shake with two quantities, each of 50 ml, of *carbon tetrachloride*, discarding the organic layers. Make the aqueous phase alkaline with *dilute ammonia solution*, add a further 5 ml of *dilute ammonia solution* and extract with two quantities, each of 40 ml, of *chloroform*. Combine the chloroform extracts, shake with 5 g of *anhydrous sodium sulphate*, filter and add sufficient *chloroform* to the filtrate to produce 100 ml. Evaporate 50 ml to dryness and dissolve the residue in 50 ml of a mixture of 1 volume of *0.1 M hydrochloric acid* and 9 volumes of *methanol*. When examined in the range 230 nm to 360 nm, the resulting solution shows absorption maxima at about 262 nm and 265 nm (2.4.7).

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. *Di-isopropyl ether* in a chromatography tank, containing 25 ml of *strong ammonia solution* in a beaker.

*Test solution*. Shake a quantity of the cream containing 20 mg of Clotrimazole with 4 ml of *dichloromethane* for 30 minutes, centrifuge and use the supernatant liquid.

*Reference solution*. A 0.5 per cent w/v solution of *clotrimazole RS* in *dichloromethane*.

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of air and spray with *dilute potassium iodobismuthate solution*. The spot in the chromatogram obtained with the test solution is reddish brown and corresponds to the spot in the chromatogram obtained with the reference solution.

## Tests

**2-Chlorotritanol**. Determine by liquid chromatography (2.4.14).

*Test solution*. Extract a quantity of the cream containing 20 mg of Clotrimazole by warming with 20 ml of *methanol* in a water-bath at 50° for 5 minutes, shaking occasionally. Remove from the water-bath, shake the mixture vigorously while cooling to room temperature, cool in ice for 15 minutes, centrifuge for 5 minutes and decant the supernatant liquid. Repeat the extraction with further quantities, each of 20 ml, of *methanol*. To the combined methanol extracts add 10 ml of *methanol* and dilute to 100.0 ml with *0.02 M phosphoric acid*. Cool in ice and filter through a membrane filter.

*Reference solution (a)*. A 0.0002 per cent w/v solution of *2-chlorotritanol RS* in a mixture of 70 volumes of *methanol* and 30 volumes of *0.02 M phosphoric acid*.

*Reference solution (b)*. Dilute 1 volume of the test solution to 50 volumes with the same solvent mixture.

Chromatographic system

- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 70 volumes of *methanol* and 30 volumes of *0.02 M phosphoric acid* adjusted to pH 7.5 with a 10 per cent w/v solution of *triethylamine* in *methanol*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume. 20 µl.

Inject reference solution (b).The column efficiency, determined using the principal peak in the chromatogram obtained is not less than 9000 theoretical plates.



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Inject the test solution and reference solution (a). Allow the chromatography to proceed for 1.5 times the retention time of the principal peak in the chromatogram obtained with the test solution. The area of any peak corresponding to 2-chlorotritanol in the chromatogram obtained with the test solution is not greater then the area of the peak in the chromatogram obtained with reference solution (a).

**Other tests**. Complies with the tests stated under Creams.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Treat a quantity of the cream containing about 20 mg of Clotrimazole as described in the test for 2-Chlorotritanol and dilute 1.0 ml of the filtrate to 5.0 ml with a mixture of 70 volumes of *methanol* and 30 volumes of *0.02 M phosphoric acid*.

*Reference solution*. Dissolve 20 mg of *clotrimazole RS* in 70 ml of *methanol*, add sufficient *0.02 M phosphoric acid* to produce 100.0 ml and dilute 1.0 ml of the resulting solution to 5.0 ml with a mixture of 70 volumes of *methanol* and 30 volumes of *0.02 M phosphoric acid*.

Chromatographic system
- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 70 volumes of *methanol* and 30 volumes of *0.02 M phosphoric acid* adjusted to pH 7.5 with a 10 per cent w/v solution of *triethylamine* in *methanol*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume. 20 µl.

Inject the reference solution. The column efficiency, determined using the peak in the chromatogram obtained, should be not less than 9000 theoretical plates.

Inject alternately the test solution and the reference solution. Allow the chromatography to proceed for 1.5 times the retention time of the principal peak in the chromatogram obtained with the test solution.

Calculate the content of $C_{22}H_{17}ClN_2$ in the cream.

**Storage**. Store at a temperature not exceeding 30°.

# Clotrimazole Pessaries

Clotrimazole Vaginal Tablets

Clotrimazole Pessaries contain Clotrimazole in a suitable base.

Clotrimazole Pessaries contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of clotrimazole, $C_{22}H_{17}ClN_2$.

**Usual strengths**. 100 mg; 200 mg; 500 mg.

## Identification

A. Mix a quantity of the powdered pessaries containing 40 mg of Clotrimazole with 20 ml of a mixture of 1 volume of *1 M sulphuric acid* and 4 volumes of *methanol* and shake with two quantities, each of 50 ml, of *carbon tetrachloride*, discarding the organic layers. Make the aqueous phase alkaline with *dilute ammonia solution*, add a further 5 ml of *dilute ammonia solution* and extract with two quantities, each of 40 ml, of *chloroform*. Combine the chloroform extracts, shake with 5 g of *anhydrous sodium sulphate*, filter and add sufficient *chloroform* to the filtrate to produce 100 ml. Evaporate 50 ml to dryness and dissolve the residue in 50 ml of a mixture of 1 volume of *0.1 M hydrochloric acid* and 9 volumes of *methanol*. When examined in the range 230 nm to 360 nm, the resulting solution shows absorption maxima at about 262 nm and 265 nm (2.4.7).

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. *Di-isopropyl ether* in a chromatography tank, containing 25 ml of *strong ammonia solution* in a beaker.

*Test solution*. Shake a quantity of the powdered pessaries containing 20 mg of Clotrimazole with 4 ml of *dichloromethane* for 30 minutes, centrifuge and use the supernatant liquid.

*Reference solution*. A 0.5 per cent w/v solution of *clotrimazole RS* in *dichloromethane*.

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of air and spray with *dilute potassium iodobismuthate solution*. The spot in the chromatogram obtained with the test solution is reddish brown and corresponds to the spot in the chromatogram obtained with the reference solution.

## Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Add 50 ml of *methanol* to a quantity of the powdered pessaries containing 0.1 g of Clotrimazole and shake for 20 minutes. Dilute to 100 ml with *methanol* and filter. To 20 ml of the filtrate add 50 ml *methanol* and sufficient *0.02 M phosphoric acid* to produce 100 ml.

*Reference solution (a)*. A 0.0002 per cent w/v solution of *2-chlorotritanol RS* in a mixture of 70 volumes of *methanol* and 30 volumes of *0.02 M phosphoric acid*.

*Reference solution (b)*. Dilute 1 volume of the test solution to 50 volumes with the same solvent mixture.

Chromatographic system
- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),



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- mobile phase: a mixture of 70 volumes of *methanol* and 30 volumes of *0.02 M phosphoric acid* adjusted to pH 7.5 with a 10 per cent w/v solution of *triethylamine* in *methanol*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume. 20 µl.

Inject reference solution (b).The column efficiency, determined using the principal peak in the chromatogram obtained is not less than 9000 theoretical plates.

Inject the test solution and reference solution (a). Allow the chromatography to proceed for 1.5 times the retention time of the principal peak in the chromatogram obtained with the test solution. The area of any peak corresponding to 2-chlorotritanol in the chromatogram obtained with the test solution is not greater then the area of the peak in the chromatogram obtained with reference solution (a).

**Other tests.** Complies with the tests stated under Pessaries.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh and powder 20 pessaries. Weigh accurately a quantity of the powder containing about 0.1 g of Clotrimazole, add 50 ml of *methanol* and shake for 20 minutes. Dilute to 250.0 ml with *methanol* and filter. To 10.0 ml of the filtrate add 60 ml of *methanol* and sufficient *0.02 M phosphoric acid* to produce 100.0 ml.

*Reference solution*. Dissolve 20 mg of *clotrimazole RS* in 70 ml of *methanol*, add sufficient *0.02 M phosphoric acid* to produce 100.0 ml and dilute 1.0 ml of the resulting solution to 5.0 ml with a mixture of 70 volumes of *methanol* and 30 volumes of *0.02 M phosphoric acid.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 70 volumes of *methanol* and 30 volumes of *0.02 M phosphoric acid* adjusted to pH 7.5 with a 10 per cent w/v solution of *triethylamine* in *methanol*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume. 20 µl.

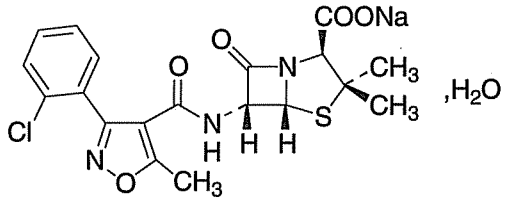
Inject the reference solution. The column efficiency, determined using the peak in the chromatogram obtained should be not less than 9000 theoretical plates.

Inject alternately the test solution and the reference solution. Allow the chromatography to proceed for 1.5 times the retention time of the principal peak in the chromatogram obtained with the test solution.

Calculate the content of $C_{22}H_{17}ClN_2$ in the pessaries.

**Storage**. Store protected from moisture and crushing.

# Cloxacillin Sodium




$C_{19}H_{17}ClN_3NaO_5S, H_2O$ Mol. Wt. 475.9

Cloxacillin Sodium is sodium (6*R*)-6-[3-(2-chlorophenyl)- 5-methylisoxazole-4-carboxamido]penicillanate monohydrate.

Cloxacillin Sodium contains not less than 95.0 per cent and not more than 101.0 per cent of $C_{19}H_{17}ClN_3NaO_5S$, calculated on the anhydrous basis.

**Category**. Antibacterial.

**Dose**. Orally, the equivalent of 500 mg of cloxacillin every 6 hours, at least 30 minutes before food; by intramuscular injection, 250 mg every 4 to 6 hours; by slow intravenous injection or by infusion, 500 mg every 4 to 6 hours.

**Description**. A white or almost white, crystalline powder; hygroscopic.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cloxacillin sodium RS* or with the reference spectrum of cloxacillin sodium.

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

C. Gives reaction A of sodium salts (2.3.1).

## Tests

**Appearance of solution**. A 10.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1); absorbance of the solution at about 430 nm, not more than 0.04 (2.4.7).

**pH** (2.4.24). 5.0 to 7.0, determined in a 10.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). +160° to +169°, determined at 20° in a 1.0 per cent w/v solution.

***N,N*-Dimethylaniline** (2.3.21). Not more than 20 ppm, determined by Method A.

**Water** (2.3.43). Not more than 4.5 per cent, determined on 0.3 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*Buffer solution.* Prepare a *0.02 M monobasic potassium phosphate* solution and adjust the pH to 6.6 with *2 M sodium hydroxide.*



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*Test solution.* Weigh accurately about 55 mg of the substance under examination and dilute to 100.0 ml with the buffer solution.

*Reference solution.* Weigh accurately a suitable quantity of *cloxacillin sodium RS* dissolve in the buffer solution and dilute to obtain a solution containing a known concentration of about 0.55 mg per ml.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a mixture of 80 volumes of the buffer solution and 20 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.8 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_{19}H_{18}ClN_3O_5S$.

*Cloxacillin Sodium intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.*

**Bacterial endotoxins** (2.2.3). Not more than 0.40 Endotoxin Unit per mg of cloxacillin.

*Cloxacillin Sodium intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.*

**Sterility** (2.2.11). Complies with the test for sterility.

**Storage**. Store protected from moisture at a temperature not exceeding 30°. If it is intended for use in the manufacture of parenteral preparations, the containers should be sterile and sealed so as to exclude micro-organisms.

**Labelling**. The label states whether or not the material is intended for use in the manufacture of parenteral preparations.

# Cloxacillin Capsules

Cloxacillin Sodium Capsules

Cloxacillin Capsules contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of cloxacillin, $C_{19}H_{18}ClN_3O_5S$.

**Usual strengths**. The equivalent of 250 mg and 500 mg of cloxacillin.

## Identification

A. Determine on the contents of the capsules by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cloxacillin sodium RS* or with the reference spectrum of cloxacillin sodium.

B. The contents of the capsules give reactions A and B of sodium salts (2.3.1).

## Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *water,*
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first 1 ml of the filtrate. Dilute the filtrate, if necessary, with the same solvent. Measure the absorbance of the resulting solution at the maximum at about 271 nm (2.4.7). Similarly measure the absorbance of a standard solution of known concentration of *cloxacillin sodium RS* in place of the contents of the capsules. Calculate the content of $C_{19}H_{18}ClN_3O_5S$.

D. Not less than 75 per cent of the stated amount of $C_{19}H_{18}ClN_3O_5S$.

**Other tests**. Comply with the tests stated under Capsules.

**Assay**. Determine by liquid chromatography (2.4.14).

*Buffer solution.* Prepare a *0.02 M monobasic potassium phosphate* solution and adjust the pH to 6.6 with *2 M sodium hydroxide.*

*Test solution.* Weigh accurately a quantity of the mixed contents of 20 capsules containing about 50 mg of Cloxacillin, dissolve in the buffer solution by stirring for 10 minutes, dilute to 100.0 ml with the buffer solution and filter.

*Reference solution.* Weigh accurately a suitable quantity of *cloxacillin sodium RS* dissolve in the buffer solution and dilute to obtain a solution containing a known concentration of about 0.55 mg per ml.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a mixture of 80 volumes of the buffer solution and 20 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.8 and the relative standard deviation for replicate injections is not more than 2.0 per cent.



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Inject alternately the test solution and the reference solution.

Calculate the content of $C_{19}H_{18}ClN_3O_5S$ in the capsules.

**Storage**. Store protected from moisture at a temperature not exceeding 30°.

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

## Cloxacillin Injection

Cloxacillin Sodium Injection

Cloxacillin Injection is a sterile material consisting of Cloxacillin Sodium with or without excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

*The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).*

**Storage**. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Cloxacillin Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cloxacillin, $C_{19}H_{18}ClN_3O_5S$.

**Usual strengths**. The equivalent of 250 mg and 500 mg of cloxacillin.

**Description**. A white or almost white powder; very hygroscopic.

*The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.*

### Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cloxacillin sodium RS* or with the reference spectrum of cloxacillin sodium.

B. Gives reaction A of sodium salts (2.3.1).

### Tests

**pH** (2.4.24). 5.0 to 7.0, determined in a 10 per cent w/v solution.

**Bacterial endotoxins** (2.2.3). Not more than 0.40 Endotoxin Unit per mg of cloxacillin.

**Water** (2.3.43). Not more than 4.5 per cent, determined on 0.3 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*Buffer solution.* Prepare a *0.02 M monobasic potassium phosphate* solution and adjust the pH to 6.6 with *2 M sodium hydroxide.*

*Test solution.* Determine the weight of the content of 10 containers. Weigh accurately a suitable quantity of the mixed contents of the 10 containers containing about 55 mg of Cloxacillin, dissolve in the buffer solution by shaking and dilute to 100.0 ml with the buffer solution.

*Reference solution.* Weigh accurately a suitable quantity of *cloxacillin sodium RS* dissolve in the buffer solution and dilute to obtain a solution containing a known concentration of about 0.55 mg per ml.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a mixture of 80 volumes of the buffer solution and 20 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.8 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_{19}H_{18}ClN_3O_5S$ in the injection.

**Storage**. Store protected from moisture at a temperature not exceeding 30°.

**Labelling**. The label states the quantity of Cloxacillin Sodium contained in the sealed container in terms of the equivalent amount of cloxacillin.

## Cloxacillin Syrup

Cloxacillin Sodium Syrup; Cloxacillin Elixir; Cloxacillin Sodium Elixir; Cloxacillin Oral Solution; Cloxacillin Sodium Oral Solution

Cloxacillin Syrup is a mixture consisting of Cloxacillin Sodium with buffering agents and other excipients. It contains a suitable flavouring agent. It is filled in a sealed container.

*The suspension is constituted by dispersing the contents of the sealed container in the specified volume of water just before use.*

Cloxacillin Syrup contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of $C_{19}H_{18}ClN_3O_5S$.

When stored at the temperature and for the period stated on the label during which the constituted suspension may be



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expected to be satisfactory for use, it contains not less than 80.0 per cent of the stated amount of cloxacillin, $C_{19}H_{18}ClN_3O_5S$.

**Usual strength**. The equivalent of 125 mg of cloxacillin per 5 ml when reconstituted with water.

### Identification

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

*The constituted suspension complies with the tests stated under Oral liquids and with the following tests.*

### Tests

**pH** (2.4.24). 4.0 to 7.0.

**Assay**. Determine by liquid chromatography (2.4.14).

*Buffer solution.* Prepare a *0.02 M monobasic potassium phosphate* solution and adjust to pH 6.6 with *1 M sodium hydroxide*.

*Test solution.* Transfer an accurately weighed quantity of the oral suspension containing about 125 mg of the cloxacillin to a 250-ml volumetric flask and dissolve in the buffer solution by stirring for 15 minutes. Dilute to 250.0 ml with the buffer solution.

*Reference solution.* Weigh accurately a suitable quantity of *cloxacillin sodium RS* dissolve in the buffer solution and dilute to obtain a solution containing a known concentration of about 0.55 mg per ml.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a mixture of 80 volumes of the buffer solution and 20 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.8 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

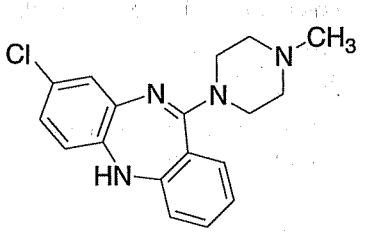
Determine the weight per ml of the oral suspension (2.4.29) and calculate the content of $C_{19}H_{18}ClN_3O_5S$ weight in volume.

Repeat the procedure using a portion of the constituted suspension that has been stored at the temperature and for the period stated on the label.

**Storage**. Store protected from moisture, at a temperature not exceeding 30°.

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

## Clozapine




$C_{18}H_{19}ClN_4$ Mol. Wt. 326.8

Clozapine is 8-chloro-11-(4-methylpiperazin-1-yl)-5*H*-dibenzo[*b*,*e*][1,4]diazepine.

Clozapine contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{18}H_{19}ClN_4$, calculated on the dried basis.

**Category**. Antipsychotic.

**Description**. A yellow crystalline powder.

### Identification

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

B. In the Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to the principal spot obtained with the reference solution (a).

### Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 75 volumes of *chloroform* and 25 volumes of *methanol*.

*Test solution (a)*. Dissolve 0.1 g of the substance under examination in 10 ml of *chloroform*.

*Reference solution (a)*. A 0.01 per cent w/v solution of *clozapine RS* in *chloroform*.

*Reference solution (b)*. Dilute 3 ml of reference solution (a) to 10 ml with *chloroform*.

*Reference solution (c)*. Dilute 1 ml of reference solution (a) to 5 ml with *chloroform*.

*Reference solution (d)*. Dilute 1 ml of reference solution (a) to 10 ml with *chloroform*.

*Reference solution (e)*. Dilute 1 ml of reference solution (a) to 20 ml with *chloroform*.

Apply to the plate 20 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. Compare the intensities of any secondary spots obtained in the chromatogram obtained with the test solution with those of the principal spots in the chromatogram



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obtained with the reference solutions. No spot in the chromatogram obtained with the test solution with an $R_F$ value of about 0.82, corresponding to 1,4-bis(8-chloro-5*H*-dibenzo[*b,e*][1,4] diazepin-11-yl)piperazine; about 0.67, corresponding to 8-chloro-5,10-dihydro-11*H* –dibenzo [*b,e*][1,4]diazepin-11-one; or about 0.10, corresponding to 8-chloro-11-(1-piperazinyl)-5*H*-dibenzo[*b,e*][1,4]diazepine, is larger or more intense than that obtained with reference solution (c), reference solution (d), or reference solution (b), respectively; no other secondary spot from the chromatogram obtained with the test solution is larger or more intense than the principal spot obtained with reference solution (d) (0.1 per cent); and the sum of the intensities of all secondary spots obtained with the test solution corresponds to not more than 0.6 per cent.

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

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 4 hours.

**Assay**. Weigh accurately about 0.115 g, dissolve in 70 ml of *glacial acetic acid* and titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01634 g of $C_{18}H_{19}ClN_4$.

**Storage**. Store protected from light.

## Clozapine Tablets

Clozapine Tablets contains not less than 90.0 percent and not more than 110.0 percent of the stated amount of clozapine, $C_{18}H_{19}ClN_4$.

**Usual strengths**. 25 mg; 50 mg; 100 mg.

### Identification

A. In the Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to the principal spot obtained with the reference solution (a).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution (a).

### Tests

**Dissolution** (2.5.2).

Apparatus No. 2,

Medium. 900 ml of acetate buffer pH 4.0, prepared by dissolving 2 g of *sodium hydroxide* in 450 ml of *water*, adjusting the pH to 4.0 with *glacial acetic acid* and diluting with *water* to 1000 ml,

Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Dilute the filtrate if necessary, with the dissolution medium. Measure the absorbance of the resulting solution at the maximum at about 290 nm (2.4.7). Similarly measure the absorbance of a standard solution of known concentration of *clozapine RS* and calculate the content of $C_{18}H_{19}ClN_4$.

D. Not less than 80 per cent of the stated amount of $C_{18}H_{19}ClN_4$

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

*Mobile phase*. A mixture of 75 volumes of *chloroform* and 25 volumes of *methanol.*

*Solvent mixture*. 80 volumes of *chloroform* and 20 volumes of *methanol.*

*Test solution*. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 125 mg of Clozapine and dissolve in 25 ml of the solvent mixture.

*Reference solution (a)*. A 0.5 per cent w/v solution of *clozapine RS* in the solvent mixture.

*Reference solution (b)*. Dilute 1 ml of reference solution (a) to 200 ml with the solvent mixture.

*Reference solution (c)*. Dilute 1 ml of reference solution (a) to 250 ml with the solvent mixture.

*Reference solution (d)*. Dilute 3 ml of reference solution (a) to 1000 ml with the solvent mixture.

*Reference solution (e)*. Dilute 1 ml of reference solution (a) to 500 ml with the solvent mixture.

*Reference solution (f)*. Dilute 1 ml of reference solution (a) to 1000 ml with the solvent mixture.

Apply to the plate 20 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. Compare the intensities of any secondary spots observed in the chromatogram obtained with the test solution with those of the principal spots in the chromatogram obtained with the reference solutions : no secondary spot from the chromatogram obtained with the test solution is larger or more intense than the principal spot obtained with reference solution (b) (0.5 per cent); and the sum of the intensities of all secondary spots obtained with the test solution corresponds to not more than 2.0 per cent.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14)

*Test solution*. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 125 mg of Clozapine,



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dissolve in 640 ml of *methanol* and add sufficient *water* to produce 1000 ml.

*Reference solution (a)*. Weigh accurately about 12.5 mg of *clozapine RS* in 80 ml of *methanol* and dilute to 100.0 ml with *water*.

*Reference solution (b)*. Weigh accurately about 10 mg of Clozapine, add 5 ml of *0.1 M hydrochloric acid* and heat for 2 hours at 90°. Cool, add 15 ml of *water*, dilute with *methanol* to 100.0 ml and mix. To 10.0 ml of this solution add 10.0 ml of reference solution (a) and mix.

Chromatographic system
- a stainless steel column 25 cm x 4.0 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: 800 volumes of *methanol*, 200 volumes of *water* and 0.75 volumes of *triethylamine*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 257 nm,
- injection volume. 10 µl.

Inject the reference solution (a). The test is not valid unless the column efficiency is not less than 1500 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

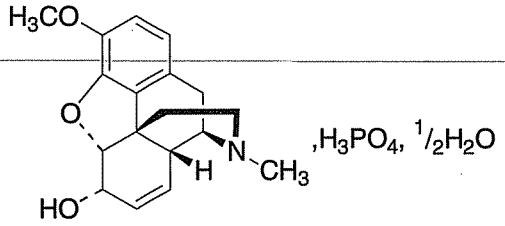
Inject reference solution (b). The resolution between the clozapine peak and any secondary peak is not less than 1.5.

Inject the test solution and reference solution (a).

Calculate the content of $C_{18}H_{19}ClN_4$ in the tablets.

# Codeine Phosphate

Codeine Phosphate Hemihydrate




$C_{18}H_{21}NO_3,H_3PO_4,½\ H_2O$ Mol. Wt. 406.4

Codeine Phosphate is *(5R,6S)*-7,8-didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6-ol dihydrogen phosphate hemihydrate, an alkaloid occurring in *Papaver somniferum*.

Codeine Phosphate contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{18}H_{21}NO_3,H_3PO_4$, calculated on the dried basis.

**Category**. Analgesic; antidiarrhoeal; cough suppressant.

**Dose**. 30 to 60 mg every 4 hours when necessary, to a maximum of 200 mg daily.

**Description**. Colourless crystals or a white, crystalline powder.

## Identification

*Test A may be omitted if tests B, C, D, E and F are carried out. Tests B, C, D and E may be omitted if tests A and F are carried out.*

A. Dissolve 0.2 g in 4 ml of *water*, add 2 ml of *2 M sodium hydroxide* and induce crystallisation, if necessary by scratching the wall of the tube with a glass rod and cooling in ice. The residue after washing with *water* and drying at 100° to 105° complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *codeine phosphate RS* treated in the same manner or with the reference spectrum of codeine.

B. To 25 ml of a 0.04 per cent w/v solution add 25 ml of *water* and 10 ml of *1 M sodium hydroxide* and dilute to 100 ml of *water*. When examined in the range 230 nm to 360 nm, the resulting solution shows an absorption maximum only at about 284 nm; absorbance at about 284 nm, about 0.38 (2.4.7).

C. On the surface of one drop of *nitric acid* place a little of the powder; a yellow but not red colour is produced (distinction from morphine).

D. Dissolve 0.1 g in 1 ml of *sulphuric acid* and 1 drop of *ferric chloride test solution* and warm gently; a bluish violet colour is produced. Add 1 drop of *dilute nitric acid*; the colour changes to red.

E. Gives the reaction of alkaloids (2.3.1).

F. Gives reaction A of phosphates (2.3.1).

## Tests

**Appearance of solution**. A 4.0 per cent w/v solution in *carbon dioxide-free water* prepared from *distilled water* is clear (2.4.1), and not more intensely coloured than reference solution YS6 (2.4.1).

**pH** (2.4.24). 4.2 to 5.0, determined in a 4.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). –98.0° to –102°, determined in a 2.0 per cent w/v solution.

**Foreign alkaloids**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 72 volumes of *ethanol*, 30 volumes of *cyclohexane* and 6 volumes of *strong ammonia solution*.

*Test solution*. Dissolve 0.5 g of the substance under examination in sufficient of a mixture of 4 volumes of *0.01 M hydrochloric acid* and 1 volume of ethanol to produce 10 ml.



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*Reference solution (a).* Dilute 1.5 ml of the test solution to 100 ml with the same solvent.

*Reference solution (b).* Dilute 1 ml of the test solution to 100 ml with the same solvent.

Apply separately to the plate 10 µl of each solution. After development, dry the plate in air and spray with *acidic potassium iodobismuthate solution.* Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot, with an Rf value higher than that of the principal spot, is more intense than the spot in the chromatogram obtained with reference solution (b).

Chlorides (2.3.12). 12.5 ml of a 2.0 per cent w/v solution in *distilled water* complies with the limit test for chlorides (0.1 per cent).

**Sulphates** (2.3.17). 7.5 ml of a 2.0 per cent w/v solution in *distilled water* complies with the limit test for sulphates (0.1 per cent).

**Morphine.** Dissolve 0.1 g in sufficient *0.1 M hydrochloric acid* to produce 5 ml, add 2 ml of a 1 per cent w/v solution of *sodium nitrite*, allow to stand for 15 minutes and add 3 ml of *6 M ammonia*. The resulting solution is not more intensely coloured than reference solution BS4 (2.4.1).

**Loss on drying** (2.4.19). Not more than 3.0 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay.** Weigh accurately about 0.4 g of the substance under examination and dissolve in a mixture of 10 ml of *anhydrous glacial acetic acid* and 20 ml of *dioxan*. Titrate with *0.1 M perchloric acid*, using a few drops of crystal violet solution as indicator. Carry out blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03974 g of $C_{18}H_{21}NO_3,H_3PO_4$.

**Storage.** Store protected from light.

# Codeine Syrup

Codeine Phosphate Syrup

Codeine Syrup is a 0.5 per cent w/v solution of Codeine Phosphate in a suitable flavoured vehicle.

Codeine Syrup contains not less than 0.48 per cent w/v and not more than 0.52 per cent w/v of codeine phosphate, $C_{18}H_{21}NO_3,H_3PO_4,½\ H_2O$.

## Identification

To 10 ml of the syrup add sufficient *dilute ammonia solution* until the solution is alkaline and extract with three quantities, each of 10 ml, of *chloroform*. Evaporate the combined chloroform extracts to dryness on a water-bath and dry the residue at 80°. The residue complies with the following tests.

A. Dissolve 0.2 g in 4 ml of *water*, add 2 ml of *2 M sodium hydroxide* and induce crystallisation, if necessary by scratching the wall of the tube with a glass rod and cooling in ice. The residue washed with *water* and dried at 100° to 105° complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *codeine phosphate RS* treated in the same manner or with the reference spectrum of codeine.

B. Dissolve 0.1 g in 1 ml of *sulphuric acid* and 1 drop of *ferric chloride test solution* and warm gently; a bluish violet colour is produced. Add 1 drop of *dilute nitric acid*; the colour changes to red.

## Tests

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

*Mobile phase.* A mixture of 72 volumes of *ethanol*, 30 volumes of *cyclohexane* and 6 volumes of *strong ammonia solution.*

*Test solution.* To 20 ml of the syrup add 20 ml of *water* and 2 ml of *strong ammonia solution* and extract with two quantities, each of 20 ml, of *chloroform.* Dry the combined extracts with *anhydrous sodium sulphate*, filter, evaporate the filtrate to dryness and dissolve the residue in 1 ml of *chloroform.*

*Reference solution (a).* Dilute 1.5 ml of the test solution to 100 ml with the same solvent.

*Reference solution (b).* Dilute 1 ml of the test solution to 100 ml with the same solvent.

Apply separately to the plate 10 µl of each solution. After development, dry the plate in air and spray with *acidic potassium iodobismuthate solution.* Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot, with an Rf value higher than that of the principal spot, is more intense than the spot in the chromatogram obtained with reference solution (b).

**Other tests.** Complies with the tests stated under Oral Liquids.

**Assay.** Weigh accurately about 10.0 g, add *dilute ammonia solution* until the solution is alkaline to *litmus paper* and extract with four quantities, each of 25 ml, of *chloroform.* Wash each extract successively with the same 10 ml of *water*; combine the chloroform extracts and evaporate to dryness on a water-bath. To the residue add 5 ml of *ethanol (95 per cent)* and again evaporate to dryness. Dissolve the residue in 5.0 ml of



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*0.05 M hydrochloric acid* and titrate the excess of acid with *0.05 M sodium hydroxide* using *methyl red solution* as indicator.

1 ml of *0.05 M hydrochloric acid* is equivalent to 0.02032 g of $C_{18}H_{21}NO_3,H_3PO_4,½\ H_2O$.

Determine the weight per ml of the syrup (2.4.29) and calculate the content of $C_{18}H_{21}NO_3,H_3PO_4,½\ H_2O$, weight in volume.

**Storage**. Store protected from light.

## Codeine Tablets

Codeine Phosphate Tablets

Codeine Phosphate Tablets contain Codeine Phosphate Hemihydrate.

Codeine Phosphate Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of codeine phosphate hemihydrate, $C_{18}H_{21}NO_3,H_3PO_4,½H_2O$.

**Usual strength**. 10 mg.

### Identification

Macerate a quantity of the powdered tablets containing 50 mg of Codeine Phosphate with 5 ml of *1 M sulphuric acid* and 15 ml of *water*. Filter, make alkaline with *5 M ammonia*, extract with successive quantities of *chloroform* and evaporate the combined chloroform extracts to dryness on a water-bath. The residue complies with the following tests.

A. Place a few mg of residue on the surface of a drop of *nitric acid*. A yellow but no red colour is produced (distinction from morphine).

B. Dissolve 0.1 g in 1 ml of *sulphuric acid* and 1 drop of *ferric chloride test solution* and warm gently; a bluish violet colour is produced. Add 1 drop of *dilute nitric acid*; the colour changes to red.

C. Gives reaction A of phosphates (2.3.1).

### Tests

**Foreign alkaloids**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 72 volumes of *ethanol*, 30 volumes of *cyclohexane* and 6 volumes of *strong ammonia solution*.

*Test solution*. Shake a quantity of the powdered tablets containing 0.25 g of Codeine Phosphate with 10 ml of a mixture of 4 volumes of *0.01 M hydrochloric acid* and 1 volume of *ethanol* for 15 minutes and filter.

*Reference solution (a)*. Dilute 1.5 ml of the test solution to 100 ml with *0.01 M hydrochloric acid*.

*Reference solution (b)*. Dilute 1 ml of the test solution to 100 ml with *0.01 M hydrochloric acid*.

Apply separately to the plate 20 µl of each solution. After development, dry the plate in air and spray with *acidic potassium iodobismuthate solution*. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) (1.5 per cent) and not more than one such spot, with an $R_f$ value higher than that of the principal spot, is more intense than the spot in the chromatogram obtained with reference solution (b) (1.0 per cent).

**Other tests**. Complies with the tests stated under Tablets.

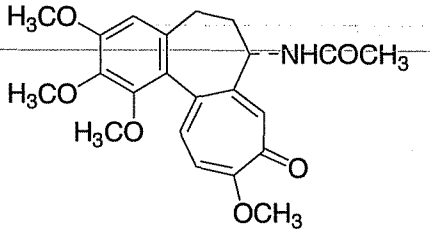
**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of powder containing 0.3 g of Codine Phosphate, dissolve in 20 ml of *0.25 M sulphuric acid*, filter and wash the residue with *0.25 M sulphuric acid*. Make alkaline with *5 M ammonia* and extract with successive quantities of *chloroform*. Wash each chloroform solution with 10 ml of *water* and evaporate the chloroform. To the residue add 5 ml of *ethanol (95 per cent)* and again evaporate to dryness. Dissolve the residue in 1 ml of neutralised *ethanol (95 per cent)*, add 10.0 ml of *0.1 M hydrochloric acid* and 10 ml of *water*. Titrate with *0.1 M sodium hydroxide* using *methyl red solution* as indicator.

1 ml of *0.1 M hydrochloric acid* is equivalent to 0.04064 g of $C_{18}H_{21}NO_3,H_3PO_4,½H_2O$.

**Storage**. Store protected from light.

**Labelling**. When the active ingredient is codeine phosphate hemihydrate, the quantity is stated in terms of the equivalent amount of codeine phosphate.

## Colchicine




$C_{22}H_{25}NO_6$ Mol. Wt. 399.4

Colchicine is (*S*)-*N*-(5,6,7,9-tetrahydro-1,2,3,10-tetramethoxy-9-oxobenzo[*a*]heptalen-7-yl)acetamide, an alkaloid which occurs in the corm and seeds of various species of *Colchicum*.

Colchicine contains not less than 97.0 per cent and not more than 103.0 per cent of $C_{22}H_{25}NO_6$, calculated on the anhydrous and solvent-free basis.



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**Category**. Gout suppressant.

**Dose**. Initial dose, 1 mg; subsequent doses, 500 µg every two hours.

**Description**. Pale yellow crystals, amorphous scales or a powder.

### Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

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

B. When examined in the range 230 nm to 400 nm, a 0.001 per cent w/v solution in *ethanol (95 per cent)* shows absorption maxima, at about 243 nm and 350 nm; absorbance at about 243 nm, about 0.73 and at about 350 nm, about 0.42 (2.4.7).

C. Dissolve 50 mg in 1.5 ml of *water;* a yellow solution is produced the colour of which is intensified on adding mineral acids.

D. Mix 1 mg with 0.2 ml of *sulphuric acid* in a white dish; a yellow colour is produced which on the addition of 0.05 ml of *nitric acid* changes to greenish-blue and then rapidly becomes reddish and finally almost colourless. On addition of an excess of *5 M sodium hydroxide* the colour changes to red.

### Tests

**Specific optical rotation** (2.4.22). -235° to -250°, determined at 20° in a 0.5 per cent w/v solution in *ethanol (95 per cent).*

**Colchiceine**. To 5 ml of a 1.0 per cent w/v solution add 0.1 ml of a 10.5 per cent w/v solution of *ferric chloride*. Any colour produced is not more intense than that obtained by mixing 2.0 ml of FCS with 1.0 ml of CCS and 2.0 ml of CSS (2.4.1).

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254.*

*Mobile phase*. A mixture of 50 volumes of *acetone*, 25 volumes of *1,2-dichloroethane* and 1 volume of *strong ammonia solution.*

*Prepare the following solutions immediately before use.*

*Test solution*. Dissolve 0.1 g of the substance under examination in 10 ml of *chloroform.*

*Reference solution (a)*. Dilute 1 ml of the test solution to 50 ml with the same solvent.

*Reference solution (b).* Dilute 1 volume of reference solution (a) with an equal volume of *chloroform.*

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in chromatogram obtained with reference solution (b).

**Solvent**. Determine by gas chromatography (2.4.13).

*Test solution.* A 1.0 per cent w/v solution of the substance under examination and the same concentration of the internal standard as in the reference solution.

*Reference solution.* A solution in *water* containing 0.1 per cent v/v of *ethanol-free chloroform*, 0.1 per cent v/v of *ethyl acetate* and either 0.1 per cent v/v (for the determination of ethyl acetate) or 0.02 per cent v/v (for the determination of chloroform) of *ethanol* (internal standard).

Chromatographic system
- a glass or stainless steel column 1.5 m x 4 mm, packed with white diatomaceous earth (100 to 120 mesh) coated with 10 per cent w/w of *polyethylene glycol 1000*,
- temperature:
  column.75°,
  inlet port. 130°,
  detector. 150°,
- a flame ionisation detector at 150°,
- flow rate. 30 ml per minute of the carrier gas.

Calculate the percentage w/w of ethyl acetate or chloroform, taking 0.901 g or 1.477 g, respectively, as the weight per ml at 20° (2.4.29).

The sum of the contents of chloroform or ethyl acetate and the percentage of water is not more than 10 per cent.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 2.0 per cent, determined on 0.5 g.

**Assay**. Weigh accurately about 50 mg and dissolve in a mixture of 10 ml of *acetic anhydride* and 20 ml of *toluene.* Titrate with *0.02 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.02 M perchloric acid* is equivalent to 0.007988 g of $C_{22}H_{25}NO_6$.

**Storage**. Store protected from light.

## Colchicine Tablets

Colchicine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of colchicine, $C_{22}H_{25}NO_6$.

**Usual strengths**. 250 µg; 500 µg.



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### Identification

A.To a quantity of the powdered tablets containing 10 mg of Colchicine add 20 ml of *water* and mix well. Filter into a separating funnel and extract with 30 ml of *chloroform*. Evaporate the *chloroform* extract to dryness using moderate heat. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spedtrum with that obtained with *colchicine RS* or with the reference spectrum of colchicine.

B. To a quantity of the powdered tablets containing 1 mg of Colchicine add 0.2 ml of *sulphuric acid* and mix; a yellow colour is produced. On adding a drop of *nitric acid* the colour changes to greenish-blue, then reddish and finally almost colourless. On the addition of an excess of *5 M sodium hydroxide* the colour changes to red.

### Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254*.

*Mobile phase*. A mixture of 50 volumes of *acetone*, 25 volumes of *1,2-dichloroethane* and 1 volume of *strong ammonia solution*.

*Prepare the following solutions immediately before use.*

*Test solution*. Extract a quantity of the powdered tablets containing 5 mg of Colchicine with 5 ml of *chloroform*, filter and evaporate the filtrate to dryness in a current of air. Dissolve the residue as completely as possible in 0.1 ml of *ethanol (95 per cent)*, centrifuge and use the supernatant liquid.

*Reference solution (a)*. Dilute 1 volume of the test solution to 20 volumes with *ethanol (95 per cent)*.

*Reference solution (b)*. Dilute 1 volume of reference solution (a) with an equal volume of *chloroform*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in chromatogram obtained with reference solution (b).

**Uniformity of content**. Comply with the test stated under Tablets.

*Protect the solutions from light throughout the test.*

Crush one tablet and transfer to a centrifuge tube with the aid of 10 ml of *ethanol*. Shake for 30 minutes, centrifuge and decant the supernatant liquid. Wash the residue with small quantities of *ethanol*, combine the extract and washings and add sufficient *ethanol* to produce a solution containing 0.001 per cent w/v of Colchicine. Measure the absorbance of the resulting solution at the maximum at about 350 nm (2.4.7). Calculate the content of $C_{22}H_{25}NO_6$ in the tablet taking 425 as the specific absorbance at 350 nm.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. *Protect the solutions from light throughout the test.*

Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.5 mg of Colchicine, add 10 ml of *ethanol* and shake for 30 minutes. Centrifuge and decant the supernatant liquid. Wash the residue with small quantities of *ethanol* and mix the extract and washings. Add sufficient *ethanol* to produce 50.0 ml and mix well. Measure the absorbance of the resulting solution at the maximum at about 350 nm (2.4.7). Calculate the content of $C_{22}H_{25}NO_6$ taking 425 as the specific absorbance at 350 nm.

**Storage**. Store protected from light.

## Colchicine and Probenecid Tablets

Colchicine and Probenecid Tablets contain not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of colchicine, $C_{22}H_{25}NO_6$, and not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of probenecid, $C_{13}H_{19}NO_4S$.

**Category**. Uricosuric agent.

**Dose**. Colchicine, 1 mg and Probenecid, 250 mg twice daily after food.

**Usual strength**. Colchicine, 1 mg and Probenecid, 250 mg.

### Identification

A.Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 100 volumes of *methanol* and 1.5 volumes of *strong ammonia solution*.

*Test solution (a)*. Shake a quantity of the powdered tablets containing 1 mg of Colchicine with 15 ml of *water*, mix, filter, extract the filtrate with 25 ml of *chloroform* and evaporate the chloroform extract to a volume of about 1 ml.

*Test solution (b)*. Shake a quantity of the powdered tablets containing 10 mg of Probenecid with 10 ml of *chloroform*, allow to settle and decant the clear supernatant liquid.

*Reference solution (a)*. A solution containing 0.1 per cent w/v of *colchicine RS*.

*Reference solution (b)*. A solution containing 0.1 per cent w/v of *probenecid RS*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm.



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The principal spot in the chromatogram obtained with test solution (a) corresponds to that in the chromatogram obtained with reference solution (a). Similarly, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

**Uniformity of content**. *For colchicine* - Comply with the test stated under Tablets using the method described under Assay.

**Other tests**. Comply with the tests stated under Tablets.

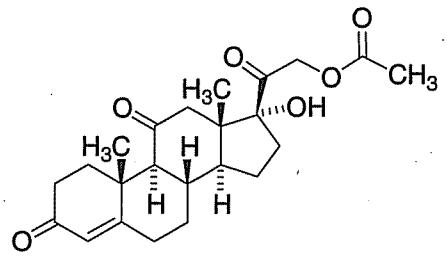
**Assay**. *For colchicine — Carry out the determination without delay, under subdued light, using low actinic glassware.*

Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 1 mg of Colchicine, shake with 75 ml of *ethanolic sodium carbonate* for 30 minutes, add sufficient of *ethanolic sodium carbonate* to produce 100.0 ml and filter. Measure the absorbance of the resulting solution at the maximum at about 350 nm (2.4.7). Calculate the content of $C_{22}H_{25}NO_6$ taking 425 as the specific absorbance at 350 nm.

*For probenecid* — Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.25 g of Probenecid, shake with 100 ml of *0.1 M sodium hydroxide* for 10 minutes, add sufficient of *0.1 M sodium hydroxide* to produce 250.0 ml, mix and filter. Dilute 10.0 ml of the filtrate to 100.0 ml with *0.1 M sodium hydroxide*. Dilute further 10.0 ml to 50.0 ml with the same solvent and measure the absorbance of the resulting solution at the maximum at about 248 nm (2.4.7). Calculate the content of $C_{13}H_{19}NO_4S$ taking 332 as the specific absorbance at 248 nm.

**Storage**. Store protected from light.

# Cortisone Acetate



$C_{23}H_{30}O_6$ Mol. Wt 402.5

Cortisone Acetate is 17α,21-dihydroxypregn-4-ene-3,11,20-trione 21-acetate.

Cortisone Acetate contains not less than 96.0 per cent and not more than 104.0 per cent of $C_{23}H_{30}O_6$, calculated on the dried basis.

**Category**. Adrenocortical steroid.

**Dose**. Orally, 25 to 37.5 mg daily, in divided doses; by intramuscular injection, 50 to 400 mg daily, in single or divided doses.

**Description**. A white or almost white, crystalline powder; odourless.

## Identification

*Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cortisone acetate RS* or with the reference spectrum of cortisone acetate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Solvent mixture*. A mixture of 90 volumes of *acetone* and 10 volumes of *formamide*.

*Mobile phase*. *Chloroform*.

*Test solution*. Dissolve 25 mg of the substance under examination in 10 ml of the same solvent mixture.

*Reference solution (a)*. Dissolve 25 mg of *cortisone acetate RS* in 10 ml of the same solvent mixture.

*Reference solution (b)*. Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v)*. Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

C. 10 mg gives the reactions of acetyl groups (2.3.1).

## Tests

**Specific optical rotation** (2.4.22). +211° to +220°, determined in a 1.0 per cent w/v solution in *dioxan*.



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**Light absorption** (2.4.7). Dissolve 50 mg in sufficient *ethanol (95 per cent)* to produce 100 ml and dilute 2 ml to 100 ml with the same solvent. Absorbance of the resulting solution at the maximum at about 240 nm, 0.375 to 0.405.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Prepare the following solutions immediately before use.*

*Test solution.* Dissolve 25.0 mg of the substance under examination in *acetonitrile* and dilute to 10.0 ml with the same solvent.

*Reference solution (a).* Dissolve 2 mg of *cortisone acetate RS* and 2 mg of *hydrocortisone acetate RS* in *acetonitrile* and dilute to 100.0 ml with the same solvent.

*Reference solution (b).* Dilute 1.0 ml of the test solution to 100.0 ml with *acetonitrile*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 400 ml of *acetonitrile and* 550 ml of *water,* allowed to equilibrate sufficient *water* added to produce 1000 ml and mixed,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Equilibrate the column with the mobile phase for 30 minutes.

Inject reference solution (b). Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram is at least 50 per cent of the full scale of the recorder.

Inject reference solution (a). The retention times are: hydrocortisone acetate, about 10 minutes and cortisone acetate, about 12 minutes. The test is not valid unless the resolution between the peaks due to hydrocortisone acetate and cortisone acetate is at least 4.2.

Inject the test solution and reference solution (b). Continue the chromatography for twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak other than the principal peak, is not greater than half the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent); the sum of the areas of all the peaks other than the principal peak, is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 0.5 g by drying in an oven at 105° for 3 hours.

**Assay**. Dissolve 0.1 g in *ethanol* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of the solution to 100.0 ml with *ethanol*. Measure the absorbance at the maximum at about 237 nm (2.4.7).

Calculate the content of $C_{23}H_{30}O_6$ taking 395 as the specific absorbance at 237 nm.

**Storage**. Store protected from light.

# Cortisone Injection

Cortisone Acetate Injection

Cortisone Injection is a sterile suspension of a very fine powder of Cortisone Acetate in Sodium Chloride Injection containing suitable dispersing agents.

Cortisone Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cortisone acetate, $C_{23}H_{30}O_6$.

**Usual strength**. 25 mg per ml.

**Description**. A white suspension, which settles on standing, but readily disperses on shaking.

## Identification

Extract a volume of the injection containing 0.1 g of Cortisone Acetate with 6 ml of *chloroform,* filter and evaporate the *chloroform.* The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cortisone acetate RS* or with the reference spectrum of cortisone acetate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

*Solvent mixture.* A mixture of 90 volumes of *acetone* and 10 volumes of *formamide.*

*Mobile phase. Chloroform.*

*Test solution.* Dissolve 25 mg of the substance under examination in 10 ml of the solvent mixture.

*Reference solution (a).* Dissolve 25 mg of *cortisone acetate RS* in 10 ml of the solvent mixture.

*Reference solution (b).* Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.



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Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v)*. Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

C. 10 mg gives the reactions of acetyl groups (2.3.1).

### Tests

**pH** (2.4.24). 5.0 to 7.2.

**Related substances.** Determine by liquid chromatography (2.4.14).

*Test solution.* Mix a quantity of the suspension containing 25 mg of Cortisone Acetate with 15 ml of *isopropyl alcohol*, evaporate to dryness on a steam bath. To the residue add 10 ml of the mobile phase, shake, mix with the aid of ultrasound and filter (such as Whatman GF/C filter).

*Reference solution (a).* Dilute 1 volume of the test solution to 100 volumes with the mobile phase.

*Reference solution (b).* A solution containing 0.002 per cent w/v each *of cortisone acetate RS* and *hydrocortisone acetate RS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with packed with octadecylsilane bonded to porous silica (5 µm) (such as Hypersil ODS),
- mobile phase: a mixture of 400 ml *of acetonitrile* and 550 ml of *water*, allowed to equilibrate and sufficient *water* added to produce 1000 ml,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (b). The retention times are: hydrocortisone acetate, about 10 minutes and cortisone acetate, about 12 minutes. The test is not valid unless the resolution between the peaks due to hydrocortisone acetate and cortisone acetate is at least 4.2.

Inject the test solution and reference solution (a). Continue the chromatography for twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not greater than half the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent); the sum of the areas of all the secondary peaks is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Add 50 ml of *methanol* to a quantity of the injection containing about 10 mg of Cortisone Acetate, shake, mix with the aid of ultrasound for 2 minutes, dilute to 100.0 ml with *water*, shake, centrifuge and use the supernatant liquid.

*Reference solution.* Dilute 50 ml of a solution in *methanol* containing 0.02 per cent w/v each of *cortisone acetate RS* and *prednisolone RS* to 100.0 ml with *water.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Hypersil ODS),
- mobile phase: *methanol (60 per cent),*
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to cortisone acetate and prednisolone in the chromatogram obtained is at least 5.0.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_{23}H_{30}O_6$ in the injection.

**Storage**. Store protected from light in single dose or multiple dose containers at a temperature not exceeding 30°. It should not be allowed to freeze.

**Labelling**. The label states (1) the name(s) of the dispersing agent(s) added; (2) that it is not meant to be given by intravenous injection; (3) that the container should be gently shaken before a dose is withdrawn.

## Cortisone Tablets

Cortisone Acetate Tablets

Cortisone Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cortisone acetate, $C_{23}H_{30}O_6$.

**Usual strengths**. 5 mg; 25 mg.

### Identification

Extract a quantity of the powdered tablets containing 0.1 g of Cortisone Acetate with 5 ml of *chloroform*, filter and evaporate the *chloroform*. The residue complies with the following tests.



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A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cortisone acetate RS* or with the reference spectrum of cortisone acetate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Solvent mixture*. A mixture of 90 volumes of *acetone* and 10 volumes of *dimethylformamide*.

*Mobile phase*. *Chloroform*.

*Test solution*. Dissolve 25 mg of the substance under examination in 10 ml of the same solvent mixture.

*Reference solution (a)*. Dissolve 25 mg of *cortisone acetate RS* in 10 ml of the same solvent mixture.

*Reference solution (b)*. Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v)*. Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

C. 10 mg gives the reactions of acetyl groups (2.3.1).

## Tests

**Related substances.** Determine by liquid chromatography (2.4.14).

*Test solution*. Mix a quantity of the powdered tablets containing 25 mg of Cortisone Acetate with 10 ml of the mobile phase, place in an ultrasonic bath for 10 minutes and filter (such as Whatman GF/C filter).

*Reference solution (a)*. Dilute 1 volume of the test solution to 100 volumes with the mobile phase.

*Reference solution (b)*. A solution containing 0.002 per cent w/v each *of cortisone acetate RS* and *hydrocortisone acetate RS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with packed with octadecylsilane bonded to porous silica (5 µm) (such as Hypersil ODS),
- mobile phase: a mixture of 400 ml *of acetonitrile* and 550 ml of *water*, allowed to equilibrate and sufficient *water* added to produce 1000 ml,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (b). The retention times are: hydrocortisone acetate, about 10 minutes and cortisone acetate, about 12 minutes. The test is not valid unless the resolution between the peaks due to hydrocortisone acetate and cortisone acetate is at least 4.2.

Inject the test solution and reference solution (a). Continue the chromatography for twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not greater than half the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent); the sum of the areas of all the secondary peaks is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

**Uniformity of content**. *For tablets containing 10 mg or less.*

Comply with the test stated under Tablets.

Powder one tablet, add 50 ml of *ethanol,* shake for 30 minutes and add sufficient *ethanol* to produce 100.0 ml. Centrifuge and dilute a suitable volume of the supernatant liquid containing 0.5 mg of Cortisone Acetate to 50.0 ml with *ethanol.* Measure the absorbance of the resulting solution at the maximum at about 240 nm (2.4.7). Calculate the content of $C_{23}H_{30}O_6$ taking 390 as the specific absorbance at 240 nm.

**Dissolution** (2.5.2).

Apparatus No. 1,

Medium. 900 ml of a mixture of a *1 per cent v/v solution of hydrochloric acid* and 30 volumes of *2-propanol,*

Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter with an average pore diameter not greater than 1.0 µm. Measure the absorbance of the filtrate, suitably diluted if necessary with the medium, at the maximum at about 242 nm (2.4.7). Calculate the content of $C_{23}H_{30}O_6$ taking 399 as the specific absorbance at 242 nm.

D. Not less than 75 per cent of the stated amount of $C_{23}H_{30}O_6$.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh and powder 20 tablets. Add 50 ml of *methanol* to a quantity of the powder containing about 10 mg



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of Cortisone Acetate, shake, mix with the aid of ultrasound for 2 minutes, dilute to 100.0ml with *water*, shake, centrifuge and use the supernatant liquid.

*Reference solution*. Dilute 50 ml of a solution in *methanol* containing 0.02 per cent w/v each of *cortisone acetate RS* and prednisolone to 100.0 ml with *water*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilylsilane bonded to porous silica (5 µm) (such as Hypersil ODS),
- mobile phase: *methanol (60 per cent)*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to cortisone acetate and prednisolone in the chromatogram obtained is at least 5.0.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_{23}H_{30}O_6$ in the tablets.

**Storage**. Store protected from light.

# Absorbent Cotton

Absorbent Cotton Wool

Absorbent Cotton consists of the new fibres or good quality new combers obtained from the seed coat of various species of the genus Gossypium Linn., cleaned, purified, bleached and carded. It does not contain any compensatory colouring matter.

**Category**. Surgical dressing.

**Description**. White, well-carded fibres of average staple length not less than 10 mm, containing not more than traces of leaf residue, seed coat and other impurities. It offers appreciable resistance when pulled and does not shed a significant quantity of dust when shaken gently; practically odourless.

## Identification

A. When examined under a microscope, each fibre is seen to consist of a single cell, up to about 4 cm long and up to 40 µm wide, in the form of a flattened tube with thick and rounded walls and often twisted.

B. Treat with *iodinated zinc chloride solution;* the fibres become violet.

C. To 0.1 g add 10 ml of *zinc chloride solution,* heat to 40° and allowed to stand for 2 ½ hours, shaking occasionally; the fibres do not dissolve.

## Tests

To 15.0 g add 150 ml of *water,* macerate for 2 hours in a closed vessel, decant the liquid, carefully squeezing out the residual liquid with a glass rod and mix. Reserve 10 ml for the test for surface-active substances and filter the remainder (solution S).

**Acidity or alkalinity**.To 25 ml of solution S add 0.1 ml of *dilute phenolphthalein solution;* to another 25 ml add *0.05* ml of *methyl orange solution.* Neither solution shows a pink colour.

**Surface-active substances**. Into a 25-ml graduated, ground-glass stoppered cylinder with external diameter of 18 to 22 mm, previously rinsed with *sulphuric acid* and then with *water,* add the 10 ml portion of solution S, shake vigorously 30 times in 10 seconds, allow to stand for 1 minute and shake again 30 times. After 5 minutes, the height of the froth does not exceed 2 mm above the surface of the liquid.

**Absorbency**

*Apparatus*. A dry, cylindrical wire basket, 80 mm high and 50 mm in diameter, fabricated from wire of diameter 0.4 mm and having a mesh aperture of 15 to 20 mm; the basket weighs 2.4 to 3.0 g.

*Sinking time*. Not more than 10 seconds, determined by the following method.

Weigh the basket to the nearest 10 mg. Take five samples, each of approximately 1 g, from different places in the material being examined, pack loosely in the basket and weigh the packed basket to the nearest 10 mg. Hold the basket with its long axis in the horizontal position and drop it from a height of about 10 mm into *water* at 25° contained in a beaker at least 12 cm in diameter and filled to a depth of 10 cm. Measure with a stopwatch the time taken by the basket to sink below the surface of the water. Repeat the procedure on two further samples and calculate the average value.

*Water-holding capacity*. Not less than 23.0 g per g, determined by the following method.

After the sinking time has been recorded in test A, remove the basket from the water, allow it to drain for exactly 30 seconds with its long axis in the horizontal position, transfer it to a tared beaker and weigh to the nearest 10 mg. Calculate the weight of water retained by the sample. Repeat the procedure on two further samples and calculate the average value.

**Foreign fibres**. When examined under a microscope, it is seen to consist exclusively of typical cotton fibres, except that occasionally a few isolated foreign fibres may be seen.

**Fluorescence**. Examine a layer about 5 mm in thickness in ultraviolet light at 365 nm. It shows only a slight, brownish-violet fluorescence and a few yellow particles. Not more than a few isolated fibres show an intense blue fluorescence.



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**Colouring matter**. Slowly extract 10 g in a narrow percolator with *ethanol (95 per cent)* until 50 ml of extract is obtained. The extract is not more intensely coloured than reference solution YS5 or GYS6 (2.4.1) or a solution prepared in the following manner. To 3.0 ml of CSS add 7.0 ml of a solution of *hydrochloric acid* containing 1.0 per cent v/v of *hydrochloric acid* and dilute 0.5 ml of the resulting solution to 10 ml with the same solution of *hydrochloric acid.*

**Ether-soluble substances**. Not more than 0.5 per cent determined by the following method. Extract 5 g with *ether* in a continuous extraction apparatus (2.1.8), for 4 hours in such a way that the rate is at least four extractions per hour. Evaporate the ether and dry the residue to constant weight at 105°.

**Water-soluble substances**. Not more than 0.5 per cent, determined by the following method. Boil 5 g with 500 ml of *water* for 30 minutes, stirring frequently and replacing the water lost by evaporation. Decant the liquid into a beaker, squeeze the residual liquid from the material carefully with a glass rod, mix the liquids and filter the extract. Evaporate 400 ml of the filtrate and dry the residue to constant weight at 105°.

**Neps**. A thin layer approximately equivalent to 0.5 g for an area of 450 sq. cm. spread uniformly between two glass plates, and viewed by the naked eye under transmitted light, does not show more neps than about an average of 250 for three tests.

**Sulphated ash** (2.3.18). Not more than 0.5 per cent, determined on 5.0 g.

**Loss on drying** (2.4.19). Not more than 8.0 per cent, determined on 5.0 g by drying in an oven at 105°.

**Storage**. Store in a dust-proof package, in a dry place.

# Cresol

Cresol is a mixture of cresols and other phenols obtained from coal tar.

**Category**. Disinfectant; pharmaceutical aid (antimicrobial preservative).

**Description**. An almost colourless to pale brownish-yellow liquid, becoming darker on keeping or on exposure to light; odour, resembling that of phenol but more tarry.

## Identification

To 0.5 ml add 300 ml of *water*, shake and filter. Divide the filtrate into two parts. To one part add *ferric chloride test solution*; a transient bluish colour is produced. To the other part add *bromine solution*; a pale yellow flocculent precipitate is produced.

## Tests

**Distillation range** (2.4.8). Not more than 2.0 per cent v/v distils below 188° and not less than 80.0 per cent v/v between 195° and 205°.

**Acidity**. A 2 per cent w/v solution is neutral to *bromocresol purple solution.*

**Weight per ml** (2.4.29). 1.029 g to 1.044 g.

**Hydrocarbons and volatile bases**. Place 50 ml in a 500-ml round-bottomed flask, add about 83 ml of a 27 per cent w/v solution of *sodium hydroxide* and 100 ml of *water* and mix thoroughly. Connect the flask to a splash-bulb and air condenser about 60 cm long, with the end of the air-condenser fitting closely into the neck of a 250-ml pear-shaped separator and passing well into the separator, which has a cylindrical graduated portion above the stopcock. Fill the graduated portion of the separator with *water.* Distil rapidly until 75 ml of distillate has been collected, cooling the separator in running *water*, if necessary. Allow the separator to stand in a vertical position until separation is complete and draw off the aqueous liquid into a titration flask.

*Hydrocarbons* — Allow the separator to stand for a short time, measure the volume of hydrocarbon oil in the graduated portion and warm if necessary in order to keep the oil in the liquid state; subtract the volume of volatile bases in the hydrocarbon oil, as determined in the following test; not more than 0.5 per cent v/v of hydrocarbon oil is present.

*Volatile bases* — To the aqueous portion of the distillate obtained in the preceding test, add any aqueous liquid still remaining in the separator and neutralise it if necessary with *0.1 M hydrochloric acid*, using *phenolphthalein solution* as indicator. Titrate with *1 M hydrochloric acid* using *methyl orange solution* as indicator. Wash the oil from the separator into the titration flask with water and again titrate with *1 M hydrochloric acid*. From the volume of additional *1 M hydrochloric acid* calculate the proportion of volatile bases in the hydrocarbon oil. From the total volume of *1 M hydrochloric acid* used in both titrations calculate the proportion of volatile bases in cresol.

1 ml of *1 M hydrochloric acid* is equivalent to 0.08 ml of volatile bases; not more than 0.15 per cent v/v of volatile bases, calculated as pyridine, are present.

**Sulphur compounds**. Place about 20 ml in a small conical flask. Moisten a piece of filter paper with a 10 per cent w/v solution of *lead acetate* and fix it on the mouth of the flask; heat the flask on a water-bath for 5 minutes; the filter paper shows not more than a light yellow colour.

**Non-volatile matter**. Not more than 0.1 per cent w/v when evaporated on a water-bath and dried to constant weight at 105°.



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**Storage**. Store protected from light.

## Cresol with Soap Solution

Lysol

Cresol with Soap Solution is prepared by the saponification of a mixture of Cresol with vegetable oils such as cotton seed, linseed, soyabean or similar oils but excluding coconut and palm kernel oils. Alternatively, the mixed fatty acids derived from these oils may be used.

Cresol with Soap Solution contains not less than 47.0 per cent v/v and not more than 53.0 per cent v/v of Cresol.

**Category**. Disinfectant.

**Description**. An amber-coloured to reddish-brown liquid; odour, that of cresol; soapy to touch.

### Tests

**Appearance of solution**. 5 ml mixed with 95 ml of *water* forms a clear solution without producing any opalescence on standing for 3 hours.

**Alkalinity**. Dilute 5 ml with 50 ml of *ethanol (95 per cent)* neutralised to phenol red solution and titrate with *1 M sulphuric acid*, using *phenol red solution* as indicator; not more than 0.6 ml is required.

**Hydrocarbons and volatile bases**. Distil 120 ml until all the water and 50 ml of cresol have been collected. Place the cresol thus recovered in a 500-ml round-bottomed flask, add about 83 ml of a 27 per cent w/v solution of *sodium hydroxide* and 100 ml of *water* and mix thoroughly. Connect the flask to a splash-bulb and air condenser about 60 cm long, with the end of the air-condenser fitting closely into the neck of a 250-ml pear-shaped separator and passing well into the separator, which has a cylindrical graduated portion above the stopcock. Fill the graduated portion of the separator with *water*. Distil rapidly until 75 ml of distillate has been collected, cooling the separator in running *water*, if necessary. Allow the separator to stand in a vertical position until separation is complete and draw off the aqueous liquid into a titration flask.

*Hydrocarbons* — Allow the separator to stand for a short time, measure the volume of hydrocarbon oil in the graduated portion and warm if necessary in order to keep the oil in the liquid state; subtract the volume of volatile bases in the hydrocarbon oil, as determined in the following test; not more than 0.5 per cent v/v of hydrocarbon oil is present.

*Volatile bases* —To the aqueous portion of the distillate obtained in the preceding test, add any aqueous liquid still remaining in the separator and neutralise it if necessary with *0.1 M hydrochloric acid*, using *phenolphthalein solution* as indicator. Titrate with *1 M hydrochloric acid* using *methyl orange solution* as indicator. Wash the oil from the separator into the titration flask with water and again titrate with *1 M hydrochloric acid*. From the volume of additional *1 M hydrochloric acid* calculate the proportion of volatile bases in the hydrocarbon oil. From the total volume of *1 M hydrochloric acid* used in both titrations calculate the proportion of volatile bases in cresol.

1 ml of *1 M hydrochloric acid* is equivalent to 0.08 ml of volatile bases; not more than 0.15 per cent v/v of volatile bases, calculated as pyridine, are present.

**Sulphur compounds**. Complies with the test for Sulphur compounds described under Cresol.

**Assay**. To 50 ml, accurately measured, add 150 ml of *kerosene*, mix and add little powdered *pumice stone* and 3 g of *sodium bicarbonate*. Distil into a separator, the rate of distillation being not more than 2 drops per second until the kerosene and cresol have completely distilled. This is indicated by the distillate being yellow in colour. Stop the distillation, add 50 ml of *kerosene* and collect a further 50 ml of the distillate. Discard the lower aqueous layer in the separator, dry the remainder with *anhydrous calcium chloride* and shake with 10 ml of *sulphuric acid (50 per cent w/w)*. Set aside for 2 hours, reject the acid layer and to the kerosene layer add 40 ml of *sodium hydroxide solution* and shake for 5 minutes. Transfer the alkaline layer to a 100-ml volumetric flask and extract the kerosene layer with 20 ml of *sodium hydroxide solution* adding the alkaline layer to that in the 100-ml volumetric flask. Add *sodium hydroxide solution* from a burette to make the volume in the flask to 100 ml. The difference between the burette reading and 40.5 is equal to the volume of cresol in 50 ml of the sample.

**Storage**. Store protected from light.

## Croscarmellose Sodium

Croscarmellose sodium (cross-linked sodium carboxymethylcellulose) is the sodium salt of a cross-linked, partly *O*-carboxymethylated cellulose.

**Category**. Excipient.

**Description**. A white or greyish-white powder.

### Identification

A. Shake 1 g with 100 ml of 0.00001 per cent w/v solution of *methylene blue* and allow to settle. The substance under examination absorbs the methylene blue and settles as a blue, fibrous mass.

B. Shake 1 g with 50 ml of *water*. Transfer 1 ml of the mixture to a test-tube, add 1 ml of *water* and 0.05 ml of a freshly prepared



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4.0 per cent w/v solution of α-*naphthol* in *methanol*. Incline the test-tube and add carefully 2 ml of *sulphuric acid* down the side so that it forms a lower layer. A reddish-violet colour develops at the interface.

C. The solution prepared from the sulphated ash in the test for Heavy metals (see Tests) gives reaction (a) of sodium salts (2.3.1)

## Tests

**pH** (2.4.24). 5.0 to 7.0, determined on 1.0 per cent w/v solution in *carbon dioxide-free water*.

**Degree of substitution**. Take 1.0 g in 500 ml conical flask, add 300 ml of a 10 per cent w/v solution of *sodium chloride*, 25.0 ml of *0.1 M sodium hydroxide*, stopper the flask and allow to stand for 5 minutes, shaking occasionally. Add 0.05 ml of *m-cresol purple solution* and about 15 ml of *0.1 M hydrochloric acid* from a burette. Insert the stopper and shake. If the solution is violet, add *0.1 M hydrochloric acid* in 1 ml portions until the solution becomes yellow, shaking after each addition. Titrate with *0.1 M sodium hydroxide* until the colour turns to violet.

Calculate the number of milliequivalents ( $M$) of base required for the neutralisation equivalent to 1 g of dried substance.

Calculate the degree of acid carboxymethyl substitution ($A$) from the expression:

$$\frac{1150\,M}{(7102 - 412\,M - 80\,C)}$$

$C$ = sulphated ash as a percentage

Calculate the degree of sodium carboxymethyl substitution ($S$) from the expression:

$$\frac{(162 + 58\,A)\,C}{(7102 - 80\,C)}$$

The degree of substitution is the sum of $A + S$ and it is between 0.60 and 0.85, calculated on the dried basis.

**Sodium chloride and sodium glycollate**. The sum of the percentage contents of sodium chloride and sodium glycollate is not more than 0.5 per cent, calculated on the dried basis.

*Sodium chloride*. Place 5.0 g in a 250 ml conical flask, add 50 ml of *water* and 5 ml of *strong hydrogen peroxide solution* and heat on a water-bath for 20 minutes stirring occasionally to ensure total hydration. Cool, add 100 ml of *water* and 10 ml of *nitric acid*. Titrate with *0.05 M silver nitrate* determining the end-point potentiometrically (2.4.25) using a silver indicator electrode and a double-junction reference electrode containing a 10 per cent w/v solution of *potassium nitrate* in the outer jacket and a standard filling solution in the inner jacket, and stirring constantly.

1 ml of *0.05 M silver nitrate* is equivalent to 0.002922 g of NaCl.

*Sodium glycollate*. Place 0.5 g of the substance under examination in a 100 ml beaker. Add 5 ml of *glacial acetic acid* and 5 ml of *water* and stir to ensure total hydration (about 15 minutes). Add 50 ml of *acetone* and 1 g of *sodium chloride*. Stir for several minutes to ensure complete precipitation of the carboxymethylcellulose. Filter through a fast filter paper impregnated with *acetone* into a volumetric flask, rinse the beaker and filter with 30 ml of *acetone* and dilute the filtrate to 100.0 ml with the same solvent. Allow to stand for 24 hours without shaking. Use the clear supernatant to prepare the test solution.

*Reference solution*. Dissolve 0.1 g of *glycollic acid* in 100 ml of *water*. Use the solution within 30 days. Transfer 1.0 ml, 2.0 ml, 3.0 ml and 4.0 ml of the solution to separate volumetric flasks; dilute the contents of each flask to 5.0 ml with *water*, add 5 ml of *glacial acetic acid*, dilute to 100.0 ml with *acetone* and mix.

Transfer 2.0 ml of the test solution and 2.0 ml of each of the reference solutions to separate 25 ml volumetric flasks. Heat the uncovered flasks for 20 minutes on a water-bath to eliminate *acetone*. Allow to cool and add 5.0 ml of *2,7-dihydroxynaphthalene solution* to each flask. Mix, add a further 15.0 ml of *2,7-dihydroxynaphthalene solution* and mix again. Close the flasks with aluminium foil and heat on a water-bath for 20 minutes. Cool and dilute to 25.0 ml with *sulphuric acid*.

Measure the absorbance (2.4.7) of each solution at 540 nm. Prepare a blank using 2.0 ml of a solution containing 5 per cent v/v each of *glacial acetic acid* and *water* in *acetone*. Prepare a standard curve using the absorbances obtained with the reference solutions. From the standard curve and the absorbance of the test solution, determine the mass, in milligrams, of glycollic acid in the substance under examination, and calculate the content of sodium glycollate from the expression:

$$\frac{10 \times 1.29 \times a}{(100 - b)\,m}$$

1.29 = the factor converting glycollic acid to sodium glycollate

$b$ = loss on drying as a percentage

$m$ = mass of the substance under examination, in grams

**Water-soluble substances**. Not more than 10.0 per cent. Disperse 10.0 g in 800.0 ml of *water* and stir for 1 minute every



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10 minutes during the first 30 minutes. Allow to stand for 1 hour and centrifuge, if necessary. Decant 200.0 ml of the supernatant liquid onto a fast filter paper in a vacuum filtration funnel, apply vacuum and collect 150.0 ml of the filtrate. Evaporate to dryness and dry the residue at 100° to 105° for 4 hours.

**Heavy metals** (2.3.13). To the residue obtained in sulphated ash add 1 ml of *hydrochloric acid* and evaporate on a water-bath. Take up the residue in 20 ml of *water*. 12 ml of the solution complies with the limit test for heavy metals, Method A (10 ppm). Prepare the reference solution using *lead standard solution (1 ppm Pb)*.

**Settling volume**. 10.0 to 30.0 ml. Place 75 ml of *water* in a 100 ml graduated cylinder and add 1.5 g of the substance under examination in 0.5 g portions, shaking vigorously after each addition. Dilute to 100.0 ml with *water* and shake again until the substance is homogeneously distributed. Allow to stand for 4 hours.

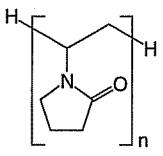
**Microbial contamination** (2.2.9). Total microbial count is not more than $10^3$ bacteria and $10^2$ fungi per gram, determined by plate count. 10 g is free from *Escherichia coli.*

**Sulphated ash** (2.3.18). 14.0 to 28.0 per cent, determined on 2.0 g, using a mixture of equal volumes of *sulphuric acid* and *water*, and calculated on the dried basis.

**Loss on drying** (2.4.19). Not more than 10.0 per cent, determined on 1.0 g by drying in an oven at 105° for 6 hours.

# Crospovidone

1-Ethenyl-2-pyrrolidinone homopolymer; 1-Vinyl-2-pyrrolidinone homopolymer




$(C_6H_9NO)_n$ Mol. Wt. $(111.1)_n$

Crospovidone is a water-insoluble synthetic crosslinked homopolymer of *N*-vinyl-2-pyrrolidinone.

Crospovidone contains not less than 11.0 per cent and not more than 12.8 per cent of nitrogen (N), calculated on the anhydrous basis.

**Category**. Excipient.

**Description**. A white to creamy white hygroscopic powder having a faint odour.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6) on specimen previously dried in vacuum at 105° for 1 hour. Compare the spectrum with that obtained with *crospovidone RS*.

B. Suspend 1 g in 10 ml of *water*, add 0.1 ml of *0.1 M iodine*, and shake for 30 seconds. Add 1 ml of *starch solution*, and shake; no blue color develops.

## Tests

**pH** (2.4.24). 5.0 and 8.0, determined in a 1.0 per cent w/v aqueous suspension.

**Water** (2.3.43). Not more than 5.0 per cent, determined on 0.5 g.

**Water-soluble substances**. Transfer 25.0 g to a 400 ml beaker, add 200 ml of *water*, and stir on a magnetic stirrer, using a 5-cm stirring bar, for 1 hour. Transfer to a 250 ml volumetric flask with the aid of about 25 ml of *water*, add *water* to volume, and mix. Allow the bulk of the solids to settle. Pass about 100 ml of the relatively clear supernatant through a membrane filter having a 0.45 mm porosity, protected against clogging by super imposing a membrane filter. Transfer 50.0 ml of the clear filtrate to a tared 100 ml beaker, evaporate to dryness, and dry at 110° for 3 hours: the weight of the residue does not exceed 75 mg (1.5 per cent).

**Impurity A**. Determine by liquid chromatography (2.4.14).

*Test solution*. Shake 1.25 g of the substance under examination in 50.0 ml of *methanol* for 60 minutes and filter.

*Reference solution (a)*. Dissolve 50 mg of *1-vinylpyrrolidin-2-one* in 100.0 ml of the *methanol*. Dilute 1.0 ml of this solution to 100.0 ml with *methanol*. Dilute 5.0 ml of this solution to 100.0 ml with the mobile phase.

*Reference solution (b)*. Dissolve 10 mg of *1-vinylpyrrolidin-2-one (crospovidone impurity A RS)* and 50 mg of *vinyl acetate* in 100.0 ml of the *methanol*. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4 mm packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 40°,
- mobile phase: a mixture of 10 volumes of *acetonitrile* and 90 volumes of *water*,
- flow rate. 1.0 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume. 50 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to crospovidone impurity A and vinyl acetate is not less than 2.0.



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Inject the test solution and reference solution (a). The relative standard deviation for replicate injections is not more than 2.0. In the chromatogram obtained with the test solution, the area of secondary peak due to crospovidone impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (10 ppm).

**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

**Vinylpyrrolidinone**. Suspend 4.0 g in 20 ml of *water*, stir for 15 minutes, centrifuge the suspension, and filter the slightly turbid upper layer through a sintered glass 10 mm filter. Stir the lower layer with 50 ml of *water*, centrifuge, and filter the upper layer through the same filter. Again stir the lower layer with 50 ml of *water*, and filter similarly. Add 0.5 g of *sodium acetate* to the combined filtrates and titrate with *0.05 M iodine* until the color of iodine no longer fades, add 3.0 ml of *0.05 M iodine*, allow to stand for 10 minutes, and titrate the excess of iodine with *0.1 M sodium thiosulphate*, adding 3 ml of *starch solution* as the end-point is approached. Carry out a blank determination, using the same total volume of the same *0.05 M iodine*, accurately measured, as was used for titrating the substance under examination. Before titrating the blank, adjust with *acetic acid* to the same pH as that of the substance under examination; not more than 0.72 ml of *0.05 M iodine* is consumed, corresponding to not more than 0.1 per cent of vinylpyrrolidinone.

**Nitrogen**. Place 0.1 g of the substance under examination (*m* mg) in a combustion flask, add 5 g of a mixture of 1 g of *copper sulphate*, 1 g of *titanium dioxide* and 33 g of *dipotassium sulphate*, and 3 glass beads. Wash any adhering particles from the neck into the flask with a small quantity of *water*. Add 7 ml of *sulphuric acid*, allowing it to run down the sides of the flask, and mix the contents by rotation. Close the mouth of the flask loosely, for example by means of a glass bulb with a short stem, to avoid excessive loss of *sulphuric acid*. Heat gradually at first, then increase the temperature until there is vigorous boiling with condensation of sulphuric acid in the neck of the flask; precautions are to be taken to prevent the upper part of the flask from becoming overheated. Continue the heating for 45 minutes. Cool, dissolve the solid material by cautiously adding to the mixture 20 ml of *water*, cool again and place in a steam-distillation apparatus. Add 30 ml of *strong sodium hydroxide solution* through the funnel, rinse the funnel cautiously with 10 ml of *water* and distil immediately by passing steam through the mixture. Collect 80-100 ml of distillate in a mixture of 30 ml of a 4.0 per cent w/v solution of *boric acid* and 0.05 ml of *bromocresol green-methyl red solution* and enough *water* to cover the tip of the condenser. Towards the end of the distillation lower the receiver so that the tip of the condenser is above the surface of the acid solution and rinse the end part of the condenser with a small quantity of *water*. Titrate the distillate with *0.025 M sulphuric acid* until the colour of the solution changes from green through pale greyish-blue to pale greyish-red-purple ($n_1$ ml of *0.025 M sulphuric acid*).

Repeat the test using about 100 mg of *glucose* in place of the substance under examination ($n_2$ ml of *0.025 M sulphuric acid*).

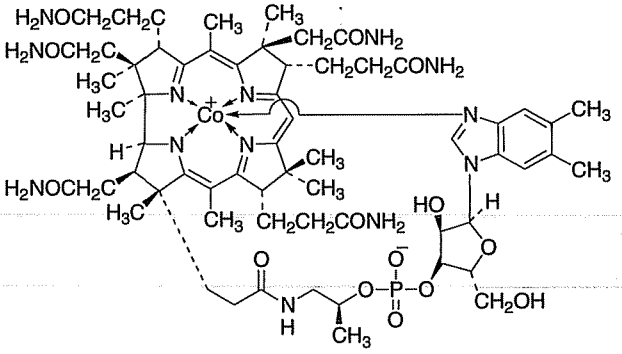
$$\text{Percent content of nitrogen} = \frac{0.7004(n_1 - n_2)}{m} \times 100$$

**Storage**. Store protected from moisture.

**Labelling**. The label states the type (type A or type B).

# Cyanocobalamin

Vitamin $B_{12}$



$C_{63}H_{88}CoN_{14}O_{14}P$ Mol. Wt. 1355.4

Cyanocobalamin is *Co*α-[α-(5,6-dimethylbenzimidazolyl)]-*Co*β-cyanocobamide.

Cyanocobalamin contains not less than 96.0 per cent and not more than 102.0 per cent of $C_{63}H_{88}CoN_{14}O_{14}P$, calculated on the dried basis.

**Category**. B-group Vitamin; haematopoietic.

**Dose**. In the treatment of megaloblastic anaemia, by intramuscular injection, 1 to 2 mg, in divided doses, in the first week. Subsequent doses, 250 µg weekly until the blood count is normal, maintenance dose, 250 µg every 3 or 4 weeks.

**Description**. A dark red, crystalline powder; very hygroscopic.

## Identification

A. When examined in the range 260 nm to 610 nm, a 0.0025 per cent w/v solution shows absorption maxima, at about 278 nm, 361 nm and 547 nm to 559 nm. The ratio of the absorbance at



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about 361 nm to that at about 547nm to 559 nm is 3.15 to 3.45 and the ratio of the absorbance at about 361 nm to that at about 278 nm is 1.70 to 1.90 (2.4.7).

B. Determine by thin-layer chromatography (2.4.17), protected form light and coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 60 volumes of *chloroform*, 40 volumes of *methanol* and 12 volumes of *6 M ammonia*. Use an unlined tank.

*Test solution*. Dissolve 20 mg of the substance under examination in 10 ml of *ethanol (50 per cent)*.

*Reference solution*. A 0.2 per cent w/v solution of *cyanocobalamin RS* in *ethanol (50 per cent)*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in air and examine in day light. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

C. Mix about 1 mg with 10 mg of *potassium sulphate* and 0.1 ml of *0.5 M sulphuric acid* and heat carefully to redness. Allow to cool, add to the residue 0.1 ml of water, 0.5 ml of saturated solution of *ammonium thiocyanate* and 0.5 ml of *benzyl alcohol* and shake; a blue colour is formed and is taken into the benzyl alcohol layer.

### Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*The following solutions should be used within 1 hour of preparation.*

*Test solution*. Dissolve 10 mg of the substance under examination in 10 ml of the mobile phase.

*Reference solution (a)*. A 0.003 per cent w/v solution of the substance under examination in the mobile phase.

*Reference solution (b)*. A 0.0001 per cent w/v solution of the substance under examination in the mobile phase.

*Reference solution (c)*. Dissolve 5 mg of the substance under examination in 2 ml of *water*, warming if necessary, allow to cool, add 1 ml of a 0.1 per cent w/v solution of *chloramine T* and 0.1 ml of *0.05 M hydrochloric acid*, dilute to 5 ml with *water*, shake and allow to stand for 5 minutes. Dilute 1 ml of this solution to 10 ml with the mobile phase. Use immediately.

Chromatographic system

- a stainless steel column 25 cm x 4 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 147 volumes of a 1.0 per cent w/v solution of *disodium hydrogen phosphate* and 53 volumes of *methanol* adjusted to pH 3.5 with *phosphoric acid* (to be used within 2 days of preparation),
- flow rate. 0.8 ml per minute,
- spectrophotometer set at 361 nm,
- injection volume. 20 µl.

Inject the test solution and reference solutions (a), (b) and (c).

Allow the chromatography to proceed for three times the retention time of the peak due to cyanocobalamin.

In the chromatogram obtained with the test solution the sum of the areas of any secondary peaks is not greater than the area of the principal peak in the chromatogram obtained with reference solution (a). Ignore any peak the area of which is less than that of the principal peak in the chromatogram obtained with reference solution (b). The test is not valid unless the chromatogram obtained with reference solution (c) exhibits two principal peaks, the resolution between these peaks is 2.5 or more and the chromatogram obtained with reference solution (a) exhibits one principal peak.

**Loss on drying** (2.4.19). Not more than 12.0 per cent, determined on 20.0 mg by drying in an oven at 105° at a pressure of 1.5 to 2.5 kPa for 2 hours.

**Assay**. Weigh accurately about 25 mg and dissolve in sufficient *water* to produce 1000.0 ml. Measure the absorbance of the solution at the maximum at about 361 nm (2.4.7). Calculate the content of $C_{63}H_{88}CoN_{14}O_{14}P$ taking 207 as the specific absorbance at 361 nm.

**Storage**. Store protected from light and moisture.

## Cyanocobalamin Injection

Vitamin $B_{12}$ Injection

Cyanocobalamin Injection is a sterile solution of Cyanocobalamin in Water for Injections containing sufficient Acetic Acid or Hydrochloric Acid to adjust the pH to about 4. It may contain suitable buffering agents.

Cyanocobalamin Injection contains not less than 95.0 per cent and not more than 110.0 per cent of the stated amount of anhydrous cyanocobalamin, $C_{63}H_{88}CoN_{14}O_{14}P$.

**Usual strength**. The equivalent of 500 µg and 1 mg of anhydrous cyanocobalamin per ml.

### Identification

When examined in the range 260 nm to 610 nm, a 0.0025 per cent w/v solution shows absorption maxima at about 278 nm, 361 nm and 547 nm to 559 nm. The ratio of the absorbance at about 361 nm to that at about 547 nm to 559 nm is 3.15 to 3.45 and the ratio of the absorbance at about 361 nm to that at about 278 nm is 1.70 to 1.90 (2.4.7).



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### Tests

**pH** (2.4.24). 3.8 to 5.5.

**Related substances**. Determine by liquid chromatography (2.4.14).

*The following solutions should be used within 1 hour of preparation.*

*Test solution (a)*. Dilute a suitable volume of the injection, if necessary, with the mobile phase to produce a solution containing 0.0001 per cent w/v of Cyanocobalamin.

*Test solution (b)*. Dilute a suitable volume of the injection, if necessary, with the mobile phase to produce a solution containing 0.05 per cent w/v of Cyanocobalamin.

*Reference solution (a)*. Dilute a suitable volume of the injection, if necessary, with the mobile phase to produce a solution containing 0.003 per cent w/v of Cyanocobalamin.

*Reference solution (b)*. Add 1 ml of a 0.1 per cent w/v solution of *chloramine T* and 0.1 ml of *0.05 M hydrochloric acid* to a volume containing 5 mg of Cyanocobalamin, dilute to 10 ml with *water,* shake and allow to stand for 5 minutes. Dilute 2 ml of this solution to 10 ml with the mobile phase and use immediately.

Chromatographic system

- a stainless steel column 25 cm x 4 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 147 volumes of a 1.0 per cent w/v solution of *disodium hydrogen phosphate* and 53 volumes of *methanol*, adjusted to pH 3.5 with *phosphoric acid* (to be used within 2 days of preparation),
- flow rate. 0.8 ml per minute,
- spectrophotometer set at 361 nm,
- injection volume. 20 µl.

Allow the chromatography to proceed for three times the retention time of the peak due to cyanocobalamin.

In the chromatogram obtained with test solution (b) the sum of the areas of any secondary peaks is not greater than the area of the principal peak in the chromatogram obtained with reference solution (a). Ignore any peak the area of which is less than that of the principal peak in the chromatogram obtained with test solution (a).

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

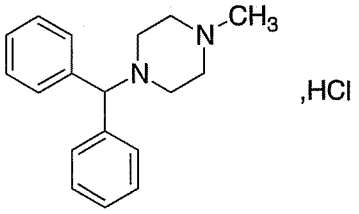
**Assay**. *Carry out the following procedure protected from light.*

Dilute the injection, if necessary, with *water* to produce a solution containing not more than the equivalent of 0.0025 per cent w/v of anhydrous cyanocobalamin and measure the absorbance at the maximum at about 361 nm (2.4.7). Calculate the content of $C_{63}H_{88}CoN_{14}O_{14}P$ taking 207 as the specific absorbance at 361 nm.

**Storage**. Store protected from light in single dose or multiple dose containers.

**Labelling**. The label states the strength in terms of the equivalent amount of anhydrous cyanocobalamin in a suitable dose-volume.

## Cyclizine Hydrochloride




$C_{18}H_{22}N_2$, HCl Mol. Wt. 302.9

Cyclizine Hydrochloride is 1-(diphenylmethyl)-4-methylpiperazine hydrochloride

Cyclizine Hydrochloride contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{18}H_{22}N_2$,HCl, calculated on the dried basis.

**Category**. Antiemetic.

**Dose**. 25 to 50 mg.

**Description**. A white, crystalline powder; almost odourless.

### Identification

A.Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cyclizine hydrochloride RS* or with the reference spectrum of cyclizine hydrochloride.

B. When examined in the range 220 nm to 360 nm, a freshly prepared 0.002 per cent w/v solution in *0.05 M sulphuric acid* shows absorption maximum only at about 225 nm; absorbance at about 225 nm, about 0.78 (2.4.7).

C. Dissolve 0.5 g in 10 ml of *ethanol (95 per cent),* warming if necessary, cool in ice, add 1 ml of *5 M sodium hydroxide* and sufficient *water* to produce 20 ml. Stir well and filter; the precipitate, after washing with *water* and drying at 60° at a pressure not exceeding 0.7 kPa for 2 hours, melts at about 107° *(2.4.21).*

D. Gives the reactions of chlorides (2.3.1).



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### Tests

***N*-Methylpiperazine**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 90 volumes of *chloroform*, 8 volumes of *methanol* and 2 volumes of *strong ammonia solution*.

*Prepare the following solutions freshly.*

*Test solution*. Dissolve 0.1 g of the substance under examination in 10 ml of *methanol*.

*Reference solution*. A 0.005 per cent w/v solution of *N-methylpiperazine RS* in *methanol*.

Apply to the plate 20 µl of each solution. After development, dry the plate in air and expose to iodine vapours for 10 minutes. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the corresponding spot in the chromatogram obtained with the reference solution.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 130°.

**Assay**. Weigh accurately about 0.1 g, dissolve in 20 ml of *anhydrous glacial acetic acid* and add 50 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01514 g of $C_{18}H_{22}N_2,HCl$.

**Storage**. Store protected from light.

## Cyclizine Tablets

Cyclizine Hydrochloride Tablets

Cyclizine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of cyclizine hydrochloride, $C_{18}H_{22}N_2,HCl$.

**Usual strength**. 50 mg.

### Identification

A. Extract a quantity of the powdered tablets containing 0.1 g of Cyclizine Hydrochloride with 10 ml of *ethanol (95 per cent)*, filter and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cyclizine hydrochloride RS* or with the reference spectrum of cyclizine hydrochloride.

B. Extract a quantity of the powdered tablets containing 0.5 g of Cyclizine Hydrochloride with 20 ml of *water* and filter. The filtrate gives reaction A of chlorides (2.3.1).

### Tests

***N*-Methylpiperazine**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 90 volumes of *chloroform*, 8 volumes of *methanol and* 2 volumes of *strong ammonia solution*.

*Prepare the following solutions freshly.*

*Test solution*. Triturate a quantity of the powdered tablets containing 0.1 g of Cyclizine Hydrochloride with 10 ml of *methanol* and filter.

*Reference solution*. A 0.005 per cent w/v solution of *N-methylpiperazine RS* in *methanol*.

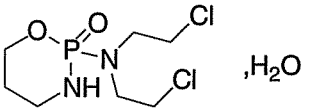
Apply to the plate 20 µl of each solution. After development, dry the plate in air and expose to iodine vapours for 10 minutes. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the corresponding spot in the chromatogram obtained with the reference solution.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.125 g of Cyclizine Hydrochloride and shake with 400 ml of *0.5 M sulphuric acid* for 15 minutes. Add sufficient *0.5 M sulphuric acid* to produce 500.0 ml and filter. Dilute 5.0 ml of the filtrate to 100.0 ml with *0.5 M sulphuric acid* and measure the absorbance of the resulting solution at the maximum at about 225 nm (2.4.7). Calculate the content of $C_{18}H_{22}N_2,HCl$ taking 390 as the specific absorbance at 225 nm.

**Storage**. Store protected from light.

## Cyclophosphamide




$C_7H_{15}Cl_2N_2O_2P, H_2O$ Mol. Wt. 279.1

Cyclophosphamide is (*RS*)-2-bis(2-chloroethyl)aminoperhydro-1,3,2-oxazaphosphorinane 2-oxide monohydrate.

Cyclophosphamide contains not less than 98.0 per cent and not more than 102.0 per cent of $C_7H_{15}Cl_2N_2O_2P$, calculated on the anhydrous basis.

**Category**. Anticancer.

**Dose**. Orally or by intravenous injection, 100 to 150 mg daily.



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**Description**. A white or almost white, crystalline powder.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

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

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

C. Dissolve 0.1 g in 10 ml of *water* and add 5 ml of *silver nitrate solution*; no precipitate is produced. Boil; a white precipitate is produced which is insoluble in *dilute nitric acid* but is soluble in *dilute ammonia solution* from which it can be reprecipitated by the addition of *dilute nitric acid.*

D. Dissolve 0.1 g in 3 ml of *nitric acid* and 1 ml of *sulphuric acid*, heat till brown fumes are evolved and the solution becomes colourless. Cool, add 10 ml of *water*, heat again up to 60° and add 10 ml of *ammonium molybdate solution*; a bright yellow precipitate is slowly formed.

## Tests

**Appearance of solution**. A 2.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and not more intensely coloured than reference solution YS6 (2.4.1).

**pH** (2.4.24). 4.0 to 6.0, determined in a 2.0 per cent w/v solution.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

*Mobile phase*. A mixture of 80 volumes of *2-butanone*, 12 volumes of *water*, 4 volumes of *acetone* and 2 volumes of *anhydrous formic acid.*

*Test solution (a)*. Dissolve 0.2 g of the substance under examination in 10 ml of *ethanol (95 per cent).*

*Test solution (b)*. Dilute 5 ml of test solution (a) to 50 ml with *ethanol (95 per cent).*

*Reference solution (a)*. Dilute 5 ml of test solution (b) to 50 ml with *ethanol (95 per cent).*

*Reference solution (b)*. A 0.2 per cent w/v solution of *cyclophosphamide RS* in *ethanol (95 per cent).*

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of warm air and heat at 110° for 10 minutes. Place the plate while hot in a tank in which is placed a dish containing equal volumes of a 5 per cent w/v solution of *potassium permanganate* and *hydrochloric acid*, close the tank and allow to stand for 2 minutes. Remove the plate and place it in a current of cold air until excess chlorine is removed and an area of coating below the line of application gives not more than a faint blue colour with *potassium iodide* and *starch solution*; do not expose long to cold air. Spray the plate with potassium iodide and starch solution and allow to stand for 5 minutes. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a). Ignore any spot remaining on the line of application.

**Heavy metals** (2.3.13). 1.0 g dissolved in 2 ml of *dilute acetic acid* and diluted to 25 ml with *water* complies with the limit test for heavy metals, Method A (20 ppm).

**Chlorides** (2.3.12). A freshly prepared solution of 0.75 g in sufficient *water* to produce 25 ml complies with the limit test for chlorides (330 ppm).

**Water** (2.3.43). 5.8 to 7.0 per cent, determined on 0.3 g.

**Assay**. Weigh accurately about 0.1 g and dissolve in 50 ml of a 0.1 per cent w/v solution of *sodium hydroxide* in *ethylene glycol*; boil under a reflux condenser for 30 minutes and allow to cool. Rinse the condenser with 25 ml of *water*, add 75 ml of *2-propanol*, 15 ml of *2 M nitric acid*, 10.0 ml of *0.1 M silver nitrate* and 2 ml of *ferric ammonium sulphate solution* and titrate with *0.1 M ammonium thiocyanate.*

1 ml of *0.1 M silver nitrate* is equivalent to 0.01305 g of $C_7H_{15}Cl_2N_2O_2P$.

*Cyclophosphamide Phosphate intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.*

**Bacterial endotoxins** (2.2.3). Not more than 0.2 Endotoxin Unit per mg of cyclophosphamide.

*Cyclophosphamide Phosphate intended for use in the manufacture of parenteral preparations without a further appropriate sterilization procedure complies with the following additional requirement.*

**Sterlity**. Complies with the test for sterility (2.2.11).

**Storage**. Store at a temperature not exceeding 30°. Avoid long exposure to temperatures above 30°.

**Labelling**. The label states whether or not the material is intended for use in the manufacture of parenteral preparations.

# Cyclophosphamide Injection

Cyclophosphamide Injection is a sterile material consisting of Cyclophosphamide with or without auxiliary substances. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.



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*The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).*

**Storage**. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Cyclophosphamide Injection contains not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of cyclophosphamide, $C_7H_{15}Cl_2N_2O_2P$.

**Usual strengths**. 100 mg; 200 mg; 500 mg; 1 g.

**Description**. A white or almost white powder.

*The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.*

### Identification

A. Shake a quantity containing 0.2 g of anhydrous cyclophosphamide with 2 ml of *chloroform* and filter. The solution complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cyclophosphamide RS* or with the reference spectrum of cyclophosphamide.

B. Extract a quantity containing 0.2 g of anhydrous cyclophosphamide with *ether* and evaporate the extract to dryness. Reserve a portion of the residue for identification test C. Dissolve 0.1 g in 10 ml of *water* and add 5 ml of *silver nitrate solution*; no precipitate is produced. Boil; a white precipitate is produced which is insoluble in *dilute nitric acid* but is soluble in *dilute ammonia solution* from which it can be reprecipitated by the addition of dilute *nitric acid*.

C. Dissolve 0.1 g of the residue from test B in 3 ml of *nitric acid* and 1 ml of *sulphuric acid*, heat till brown fumes are evolved and the solution becomes colourless. Cool, add 10 ml of *water*, heat again up to 60° and add 10 ml of *ammonium molybdate solution*; a bright yellow precipitate is slowly formed.

### Tests

**pH** (2.4.24). 4.0 to 6.0, determined in a 2.0 per cent w/v solution immediately after preparation.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 80 volumes of *2-butanone*, 12 volumes of *water*, 4 volumes of *acetone* and 2 volumes of *anhydrous formic acid*.

*Test solution*. Dissolve a quantity of the contents of the sealed container containing 0.2 g of anhydrous cyclophosphamide in sufficient *ethanol (95 per cent)* to produce 10 ml and filter.

*Reference solution*. Dilute 1 volume of solution (1) to 100 volumes with *ethanol (95 per cent)*.

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of warm air and heat at 110° for 10 minutes. Place the plate while hot in a tank in which is placed a dish containing equal volumes of a 5 per cent w/v solution of *potassium permanganate* and *hydrochloric acid*, close the tank and allow to stand for 2 minutes. Remove the plate and place it in a current of cold air until excess chlorine is removed and an area of coating below the line of application gives not more than a faint blue colour with *potassium iodide* and *starch solution*; do not expose long to cold air. Spray the plate with *potassium iodide* and *starch solution* and allow to stand for 5 minutes. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution. Ignore any spot remaining on the line of application.

**Bacterial endotoxins** (2.2.3). Not more than 0.2 Endotoxin Unit per mg of cyclophosphamide.

**Assay**. Determine the weight of the contents of 10 containers. Shake vigorously a quantity of the mixed contents of the 10 containers containing about 0.1 g of anhydrous cyclophosphamide in 30 ml of *chloroform* for 15 minutes, filter and wash the filter with 15 ml of *chloroform*. Evaporate the combined filtrate and washings to dryness and dissolve the residue in 50 ml of a 0.1 per cent w/v solution of *sodium hydroxide* in *1,2-ethanediol*. Boil the solution under a reflux condenser for 30 minutes, allow to cool and rinse the condenser with 25 ml of *water*. Add 75 ml of *2-propanol*, 15 ml of *2 M nitric acid*, 10 ml of *0.1 M silver nitrate* and 2 ml of *ferric ammonium sulphate solution* and titrate with *0.1 M ammonium thiocyanate*.

1 ml of *0.1 M silver nitrate* is equivalent to 0.01305 g of $C_7H_{15}Cl_2N_2O_2P$.

**Storage**. Store at a temperature not exceeding 30°. Avoid long exposure to temperatures above 30°. The solution should be used immediately after preparation as it deteriorates on storage.

**Labelling**. The label states (1) the quantity of Cyclophosphamide in terms of the equivalent amount of anhydrous cyclophosphamide; (2) the volume of Water for Injections to be added; (3) that the solution should be used immediately after preparation.

## Cyclophosphamide Tablets

Cyclophosphamide Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of anhydrous cyclophosphamide, $C_7H_{15}Cl_2N_2O_2P$. The tablets are coated.



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**Usual strengths**. 10 mg; 50 mg.

## Identification

A. Shake a quantity of the powdered tablets containing 0.2 g of anhydrous cyclophosphamide with 2 ml of *chloroform* and filter. The resulting solution complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cyclophosphamide RS* or with the reference spectrum of cyclophosphamide.

B. Extract a quantity of the powdered tablets containing 0.25 g of anhydrous cyclophosphamide with *ether* and evaporate the extract to dryness. Preserve a portion of the residue for identification test C. Dissolve 0.1 g in 10 ml of *water* and add 5 ml of *silver nitrate solution*; no precipitate is produced. Boil; a white precipitate is produced which is insoluble in *dilute nitric acid* but is soluble in *dilute ammonia solution* from which it can be reprecipitated by the addition of *dilute nitric acid*.

C. Dissolve 0.1 g of the residue from test B in 3 ml of nitric acid and 1 ml of *sulphuric acid*, heat till brown fumes are evolved and the solution becomes colourless. Cool, add 10 ml of *water*, heat again up to 60° and add 10 ml of *ammonium molybdate solution*; a bright yellow precipitate is slowly formed.

## Tests

**Acidity**. Shake a quantity of the powdered tablets containing 0.25 g of anhydrous cyclophosphamide with 20 ml of *carbon dioxide-free water*, filter and titrate the filtrate with *0.1 M sodium hydroxide* using *phenolphthalein solution* as indicator; not more than 0.2 ml of *0.1 M sodium hydroxide* is required to change the colour of the solution.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 80 volumes of *2-butanone*, 12 volumes of *water*, 4 volumes of *acetone* and 2 volumes of *anhydrous formic acid*.

*Test solution*. Shake vigorously a quantity of the powdered tablets containing 0.2 g of anhydrous cyclophosphamide with 50 ml of *chloroform* for 15 minutes, filter, evaporate the filtrate to dryness and dissolve the residue in 10 ml of *ethanol (95 per cent)*.

*Reference solution*. Dilute 1 volume of solution (1) to 100 volumes with *ethanol (95 per cent)*.

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of warm air and heat at 110° for 10 minutes. Place the plate while hot in a tank in which is placed a dish containing equal volumes of a 5 per cent w/v solution of *potassium permanganate* and *hydrochloric acid*, close the tank and allow to stand for 2 minutes. Remove the plate and place it in a current of cold air until excess chlorine is removed and an area of coating below the line of application gives not more than a faint blue colour with *potassium iodide* and *starch solution*; do not expose long to cold air. Spray the plate with *potassium iodide* and *starch solution* and allow to stand for 5 minutes. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution. Ignore any spot remaining on the line of application.

**Disintegration** (2.5.1). Not more than 30 minutes.

**Uniformity of content** *(for tablets containing 10 mg or less)*. Comply with the test stated under Tablets.

Place one tablet in a 10-ml volumetric flask, add about 7 ml of *water*, shake until the tablet is completely disintegrated, dilute with *water* to volume and filter. Wash the filter quantitatively with 10 ml of water and combine the filtrate and washings (test solution). In another volumetric flask dissolve an accurately weighed quantity of *cyclophosphamide RS* in *water* to obtain a solution of known concentration of about 500 µg per ml (reference solution). Place in separate test-tubes (170 mm x 25 mm) 2.0 ml of the test solution, 2.0 ml of the reference solution and 2.0 ml of water as the blank. Treat each tube in the following manner. Add 0.7 ml of a 2.35 per cent v/v solution of perchloric acid in water, mix and heat on a water-bath for 10 minutes. Cool, add 1 ml of *0.1 M sodium acetate* and mix. Add 1.6 ml of a 0.75 per cent w/v solution of *4-(4-nitrobenzyl) pyridine* in *1,2-ethanediol*, mix and heat on a water-bath for 10 minutes. Cool, add 8.0 ml of a 2 per cent w/v solution of *sodium hydroxide* in *ethanol (95 per cent)*. Measure the absorbances of the solutions against the blank within 4 minutes at the maximum at about 560 nm (2.4.7).

Calculate the content of $C_7H_{15}Cl_2N_2O_2P$ in the tablet.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. To an accurately weighed quantity of the powder containing about 0.1 g of anhydrous cyclophosphamide add 30 ml of *chloroform*, shake vigorously for 15 minutes, filter and wash the filter with 15 ml of *chloroform*. Evaporate the combined filtrate and washings to dryness and dissolve the residue in 50 ml of a 0.1 per cent w/v solution of *sodium hydroxide* in *1,2-ethanediol*. Boil the solution under a reflux condenser for 30 minutes, allow to cool and rinse the condenser with 25 ml of water. Add 75 ml of 2-propanol, 15 ml of *2 M nitric acid*, 10 ml of *0.1 M silver nitrate* and 2 ml of *ferric ammonium sulphate solution* and titrate with *0.1 M ammonium thiocyanate*.

1 ml of *0.1 M silver nitrate* is equivalent to 0.01305 g of $C_7H_{15}Cl_2N_2O_2P$.

**Storage**. Store at a temperature not exceeding 30°.



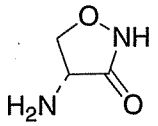
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**Labelling**. The label states the strength in terms of the equivalent amount of anhydrous cyclophosphamide.

## Cycloserine



$C_3H_6N_2O_2$ Mol. Wt. 102.1

Cycloserine is (*R*)-4-aminoisoxazolidin-3-one, an antimicrobial substance produced by the growth of certain strains of *Streptomyces orchidaceous* or *S. garyphalus* or obtained by synthesis.

Cycloserine contains not less than 98.0 per cent and not more than 100.5 per cent of $C_3H_6N_2O_2$, calculated on the dried basis.

**Category**. Antibacterial.

**Dose**. 500 mg to 1 g daily, in divided doses.

**Description**. A white or pale yellow, crystalline powder; hygroscopic.

### Identification

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

B. To 1 ml of a 0.01 per cent w/v solution in *0.1 M sodium hydroxide* add 3 ml of *1 M acetic acid* and 1 ml of a freshly prepared mixture of equal volumes of a 4 per cent w/v solution of *sodium nitroprusside* and *5 M sodium hydroxide*; a blue colour is produced slowly.

### Tests

**pH** (2.4.24). 5.5 to 6.5, determined in a 10.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). +108° to +114°, determined in a 5.0 per cent w/v solution in *2 M sodium hydroxide*.

**Heavy metals** (2.3.13). 2.0 g complies with limit test for heavy metals, Method B (10 ppm).

**Condensation products**. Absorbance of a 0.04 per cent w/v solution in *0.1 M sodium hydroxide* at about 285 nm, not more than 0.32 (2.4.7).

**Sulphated ash** (2.3.18). Not more than 0.5 per cent.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa for 3 hours.

**Assay**. Determine by liquid chromatography (2.4.14)

*Test solution*. Dissolve about 10 mg of the substance under examination in 20.0 ml of the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

*Reference solution*. Dissolve 10 mg of the *cycloserine RS* in 20.0 ml of the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm) (such as Wakosil C8 RS),
- mobile phase: 0.1 per cent w/v of *methane sulphonic acid* and 0.78 per cent w/v of *sodium dihydrogen orthophosphate* in *water*, the pH adjusted to 6.0 with *dilute sodium hydroxide* and filtered,
- flow rate. 1 ml per minute,
- spectrophotometer set at 227 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternatively the test solution and the reference solution.

Calculate the content of $C_3H_6N_2O_2$.

**Storage**. Store protected from moisture

## Cycloserine Capsules

Cycloserine Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cycloserine, $C_3H_6N_2O_2$.

**Usual strengths**. 125 mg; 250 mg.

### Identification

A. Shake a quantity of the contents of the capsules containing 10 mg of Cycloserine with 100 ml of *1 M sodium hydroxide* and filter. To 1 ml of the filtrate add 3 ml of *1 M acetic acid* and 1 ml of a freshly prepared mixture of equal volumes of a 4 per cent w/v solution of *sodium nitroprusside* and *5 M sodium hydroxide*; a blue colour is produced slowly.

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

### Tests

**Dissolution** (2.5.2).

Apparatus. No 2
Medium. 900 ml of phosphate buffer pH 6.8.



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Speed and time. 100 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

*Test solution.* The filtrate diluted to produce a 0.028 per cent w/v solution.

*Reference solution.* A 0.028 per cent w/v solution of *cycloserine RS* in the dissolution medium.

Use the chromatographic system described under Assay.

Calculate the content of $C_3H_6N_2O_2$.

D. Not less than 80 per cent of the stated amount of $C_3H_6N_2O_2$.

**Condensation products**. Weigh the contents of the capsules containing about 0.5 g of Cycloserine, dissolve in 250 ml of *0.1 M sodium hydroxide* solution, disperse with the aid of ultrasound for 5 minutes. Dilute 5 ml of this solution to 25 ml with *0.1 M sodium hydroxide.* Absorbance of the resulting solution at about 285 nm, not more than 0.32 (2.4.7).

**Loss on drying** (2.4.19). Not more than 2.0 per cent, determined on 1.0 g of the contents of the capsules, by drying in an oven at 60° at a pressure not exceeding 0.7 kPa for 3 hours.

**Other tests**. Comply with the tests stated under Capsules.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Mix the contents of 20 capsules. Weigh accurately a quantity of the mixed contents of the capsules containing about 250.0 mg of Cycloserine dissolve in *phosphate buffer pH 6.8,* dilute to 250.0 ml with the same solvent and filter. Dilute 5.0 ml of the filtrate to 25.0 ml with *phosphate buffer pH 6.8.*

*Reference solution.* A 0.02 per cent w/v solution of *cycloserine RS* in *phosphate buffer pH 6.8.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm) (such as Warkosil C8 RS),
- mobile phase: dissolve 1.0 g of *methane sulphonic acid* and 7.8 g of *sodium dihydrogen orthophosphate dihydrate* in 1000 ml of *water* and adjust pH to 6.0 with *dilute sodium hydroxide*, filter,
- flow rate. 1 ml per minute,
- spectrophotometer set at 227 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_3H_6N_2O_2$ in the capsules.

**Storage**. Store protected from moisture.

# Cycloserine Tablets

Cycloserine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of cycloserine, $C_3H_6N_2O_2$.

**Usual strengths**. 125 mg; 250 mg.

## Identification

A. Shake a quantity of the powdered tablets containing 0.5 g of Cycloserine with 25 ml of *1 M sodium hydroxide* for 5 minutes and filter. The optical rotation of the filtrate is about +2.2° (2.4.22).

B. To 0.2 ml of the filtrate obtained in test A add 3 ml of *1 M acetic acid* and 1 ml of a freshly prepared mixture of equal volumes of a 4 per cent w/v solution of *sodium nitroprusside* and *5 M sodium hydroxide*; a blue colour is produced slowly.

## Tests

**Light absorption**. Shake a quantity of the powdered tablets containing 0.25 g of Cycloserine with 80 ml of *0.1 M sodium hydroxide* for 10 minutes, add sufficient *0.1 M sodium hydroxide* to produce 100.0 ml, mix and filter. Dilute a suitable volume of the filtrate with sufficient *0.1 M hydrochloric acid* to produce a solution containing 0.0025 per cent w/v of Cycloserine. Absorbance of the resulting solution, measured within 15 minutes of preparing the final solution, at the maximum at about 219 nm, 0.78 to 0.96 (2.4.7).

**Disintegration** (2.5.1). Not more than 30 minutes.

**Loss on drying** (2.4.19). Not more than 2.0 per cent, determined on 1.0 g of the powdered tablets, by drying in an oven at about 60° at a pressure not exceeding 0.7 kPa for 3 hours.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.1 g of Cycloserine, shake with 150 ml of *water* for 30 minutes, add sufficient *water* to produce 200.0 ml and filter. To 10.0 ml of the filtrate add 10 ml of *water* and 25 ml of *0.2 M sodium hydroxide*, dilute to 50.0 ml with *water* and mix. To 4.0 ml of the mixture add 10 ml of *1 M acetic acid* and 4 ml of *sodium nitroprusside solution*, dilute to 20 ml with *1 M acetic acid*, mix and allow to stand for 15 minutes. Measure the absorbance of the resulting solution at the maximum at about 625 nm (2.4.7), using as the blank a solution prepared by treating 4.0 ml of *0.1 M sodium hydroxide* in the same manner beginning at the words "add 10 ml of *1 M acetic acid...*" Calculate the content of $C_3H_6N_2O_2$ from the absorbance obtained by repeating the operation using *cycloserine RS* in place of the powdered tablets.

**Storage**. Store at a temperature not exceeding 30°.

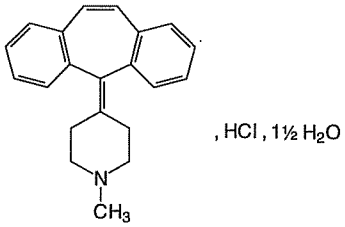


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# Cyproheptadine Hydrochloride




$C_{21}H_{21}N$, HCl,1½$H_2O$ Mol. Wt. 350.9

Cyproheptadine Hydrochloride is 4-(5*H*-dibenzo[*a,d*]-cyclohepten-5-ylidene)-1-methylpiperidine hydrochloride sesquihydrate.

Cyproheptadine Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{21}H_{21}N$,HCl, calculated on the dried basis.

**Category**. Histamine $H_1$-receptor antagonist.

**Dose**. 4 to 20 mg daily, in divided doses.

**Description**. A white or slightly yellow, crystalline powder.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.*

A. Dissolve 0.1 g in 10 ml of water, make alkaline with *1 M sodium hydroxide*, extract with 5 ml of *dichloromethane*, dry over *anhydrous sodium sulphate* and remove the solvent with the aid of a current of nitrogen. The oily residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cyproheptadine hydrochloride RS* treated in the same manner or with the reference spectrum of cyproheptadine.

B. When examined in the range 230 nm to 360 nm, a 0.002 per cent w/v solution in *ethanol (95 per cent)* shows an absorption maximum only at about 286 nm; absorbance at about 286 nm, about 0.67 (2.4.7).

C. Determine by thin-layer chromatography (2.4.17) coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 75 volumes of *cyclohexane*, 20 volumes of *ether* and 5 volumes of *diethylamine*.

*Test solution*. Dissolve 0.1 g of the substance under examination in 100 ml of *methanol*.

*Reference solution (a)*. A 0.1 per cent w/v solution of *cyproheptadine hydrochloride RS* in *methanol*.

*Reference solution (b)*. A solution containing 0.05 per cent w/v of each of *imipramine hydrochloride RS* and *cyproheptadine hydrochloride RS* in *methanol*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated principal spots.

D. A saturated solution gives reaction A of chlorides (2.3.1).

## Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17) coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 90 volumes of *dichloromethane* and 10 volumes of *methanol*.

*Solvent mixture*. A mixture of 9 volumes of *dichloromethane* and 1 volumes of *methanol*.

*Test solution*. Dissolve 0.1 g of the substance under examination in 10 ml with solvent mixture.

*Reference solution (a)*. A 0.001 per cent w/v solution of the substance under examination in the same solvent mixture.

*Reference solution (b)*. A solution containing 0.002 per cent w/v of *dibenzocycloheptatriene RS* in the same solvent mixture.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with *ethanolic sulphuric acid (20 per cent)*, heat at 110° for 30 minutes. Allow to cool and examine in ultraviolet light at 365 nm. Any spot corresponding to dibenzocycloheptatriene in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b) and any other secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). 7.0 to 9.0 per cent, determined on 1.0 g by drying in an oven at 100° at a pressure not exceeding 0.7 kPa.

**Assay**. Weigh accurately about 0.5 g, dissolve in 0.5 ml of *acetic anhydride* and 20 ml of *anhydrous glacial acetic acid* and add 10 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03239 g of $C_{21}H_{21}N$,HCl.

**Storage**. Store protected from light.



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# Cyproheptadine Syrup

Cyproheptadine Hydrochloride Syrup

Cyproheptadine Syrup contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cyproheptadine hydrochloride, $C_{21}H_{21}N,HCl$.

**Usual strength**. 2 mg in 5 ml.

## Identification

To 5 ml add 5 ml of a *1 per cent w/v solution of sodium bicarbonate* and extract with three quantities, each of 15 ml, of *2,2,4-trimethylpentane*. Wash the combined 2,2,4-trimethylpentane extracts with 5 ml of the sodium bicarbonate solution and discard the washings. Evaporate the 2,2,4-trimethylpentane solution to dryness on a water-bath and dissolve the residue in 100 ml of *ethanol (95 per cent)*. When examined in the range 230 nm to 360 nm, the resulting solution shows an absorption maximum only at about 286 nm (2.4.7).

## Tests

**pH** (2.4.24). 3.5 to 4.5.

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

**Assay**. To an accurately measured volume of the syrup containing about 2 mg of Cyproheptadine Hydrochloride add 20 ml of a 1 per cent w/v solution of *sodium bicarbonate* and extract with two quantities, each of 25 ml, of *2,2,4-trimethylpentane*. Wash the combined 2,2,4-trimethylpentane extracts with 5 ml of the *sodium bicarbonate solution* and discard the washings. Extract the 2,2,4-trimethylpentane solution with 50 ml of *0.05 M sulphuric acid* and collect the aqueous extract in a 100-ml volumetric flask. Dilute to volume with *0.05 M sulphuric acid* and mix. Filter a portion of the solution through a dry filter paper and discard the first 20 ml of the filtrate. Measure the absorbance of the filtrate at the maximum at about 286 nm (2.4.7), using *0.05 M sulphuric acid* as the blank. Calculate the content of $C_{21}H_{21}N,HCl$ taking 355 as the specific absorbance at 286 nm.

# Cyproheptadine Tablets

Cyproheptadine Hydrochloride Tablets

Cyproheptadine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cyproheptadine hydrochloride, $C_{21}H_{21}N,HCl$.

**Usual strength**. 4 mg.

## Identification

A. To a quantity of the powdered tablets containing 20 mg of Cyproheptadine Hydrochloride add 10 ml of *water* and 2.5 ml of *0.1 M sodium hydroxide*, extract with 10 ml of *dichloromethane*, filter through *anhydrous sodium sulphate* placed over absorbent cotton moistened with *dichloromethane* and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cyproheptadine hydrochloride RS* treated in the same manner or with the reference spectrum of cyproheptadine.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

C. Extract a quantity of the powdered tablets containing 20 mg of Cyproheptadine Hydrochloride with 7 ml of *water*, filter, add 0.3 ml of *5 M ammonia* to the filtrate and filter again. The filtrate gives reaction A of chlorides (2.3.1).

## Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), using a precoated silica gel plate.

*Mobile phase*. A mixture of 90 volumes of *dichloromethane* and 10 volumes of *methanol*.

*Test solution (a)*. Shake mechanically for 10 minutes a quantity of the powdered tablets containing 50 mg of Cyproheptadine Hydrochloride with 5 ml of the mobile phase and filter (such as Whatman GF/C filter paper).

*Test solution (b)*. Dilute 1 volume of test solution (a) to 10 volumes with the mobile phase.

*Reference solution (a)*. Dilute 1 volume of test solution (a) serially in two steps to 1000 volumes with the mobile phase.

*Reference solution (b)*. A solution containing 0.1 per cent w/v of *cyproheptadine hydrochloride RS* in the mobile phase.

*Reference solution (c)*. A solution containing 0.002 per cent w/v of *dibenzocycloheptatriene RS* in the mobile phase.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with *ethanolic sulphuric acid (20 per cent)*. Heat at 110° for 30 minutes and examine in ultraviolet light at 365 nm. In the chromatogram obtained with test solution (a) any spot corresponding to dibenzocycloheptatriene is not more intense than the spot in the chromatogram obtained with reference solution (c) and any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (a).

**Uniformity of content**. Comply with the test stated under Tablets.

Powder one tablet, warm with 20 ml of *ethanol (95 per cent)* and centrifuge. Repeat the extraction with three further



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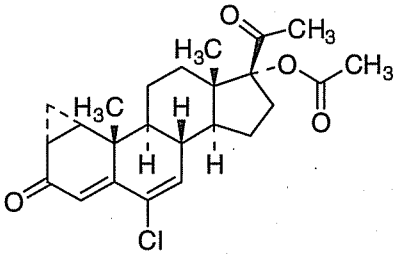


quantities, each of 20 ml, of *ethanol (95 per cent)*. Cool the combined extracts and add sufficient *ethanol (95 per cent)* to produce 200.0 ml. Measure the absorbance of the resulting solution at the maximum at about 286 nm (2.4.7). Calculate the content of $C_{21}H_{21}N$,HCl taking 355 as the specific absorbance at 286 nm.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 1.5 mg of Cyproheptadine Hydrochloride, add sufficient *ethanol (95 per cent)* to produce 100.0 ml, mix well and filter. Measure the absorbance of the filtrate at the maximum at about 286 nm (2.4.7). Calculate the content of $C_{21}H_{21}N$,HCl taking 355 as the specific absorbance at 286 nm.

# Cyproterone Acetate



$C_{24}H_{29}ClO_4$ Mol. Wt. 416.9

Cyproterone Acetate is (1$\beta$, 2$\beta$)-6-chloro-1,2-dihydro-17-acetyloxy-3'*H*-cyclopropa[1,2]pregna-1,4,6-triene-3,20-dione.

Cyproterone Acetate contains not less than 97.0 per cent and not more than 103.0 per cent of $C_{24}H_{29}ClO_4$, calculated on the dried basis.

**Category**. Anticancer.

**Description**. A white or almost white, crystalline powder.

## Identification

*Test A may be omitted if tests B, C, D and E are carried out. Tests B, C and D may be omitted if tests A and E are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cyproterone acetate RS* or with the reference spectrum of cyproterone acetate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. a mixture of equal volumes of *cyclohexane* and *ethyl acetate*.

*Test solution*. Dissolve 0.02 g of the substance under examination in *dichloromethane* and dilute to 10 ml with the same solvent.

*Reference solution*. A 0.2 per cent w/v solution of *cyproterone acetate RS* in *dichloromethane*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

C. Dissolve about 1.0 mg with 2 ml of *sulphuric acid* and heat on a water-bath for 2 minutes. A red colour develops. Cool and add this solution cautiously to 4 ml of *water* and shake. The solution becomes violet.

D. Incinerate about 30 mg with 0.3 g of *anhydrous sodium carbonate* over a naked flame for about 10 minutes. Cool, dissolve the residue in 5 ml of *dilute nitric acid* and filter. To 1 ml of the filtrate, add 1 ml of *water*. The solution gives reaction A of chlorides (2.3.1).

E. It gives the reactions of acetyl groups (2.3.1).

## Test

**Specific optical rotation** (2.4.22). +152° to +157°, determined in a 1.0 per cent w/v solution in *acetone*.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 10 mg of the substance under examination in *acetonitrile* and dilute to 10.0 ml with the same solvent.

*Reference solution (a)*. Dilute 1.0 ml of the test solution to 100.0 ml with *acetonitrile*.

*Reference solution (b)*. Dissolve 5 mg of *medroxyprogesterone acetate RS* in *acetonitrile* and dilute to 50.0 ml with the same solvent. Dilute 1.0 ml of this solution to 10.0 ml with reference solution (a).

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- mobile phase: a mixture of 40 volumes of *acetonitrile* and 60 volumes of *water*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to cyproterone acetate and medroxyprogesterone acetate is not less than 3.0.



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Inject reference solution (a) and the test solution. Run the chromatogram at least twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the sum of the areas of all the secondary peaks is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying at 80° at a pressure not exceeding 0.7 kPa.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Assay**. Weigh accurately about 0.05 g, dissolve in *methanol* and dilute to 50.0 ml with the same solvent. Dilute 1.0 ml of this solution to 100.0 ml with *methanol* and measure the absorbance of the resulting solution at the maximum at about 282 nm (2.4.7). Calculate the content of $C_{24}H_{29}ClO_4$ taking 414 as the specific absorbance at 282 nm.

**Storage**. Store protected from light.

# Cyproterone Tablets

Cyproterone Acetate Tablets

Cyproterone Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of cyproterone acetate, $C_{24}H_{29}ClO_4$.

**Usual strength**. 50 mg.

## Identification

A. Shake a quantity of powdered tablets containing about 100 mg of cyproterone acetate with 20 ml of *dichloromethane*, filter and evaporate the filtrate to dryness using a rotary evaporator and a water-bath at 40°. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cyproterone acetate RS* or with the reference spectrum of cyproterone acetate.

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

## Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of 0.07 per cent w/v solution of *sodium dodecyl sulphate* in *0.1M hydrochloric acid* (For tablets containing less than 100 mg of Cyproterone Acetate); 900 ml of 0.1 per cent w/v solution of *sodium dodecyl sulphate* in *0.1M hydrochloric acid* (For tablets containing more than 100 mg of Cyproterone Acetate),

Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter. Measure the absorbance of the filtrate, suitably diluted if necessary with dissolution medium at 285 nm (2.4.7). Calculate the content of cyproterone acetate, $C_{24}H_{29}ClO_4$ in the medium from the absorbances obtained from a solution of known concentration of *cyproterone acetate RS*.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. 80 volumes of *acetonitrile* and 20 volumes of *water*.

*Test solution*. Shake a quantity of powdered tablets containing about 0.1 g of Cyproterone Acetate in 50 ml of the solvent mixture for 5 minutes, dilute to 100 ml with *water* and filter.

*Reference solution (a)*. Dilute 1 ml of the test solution to 100 ml with the mobile phase.

*Reference solution (b)*. A solution containing 0.01 per cent w/v each of *cyproterone acetate RS* and *medroxyprogesterone acetate RS* in the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 12.5 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm) (such as Spherisorb ODS 2),
- mobile phase: a mixture of 40 volumes of *acetonitrile* and 60 volumes of *water*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to cyproterone acetate and medroxyprogesterone acetate is not less than 3.0.

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

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Shake a quantity of powdered tablets containing about 50 mg of Cyproterone Acetate in 100 ml of the solvent



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mixture and filter. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

*Reference solution (a)*. A 0.05 per cent w/v solution of *cyproterone acetate RS* in the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

*Reference solution (b)*. A solution containing 0.01 per cent w/v each of *cyproterone acetate RS* and *medroxyprogesterone acetate RS* in the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Use the chromatographic system as described under Related substances.

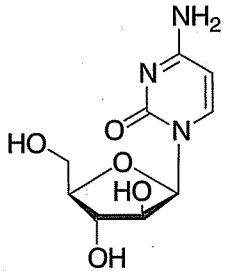
Inject reference solution (b). The test is not valid unless the resolution between the peak due to cyproterone and medroxyprogesterone is not less than 3.0.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{24}H_{29}ClO_4$ in the tablets.

# Cytarabine

β-Cytosine Arabinoside




$C_9H_{13}N_3O_5$ Mol. Wt. 243.2

Cytarabine is 1-β-D-arabinofuranosylcytosine.

Cytarabine contains not less than 99.0 per cent and not more than 100.5 per cent of $C_9H_{13}N_3O_5$, calculated on the dried basis.

**Category**. Cytotoxic.

**Dose**. To be determined by the physician.

**Description**. A white or almost white, crystalline powder.

*CAUTION — Cytarabine is very poisonous. Great care should be taken to avoid inhaling the particles of cytarabine and exposing the skin to the dried substance.*

## Identification

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

B. When examined in the range 230 nm to 360 nm, a 0.001 per cent w/v solution in *0.1 M hydrochloric acid* shows an absorption maximum only at about 280 nm; absorbance at about 280 nm, about 0.55 (2.4.7).

C. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

## Tests

**Specific optical rotation** (2.4.22). +154° to +160°, determined in a 1.0 per cent w/v solution.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 65 volumes of *2-butanone*, 20 volumes of *acetone* and 15 volumes of *water*.

*Test solution (a)*. A 5 per cent w/v solution of the substance under examination in *water*.

*Test solution (b)*. A 0.2 per cent w/v solution of the substance under examination in *water*.

*Reference solution (a)*. A 0.025 per cent w/v solution of the substance under examination in *water*.

*Reference solution (b)*. A solution containing 0.2 per cent w/v solution of *cytarabine RS* in *water*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent).

**Sulphated ash** (2.3.18). Not more than 0.5 per cent.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven over *phosphorus pentoxide* at 60° for 3 hours at a pressure of 0.2 kPa to 0.7 kPa.

**Assay**. Weigh accurately about 0.5 g and dissolve in 40 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, using *1-naphtholbenzein solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02432 g of $C_9H_{13}N_3O_5$.

*Cytarabine intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.*

**Bacterial endotoxins** (2.2.3). Not more than 0.07 Endotoxin Unit per mg.



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*Cytarabine intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.*

**Sterility**. Complies with the test for sterility (2.2.11).

**Storage**. Store protected from light. If it is intended for use in the manufacture of parenteral preparations, the container should be sterile, tamper-evident and sealed so as to exclude micro-organisms.

**Labeling**. The label states whether or not the material is intended for use in the manufacture of parenteral preparations.

# Cytarabine Injection

β-Cytosine Arabinoside Injection

Cytarabine for Injection is a sterile material consisting of Cytarabine with or without excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

*The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).*

**Storage**. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Cytarabine Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of cytarabine, $C_9H_{13}N_3O_5$.

**Usual strength**. 100 mg.

**Description**. A white or almost white powder.

*The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.*

## Identification

Mix 0.1 g of the substance under examination with 10 ml of hot *ethanol (95 per cent)*, filter, allow the filtrate to cool and induce crystallisation if necessary. Filter, wash the crystals with 2 ml of *ethanol (95 per cent)* and dry at 60° at a pressure of 0.7 kPa. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cytarabine RS* or with the reference spectrum of cytarabine.

## Tests

**pH** (2.4.24). 4.0 to 6.0, determined in a 2.0 per cent w/v solution in the solvent stated on the label.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 65 volumes of *2-butanone*, 20 volumes of *acetone* and 15 volumes of *water*.

*Test solution*. A 4 per cent w/v solution of the substance under examination in *water*.

*Reference solution (a)*. A 0.02 per cent w/v solution of the substance under examination *water*.

*Reference solution (b)*. A solution containing 0.04 per cent w/v solution of *uridine water*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution with an $R_f$ value of about 1.1 relative to the spot in the chromatogram obtained with reference solution (b) is not more intense than the spot in the chromatogram obtained with reference solution (b). Any other secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a).

**Water** (2.3.43). Not more than 3.0 per cent, determined on 0.8 g.

**Bacterial endotoxins** (2.2.3). Not more than 0.07 Endotoxin unit per mg.

**Assay**. Determine the weight of the contents of 10 containers. Weigh accurately about 0.5 g of the mixed contents of the 10 containers and dissolve by heating, if necessary, in 80 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, using *1-naphtholbenzein solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02432 g of $C_9H_{13}N_3O_5$.

**Storage**. Store protected from light.



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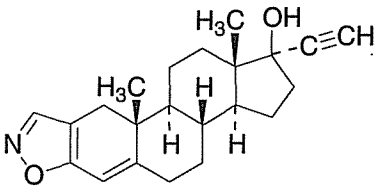


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# Danazol



$C_{22}H_{27}NO_2$ Mol. Wt. 337.5

Danazol is 17α-pregna-2,4-diene-20-yno[2,3-*d*]isoxazol-17-ol.

Danazol contains not less than 97.0 per cent and not more than 102.0 per cent of $C_{22}H_{27}NO_2$, calculated on the dried basis.

**Category**. Antigonadotrophin.

**Dose**. 200 to 800 mg daily , in divided doses.

**Description**. A white to pale yellow, crystalline powder.

## Identification

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

B. When examined in the range 230 nm to 360 nm (2.4.7), the final solution obtained in the Assay shows an absorption maximum only at about 285 nm.

## Tests

**Specific optical rotation** (2.4.22). +21.0° to +27.0°, determined in a 1.0 per cent w/v solution in *chloroform*.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Solvent mixture*. 9 volumes of *chloroform* and 1 volume of *methanol*.

*Mobile phase*. A mixture of 70 volumes of *cyclohexane* and 30 volumes of *ethyl acetate*.

*Test solution*. Dissolve 0.5 g of the substance under examination in 10.0 ml of the solvent mixture.

*Reference solution (a)*. Dissolve 50 mg of *danazol RS* in 100 ml of the solvent mixture.

*Reference solution (b)*. Dilute 10 ml of reference solution (a) to 20 ml with the solvent mixture.

Apply to the plate 5 µl of each solution. After development, dry the plate in warm air and examine in ultraviolet light at 254 nm. Expose the plate to the vapour of iodine for 5 minutes and examine the plate again. By both methods of visualisation, any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

**Loss on drying** (2.4.19). Not more than 2.0 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 2.7 kPa.

**Assay**. Weigh accurately about 0.1 g, previously dried, dissolve in 50 ml of *ethanol (95 per cent)*, swirling until dissolved, and dilute to 100.0 ml with *ethanol (95 per cent)*. Dilute 2.0 ml of this solution to 100.0 ml with *ethanol (95 per cent)*. Measure the absorbance of the resulting solution at the maximum at about 285 nm (2.4.7). Calculate the content of $C_{22}H_{27}NO_2$ from the absorbance obtained by repeating the procedure using a solution containing 0.002 per cent w/v of *danazol RS* in place of the substance under examination.

**Storage**. Store protected from light.

# Danazol Capsules

Danazol Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of danazol, $C_{22}H_{27}NO_2$.

**Usual strengths**. 50 mg; 100 mg; 200 mg.

## Identification

A. Extract the contents of the capsules containing about 50 mg of Danazol with 50 ml of *chloroform*, filter and evaporate the filtrate to dryness on a water-bath in a stream of nitrogen. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *danazol RS* or with the reference spectrum of danazol.

## Tests

**Dissolution** (2.5.2).

Apparatus No. 2,
Medium. 900 ml of 0.75 per cent w/v solution of *sodium lauryl sulphate*,
Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter. Measure the absorbance (2.4.7) of the filtrate, suitably diluted if necessary with dissolution medium at 286 nm. Calculate the content of danazol, $C_{22}H_{27}NO_2$, in the medium from the absorbance obtained from a solution of known concentration of *danazol RS*, prepared by dissolving in minimum quantity of *isopropyl alcohol* and diluted with dissolution medium.

D. Not less than 75 per cent of the stated amount of $C_{22}H_{27}NO_2$.

**Other tests**. Comply with the tests stated under Capsules.

**Assay**. Determine by liquid chromatography (2.4.14).



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*Test solution.* Shake a quantity of the mixed contents of 20 capsules containing 100 mg of Danazol in 50 ml of the mobile phase for 10 minutes and dilute to 100.0 ml with the mobile phase and filter. Dilute 5.0 ml of the filtrate to 25.0 ml with the mobile phase.

*Reference solution.* A 0.02 per cent w/v solution of *danazol RS* in the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (4 µm),
- mobile phase: a mixture of 40 volumes of *acetonitrile,* 30 volumes of *methanol* and 30 volumes of *water,*
- flow rate. 1.5 ml per minute.
- spectrophotometer set at 270 nm,
- injection volume. 20 µl.

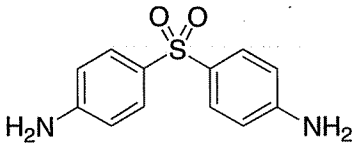
Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{22}H_{27}NO_2$ in the capsules.

**Storage**. Store protected from light.

# Dapsone



$C_{12}H_{12}N_2O_2S$ Mol. Wt. 248.3

Dapsone is the bis(4-aminophenyl)sulphone.

Dapsone contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{12}H_{12}N_2O_2S$, calculated on the dried basis.

**Category**. Antileprotic.

**Dose**. 100 mg daily.

**Description**. A white or creamy-white, crystalline powder.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

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

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.0005 per cent w/v solution in *methanol* shows absorption maxima at about 260 nm and 295 nm; absorbance at about 260 nm, about 0.36 and at about 295 nm, about 0.6.

C. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (c).

D. 2 ml of a 0.005 per cent w/v solution in *0.1 M hydrochloric acid* gives the reaction of primary aromatic amines (2.3.1).

## Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

*Mobile phase.* A mixture of 20 volumes of *n-heptane,* 20 volumes of *ethyl acetate,* 6 volumes of *methanol* and 1 volume of *strong ammonia solution.*

*Test solution (a).* Dissolve 0.1 g of the substance under examination in 10 ml of *methanol.*

*Test solution (b).* Dilute 1 ml of test solution (a) to 10 ml with *methanol.*

*Reference solution (a).* Dilute 1 ml of test solution (b) to 10 ml with *methanol.*

*Reference solution (b).* Dilute 1 ml of reference solution (a) to 50 ml with *methanol.*

*Reference solution (c).* A 0.1 per cent w/v solution of *dapsone RS* in *methanol.*

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with a 0.1 per cent w/v solution of *4-dimethylaminocinnamaldehyde* in a mixture of 99 volumes of *ethanol (95 per cent)* and 1 volume of *hydrochloric acid* and examine in daylight. Any secondary spot in the chromatogram obtained with the test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than two such spots are more intense than the spot in the chromatogram obtained with reference solution (b).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 1.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.3 g and dissolve in a mixture of 20 ml of *water* and 20 ml of *hydrochloric acid.* Cool the solution to about 15° and determine by the nitrite titration (2.3.31). Carry out a blank titration.

1 ml of *0.1 M sodium nitrite* is equivalent to 0.01242 g of $C_{12}H_{12}N_2O_2S$.

**Storage**. Store protected from light.



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# Dapsone Tablets

Dapsone Tablets contain not less than 93.0 per cent and not more than 107.0 per cent of the stated amount of dapsone, $C_{12}H_{12}N_2O_2S$.

**Usual strengths.** 25 mg; 50 mg; 100 mg.

## Identification

A. Shake a quantity of the powdered tablets containing 0.1g of Dapsone with 10 ml of *acetone*, filter and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dapsone RS* or with the reference spectrum of dapsone.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (c).

## Tests

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 20 volumes of *n-heptane*, 20 volumes of *ethyl acetate*, 6 volumes of *methanol* and 1 volume of *strong ammonia solution*.

*Test solution (a)*. Shake a quantity of the powdered tablets containing 0.1 g of Dapsone with 10 ml of *methanol* and filter.

*Test solution (b)*. Dilute 1 ml of test solution (a) to 10 ml with *methanol*.

*Reference solution (a)*. Dilute 1 ml of test solution (b) to 10 ml with *methanol*.

*Reference solution (b)*. Dilute 2 ml of reference solution (a) to 10 ml with *methanol*.

*Reference solution (c)*. A 0.1 per cent w/v solution of *dapsone RS* in *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with a 0.1 per cent w/v solution of *4-dimethylaminocinnamaldehyde* in a mixture of 99 volumes of *ethanol (95 per cent)* and 1 volume of *hydrochloric acid* and examine in daylight. Any secondary spot in the chromatogram obtained with the test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than two such spots are more intense than the spot in the chromatogram obtained with reference solution (b).

**Dissolution** (2.5.2).

Apparatus No. 1,

Medium. 900 ml of a 2 per cent w/v solution of *hydrochloric acid*,

Speed and time. 100 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter disc with an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate. Transfer an accurately measured volume of the filtrate containing about 0.2 mg of Dapsone to a 25-ml volumetric flask, add 5 ml of *1 M sodium hydroxide*, dilute to volume with *water* and mix. Measure the absorbance of the resulting solution at the maximum at about 290 nm (2.4.7). Calculate the content of $C_{12}H_{12}N_2O_2S$ from the absorbance obtained from a solution prepared by adding 5 ml of *1 M sodium hydroxide* to 20 ml of a 2 per cent v/v solution of *hydrochloric acid* containing 0.2 mg of *dapsone RS* and adding sufficient *water* to produce 25.0 ml.

D. Not less than 75 per cent of the stated amount of $C_{12}H_{12}N_2O_2S$.

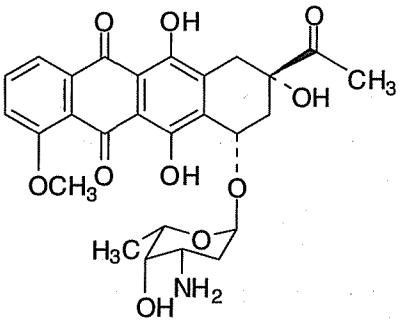
**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.25 g of Dapsone and dissolve in a mixture of 15 ml of *water* and 15 ml of *2 M hydrochloric acid*. Cool the solution to about 15° and determine by the nitrite titration (2.3.31). Carry out a blank titration.

1 ml of *0.1 M sodium nitrite* is equivalent to 0.01242 g of $C_{12}H_{12}N_2O_2S$.

**Storage.** Store protected from light.

# Daunorubicin Hydrochloride




$C_{27}H_{30}ClNO_{10}$ Mol. Wt. 564.0

Daunorubicin Hydrochloride is (8*S-cis*)-8-acetyl-10-[(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacenedione hydrochloride.



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Daunorubicin Hydrochloride contains not less than 95.0 per cent and not more than 102.0 per cent of $C_{27}H_{30}ClNO_{10}$, calculated on the anhydrous basis.

**Category**. Anticancer.

**Description**. A crystalline, orange-red powder, hygroscopic.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *daunorubicin hydrochloride RS* or with the reference spectrum of daunorubicin hydrochloride.

B. Dissolve about 10 mg in 0.5 ml of *nitric acid*, add 0.5 ml of *water* and heat over a flame for 2 minutes. Allow to cool and add 0.5 ml of *silver nitrate solution*; a white precipitate is formed.

## Tests

**pH** (2.4.24). 4.5 to 6.5, determined in a 0.5 per cent w/v solution in *carbon dioxide-free water*.

**Related substances**. Determine by liquid chromatography (2.4.14).

*NOTE—Prepare the solutions immediately before use.*

*Test solution*. Dissolve 50 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the mobile phase.

*Reference solution (a)*. A 0.1 per cent w/v solution of *daunorubicin hydrochloride RS* in the mobile phase.

*Reference solution (b)*. Dissolve 10 mg of *doxorubicin hydrochloride RS* and 10 mg of *epirubicin hydrochloride RS* in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

*Reference solution (c)*. Dissolve 5.0 mg of *daunorubicinone RS* and 5.0 mg of *doxorubicin hydrochloride RS* in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

*Reference solution (d)*. Dilute 1.0 ml of reference solution (a) to 200.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm, packed with end-capped octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of equal volumes of *acetonitrile* and a solution containing 0.288 per cent w/v of *sodium laurylsulphate* and 0.225 per cent w/v of *orthophosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 5 µl.

The relative retention time with reference to daunorubicin for (8*S*,10*S*)-8-acetyl-6,8,10,11-tetrahydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione (daunorubicin impurity A) is about 0.4, for doxorubicin (daunorubicin impurity D) is about 0.5, for epirubicin is about 0.6 and for (8*S*,10*S*)-10-[(3-amino-2,3,6-trideoxy-a-L-*lyxo*-hexopyranosyl)oxy]-6,8,11-trihydroxy-8-[(1*RS*)-1-hydroxyethyl]-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione (daunorubicin impurity B) is about 0.7.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to doxorubicin hydrochloride and epirubicin hydrochloride is not less than 2.0.

Inject reference solution (b), (c),(d) and the test solution. Run the chromatogram twice the retention time of daunorubicin peak. In the chromatogram obtained with test solution, the area of secondary peak corresponding to daunorubicin impurity A is not more than the area of corresponding peak in the chromatogram obtained with reference solution (c) (0.5 per cent), the area of secondary peak corresponding to daunorubicin impurity B is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (d) (1.5 per cent), the area of secondary peak corresponding to daunorubicin impurity D is not more than the area of corresponding peak in the chromatogram obtained with reference solution (c) (0.5 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (0.5 per cent) and sum of areas of all other secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (d) (2.5 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.05 per cent).

**Butanol** (5.4). Not more than 1.0 per cent.

**Water** (2.3.43). Not more than 3.0 per cent, determined on 0.1 g.

*Daunorubicin Hydrochloride intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.*

**Bacterial endotoxins** (2.2.3). Not more than 4.3 Endotoxin Units per mg of daunorubicin hydrochloride.

**Assay**. Determine by liquid chromatography (2.4.14) as described under Related substances.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{27}H_{30}ClNO_{10}$.

**Storage**. Store protected from light and moisture.



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# Daunorubicin Injection

Daunorubicin Hydrochloride Injection

Daunorubicin Injection is a sterile material consisting of Daunorubicin Hydrochloride and Mannitol, with or without buffering agents and other excipients. It is filled in a sealed container.

*The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.*

*The constituted solution complies with the requirement for the Appearance of solution and Particulate matter stated under Parenteral Preparations (Injections).*

**Usual strength**. 20 mg.

**Storage**. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Daunorubicin Injection contains not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of daunorubicin, $C_{27}H_{29}NO_{10}$.

## Identification

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

## Tests

**pH** (2.4.24). 4.5 to 6.5, determined on the constituted solution.

**Water** (2.3.43). Not more than 3.0 per cent, determined on 0.1 g.

**Bacterial endotoxins** (2.2.3). Not more than 4.3 Endotoxin Units per mg of daunorubicin.

**Other tests.** Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Determine the weight of the contents of 10 containers. Dissolve an accurately weighed quantity of the mixed contents of 10 containers containing about 25 mg daunorubicin to 100.0 ml with the mobile phase.

*Reference solution (a).* A 0.025 per cent w/v solution of *daunorubicin hydrochloride RS* in the mobile phase.

*Reference solution (b).* A solution containing 25 mg of *doxorubicin* in 100.0 ml of reference solution (a).

Chromatographic system

- a stainless steel column 30 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 62 volumes of *water* and 38 volumes of *acetonitrile,* adjusted to pH 2.2 with *orthophosphoric acid,*
- flow rate 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 5 µl.

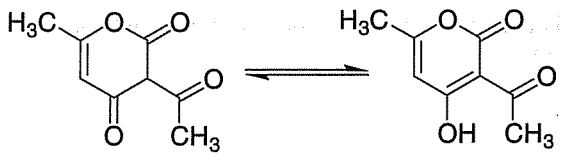
Inject reference solution (b). The test is not valid unless the resolution between the peaks due to *doxorubicin* and the *daunorubicin* is not less than 3 and the relative standard deviation for replicate injections is not more than 2.0 per cent. The relative retention time with reference to *daunorubicin* for *doxorubicin* is about 0.7.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{27}H_{29}NO_{10}$ in the injection.

**Storage**. Store protected from light.

# Dehydroacetic Acid



$C_8H_8O_4$ Mol. Wt. 168.1

Dehydroacetic Acid is a tautomeric mixture of 3-acetyl-6-methyl-2*H*-pyran-2,4(3*H*)-dione and 3-acetyl-4-hydroxy-6-methyl-2*H*-pyran-2-one

Dehydroacetic Acid contains not less than 98.0 per cent and not more than 100.5 per cent of $C_8H_8O_4$, calculated on the anhydrous basis.

**Category**. Pharmaceutical aid (antimicrobial preservative).

**Description**. A white or almost white, crystalline powder; odourless or practically odourless.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dehydroacetic acid RS*.

B. Melts at 109° to 111° (2.4.21).

## Tests

**Arsenic** (2.3.10). Heat gently 3.3 g with 2 ml of *nitric acid* and 0.5 ml of *sulphuric acid* in a long-necked flask until the first reaction has subsided, cool, add carefully and in small portions, 15 ml of *nitric acid* and 6 ml of *sulphuric acid*, taking care to



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avoid excessive foaming. Continue heating, adding further small portions of *nitric acid*, if necessary, until white fumes are evolved and the solution becomes colourless or almost colourless. Cool, add carefully 10 ml of *water*, evaporate until white fumes are evolved. Repeat the addition of *water* and evaporation until all the *nitric acid* has been removed, cool, dilute to 50 ml with *water* and add 10 ml of *stannated hydrochloric acid AsT*. The resulting solution complies with the limit test for arsenic (3 ppm).

**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

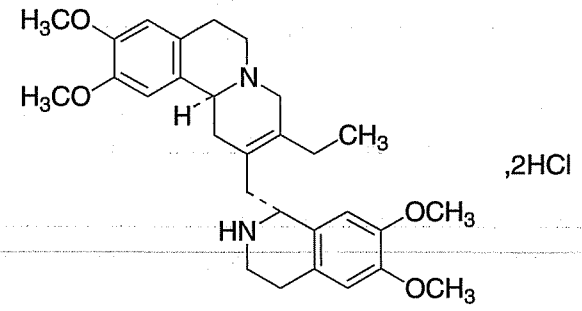
**Sulphated ash** (2.3.18). Not more than 0.1 per cent, determined on 2.0 g.

**Water** (2.3.43). Not more than 1.0 per cent, determined on 2.0 g.

**Assay**. Weigh accurately about 0.5 g, dissolve in 75 ml of previously neutralised *ethanol (95 per cent)*, add *phenolphthalein solution* and titrate with *0.1 M sodium hydroxide* to a pink end-point that persists for not less than 30 seconds.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.01681 g of $C_8H_8O_4$.

## Dehydroemetine Hydrochloride




$C_{29}H_{38}N_2O_4,2HCl$ Mol. Wt. 551.6

Dehydroemetine Hydrochloride is 2,3-didehydro-6′,7′,10, 11-tetramethoxyemetan dihydrochloride

Dehydroemetine Hydrochloride contains not less than 98.5 per cent and not more than 101.5 per cent of $C_{29}H_{38}N_2O_4,2HCl$, calculated on the dried basis.

**Category**. Antiamoebic.

**Dose**. By deep intramuscular injection, 60 to 90 mg daily.

**Description**. A white to yellowish-white, crystalline powder; odourless.

### Identification

A. When examined in the range 230 nm to 360 nm (2.4.7), a 0.005 per cent w/v solution in *0.1 M hydrochloric acid* shows an absorption maximum only at about 282 nm; absorbance at about 282 nm, about 0.62.

B. Sprinkle 5 mg on the surface of a 5 per cent w/v solution of *ammonium molybdate* in *sulphuric acid*; a green colour develops.

C. Gives reaction A of chlorides (2.3.1).

### Tests

**Appearance of solution**. A 5.0 per cent w/v solution is clear (2.4.1), and not more intensely coloured than reference solution YS5 or BYS6 (2.4.1).

**pH** (2.4.24). 3.5 to 5.0, determined in a 3.0 per cent w/v solution.

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

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 7.0 per cent, determined on 1.0 g by drying in an oven at 100° at a pressure not exceeding 0.7 kPa for 4 hours.

**Assay**. Weigh accurately about 0.4 g, dissolve in 40 ml of *anhydrous glacial acetic acid* and add 15 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02758 g of $C_{29}H_{38}N_2O_4,2HCl$.

**Storage**. Store protected from light.

## Dehydroemetine Injection

Dehydroemetine Hydrochloride Injection

Dehydroemetine Injection is a sterile solution of Dehydroemetine Hydrochloride in Water for Injections.

Dehydroemetine Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of dehydroemetine hydrochloride, $C_{29}H_{38}N_2O_4,2HCl$.

**Usual strength**. 30 mg per ml.

**Description**. A clear, almost colourless solution.

### Identification

A. To a volume containing 30 mg of Dehydroemetine Hydrochloride add 1 ml of *0.05 M iodine*; a yellowish-brown precipitate is produced.



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B. To a volume containing 15 mg of Dehydroemetine Hydrochloride add 1 ml of *potassium mercuri-iodide solution*; a white precipitate is produced.

### Tests

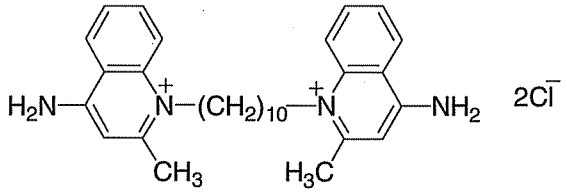
**pH** (2.4.24). 2.8 to 5.0.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. To an accurately measured volume containing 60 mg of Dehydroemetine Hydrochloride add sufficient *0.1 M hydrochloric acid* to produce 100.0 ml. Dilute 5.0 ml to 100.0 ml with *0.1 M hydrochloric acid*, mix and measure the absorbance of the resulting solution at the maximum at about 282 nm (2.4.7). Calculate the content of $C_{29}H_{38}N_2O_4,2HCl$ taking 123 as the specific absorbance at the maximum at about 282 nm.

**Storage**. Store protected from light, in single dose container.

## Dequalinium Chloride



$C_{30}H_{40}Cl_2N_4$ Mol. Wt. 527.7

Dequalinium Chloride is 4,4′-diamino-2,2′-dimethyl-*N,N′*-decamethylenedi(quinolinium chloride).

Dequalinium Chloride contains not less than 95.0 per cent and not more than 101.0 per cent of $C_{30}H_{40}Cl_2N_4$, calculated on the dried basis.

**Category**. Antiseptic.

**Description**. A creamy white powder; odourless or almost odourless.

### Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dequalinium chloride RS* or with the reference spectrum of dequalinium chloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.0008 per cent w/v solution shows absorption maxima at about 240 nm, 326 nm and 335 nm; absorbance at about 240 nm, about 0.65, at about 326 nm, about 0.4 and at about 335 nm, about 0.35.

C. Gives reaction A of chlorides (2.3.1).

### Tests

**Acidity or alkalinity**. Shake 0.1 g for 10 minutes with 100 ml of *carbon dioxide-free water* and add 0.5 ml of *bromocresol purple solution*. Not more than 0.2 ml of *0.1 M hydrochloric acid* or *0.1 M sodium hydroxide* is required to change the colour of the solution.

**Non-quaternised amines**. Not more than 1.0 per cent, calculated as 4-aminoquinaldine, $C_{10}H_{10}N_2$, on the dried basis and determined by the following method. Shake 1.0 g with 45 ml of *water* for 5 minutes, add 5 ml of *dilute nitric acid* and shake for 10 minutes. Filter through cotton wool. Transfer 20.0 ml of the filtrate to a separator, add 20 ml of *1 M sodium hydroxide*, extract with two quantities, each of 50 ml, of *ether*, washing each extract in turn with the same 5 ml of *water*, and then extract each ether extract successively with 20 ml, 20 ml and 5 ml of *1 M hydrochloric acid*. Combine the acid extracts, dilute to 50.0 ml with *1 M hydrochloric acid* and measure the absorbance of the resulting solution at the maximum at about 319 nm and 326.5 nm (2.4.7). The ratio of the absorbance at 319 nm to that at 326.5 nm is not less than 1.0. Calculate the percentage of $C_{10}H_{10}N_2$ from the expression 0.387a - 0.306b, where a and b are the specific absorbances at about 319 nm and 326.5 nm respectively.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

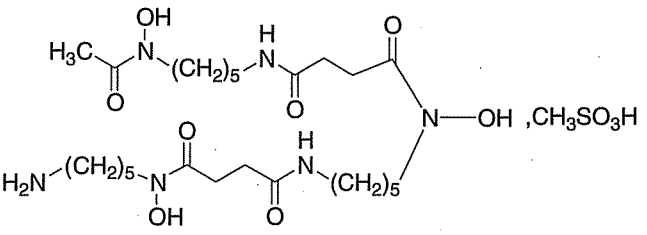
**Loss on drying** (2.4.19). Not more than 5.0 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours at a pressure not exceeding 0.7 kPa.

**Assay**. Weigh accurately about 0.5 g, dissolve in a mixture of 80 ml of *anhydrous glacial acetic acid* and 20 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02638 g of $C_{30}H_{40}Cl_2N_4$.

## Desferrioxamine Mesylate

Deferoxamine Mesylate; Deferoxamine Mesilate



$C_{25}H_{48}N_6O_8,CH_4SO_3$ Mol. Wt. 656.8

Desferrioxamine Mesylate is 30-amino-3,14,25-trihydroxy-3,9,14,20,25-pentaazatriacontane-2,10,13,21,24-pentaone methanesulphonate.



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Desferrioxamine Mesylate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{25}H_{48}N_6O_8,CH_4SO_3$, calculated on the anhydrous basis.

**Category**. Iron-chelating agent.

**Dose**. Orally, after gastric lavage, 5 to 10 g in 50 to 100 ml of liquid; by intramuscular injection, 1 to 2 g in 10 to 20 ml of Water for Injection every 3 to 12 hours; by continuous intravenous infusion, upto 15 mg per kg per hour with a maximum of 80 mg per kg in 24 hours.

**Description**. A white or almost white powder.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *desferrioxamine mesylate RS* or with the reference spectrum of desferrioxamine mesylate.

B. The titrated solution (solution A) obtained in the Assay is reddish brown. The colour is extracted by *benzyl alcohol* but not by *ether*.

C. Dissolve 5 mg in 5 ml of *water*, add 2 ml of a 0.5 per cent w/v solution of *tribasic sodium phosphate*, mix and then add 0.5 ml of a 2.5 per cent w/v solution of *sodium 1,2- naphthoquinone-4- sulphonate*; a blackish brown colour is produced.

D. Dissolve 0.1 g in 5 ml of *2 M hydrochloric acid* and add 1 ml of *barium chloride solution*; the solution remains clear. In a porcelain crucible mix 0.1 g with 1 g of *anhydrous sodium carbonate*, heat and ignite over a Bunsen flame. Allow to cool, dissolve the residue in 10 ml of *water* by heating if necessary and filter; the filtrate gives reaction A of sulphates (2.3.1).

## Tests

**Appearance of solution**. A 10.0 per cent w/v solution is not more opalescent than opalescence standard OS2 (2.4.1), and the absorbance of the solution at about 425 nm is not more than 0.10 (2.4.7).

**pH** (2.4.24). 3.7 to 5.5, determined in a freshly prepared 10.0 per cent w/v solution.

**Related substances**. Determine by liquid chromatography (2.4.14).

*NOTE—Prepare the solutions immediately before use.*

*Test solution*. Dissolve 50 mg of the substance under examination in 50.0 ml of the mobile phase.

*Reference solution (a)*. Dissolve 10 mg of *deferoxamine mesilate RS* in 10.0 ml of the mobile phase.

*Reference solution (b)*. Dilute 1.0 ml of the test solution in 25.0 ml of the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: dissolve 1.32 g of *ammonium phosphate* and 0.37 g of *sodium edetate* in 950 ml of *water*, adjust the pH to 2.8 with *orthophosphoric acid* and 55 volumes of *tetrahydrofuran*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 20 µl.

Inject reference solution (a). The test is not valid unless the resolution between the peaks with the relative retention time of about 0.8 and the principal peak is not less than 1.0.

Inject the test solution and reference solution (b). Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of secondary peak corresponding to deferoxamine mesilate impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (4.0 per cent). The sum of all the secondary peaks is not more than 1.75 times the area of the principal peak in the chromatogram obtained with reference solution (b) (7.0 per cent). Ignore any peak with an area less than 0.02 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.08 per cent).

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (10 ppm).

**Chlorides** (2.3.12). 0.75 g complies with the limit test for chlorides (330 ppm).

**Sulphates** (2.3.17). 0.25 g complies with the limit test for sulphates (600 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 2.0 per cent, determined on 1.0 g.

**Assay**. Weigh accurately about 50 mg of the substance under examination in 50.0 ml of *water*. To 2 ml of this solution, add 3 ml of *ferric chloride* solution prepared by dissolving 6.7 g of *ferric chloride* in 100 ml of 1 per cent v/v solution of *hydrochloric acid* and dilute to 25 ml with *water*. Measure the absorbance of the resulting solution at the maximum at about 485 nm (2.4.7). Calculate the content of $C_{25}H_{48}N_6O_8,CH_4O_3S$ from the absorbance obtained from a solution of known concentration of *deferoxamine mesylate RS*.

*Desferrioxamine Mesylate intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.*



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**Bacterial endotoxins** (2.2.3). Not more than 0.025 Endotoxin Unit per mg of desferrioxamine.

*Desferrioxamine Mesylate intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.*

**Sterility** (2.2.11). Complies with the test for sterility.

**Storage**. Store protected from light in a refrigerator (2° to 8°). Do not freeze. If the substance is sterile, store in sterile, air-tight, tamper-evident containers sealed so as to exclude micro-organisms.

**Labelling**. The label states where applicable, that the substance is sterile.

# Desferrioxamine Injection

Desferrioxamine Mesylate Injection; Desferrioxamine Injection

Desferrioxamine Mesylate Injection is a sterile material consisting of Desferrioxamine Mesylate with or without excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

*The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).*

**Storage**. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Desferrioxamine Mesylate Injection contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of desferrioxamine mesylate, $C_{25}H_{48}N_6O_8,CH_4SO_3$.

**Usual strength**. 0.5 g.

**Description**. A white or almost white powder; very hygroscopic.

*The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.*

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *desferrioxamine mesylate RS* or with the reference spectrum of desferrioxamine mesylate.

B. The titrated solution (solution A) obtained in the Assay is reddish brown. The colour is extracted by *benzyl alcohol* but not by *ether*.

## Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*NOTE—Prepare the solutions immediately before use.*

*Test solution*. Dissolve an accurately weighed quantity containing about 75 mg of Desferrioxamine Mesilate in 50.0 ml of the mobile phase.

*Reference solution (a)*. Dissolve 1.0 ml of the test solution in 25.0 ml of the mobile phase.

*Reference solution (b)*. A 0.15 per cent w/v solution of *desferrioxamine mesilate RS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (10 µm) (such as Nucleosil C18),
- mobile phase: a mixture of 95 volumes of solution containing 0.039 per cent w/v of *disodium edetate* and 0.139 per cent w/v of *ammonium phosphate* adjust the pH to 2.8 with *orthophosphoric acid* and 5.5 volumes of *tetrahydrofuran*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks with the relative retention time of about 0.8 and the principal peak is not less than 1.0.

Inject the test solution and reference solution (a). Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (4.0 per cent). The sum of all secondary peaks is not more than 1.75 times the area of the principal peak in the chromatogram obtained with reference solution (a) (7.0 per cent). Ignore any peak with an area less than 0.02 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.08 per cent).

**Bacterial endotoxins** (2.2.3). Not more than 0.025 Endotoxin Unit per mg of desferrioxamine.

**Sterility** (2.2.11). Complies with the test for sterility.

**Assay**. Weigh accurately a quantity of powder containing about 50 mg of desferrioxamine mesylate in 50.0 ml of *water*. To 2 ml of this solution, add 3 ml of *ferric chloride solution* prepared by dissolving 6.7 g of *ferric chloride* in 100 ml of 1 per cent v/v solution of *hydrochloric acid* and dilute to 25 ml with *water*. Measure the absorbance of the resulting solution



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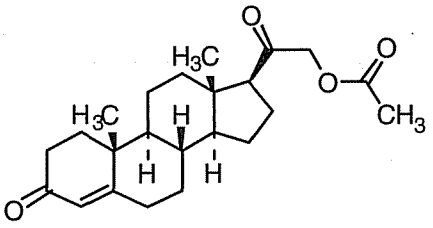


at the maximum at about 485 nm (2.4.7). Calculate the content of $C_{25}H_{48}N_6O_8.CH_4O_3S$ in the injection from the absorbance obtained from a solution of known concentration of *desferrioxamine mesylate RS*.

**Storage**. Store protected from light in a refrigerator (2° to 8°). Do not freeze.

## Desoxycortone Acetate

Desoxycorticosterone Acetate; Deoxycorticosterone Acetate; Deoxycortone Acetate



$C_{23}H_{32}O_4$ Mol. Wt. 372.5

Desoxycortone Acetate is 3,20-dioxo-4-pregnen-21-yl acetate.

Desoxycortone Acetate contains not less than 96.0 per cent and not more than 104.0 per cent of $C_{23}H_{32}O_4$, calculated on the dried basis.

**Category**. Adrenocortical steroid.

**Dose**. By intramuscular injection, 2 to 5 mg daily.

**Description**. A white or creamy-white, crystalline powder; odourless.

### Identification

*Test A may be omitted if tests B, C, D and E are carried out. Tests B, D and E may be omitted if tests A and C are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *desoxycortone acetate RS*.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *ethanol (95 per cent)* shows an absorption maximum at about 240 nm; absorbance at about 240 nm, 0.43 to 0.46.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Solvent mixture*. A mixture of 90 volumes of *acetone* and 10 volumes of *1,2-propanediol*.

*Mobile phase*. A mixture of equal volumes of *cyclohexane* and *light petroleum (40° to 60°)*.

*Test solution*. Dissolve 25 mg of the substance under examination in 10 ml of the solvent mixture.

*Reference solution (a)*. Dissolve 25 mg of *desoxycortone acetate RS* in 10 ml of the solvent mixture.

*Reference solution (b)*. Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the liquid to ascend to the top, remove the plate from the tank and allow the solvents to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v)*. Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

D. Dissolve 40 mg in 1 ml of *methanol*, warm and add 1 ml of *alkaline cupritartrate solution*; a red precipitate is formed.

E. Dissolve 5 mg in 0.5 ml of *methanol*, add 0.5 ml of *ammoniacal silver nitrate solution*; a black precipitate is slowly produced in the cold but is rapidly produced on warming.

### Tests

**Specific optical rotation** (2.4.22). +171.0° to +179.0°, determined in a 1.0 per cent w/v solution in *dioxan*.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 25 mg of the substance under examination in the mobile phase and dilute to 10 ml with the mobile phase.

*Reference solution (a)*. Dissolve 2 mg of *desoxycortone acetate RS* and 2 mg of *betamethasone 17-valerate RS* in the mobile phase and dilute to 200 ml with the mobile phase.

*Reference solution (b)*. Dilute 1 ml of the test solution to 200 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 350 ml of *water* and 600 ml of *acetonitrile*, allowed to equilibrate, diluted to 1000 ml with *water* and mixed again,



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- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Equilibrate the column with the mobile phase for about 30 minutes.

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (b) is not less than 50 per cent of the full scale of the recorder.

Inject reference solution (a). The retention times are: betamethasone 17-valerate, about 7.5 minutes and desoxycortone acetate about 9.5 minutes. The test is not valid unless the resolution between the peaks corresponding to betamethasone 17-valerate and desoxycortone acetate is at least 4.5. If necessary, adjust the concentration of acetonitrile in the mobile phase.

Inject the test solution and reference solution (b). Continue the chromatography for three times the retention time of the principal peak. In the chromatogram obtained with the test solution, the sum of the areas of all the peaks other than the principal peak is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.1 times that of the principal peak in the chromatogram obtained with reference solution (b).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.1 g, dissolve in sufficient *ethanol* to produce 100.0 ml. Dilute 2.0 ml of this solution to 100.0 ml with *ethanol* and mix. Measure the absorbance of the resulting solution (2.4.7) at the maximum at about 240 nm. Calculate the content of $C_{23}H_{32}O_4$ taking 450 as the specific absorbance at 240 nm.

**Storage**. Store protected from light.

# Desoxycortone Acetate Injection

Desoxycorticosterone Acetate Injection; Deoxycortone Acetate Injection

Desoxycortone Acetate Injection is a sterile solution of Desoxycortone Acetate in Ethyl Oleate or other suitable ester, in a suitable fixed oil, or in any mixture of these. It may contain suitable alcohols.

Desoxycortone Acetate Injection contains not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of desoxycortone acetate, $C_{23}H_{32}O_4$.

**Usual strength**. 5 mg per ml.

## Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 70 volumes of *n-heptane* and 30 volumes of *acetone*.

*Test solution*. Dilute the injection with *carbon tetrachloride* to give a solution containing 0.25 per cent w/v of Desoxycortone Acetate.

*Reference solution*. A 0.25 per cent w/v solution of *desoxycortone acetate RS* in *carbon tetrachloride*.

Apply to the plate 1 µl of each solution. After development, dry the plate in air until the odour of solvent is no longer detectable, spray with *ethanolic sulphuric acid (10 per cent v/v)*, heat at 105° for 30 minutes and examine in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution. Ignore any spots due to the vehicle.

## Tests

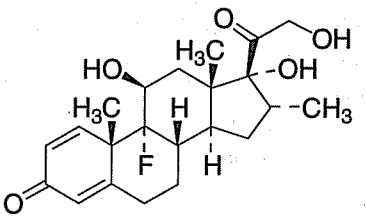
**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. To an accurately measured volume containing 10 mg of Desoxycortone Acetate add sufficient *ethanol* to produce 100.0 ml. Dilute 2.0 ml of this solution to 100.0 ml with *ethanol* and mix. Measure the absorbance of the resulting solution (2.4.7) at the maximum at about 240 nm. Calculate the content of $C_{23}H_{32}O_4$ taking 450 as the specific absorbance at 240 nm.

**Storage**. Store protected from light.

**Labelling**. The label states (1) the composition of the solvent; (2) that it is meant for intramuscular injection only; (3) that any sediment should be dissolved by warming before use.

# Dexamethasone




$C_{22}H_{29}FO_5$ Mol. Wt. 392.5

Dexamethasone is 9α-fluoro-11β,17α,21-trihydroxy-16α-methyl-1,4-pregnadiene-3,20-dione.

Dexamethasone contains not less than 96.0 per cent and not more than 104.0 per cent of $C_{22}H_{29}FO_5$, calculated on the dried basis.



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**Category**. Adrenocortical steroid (anti-inflammatory).

**Dose**. 500 µ g to 10 mg daily, in divided doses.

**Description**. White or almost white crystals or a crystalline powder; odourless.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests C and D may be omitted if tests A and B are carried out.*

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

B. Determine by thin-layer chromatography (2.4.17), coating the plate with a suitable silica gel containing a fluorescent indicator with an optimal intensity at about 254 nm.

*Mobile phase*. A mixture of 85 volumes of *ether*, 10 volumes of *toluene* and 5 volumes of *1-butanol* saturated with *water*.

*Solvent mixture*. A mixture of 9 volumes of *chloroform* and 1 volume of *methanol*.

*Test solution*. Dissolve 25 mg of the substance under examination in 10 ml of the solvent mixture.

*Reference solution (a)*. A 0.25 per cent w/v solution of *dexamethasone RS* in the solvent mixture.

*Reference solution (b)*. A solution containing 0.125 per cent w/v each of the substance under examination and *dexamethasone RS* in the solvent mixture.

*Reference solution (c)*. A solution containing 0.125 per cent w/v each of *dexamethasone RS* and *betamethasone RS* in the solvent mixture.

Apply to the plate 2 µl of each solution. After development, dry the plate in air, spray with *ethanolic sulphuric acid (20 per cent v/v)*, heat at 120° for 10 minutes or until spots appear, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution is similar in colour in day-light, fluorescence in ultraviolet light at 365 nm, in position and size to that in the chromatogram obtained with reference solution (a) and the chromatogram obtained with reference solution (b) shows only one spot. The test is not valid unless the chromatogram obtained with reference solution (c) shows two spots that are close to one another but separated.

C. Place 2 ml of a 0.01 per cent w/v solution in *ethanol* in a stoppered tube, add 10 ml of *phenylhydrazine-sulphuric acid solution*, mix, place in a water-bath at 60° for 20 minutes and cool immediately. Absorbance of the resulting solution at the maximum at about 419 nm, not less than 0.4 (2.4.7).

D. To 2 ml of *sulphuric acid* add 2 mg and shake to dissolve; a faint reddish brown colour is produced within 5 minutes. Add 10 ml of *water* and mix; the colour is discharged.

## Tests

**Specific optical rotation** (2.4.22). +75.0° to +80.0°, determined in a 1.0 per cent w/v solution in *dioxan*.

**Light absorption** (2.4.7). Absorbance of a 0.001 per cent w/v solution in *ethanol (95 per cent)* at the maximum at about 240 nm, 0.38 to 0.41.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. To 25 mg of the substance under examination add 1.5 ml of *acetonitrile* and 5 ml of mobile phase A. Mix with the aid of ultrasonic bath until the solids are completely dissolved and add sufficient of mobile phase A to produce 10 ml and mix well.

*Reference solution (a)*. Dissolve 2 mg of *dexamethasone RS* and 2 mg of *methylprednisolone RS* in sufficient of mobile phase A to produce 100 ml.

*Reference solution (b)*. Dilute 1 ml of the test solution to 100 ml with mobile phase A.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 45°,
- mobile phase: A. 250 volumes of *acetonitrile* and 700 volumes of *water* mixed, allowed to equilibrate and adjusted to 1000 volumes with *water* and mixed,
  B. *acetonitrile*,
- flow rate. 2 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

| Time (in min) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) | Comment |
|---|---|---|---|
| 0 | 100 | 0 | isocratic |
| 15 | 100→0 | 0→100 | begin linear gradient |
| 40 | 0 | 100 | end chromatogram, return to 100 A |
| 41 | 100 | 0 | begin equilibration with A |
| 46 | 100 | 0 | end equilibration, begin next chromatogram |

Equilibrate the column for at least 30 minutes with mobile phase B and then with mobile phase A for 5 minutes. For subsequent operations use the conditions described from 40 to 46 minutes.



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Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (b) is at least 50 per cent of the full scale of the recorder.

Inject reference solution (a). The retention times are; methylprednisolone about 11.5 minutes, and dexamethasone about 13 minutes. The test is not valid unless the resolution between the peaks corresponding to methylprednisolone and dexamethasone is at least 2.8; if necessary, adjust the concentration of acetonitrile in mobile phase A.

Inject mobile phase A as the blank, the test solution and reference solution (b). Record the chromatogram of the test solution for twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak other than the principal peak, is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent); the sum of the areas of all the peaks other than the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent). Ignore any peak due to the blank and any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° at a pressure not exceeding 0.7 kPa for 3 hours.

**Assay**. Weigh accurately about 0.1 g and dissolve in sufficient *ethanol* to produce 100.0 ml and mix. Dilute 2.0 ml of this solution to 100.0 ml with *ethanol* and mix well. Determine the absorbance of the resulting solution (2.4.7) at the maximum at about 238 nm. Calculate the content of $C_{22}H_{29}FO_5$ taking 394 as the specific absorbance at 238 nm.

**Storage**. Store protected from light.

# Dexamethasone Tablets

Dexamethasone Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of dexamethasone, $C_{22}H_{29}FO_5$.

**Usual strength**. 0.5 mg.

## Identification

Shake a quantity of the powdered tablets containing 20 mg of Dexamethasone with 50 ml of *chloroform* for 30 minutes, filter and evaporate the filtrate to dryness at 105° for 2 hours. The residue complies with the following tests.

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

B. Determine by thin-layer chromatography (2.4.17), coating the plate with a suitable silica gel containing a fluorescent indicator with an optimal intensity at about 254 nm.

*Solvent mixture*. A mixture of 9 volumes of *chloroform* and 1 volume of *methanol*.

*Mobile phase*. A mixture of 85 volumes of *ether*, 10 volumes of *toluene* and 5 volumes of *1-butanol* saturated with *water*.

*Test solution*. Dissolve 25 mg of the substance under examination in 10 ml of the solvent mixture.

*Reference solution (a)*. A 0.25 per cent w/v solution of *dexamethasone RS* in the solvent mixture.

*Reference solution (b)*. A solution containing 0.125 per cent w/v each of the substance under examination and *dexamethasone RS* in the solvent mixture.

*Reference solution (c)*. A solution containing 0.125 per cent w/v each of *dexamethasone RS* and *betamethasone RS* in the solvent mixture.

Apply to the plate 2 µl of each solution. After development, dry the plate in air, spray with *ethanolic sulphuric acid (20 per cent v/v)*, heat at 120° for 10 minutes or until spots appear, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution is similar in colour in day-light, fluorescence in ultraviolet light at 365 nm, in position and size to that in the chromatogram obtained with reference solution (a) and the chromatogram obtained with reference solution (b) shows only one spot. The test is not valid unless the chromatogram obtained with reference solution (c) shows two spots that are close to one another but separated.

C. To 2 ml of *sulphuric acid* add 2 mg and shake to dissolve; a faint reddish brown colour is produced within 5 minutes. Add 10 ml of *water* and mix; the colour is discharged.

## Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. To a weighed quantity of the tablets containing 2.5 mg of Dexamethasone add 10 ml of *acetonitrile*, mix with the aid of ultrasound and filter through a 0.45 µm filter. Dilute 4 ml of the filtrate to 10 ml with *water*.



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*Reference solution (a)*. Dilute 1 ml of the test solution to 100 ml with mobile phase A.

*Reference solution (b)*. Dissolve 2 mg of *dexamethasone RS* and 2 mg of *methylprednisolone RS* in mobile phase A and dilute to 100 ml with the same solvent.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Hypersil ODS),
- column temperature. 45°,
- mobile phase: A. 15 per cent v/v of *acetonitrile*,
  B. *acetonitrile*,
- flow rate. 2.5 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

| Time (in min) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) | Comment |
|---|---|---|---|
| 0 | 100 | 0 | isocratic |
| 15 | 100→0 | 0→100 | begin linear gradient |
| 40 | 0 | 100 | end chromatogram, return to 100 A |
| 41 | 100 | 0 | begin equilibration with A |
| 46 | 100 | 0 | end equilibration, begin next chromatogram |

Inject reference solution (b). When the chromatograms are recorded, the retention times are; methylprednisolone about 13 minutes, and dexamethasone about 16 minutes. The test is not valid unless the resolution between the peaks corresponding to methylprednisolone and dexamethasone is at least 2.8; if necessary, adjust the concentration of acetonitrile in mobile phase A.

Inject mobile phase A, the test solution and reference solution (a). In the chromatogram obtained with the test solution, the area of any secondary peak is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent); the sum of the areas of all secondary peaks is not greater than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak due to mobile phase A and any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

**Uniformity of content**. Comply with the test stated under Tablets.

*Test solution*. Finely crush one tablet, add sufficient quantity of a 0.002 per cent w/v solution of *hydrocortisone* in *methanol (50 per cent)* to produce a solution containing 0.0025 per cent w/v solution of Dexamethasone, shake for 10 minutes and filter through a glass-fibre filter paper (such as Whatman GF/C).

*Reference solution*. A solution containing 0.0025 per cent w/v of *dexamethasone RS* and 0.002 per cent w/v of *hydrocortisone* (internal standard) in *methanol (50 per cent)*.

Use chromatographic system as described under Assay.

Calculate the content of $C_{22}H_{29}FO_5$ in the tablet.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**—Determine by liquid chromatography (2.4.14), protected from light.

*Test solution (a)*. Weigh and powder 20 tablets. To a quantity of the powder containing about 2.5 mg of Dexamethasone add 20.0 ml of *methanol (50 per cent)*, shake for 20 minutes and filter through a glass-fibre filter paper (such as Whatman GF/C).

*Test solution (b)*. Prepare in the same manner as test solution (a) but use 20.0 ml of a 0.01 per cent w/v solution of *hydrocortisone* in *methanol (50 per cent)* in place of 20.0 ml of *methanol (50 per cent)*.

*Reference solution*. A solution containing 0.0125 per cent w/v of *dexamethasone RS* and 0.01 per cent w/v of *hydrocortisone* (internal standard) in *methanol (50 per cent)*.

Chromatographic system

- a stainless steel column 20 cm x 5 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as (Spherisorb ODS 1),
- mobile phase: a mixture of 53 volumes of *water* and 47 volumes of *methanol*,
- flow rate. 1.4 ml per minute,
- spectrophotometer set at 238 nm,
- injection volume. 20 µl.

Calculate the content of $C_{22}H_{29}FO_5$ in the tablets.

**Storage**. Store protected from light.

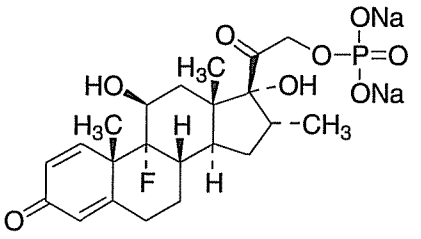


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# Dexamethasone Sodium Phosphate



$C_{22}H_{28}FNa_2O_8P$ Mol. Wt. 516.4

Dexamethasone Sodium Phosphate is disodium 9α-fluoro-11β,17α-dihydroxy-16α-methyl-3,20-dioxo-1,4-pregnadien-21-yl phosphate.

Dexamethasone Sodium Phosphate contains notless than 97.0 per cent and not more than 103.0 per cent of $C_{22}H_{28}FNa_2O_8P$, calculated on the anhydrous, and ethanol-free or solvent-free basis.

**Category**. Adrenocortical steroid (anti-inflammatory).

**Dose**. In the treatment of adrenal insufficiency, by intramuscular or slow intravenous injection or infusion, 0.5 to 10 mg. In shock, by intravenous injection or infusion, 2 to 6 mg per kg body weight, repeated if necessary after 2 to 6 hours. In cerebral oedema, by intravenous injection, 10 mg initially, then 4 mg by intramuscular injection every 6 hours as required for 2 to 10 days. (All doses are in terms of the equivalent amount of dexamethasone phosphate).

**Description**. A white or slightly yellow, crystalline powder; almost odourless; very hygroscopic. It shows polymorphism.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B and D may be omitted if Tests A and C are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dexamethasone sodium phosphate RS* or with the reference spectrum of dexamethasone sodium phosphate.

B. Dissolve 10 mg in 5 ml of *water* and dilute to 100 ml with *ethanol*. To 2 ml of the resulting solution in a glass-stoppered tube add 10 ml of *phenylhydrazine- sulphuric acid solution*, mix, heat in a water-bath at 60° for 20 minutes and cool immediately. Absorbance of the resulting solution at the maximum at about 419 nm, not less than 0.20 (2.4.7).

C. In the test for Related substances, the principal peak in the chromatogram obtained with reference solution (b) corresponds to the peak in the chromatogram obtained with reference solution (c).

D. Heat gently 40 mg with 2 ml of *sulphuric acid* until white fumes are evolved, add *nitric acid* dropwise until oxidation is complete and cool. Add 2 ml of *water*, heat until white fumes are evolved again, cool, add 10 ml of *water* and neutralise to *litmus paper* with *5 M ammonia*. The solution gives reaction A of sodium salts and reaction B of phosphates (2.3.1).

## Tests

**pH** (2.4.24). 7.5 to 9.5, determined in a 1.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). +75.0° to +83.0°, determined in a 1.0 per cent w/v solution.

**Inorganic phosphates**. Not more than 0.5 per cent, calculated as $PO_4$, determined by the following method. Weigh accurately about 25 mg, dissolve in 10 ml of *water*, add 4 ml of *dilute sulphuric acid*, 1 ml of *ammonium molybdate solution* and 2 ml of *methylaminophenol with sulphite solution* and allow to stand for 15 minutes. Add sufficient *water* to produce 25.0 ml, allow to stand for further 15 minutes and measure the absorbance of the resulting solution at the maximum at about 730 nm (2.4.7). Calculate the content of phosphate from a calibration curve prepared by treating suitable aliquots of a 0.00143 per cent w/v solution of *potassium dihydrogen phosphate* in a similar manner.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 25 mg of the substance under examination in the mobile phase and dilute to 10 ml with the mobile phase.

*Reference solution (a)*. Dissolve 2 mg of *dexamethasone sodium phosphate RS* and 2 mg of *betamethasone sodium phosphate RS* in the mobile phase and dilute to 100 ml with the mobile phase.

*Reference solution (b)*. Dilute 1 ml of the test solution to 100 ml with the mobile phase.

*Reference solution (c)*. A 0.0025 per cent w/v solution of *dexamethasone sodium phosphate RS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 1.360 g of *potassium dihydrogen phosphate* and 0.60 g of *hexylamine* allowed to stand for 10 minutes and then dissolved in 182.5 ml of *water* and 67.5 ml of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Equilibrate the column with the mobile phase for about 45 minutes.

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference



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solution (b) is at least 50 per cent of the full scale of the recorder.

Inject reference solution (a). The retention times are: betamethasone sodium phosphate, about 12.5 minutes and dexamethasone sodium phosphate about 14 minutes. The test is not valid unless the resolution between the peaks corresponding to betamethasone sodium phosphate and dexamethasone sodium phosphate is at least 2.2. If necessary, adjust the concentration of acetonitrile or increase the concentration of water in the mobile phase.

Inject the test solution and reference solution (b). Continue the chromatography for twice the retention time of the principal peak. In the chromatogram obtained with the test solution: the area of any peak other than the principal peak, is not greater than half the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent); the sum of the areas of all such peaks is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent). Ignore any peak with an area less than 0.05 times that of the principal peak in the chromatogram obtained with reference solution (b).

**Ethanol**. Not more than 8.0 per cent w/w, determined by gas chromatography (2.4.13).

*Internal standard*. A 1.0 per cent v/v solution of *1-propanol*.

*Test solution* (a). A 10.0 per cent w/v solution of the substance under examination in *water*.

*Test solution (b)*. A solution containing 10.0 per cent w/v of the substance under examination and 1.0 per cent v/v of the internal standard.

*Reference solution*. A solution containing 1.0 per cent v/v of *1-propanol* and 1.0 per cent v/v of *ethanol*. Adjust the content of ethanol to produce a peak of similar height to the corresponding peak in the chromatogram obtained with test solution (a).

Chromatographic system

- a glass column 1 m x 3.2 mm, packed with porous polymer beads (150 to 180 µm),
- temperature:
  column. 150°,
  inlet port. 250°,
  detector. 280°,
- flow rate. 30 ml per minute of the carrier gas.

Inject 2 µl of each solution.

Calculate the percentage w/w of ethanol assuming the weight per ml at 25° to be 0.787 g.

**Total ethanol and water**. Determine the content of water (2.3.43), using 0.2 g. Not more than 16.0 per cent w/w, calculated from the sum of the percentage of ethanol determined by the method described above and the percentage of water.

**Assay**. Weigh accurately about 0.1 g and dissolve in sufficient *water* to produce 100.0 ml. Dilute 5.0 ml of this solution to 250.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 240 nm (2.4.7). Calculate the content of $C_{22}H_{28}FNa_2O_8P$, taking 297 as the specific absorbance at 240 nm.

**Storage**. Store protected from light.

# Dexamethasone Injection

Dexamethasone Sodium Phosphate Injection

Dexamethasone Injection is a sterile solution of Dexamethasone Sodium Phosphate in Water for Injections.

Dexamethasone Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of dexamethasone phosphate, $C_{22}H_{30}FO_8P$.

**Usual strength**. The equivalent of 4 mg of dexamethasone phosphate per ml. (4.4 mg of dexamethasone sodium phosphate is approximately equivalent to 4 mg of dexamethasone phosphate).

## Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 50 volumes of *chloroform*, 50 volumes of *acetone* and 1 volume of *water*.

*Test solution*. Dilute a quantity of the injection containing about 5 mg of dexamethasone phosphate with 25 ml of *water* and extract with two quantities, each of 25 ml, of *dichloromethane*. Discard the dichloromethane each time and transfer the aqueous layer to a 50-ml volumetric flask, dilute to volume with *water* and mix. Pipette 5 ml into a 50-ml glass-stoppered tube and incubate at 37° for 45 minutes with 5 ml of *alkaline phosphatase solution*. Extract with 25 ml of *dichloromethane*, evaporate 15 ml of the dichloromethane extract to dryness and dissolve the residue in 1 ml of *dichloromethane*.

*Reference solution*. Dissolve 3 mg of *dexamethasone RS* in sufficient *dichloromethane* to produce 10 ml.

Apply to the plate 5 µl of each solution. After development, dry the plate in air until the odour of solvent is no longer detectable, spray with a 50 per cent w/v solution of *sulphuric acid*, heat at 105° until brown and black spots appear. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

## Tests

**pH** (2.4.24). 7.0 to 8.5.



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**Free dexamethasone**. Determine by liquid chromatography (2.4.14).

*Test solution.* Dilute a volume of the injection with the mobile phase to produce a solution containing the equivalent of 0.25 per cent w/v of dexamethasone phosphate.

*Reference solution (a).* A 0.00125 per cent w/v solution of *dexamethasone RS* in the mobile phase.

*Reference solution (b).* A solution containing 0.25 per cent w/v of *dexamethasone phosphate RS*, 0.01 per cent w/v of *propyl hydroxybenzoate* and 0.001 per cent w/v of *dexamethasone RS* in the mobile phase.

*Reference solution (c).* A solution containing 0.01 per cent w/v of *propyl hydroxybenzoate* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Hypersil ODS),
- mobile phase: a mixture of 1.360 g of *potassium dihydrogen phosphate* and 0.60 g of *hexylamine* allowed to stand for 10 minutes and then dissolved in 182.5 ml of *water* and 67.5 ml of *acetonitrile*, mixed and filtered,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Equilibrate the column with the mobile phase for about 45 minutes.

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (b) is at least 50 per cent of the full scale of the recorder.

Inject reference solution (b). The test is not valid unless the peak corresponding to dexamethasone is completely separated from the peaks due to dexamethasone phosphate and propyl hydroxybenzoate. If necessary, adjust the concentration of acetonitrile or increase the concentration of water in the mobile phase.

Inject the test solution and reference solution (a). Continue the chromatography for twice the retention time of the principal peak. In the chromatogram obtained with the test solution the area of the peak corresponding to dexamethasone is not greater than that of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent).

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Dilute an accurately measured volume of the injection containing about 8 mg of dexamethasone phosphate to 100.0 ml with the mobile phase and mix.

*Reference solution (a).* A 0.008 per cent w/v solution of *dexamethasone phosphate RS* in the mobile phase.

*Reference solution (b).* A solution containing 0.002 per cent w/v each of *dexamethasone phosphate RS* and *betamethasone sodium phosphate RS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Hypersil ODS),
- mobile phase: a mixture of 1.360 g of *potassium dihydrogen phosphate* and 0.6 g of *hexylamine* allowed to stand for 10 minutes and then dissolved in 182.5 ml of *water* and 67.5 ml of *acetonitrile*, mixed and filtered,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks corresponding to betamethasone sodium phosphate and dexamethasone phosphate is at least 2.2. If necessary, adjust the concentration of acetonitrile or increase the concentration of water in the mobile phase.

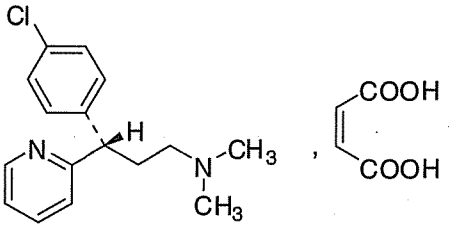
Inject alternately the test solution and reference solution (a).

Calculate the content of $C_{22}H_{30}FO_8P$ in the injection.

**Storage**. Store protected from light at a temperature not exceeding 30°.

**Labelling**. The label states the strength in terms of the equivalent amount of dexamethasone phosphate in a suitable dose-volume.

# Dexchlorpheniramine Maleate




$C_{16}H_{19}ClN_2,C_4H_4O_4$ Mol. Wt.390.9

Dexchlorpheniramine Maleate is (3*S*)-3-(4-chlorophenyl)-*N*,*N*-dimethyl-3-(pyridin-2-yl)propan-1-amine maleate.

Dexchlorpheniramine Maleate contains not less than 98.0 per cent and not more than 100.5 per cent of $C_{16}H_{19}ClN_2,C_4H_4O_4$, calculated on the dried basis.

**Category**. Antihistaminic.

**Description**. A white or almost white, crystalline powder.



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## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B and D may be omitted if tests A and C are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dexchlorpheniramine maleate RS* or with the reference spectrum of dexchlorpheniramine maleate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 3 volumes of *water*, 7 volumes of *anhydrous formic acid*, 20 volumes of *methanol* and 70 volumes of *di-isopropyl ether*.

*Test solution*. Dissolve 0.1 g of the substance under examination in *methanol* and dilute to 5.0 ml with the same solvent.

*Reference solution*. Dissolve 56 mg of *maleic acid* in *methanol* and dilute to 10 ml with the same solvent.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the spot in the chromatogram obtained with the reference solution.

C. To 0.15 g in a porcelain crucible, add 0.5 g of *anhydrous sodium carbonate*. Heat over an open flame for 10 minutes, allow to cool, take up the residue with 10 ml of *dilute nitric acid* and filter. To 1 ml of the filtrate add 1 ml of *water*; the resulting solution gives the reactions of chlorides (2.3.1).

D. Melting range (2.4.21). 110° to 115°.

## Tests

**Appearance of solution**. A 10.0 per cent w/v solution in *water* (solution A) is clear (2.4.1) and not more intensely coloured than reference solution BYS6 (2.4.1).

**pH** (2.4.24). 4.5 to 5.5, determined in a 1 per cent w/v solution in *water*.

**Specific optical rotation** (2.4.22). +22° to +23°, determined in solution A.

**Related substances**. Determine by gas chromatography (2.4.13).

*Test solution*. Dissolve 10 mg of the substance under examination in 1.0 ml of *dichloromethane*.

*Reference solution*. Dissolve 5 mg of *brompheniramine maleate RS* in 0.5 ml of *dichloromethane* and add 0.5 ml of the test solution. Dilute 0.5 ml of this solution to 50.0 ml with *dichloromethane*.

Chromatographic system
- a stainless steel column 2.3 m x 2 mm, packed with acid- and base- washed *silanised diatomaceous earth for gas chromatography* (135-175 µm) impregnated with 3 per cent w/w of a mixture of 50 per cent of poly(dimethyl)siloxane and 50 per cent of poly(diphenyl)siloxane,
- temperature:
  column. 205°,
  inlet port and detector 250°,
- a flame ionisation detector,
- flow rate. 20 ml per minute, using nitrogen as carrier gas.

Inject 1µl of the reference solution. The test is not valid unless the resolution between the peaks due to dexchlorpheniramine and brompheniramine is not less than 1.0.

Inject 1µl of the reference solution and the test solution. Run the chromatogram 2.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.8 times the area of the peak in the chromatogram obtained with the reference solution (0.4 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with the reference solution (1.0 per cent).

**Enantiomeric purity**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 10 mg of the substance under examination in 3 ml of *water*. Add a few drops of *ammonia* until an alkaline reaction is produced. Shake with 5 ml of *dichloromethane*. Separate the layers. Evaporate the lower, dichloromethane layer to an oily residue on a water-bath. Dissolve the oily residue in *2-propanol* and dilute to 10.0 ml with the same solvent.

*Reference solution (a)*. Dissolve 10 mg of *dexchlorpheniramine maleate RS* in 3 ml of *water*. Add a few drops of *ammonia* until an alkaline reaction is produced. Shake with 5 ml of *dichloromethane*. Separate the layers. Evaporate the lower, dichloromethane layer to an oily residue on a water-bath. Dissolve the oily residue in *2-propanol* and dilute to 10.0 ml with the same solvent.

*Reference solution (b)*. Dissolve 10 mg of *chlorpheniramine maleate RS* in 3 ml of *water*. Add a few drops of *ammonia* until an alkaline reaction is produced. Shake with 5 ml of *dichloromethane*. Separate the layers. Evaporate the lower, dichloromethane layer to an oily residue on a water-bath. Dissolve the oily residue in *2-propanol* and dilute to 10.0 ml with the same solvent.

*Reference solution (c)*. Dilute 1.0 ml of the test solution to 50 ml with *2-propanol*.



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Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with amylose derivative of silica,
- column temperature. 40°,
- mobile phase: a mixture of 0.3 volumes of *diethylamine*, 2.0 volumes of *2-propanol* and 98 volumes of *hexane*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to (R) –enantiomer and (S)-enantiomer is not less than 1.5.

Inject reference solution (c) and the test solution. In the chromatogram obtained with the test solution, the area of the peak corresponding to the (R)-enantiomer is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (2.0 per cent); the area of any other secondary peak is not more than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent).

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

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in vacuum oven at 65° for 4 hours.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Assay**.Weigh accurately about 0.15 g and dissolve in 25 ml of *anhydrous acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01954 g of $C_{20}H_{23}ClN_2O_4$.

**Storage**. Store protected from light.

# Dexchlorpheniramine Oral Solution

## Dexchlorpheniramine Maleate Oral Solution

Dexchlorpheniramine Oral Solution is a sterile material consisting of Dexchlorpheniramine with buffering agents and other excipients. It contains a suitable flavouring agent. It is filled in a sealed container.

*The oral solution is constituted by dispersing the contents of the sealed container in the specific volume of water just before use.*

Dexchlorpheniramine Oral Solution contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of dexchlorpheniramine, $C_{16}H_{19}ClN_2,C_4H_4O_4$.

**Usual strength**. 2 mg per 5 ml.

**Storage**. Store the constituted solution in a refrigerator (2° to 8°). Discard any unused portion after 30 days of reconstitution.

*The contents of the sealed container comply with the test requirements stated under Oral Liquids and with the following requirements.*

### Identification

A. In the Assay, the spectrum obtained in the test solution corresponds to the spectra obtained with the reference solution.

B. Evaporate the remaining extract from the Assay on a steam bath to a small volume, then transfer it to a smaller, more suitable vessel, and evaporate just to the point where hexane vapors are no longer perceptible. Transfer the oily residue, with the aid of four 3 ml portions of *dimethylformamide*, to a suitable glass-stoppered graduated cylinder, dilute with *dimethylformamide* to 15.0 ml, and mix, the optical rotation (2.4.22) of the solution is between +0.06° and +0.11°.

### Tests

**Other tests**. Complies with the tests stated under Oral Liquids.

**Assay**. Weigh accurately a measured volume of oral solution, containing about 40 mg of Dexchlorpheniramine Maleate, in 250 ml of *water*, adjust to pH 11.0 with *1 M sodium hydroxide*, cool. Extract with five 70 ml portions of *hexane*, combine the *hexane* extracts in a 500 ml separator, and wash the *hexane* with two 10 ml portions of *sodium hydroxide* (1 in 250). Extract the combined alkaline washings with two 20 ml portions of *hexane*, and add these extracts to the bulk of the alkali-washed *hexane*. Filter the hexane through a pledged of cotton that previously has been saturated with *hexane* into a 500 ml volumetric flask, rinse the separator with portions of solvent hexane, pass the rinsing through the filter to add to volume, and mix. Transfer 50.0 ml of this solution to a separator. Extract the *hexane* with two 40 ml portions of *dilute hydrochloric acid* (1 in 120), combine the acid extracts in a 100 ml volumetric flask, add *dilute hydrochloric acid* (1 in 120) to volume, and mix. Filter the solution into a glass-stoppered conical flask, discarding the first few ml of the filtrate. The concentration of *dexchlorpheniramine maleate RS* is about 40 µg per ml. Measure the absorbance of the resulting solution at the maximum at about 264 nm (2.4.7). Calculate the content of $C_{16}H_{19}ClN_2,C_4H_4O_4$ from the absorbance obtained by repeating the operation with *dexchlorpheniramine maleate RS* equivalent to 40 mg of dexchlorpheniramine prepared in the same manner.

Determine the weight per ml (2.4.29) of the oral solution and calculate the content of $C_{16}H_{19}ClN_2,C_4H_4O_4$, weight in volume.

**Storage**. Store protected from light and moisture, at a temperature not exceeding 30°.



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# Dexchlorpheniramine Tablets

Dexchlorpheniramine Maleate Tablets

Dexchlorpheniramine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of dexchlorpheniramine maleate, $C_{16}H_{19}ClN_2,C_4H_4O_4$.

**Usual strength**. 2 mg.

## Identification

A. Shake a quantity of powdered tablets containing about 50 mg of Dexchlorpheniramine Maleate with 25 ml of *0.01 M hydrochloric acid* for 10 minutes. Transfer the liquid to a separator, if necessary filtering it and washing the filter and the residue with several small portions of *water*. In a second separator dissolve 50 mg of *dexchlorpheniramine maleate RS* in 25 ml of *0.01 M hydrochloric acid*. Treat each solution as follows. Add 2 ml of *1 M sodium hydroxide* and 4 ml of *carbon disulphide*, and shake for 2 minutes. Centrifuge if necessary to clarify the lower phase, and filter it through a dry filter, collecting the filtrate in a small flask provided with a glass stopper. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dexchlorpheniramine maleate RS* or with the reference spectrum of dexchlorpheniramine maleate.

B. Shake a quantity of finely powdered tablets containing about 150 mg of Dexchlorpheniramine Maleate with 100 ml of *1 M acetic acid* for 10 minutes, filter through a sintered-glass funnel into a suitable vessel, adjust the filtrate with 10 per cent w/v *sodium hydroxide solution* to a pH of 11, and extract the solution with six 100-ml portions of hexane, filtering each hexane extract using suitable means to effect separation of the hexane layer from the aqueous layer. Concentrate the combined extracts on a steam bath to a small volume, transfer to a smaller, more suitable vessel, and evaporate just to the point where hexane vapours are no longer perceptible. Transfer the oily residue, with the aid of four 3 ml portions of *dimethylformamide*, to a suitable glass-stoppered graduated cylinder, dilute with *dimethylformamide* to 15.0 ml, mix, and centrifuge if necessary: the optical rotation of the solution is between +0.24° and +0.35°.

## Tests

**Dissolution** (2.5.2).

Apparatus No. 1,

Medium. 500 ml of *water*,

Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by gas chromatography (2.4.13).

*Internal standard solution.* Dissolve 9 mg of the *dexbrompheniramine maleate RS* in 100 ml of *water*.

*Test solution.* Dilute 15.0 ml of the dissolution medium to 50 ml with *water*, add 1.0 ml of internal standard solution and mix. Adjust to pH 11.0 with 50 per cent w/v solution of *sodium hydroxide*, add 3.0 ml of *hexane* and sonicate for 3 minutes, centrifuge, and use the clear supernatant hexane layer.

*Reference solution.* A 0.00125 per cent w/v solution of *dexchlorpheniramine maleate RS* in *water*. Dilute 5.0 ml of this solution to 10.0 ml with *water*, add 1.0 ml of internal standard solution and mix. Adjust to pH 11.0 with 50 per cent w/v solution of *sodium hydroxide*, add 3.0 ml of *hexane* and sonicate for 3 minutes, centrifuge, and use the clear supernatant hexane layer.

Chromatographic system

- a glass column 1.8 m x 2 mm, packed with 1.2 per cent phase G16 and 0.5 per cent potassium hydroxide support with S1AB,
- temperature:
  column. 205°,
  inlet port and detector at 250°,
- flow rate. 60 ml per minute using nitrogen as carrier gas.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to dexchlorpheniramine and dexbrompheniramine is not less than 1.9 and the relative standard deviation for replicate injections is not more than 2 per cent. The relative retention time with reference to dexbrompheniramine for dexchlorpheniramine is about 0.7.

Inject the reference solution and the test solution.

Calculate the content of $C_{16}H_{19}ClN_2,C_4H_4O_4$ in the tablet.

D. Not less than 75 per cent of the stated amount of $C_{16}H_{19}ClN_2,C_4H_4O_4$.

**Uniformity of content**. Comply with the test stated under Tablets.

To 1 tablet, add 10 ml of *water* and shake, adjust to pH 11.0 with 10 per cent w/v solution of *sodium hydroxide*, Extract the mixture with two 7.5 ml portions of *hexane*, and combine the extracts in a separator. Extract the *hexane* with three 5.0 ml portions of *dilute hydrochloric acid* (1 in 120), combining the acid extracts in a 25 ml volumetric flask. Add dilute *hydrochloric acid* (1 in 120) to volume, and mix. Measure the absorbance of the resulting solution at the maximum at about 264 nm (2.4.7). Calculate the content of $C_{16}H_{19}ClN_2,C_4H_4O_4$ from the absorbance obtained by repeating the operation with *dexchlorpheniramine maleate RS* equivalent to 40 mg of dexchlorpheniramine prepared in the same manner.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 8.0 mg of Dexchlorpheniramine in 50 ml of *water*, adjusted to pH 11.0 with *sodium hydroxide* ( 1 in 10). Extract the mixture with two



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75 ml portions of *hexane*, and combine the extracts in a separator. Extract the *hexane* with three 50 ml portions of *dilute hydrochloric acid* (1 in 120), combining the acid extracts in a 200 ml volumetric flask. Add dilute *hydrochloric acid* (1 in 120) to volume, and mix. Measure the absorbance of the resulting solution at the maximum at about 264 nm (2.4.7). Calculate the content of $C_{16}H_{19}ClN_2,C_4H_4O_4$ from the absorbance obtained by repeating the operation with *dexchlorpheniramine maleate RS* equivalent to 40 mg of dexchlorpheniramine prepared in the same manner.

**Storage**. Store protected from moisture.

## Dextran 40 Injection

Dextran 40 Intravenous Infusion

Dextran 40 Injection is a sterile solution, in Dextrose Injection or in Sodium Chloride Injection, of dextrans of average molecular weight of about 40,000, derived from the dextrans produced by the fermentation of sucrose by means of a certain strain of *Leuconostoc mesenteroides*. The dextrans are polymers of dextrose in which the linkages between the dextrose units are almost entirely of the α-1→6 type.

Dextran 40 Injection contains not less than 9.0 per cent and not more than 11.0 per cent w/v of dextrans.

**Category**. Plasma substitute.

**Description**. An almost colourless, slightly viscous solution.

### Tests

**pH** (2.4.24). 3.5 to 6.5 for solutions in Dextrose Injection; 4.0 to 7.0 for solutions in Sodium Chloride Injection.

**Molecular size**. For solutions in Dextrose Injection, before proceeding with tests A, B and C add 4 volumes of *ethanol (95 per cent)*, centrifuge and dissolve the residue in a volume of Sodium Chloride Injection sufficient to restore the original volume.

A. Determine the viscosity (2.4.28) ratios by Method A, using size C U-tube viscometer at 37°, of solutions in *saline solution* containing about 3.5, 2.5, 1.5 and 0.75 per cent w/v of dextrans, accurately determined. For each solution, plot (viscosity ratio – 1.00)/concentration (in per cent w/v) against concentration (in per cent w/v). The intercept on the viscosity axis of the straight line joining the points represents the intrinsic viscosity; the intrinsic viscosity is 0.16 to 0.20.

B. Place in each of five stoppered flasks 100 ml of a solution in *saline solution* containing 6 per cent w/v of dextrans and add slowly, with continuous stirring, sufficient *ethanol* to produce a faint cloudiness (about 45 ml is usually required). Add 0.5, 1.0, 1.5, 2.0 and 2.5 ml of *ethanol* to the separate flasks, stopper the flasks and immerse in a water-bath at about 35° with occasional shaking until clear solutions are obtained. Transfer the flasks to a water-bath maintained at 25.0° ± 0.1° and allow to stand overnight or until two clear liquid phases are formed. Reject the supernatant liquids, dissolve separately the syrupy residues in sufficient *saline solution* to produce 25.0 ml, remove the ethanol by evaporation at a pressure of about 2 kPa, dilute to 25.0 ml with *water* and determine the optical rotation (2.4.22). From the optical rotations calculate the amount of dextrans precipitated as described in the Assay. Choose that fraction containing as nearly as possible but not more than 10 per cent of the dextrans present in the injection and determine its intrinsic viscosity by the method described under test A; the intrinsic viscosity is not more than 0.27.

C. Place in each of four stoppered flasks 100 ml of a solution in *saline solution* containing 6 per cent w/v of dextrans and add slowly, with continuous stirring, 80, 90, 100 and 110 ml respectively of *ethanol*. Stopper the flasks, transfer to a water-bath maintained at 25.0° ± 0.1° and allow to stand overnight or until two clear liquid phases are formed. Separate the supernatant solution from the syrupy residues. Remove the *ethanol* from each supernatant solution separately by evaporation at a pressure of 2 kPa, dialyse in cellophane tubing against *water* to remove sodium chloride, adjust the volume to 25.0 ml with *water*, add sufficient *sodium chloride* to produce solutions containing 0.9 per cent w/v and determine the optical rotation (2.4.22). From the optical rotations, calculate the amounts of dextrans present as described in the Assay. Choose that fraction containing as nearly as possible but not more than 10 per cent of the dextrans present in the injection and determine the intrinsic viscosity by the method in test A above; the intrinsic viscosity is not less than 0.08.

**Content of dextrose**. For solutions in Dextrose Injection, 4.5 to 5.5 per cent w/v, determined by the following method. Dilute 15.0 ml to 50.0 ml with *water*. To 5.0 ml in a stoppered flask add 25 ml of a buffer solution containing 14.3 per cent w/v of *sodium carbonate* and 4.0 per cent w/v of *potassium iodide* and 25.0 ml of *0.05 M iodine*. Stopper the flask and allow to stand for exactly 30 minutes at 20°, add 30 ml of *dilute hydrochloric acid* and titrate immediately with *0.1 M sodium thiosulphate*. Repeat the operation beginning at the words "add 25 ml of a buffer solution..." but using 5 ml of *water* in place of 5 ml of the preparation under examination. The difference between the titrations represents the amount of iodine required to oxidise the dextrose.

1 ml of *0.05 M iodine* is equivalent to a 0.00901 g of dextrose.

**Acetone**. To 10 ml add sufficient *ammonium sulphate* to give a saturated solution, add 1 ml of *sodium nitroprusside solution* and 5 ml of *strong ammonia solution*, and allow to stand for 10 minutes. Any purple colour produced is not more intense than that produced by treating in the same manner 10 ml of a 0.02 per cent v/v solution of *acetone*.



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**Ethanol**. Distil 100 ml, collect the first 45 ml of distillate and dilute to 50 ml with *water*. Mix 10 ml of *0.0167 M potassium dichromate* and 10 ml of *sulphuric acid* in a stoppered boiling tube, immediately add 5 ml of the distillate, mix, stopper the tube, and allow to stand for 5 minutes. Transfer to a 500-ml flask, dilute to about 300 ml with *carbon dioxide-free water*, add 2 g of *potassium iodide* and 1 ml of a 10 per cent w/v solution of *potassium thiocyanate*, allow to stand for 5 minutes and titrate the liberated iodine with *0.1 M sodium thiosulphate* using *starch solution*, added towards the end of the titration, as indicator. Repeat the determination beginning at the words "Mix 10 ml of *0.0167 M potassium dichromate*.." but using 5 ml of *water* in place of 5 ml of the distillate. The difference between the titrations is not more than 4.2 ml.

**Heavy metals** (2.3.13). To 4.0 ml add 5 ml of *dilute acetic acid* and sufficient *water* to produce 25.0 ml. The resulting solution complies with the limit test for heavy metals, Method A (5 ppm).

**Nitrogen** (2.3.30). Determine by Method B, using 50 ml. For solutions in Dextrose Injection, use 30 ml of *nitrogen-free sulphuric acid*. For solutions in Sodium Chloride Injection use 20 ml of *nitrogen-free sulphuric acid*.

Not more than 0.35 ml of *0.05 M sulphuric acid* is required.

**Sulphated ash**. Titrate 25 ml with *0.1 M silver nitrate* using *potassium chromate solution* as indicator. Deduct the theoretical value of the sulphated ash due to the sodium chloride present.

1 ml of *0.1 M silver nitrate* is equivalent to 0.007102 g of sulphated ash (0.05 per cent w/v).

**Foreign protein**. Inject 0.5 ml on three occasions at intervals of 2 days into the peritoneal cavity of each of six healthy guinea-pigs weighing not less than 250 g, which have not previously been treated with any material that will interfere with the test. Inject 0.2 ml intravenously into each of the three guinea-pigs 14 days after the first intra-peritoneal injection, and into each of the other three guinea-pigs 21 days after the first intra-peritoneal injection. Observe the guinea-pigs for 30 minutes after each intravenous injection and again 24 hours later; the animals exhibit no signs of anaphylaxis such as coughing, bristling of hair or respiratory distress.

**Bacterial endotoxins** (2.2.3). Not more than 1.25 Endotoxin Units per ml.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Infusions).

**Assay**. *For solutions in Dextrose Injection* — Add a drop of *dilute ammonia solution* to the required volume and determine the optical rotation (2.4.22). Calculate the content of dextrans from the following expression $0.5076(\alpha - 0.528D)$, where $\alpha$ is the observed angular rotation and D the content of dextrose per cent w/v, determined in the test for Content of dextrose.

*For solutions in Sodium Chloride Injection* — Measure the optical rotation (2.4.22), and multiply the value obtained by 0.5076.

**Storage**. Store at a temperature not exceeding 30°. The injection should not be exposed to undue fluctuations of temperature.

**Labelling**. The label states (1) the strength as the percentage w/v of dextrans; (2) the name of the solvent; (3) the strain of *Leuconostoc mesenteroides* used; (4) that the injection should not be used if it is cloudy or if a deposit is present.

## Dextran 70 Injection

Dextran 70 Intravenous Infusion

Dextran 70 Injection is a sterile solution, in Dextrose Injection or in Sodium Chloride Injection, of dextrans of average molecular weight about 70,000, derived from the dextrans produced by the fermentation of sucrose by means of a certain strain of *Leuconostoc mesenteroides*. The dextrans are polymers of dextrose in which the linkages between the dextrose units are almost entirely of the $\alpha$-1→6 type.

Dextran 70 Injection contains not less than 5.5 per cent and not more than 6.5 per cent w/v of dextrans.

**Category**. Plasma substitute.

**Description**. An almost colourless, slightly viscous solution.

### Tests

**pH** (2.4.24). 3.5 to 6.5 for solutions in Dextrose Injection; 5.0 to 7.0 for solutions in Sodium Chloride Injection.

**Molecular size**. For solutions in Dextrose Injection, before proceeding with tests A, B and C, add 4 volumes of *ethanol (95 per cent)*, centrifuge and dissolve the residue in sufficient Sodium Chloride Injection to restore the original volume.

A. Determine the viscosity (2.4.28) ratios by Method A, using a size C U-tube viscometer at 37°, of solutions in saline solution containing about 3.5, 2.5, 1.5 and 0.75 per cent w/v of dextrans, accurately determined. For each solution, plot (viscosity ratio −1.00)/concentration (in percentage w/v) against concentration (in per cent w/v). The intercept on the viscosity ratio axis of a straight line through the points represents the intrinsic viscosity. The intrinsic viscosity is 0.22 to 0.27.

B. Place 100 ml in each of five stoppered flasks and adjust the temperature to 25.0 ± 0.1°. Maintaining this temperature, add slowly with continuous stirring sufficient *ethanol* to produce a faint cloudiness (about 45 ml). To the separate flasks add 0.5, 1.0, 1.5, 2.0 and 2.5 ml of *ethanol*, stopper the flasks and



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immerse in a water-bath at about 35°, shaking occasionally, until clear solutions are obtained. Transfer the flasks to a water-bath maintained at 25.0° ± 0.1° and allow to stand overnight or until two clear liquid phases are formed. Discard the supernatant liquids, dissolve separately the syrupy residues in sufficient saline solution to produce 25.0 ml, remove the ethanol by evaporation at a pressure of 2 kPa, dilute to 25.0 ml with *water* and determine the optical rotation (2.4.22). From the optical rotations, calculate the amounts of dextrans precipitated as described in the Assay. Choose that fraction containing as nearly as possible but not more than 10 per cent of the dextrans present in the injection and determine its intrinsic viscosity by the method described under test A using a U-tube viscometer (size A). The intrinsic viscosity is not more than 0.36.

C. Place in each of four stoppered flasks 100 ml of a solution in saline solution containing 6 per cent w/v of dextrans and add slowly, with continuous stirring, 80, 90, 100 and 110 ml respectively of *ethanol*. Stopper the flasks, transfer to a water-bath maintained at 25.0° ± 0.1° and allow to stand overnight or until two clear liquid phases are formed. Separate the supernatant solutions from the syrupy residues. Remove the ethanol from each supernatant solution separately by evaporation at a pressure of 2 kPa, dialyse in cellophane tubing against water to remove sodium chloride, adjust the volume to 25.0 ml with *water*, add sufficient *sodium chloride* to produce solutions containing 0.9 per cent w/v of sodium chloride and determine the optical rotation (2.4.22). From the optical rotations, calculate the amounts of dextrans present as described in the Assay. Choose that fraction containing as nearly as possible but not more than 10 per cent of the dextrans present in the injection and determine the intrinsic viscosity by the method in test A. The intrinsic viscosity is not less than 0.13.

**Content of dextrose** *(if present)*. For solutions in Dextrose Injection, between 4.5 and 5.5 per cent w/v, determined by the following method. Dilute 15.0 ml to 50.0 ml with *water*. To 5.0 ml in a stoppered flask, add 25 ml of a buffer solution containing 14.3 per cent w/v of *sodium carbonate* and 4.0 per cent w/v of *potassium iodide*, and 25.0 ml of *0.05 M iodine*. Stopper the flask, allow to stand for exactly 30 minutes at 20°, add 35 ml of *dilute hydrochloric acid* and titrate immediately with *0.1 M sodium thiosulphate*. Repeat the operation using 5 ml of *water* and beginning at the words 'add 25 ml of a buffer solution.....'. The difference between the titrations represents the amount of iodine required to oxidise the dextrose.

1 ml of *0.05 M iodine* is equivalent to 0.00901 g of $C_6H_{12}O_6$.

**Acetone**. To 10 ml add sufficient *ammonium sulphate* to give a saturated solution, add 1 ml of *sodium nitroprusside solution* and 5 ml of *strong ammonia solution*, and allow to stand for 10 minutes. Any purple colour produced is not more intense than that produced by treating in the same manner 10 ml of a 0.02 per cent v/v solution of *acetone* in the same solvent.

**Content of sodium chloride** *(if present)*. For solutions in Sodium Chloride Injection, 0.81 to 0.99 per cent w/v, determined by the following method. Titrate an accurately measured volume containing 0.1 g of sodium chloride with *0.1 M silver nitrate* using *potassium chromate solution* as indicator.

1 ml of *0.1 M silver nitrate* is equivalent to 0.005844 g of NaCl.

**5-Hydroxymethylfurfural and related substances** *(if dextrose is present)*. Dilute a volume containing 1.0 g of Dextrose to 500.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 284 nm; absorbance at about 284 nm, not more than 0.25 (2.4.7).

**Ethanol**. Distil 100 ml, collect the first 45 ml of the distillate and dilute to 50 ml with *water*. Mix 10 ml of *0.0167 M potassium dichromate* and 10 ml of *sulphuric acid* in a stoppered boiling tube, immediately add 5.0 ml of the distillate, mix, stopper the tube, and allow to stand for 5 minutes. Transfer to a 500-ml flask, dilute to about 300 ml with *carbon dioxide-free water*, add 2 g of *potassium iodide* and 1 ml of 10 per cent w/v solution of *potassium thiocyanate*, allow to stand for 5 minutes and titrate the liberated iodine with *0.1 M sodium thiosulphate* using *starch solution*, added towards the end of the titration, as indicator. Repeat the determination beginning at the words 'Mix 10 ml of *0.0167 M potassium dichromate*.....' but using 5.0 ml of *water* in place of 5.0 ml of the distillate. The difference between the titrations is not more than 4.2 ml.

**Heavy metals** (2.3.13). Not more than 5 ppm, determined by Method A, on 4.0 ml to which 5 ml of *dilute acetic acid* and sufficient *water* are added to produce 25.0 ml.

**Nitrogen**. Carry out Method B for the determination of nitrogen (2.3.30), using 50.0 ml. For solutions in Dextrose Injection, use 30 ml of *nitrogen-free sulphuric acid*. For solutions in Sodium Chloride Injection, use 20 ml of *nitrogen-free sulphuric acid*. Not more than 0.35 ml of *0.05 M sulphuric acid* is required.

**Sulphated ash** (2.3.18). Not more than 0.05 per cent w/v, determined by titrating 25.0 ml with *0.1 M silver nitrate* using *potassium chromate solution* as indicator and deducting the theoretical value of the sulphated ash present due to the sodium chloride.

1 ml of *0.1 M silver nitrate* is equivalent to 0.007102 g of Sulphated ash.

**Foreign protein**. Inject 0.5 ml on three occasions at intervals of 2 days into the peritoneal cavity of each of six healthy guinea-pigs, weighing not less than 250 g, which have not previously been treated with any material, which will interfere with the test. Inject 0.2 ml intravenously into each of the three guinea-pigs 14 days after the first intra-peritoneal injection, and into each of the other three guinea-pigs 21 days after the



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first intraperitoneal injection. Observe the guinea-pigs for 30 minutes after each intravenous injection and again 24 hours later; the animals exhibit no signs of anaphylaxis such as coughing, bristling of hair or respiratory distress.

**Bacterial endotoxins** (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Infusions).

**Assay**. *For solutions in Dextrose Injection* — Add a drop of *dilute ammonia solution* to 25.0 ml of the injection under examination and determine the optical rotation (2.4.22). Calculate the content of dextrans from the expression $0.5076(\alpha - 0.528)$ D, where $\alpha$ is the observed angular rotation and D the content of dextrose, in per cent w/v, determined in the test for Content of dextrose.

*For solutions in Sodium Chloride Injection* — Measure the optical rotation (2.4.22), and multiply the value obtained by 0.5076.

**Storage**. Store in single dose containers in a cool place. The injection should not be exposed to undue fluctuations of temperature.

**Labelling**. The label states (1) the strength as the percentage w/v of dextrans; (2) the name of the solvent; (3) the strain of *Leuconostoc mesenteroides* used; (4) that the injection should not be used if it is cloudy or if a deposit is present.

# Dextran 110 Injection

Dextran 110 Intravenous Infusion

Dextran 110 Injection is a sterile solution, in Dextrose Injection or in Sodium Chloride Injection, of dextrans of average molecular weight of about 110,000, derived from the dextrans produced by the fermentation of sucrose by means of a certain strain of *Leuconostoc mesenteroides*. The dextrans are polymers of dextrose in which the linkages between the dextrose units are almost entirely of the $\alpha$-1→6 type.

Dextran 110 Injection contains not less than 5.5 per cent and not more than 6.5 per cent w/v of dextrans.

**Category**. Plasma substitute.

**Description**. An almost colourless, slightly viscous solution.

## Tests

**pH** (2.4.24). 3.5 to 6.5 for solutions in Dextrose Injection; 5.0 to 7.0 for solutions in Sodium Chloride Injection.

**Molecular size**. For solutions in Dextrose Injection, before proceeding with tests A and B, add 4 volumes of *ethanol (95 per cent)*, centrifuge and dissolve the residue in a volume of *Sodium Chloride Injection* sufficient to restore the original volume.

A. Determine the viscosity (2.4.28) ratios by Method A, using size C U-tube viscometer at 37°, of solutions in *saline solution* containing about 2.0, 1.0, 0.5 and 0.25 per cent w/v of dextrans, accurately determined. For each solution, plot (viscosity ratio - 1.00)/concentration (in per cent w/v) against concentration (in per cent w/v). The intercept on the viscosity ratio axis of the straight line joining the points represents the intrinsic viscosity; the intrinsic viscosity is 0.27 to 0.32.

B. Place 100 ml in each of five stoppered flasks and adjust the temperature to 25.0° ± 0.1°. With precautions to maintain this temperature, add slowly with continuous stirring sufficient *ethanol* to produce a faint cloudiness (about 45 ml is usually required). Add 0.5, 1.0, 1.5, 2.0 and 2.5 ml of *ethanol* to the separate flasks, stopper the flasks and immerse in a water-bath at about 35° with occasional shaking until clear solutions are obtained. Transfer the flasks to a water-bath maintained at 25.0° ± 0.1° and allow to stand overnight or until two clear liquid phases are formed. Reject the supernatant liquids, dissolve separately the syrupy residues in sufficient *saline solution* to produce 25.0 ml, remove the *ethanol* by evaporation at a pressure of about 2 kPa, dilute to 25.0 ml with *water* and determine the optical rotation (2.4.22). From the optical rotations calculate the amount of dextrans precipitated as described in the Assay. Choose that fraction containing as nearly as possible but not more than 10 per cent of the dextrans present in the injection and determine its intrinsic viscosity by the method described under test A; the intrinsic viscosity is not more than 0.40.

**Content of dextrose**. For solutions in Dextrose Injection, between 4.5 and 5.5 per cent w/v determined by the following method. Dilute 15.0 ml to 50.0 ml with *water*. To 5.0 ml in a stoppered flask add 25 ml of a buffer solution containing 14.3 per cent *w/v of sodium carbonate* and 4.0 per cent *w/v* of *potassium iodide* and 25.0 ml of *0.05 M iodine*. Stopper the flask and allow to stand for exactly 30 minutes at 20°, add 30 ml of *dilute hydrochloric acid* and titrate immediately with *0.1 M sodium thiosulphate*. Repeat the operation beginning at the words "add 25 ml of a buffer solution..." but using 5 ml of *water* in place of 5 ml of the preparation under examination. The difference between the titrations represents the amount of iodine required to oxidise the dextrose.

1 ml of *0.05 M iodine* is equivalent to a 0.00901 g of dextrose.

**Acetone**. To 10 ml add sufficient *ammonium sulphate* to give a saturated solution, add 1 ml of *sodium nitroprusside solution* and 5 ml of *strong ammonia solution*, and allow to stand for 10 minutes. Any purple colour produced is not more intense than that produced by treating in the same manner 10 ml of a 0.02 per cent v/v solution of *acetone*.



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**Ethanol**. Distil 100 ml, collect the first 45 ml of distillate and dilute to 50 ml with *water*. Mix 10 ml of *0.0167 M potassium dichromate* and 10 ml of *sulphuric acid* in a stoppered boiling tube, immediately add 5 ml of the distillate, mix, stopper the tube, and allow to stand for 5 minutes. Transfer to a 500-ml flask, dilute to about 300 ml with *carbon dioxide-free water*, add 2 g of *potassium iodide* and 1 ml of a 10 per cent w/v solution of *potassium thiocyanate*, allow to stand for 5 minutes and titrate the liberated iodine with *0.1 M sodium thiosulphate* using *starch solution*, added towards the end of the titration, as indicator. Repeat the determination beginning at the words "Mix 10 ml of *0.0167 M potassium dichromate..*" but using 5 ml of *water* in place of 5 ml of the distillate. The difference between the titrations is not more than 4.2 ml.

**Heavy metals** (2.3.13). To 4.0 ml add 5 ml of *dilute acetic acid* and sufficient *water* to produce 25.0 ml. The resulting solution complies with the limit test for heavy metals, Method A (5 ppm).

**Nitrogen** (2.3.30). Determine by Method B, using 50 ml. For solutions in Dextrose Injection, use 30 ml of *nitrogen-free sulphuric acid.* For solutions in Sodium Chloride Injection use 20 ml of *nitrogen-free sulphuric acid.*

Not more than 0.35 ml of *0.05 M sulphuric acid* is required.

**Sulphated ash**. Titrate 25 ml with *0.1 M silver nitrate* using *potassium chromate solution* as indicator. Deduct the theoretical value of the sulphated ash due to the sodium chloride present.

1 ml of *0.1 M silver nitrate* is equivalent to 0.007102 g of sulphated ash (0.05 per cent w/v).

**Foreign protein**. Inject 0.5 ml on three occasions at intervals of 2 days into the peritoneal cavity of each of six healthy guinea-pigs weighing not less than 250 g that have not previously been treated with any material that will interfere with the test. Inject 0.2 ml intravenously into each of the three guinea-pigs 14 days after the first intra-peritoneal injection, and into each of the other three guinea-pigs 21 days after the first intra-peritoneal injection. Observe the guinea-pigs for 30 minutes after each intravenous injection and again 24 hours later; the animals exhibit no signs of anaphylaxis such as coughing, bristling of hair or respiratory distress.

**Bacterial endotoxins** (2.2.3). Not more than 1.25 Endotoxin Units per ml.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Infusions).

**Assay**. *For solutions in Dextrose Injection* — Add a drop of *dilute ammonia solution* to the required volume and determine the optical rotation (2.4.22). Calculate the content of dextrans from the following expression $0.5076(\alpha - 0.528D)$, where $\alpha$ is the observed angular rotation and D the content of dextrose per cent w/v, determined in the test for Content of dextrose.

*For solutions in Sodium Chloride Injection* — Measure the optical rotation (2.4.22), and multiply the value obtained by 0.5076.

**Storage**. Store at a temperature not exceeding 30°. The injection should not be exposed to undue fluctuations of temperature.

**Labelling**. The label states (1) the strength as the percentage w/v of dextrans; (2) the name of the solvent; (3) the strain of *Leuconostoc mesenteroides* used; (4) that the injection should not be used if it is cloudy or if a deposit is present.

# Dextrin

Dextrin is starch partially hydrolysed by heat with or without the aid of suitable acids and buffers.

**Category**. Pharmaceutical aid (tablet excipient).

**Description**. A white or pale yellow powder; odour, slight and characteristic.

## Identification

A. *Microscopic characteristics* - Granules have similar appearance to the starch from which the dextrin has been prepared. In dextrin prepared from maize starch many of the granules show concentric striations and in dextrin prepared from potato starch concentric striations are not clearly visible; the hilum may be bicleft and some of the granules may be distorted.

B. Boil 1 g in 50 ml of *water*, cool. To 5 ml of the cloudy suspension, add a drop of *iodine solution* and mix; a purple colour is produced.

C. To 5 ml of the suspension produced in test B add 2 ml of *2 M sodium hydroxide*, mix, add dropwise with shaking 0.5 ml of *cupric sulphate solution* and boil; a red precipitate is produced.

## Tests

**Acidity**. Add 10 g to 100 ml of *ethanol (70 per cent)*, previously neutralised to *phenolphthalein solution*, shake for 1 hour, filter and titrate 50 ml of the filtrate with *0.1 M sodium hydroxide* using *phenolphthalein solution* as indicator. Not more than 1 ml of *0.1 M sodium hydroxide* is required to change the colour of the solution.

**Heavy metals** (2.3.13). 0.5 g complies with the limit test for heavy metals, Method A (40 ppm).

**Chlorides** (2.3.12). Dissolve 2.5 g in 50 ml of boiling *water*, cool, dilute to 100 ml with *water* and filter. 5 ml of the filtrate



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diluted to 15 ml complies with the limit test for chlorides (0.2 per cent).

**Ethanol-soluble substances**. Not more than 1 per cent, determined by the following method. Boil under a reflux condenser 1 g with 20 ml of *ethanol (95 per cent)* for 5 minutes and filter while hot. Evaporate 10 ml of the filtrate on a water-bath, dry the residue at 105° and weigh.

**Protein**. Not more than 0.5 per cent, determined by the following method. Carry out Method A for the determination of nitrogen (2.3.30), using 5 g, accurately weighed, and 30 ml of *nitrogen-free sulphuric acid*. Calculate the content of protein by multiplying the percentage of nitrogen in the substance under examination by 6.25.

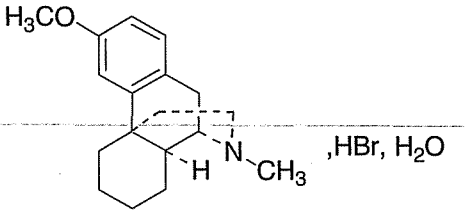
**Reducing substances**. Not more than 10 per cent, calculated as dextrose, $C_6H_{12}O_6$, determined by the following method. Weigh accurately a quantity containing 2 g of the dried substance, add 100 ml of *water*, shake for 30 minutes, dilute to 200.0 ml with *water* and filter. To 10 ml of *cupri-tartaric solution* add 20.0 ml of the filtrate, mix and heat at a rate such that the solution is brought to boil in 3 minutes. Boil for a further 2 minutes and cool quickly. Add 5 ml of a 30 per cent w/v solution of *potassium iodide* and 10 ml of *1 M sulphuric acid*, mix and titrate immediately with *0.1 M sodium thiosulphate* using *starch solution*, added towards the end of the titration, as indicator. Repeat the procedure using 20.0 ml of a 0.1 per cent w/v solution of *dextrose* in place of the filtrate beginning at the words "To 10 ml of...". Carry out a blank titration using 20 ml of *water* in place of 20.0 ml of the sample filtrate. The titre obtained with the sample filtrate is not greater than the titre obtained with the dextrose solution.

**Ash** (2.3.19). Not more than 1 per cent, determined on 1.0 g.

**Loss on drying** (2.4.19). Not more than 12 per cent, determined on 1.0 g by drying in an oven at 110°.

**Storage**. Store protected from moisture.

# Dextromethorphan Hydrobromide




$C_{18}H_{25}NO,HBr,H_2O$ Mol. Wt. 370.3

Dextromethorphan Hydrobromide is *ent*-3-methoxy-9a-methylmorphinan hydrobromide monohydrate.

Dextromethorphan Hydrobromide contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{18}H_{25}NO,HBr$ calculated on the anhydrous basis.

**Category**. Cough suppressant.

**Dose**. 10 to 30 mg every 4 to 8 hours.

**Description**. An almost white crystalline powder.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dextromethorphan hydrobromide RS* or with the reference spectrum of dextromethorphan hydrobromide.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.01 per cent w/v solution in *0.1 M hydrochloric acid* shows an absorption maximum only at about 278 nm.

C. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (c).

D. Gives the reaction of bromides (2.3.1).

## Tests

**Appearance of solution**. A 5.0 per cent w/v solution in *ethanol (95 per cent)* is clear (2.4.1), and colourless (2.4.1).

**Acidity or alkalinity**. Dissolve 0.4 g in *carbon dioxide-free water* with gentle heat, cool and dilute to 20 ml with the same solvent. Add 0.1 ml of *methyl red solution* and 0.2 ml of *0.01 M sodium hydroxide*. The solution is yellow and not more than 0.4 ml of *0.01 M hydrochloric acid* is required to change the colour to red.

**Specific optical rotation** (2.4.22). +28.0° to +30.0°, determined in a 2.0 per cent w/v solution in *0.1 M hydrochloric acid*.

***N,N*-Dimethylaniline**. Dissolve 0.5 g in 20 ml *water* with the help of gentle heat on a water-bath, cool and add 2 ml of *2 M acetic acid*, 1 ml of a 1 per cent w/v solution of *sodium nitrite* and sufficient *water* to produce 25 ml. The resulting solution is not more intensely coloured than that obtained by treating at the same time and in the same manner a solution containing 5 µg of *N,N-dimethylaniline* in 20 ml of *water*.

**Related substances**. Determine by liquid chromatography (2.4.14).

*NOTE—Use freshly prepared solutions.*

*Test solution*. Dissolve 10 mg of the substance under examination in 10.0 ml of the mobile phase.

*Reference solution*. Dilute 1.0 ml of the test solution to 200 ml with the mobile phase.



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Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: dissolve 3.11 g of *docusate sodium* in a mixture of 400 ml of *water* and 600 ml of *acetonitrile*. Add 0.56 g of *ammonium nitrate*, adjust the pH to 2.0 with *glacial acetic acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume. 20 µl.

Inject the test solution and reference solution (b). Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). The area of one such peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent), and sum of all other secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). 4.0 to 5.5 per cent, determined on 0.2 g.

**Assay**. Weigh accurately about 0.3 g and dissolve in 20 ml of *ethanol (95 per cent)* and titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25).

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.03523 g of $C_{18}H_{25}NO,HBr$.

**Storage**. Store protected from light.

## Dextromethorphan Hydrobromide Syrup

Dextromethorphan Hydrobromide Syrup is a solution of Dextromethorphan Hydrobromide in a suitable flavoured vehicle.

Dextromethorphan Hydrobromide Syrup contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of dextromethorphan hydrobromide, $C_{18}H_{25}NO,HBr,H_2O$.

**Usual strength**. 13.5 mg in 5 ml.

### Identification

A. To 50 ml, add 20 ml of *water*, 5 ml of *2.5 M sodium hydroxide* and extract with three quantities, each of 40 ml of *hexane*, collect the *hexane layer* and filter through *anhydrous sodium sulphate* placed over absorbent cotton wetted with *hexane*. Evaporate the combined extracts at 50° under nitrogen to dryness, dissolve and dilute the residue in 10 ml of *chloroform*; the solution is dextrorotatory (2.4.22). Retain the chloroform solution for test B.

B. Evaporate the chloroform solution obtained from test A on a water-bath to dryness, dissolve the residue in 2 ml of *1 M sulphuric acid* and add 1 ml of a solution prepared freshly by dissolving 700 mg of *mercuric nitrate* in 4 ml of *water*, adding 100 mg of *sodium nitrate*, mixing and filtering; the solution gives no colour, but after heating, a yellow to red colour develops in about 15 minutes.

### Tests

**Other tests**. Complies with the tests stated under Oral Liquids.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dilute a volume of the syrup containing about 10 mg of Dextromethorphan Hydrobromide to 100.0 ml with *water*.

*Reference solution*. A 0.01 per cent w/v solution of *dextromethorphan hydrobromide RS* in *water*.

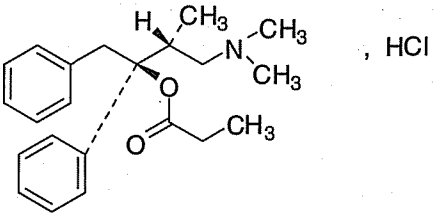
Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a filtered and degassed solution of *0.007 M ammonium nitrate* in a mixture of 70 volumes of *acetonitrile and* 30 volumes of *water* adjusted to pH 3.4 with *glacial acetic acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume. 20 µl.

Calculate the content of $C_{18}H_{25}NO,HBr,H_2O$ in the syrup.

**Storage**. Store protected from light.

## Dextropropoxyphene Hydrochloride

Propoxyphene Hydrochloride




$C_{22}H_{29}NO_2,HCl$ Mol. Wt. 375.9

Dextropropoxyphene Hydrochloride is (1*S*,2*R*)-1-benzyl-3-dimethylamino-2-methyl-1-phenylpropyl propionate hydrochloride.



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Dextropropoxyphene Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{22}H_{29}NO_2$,HCl, calculated on the dried basis.

**Category**. Analgesic.

**Description**. A white or almost white, crystalline powder.

## Identification

*Test A may be omitted if tests B and C are carried out. Test B may be omitted if tests A and C are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dextropropoxyphene hydrochloride RS* or with the reference spectrum of dextropropoxyphene hydrochloride.

B. When examined in the range 220 nm to 360 nm (2.4.7), a 0.05 per cent w/v solution in *0.01 M hydrochloric acid*, shows three absorption maxima, at 252 nm, 257 nm and 263 nm and two shoulders, at 240 nm and 246 nm. The ratio of the absorbance at the maximum at 257 nm to that at 252 nm is 1.22 to 1.28. The ratio of the absorbance at the maximum at 257 nm to that at 263 nm is 1.29 to 1.35.

C. Solution A gives the reaction (A) of chlorides (2.3.1).

## Tests

**Appearance of solution**. A 5.0 per cent w/v solution in *carbon dioxide-free water* (solution A) is clear (2.4.1) and colourless (2.4.1).

**Acidity or alkalinity**. Dilute 10 ml of solution A to 25 ml with *carbon dioxide-free water*. To 10 ml of this solution, add 0.1 ml of *methyl red solution* and 0.2 ml of *0.01 M sodium hydroxide*, the solution is yellow. Add 0.4 ml of *0.01 M hydrochloric acid*, the solution is red.

**Specific optical rotation** (2.4.22). +52° to +57°, determined in a 1.0 per cent w/v solution in *water*.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 50 mg of the substance under examination in 10.0 ml of the mobile phase.

*Reference solution (a)*. Dilute 0.5 ml of the test solution to 100.0 ml with the mobile phase.

*Reference solution (b)*. Dissolve 50 mg of the substance under examination in 2.5 ml of *2 M ethanolic potassium hydroxide*. Add 2.5 ml of *water* and boil under a reflux condenser for 30 minutes. Add 2.5 ml of *dilute hydrochloric acid* and dilute to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 12.5 cm x 4.6 mm, packed with silica gel (5 µm),
- mobile phase: a mixture of 5 volumes of *0.2 M phosphate buffer solution pH 7.5*, 8.4 volumes of *tetrahydrofuran*, 35 volumes of *methanol* and 51.6 volumes of 0.09 per cent w/v solution of *cetyltrimethylammonium bromide* in *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 20 µl.

Equilibrate the chromatographic system by passage of the mobile phase for 16 hours.

Inject reference solution (a) and (b). Run the chromatogram twice the retention time of the principal peak. The test is not valid unless the chromatogram obtained with reference solution (a) shows signal-to-noise ratio of the principal peak is not less than 5 and the chromatogram obtained with reference solution (b) shows two peaks with a resolution of not less than 2.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent).

**Heavy metals** (2.3.13). 12 ml of solution A complies with the limit test for heavy metals, Method D (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.27 g, dissolve in 60 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03759 g of $C_{22}H_{30}ClNO_2$.

**Storage**. Store protected from light.

# Dextropropoxyphene Capsules

Dextropropoxyphene Capsules contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of dextropropoxyphene, $C_{22}H_{29}NO_2$.

**Usual strength**. 65 mg.

## Identification

Shake a quantity of the content of capsules containing about 0.15 g of Dextropropoxyphene with 5 ml of *chloroform* and filter. The filtrate complies with the following tests.

A. Evaporate 3 ml to dryness and dry the residue at 105° for 1 hour. On the resiude, determine by infrared absorption



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spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dextropropoxyphene napsilate RS* or with the reference spectrum of dextropropoxyphene napsilate.

B. Evaporate 0.05 ml in a porcelain dish and streak the spot with 5 per cent v/v solution of *formaldehyde* in *sulphuric acid;* a purple colour is produced.

C. Evaporate 0.4 ml on a piece of filter paper and burn the residue by the method for oxygen-flask combustion (2.3.34), using 5 ml of *1.25 M sodium hydroxide* as the absorbing liquid. When the process is complete, dilute the liquid to 25 ml with *water*. To 5 ml of the solution, add 1 ml of *hydrogen peroxide solution (100 vol)* and 1 ml of *1 M hydrochloric acid*, mix and add 0.05 ml of *barium chloride solution*. The solution becomes turbid.

## Tests

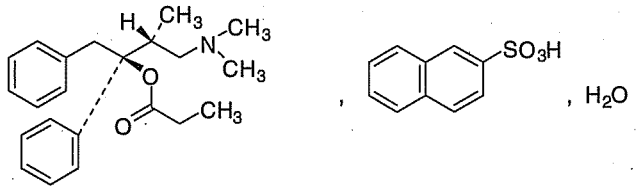
**Other tests**. Comply with the tests stated under Capsules.

**Assay**. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 0.5 g of dextropropoxyphene with 25 ml of *chloroform* and filter through absorbent cotton, washing the flask and filter with small quantities of *chloroform*. Add to the combined filtrates a mixture of 50 ml of *water* and 5 ml of *5 M sodium hydroxide*. Shake, allow the layers to separate and wash the chloroform extract with 25 ml of *water*. Extract the aqueous layer with five 25 ml quantities of *chloroform*, washing each extract with the 25 ml of *water* and adding it to the original extract. Dry the combined extracts with *anhydrous sodium sulphate*, evaporate to about 3 ml on a water-bath in a current of air, remove from the water-bath and allow to evaporate to dryness at room temperature. Titrate with *0.1 M perchloric acid*, using a few drops of *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03395 g of $C_{22}H_{29}NO_2$.

**Labelling**. The lable states the quantity of active ingredient in terms of the equivalent amount of dextropropoxyphene.

# Dextropropoxyphene Napsilate



$C_{22}H_{29}NO_2,C_{10}H_8O_3S,H_2O$ Mol. Wt. 565.8

Dextropropoxyphene Napsylate is (1*S*,2*R*)-1-benzyl-3-dimethylamino-2-methyl-1-phenylpropyl propionate naphthalene-2-sulphonate monohydrate.

Dextropropoxyphene Napsilate contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{22}H_{29}NO_2,C_{10}H_8O_3S$, calculated on the anhydrous basis.

**Category**. Analgesic.

**Description**. A white powder. It exhibits polymorphism.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dextropropoxyphene napsilate RS* or with the reference spectrum of dextropropoxyphene napsilate.

B. Dissolve 25 mg in 5 ml of *dichloromethane*, evaporate 0.05 ml of the solution in a porcelain dish and streak the spot with 5 per cent v/v solution of *formaldehyde solution* in *sulphuric acid*; a purple colour is produced.

C. Determine by the oxygen-flask method (2.3.34), burning 0.02 g, using 5 ml of *1.25 M sodium hydroxide* as the absorbing liquid. When the process is complete, dilute the liquid to 25 ml with *water*. To 5 ml of the solution so obtained add 1 ml of *hydrogen peroxide solution (100 vol)* and 1 ml of *1 M hydrochloric acid*, mix and add 0.05 ml of *barium chloride solution*. The solution becomes turbid.

## Tests

**Specific optical rotation** (2.4.22). +26.0° to +31.0°, determined in a 5.0 per cent w/v solution in *ethanol (95 per cent)*.

**Related substances**. Determine by gas chromatography (2.4.13).

*Solution A*. Dissolve 10 mg of *triphenylamine* (internal standard) in 50 ml of *dichloromethane*.

*Test solution (a)*. Dissolve 0.3 g of the substance under examination in 5 ml of *dichloromethane*, add 10 ml of *water*, 2 ml of *1.25 M sodium hydroxide* and 15 ml of *dichloromethane* and shake. Extract the aqueous layer with two 20 ml quantities of *dichloromethane*. Shake the combined dichloromethane extracts with 5 g of *anhydrous sodium sulphate*, filter and evaporate to dryness at a temperature not exceeding 40° using a rotary evaporator. Dissolve the residue in 10 ml of *dichloromethane*.

*Test solution (b)*. Prepare in the same manner as test solution (a) but add 5 ml of solution A to the initial solution of the substance under examination.

*Reference solution*. Add 5 ml of solution A, 10 ml of *water*, 2 ml of *1.25 M sodium hydroxide* and 15 ml of *dichloromethane* to 5 ml of a solution in *dichloromethane* containing 0.022 per cent w/v of *(1S,2R)-1-benzyl-3-dimethylamino-2-methyl-1-phenylpropyl acetate RS* and 0.02 per cent w/v of *4-dimethylamino-3-methyl-1,2-diphenylbutan-2-ol hydrochloride RS* and shake. Extract the aqueous layer with two 20 ml quantities of *dichloromethane*. Shake the combined



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dichloromethane extracts with 5 g of *anhydrous sodium sulphate,* filter and evaporate to dryness at a temperature not exceeding 40° using a rotary evaporator. Dissolve the residue in 10 ml of *dichloromethane.*

Chromatographic system
- a glass column 0.6 m x 3 mm, packed with acid-washed, silanised diatomaceous support (100 to 120 mesh) coated with 3 per cent w/w of dimethyl silicone fluid,
- temperature:
  column 160°,
  inlet port and detector at 150°,
- flow rate. 60 ml per minute of the carrier gas.

The peaks, other than the solvent peak, in the chromatogram obtained with the reference solution are due, in order of emergence, to (a) the internal standard, (b) (1*S*,2*R*)-1-benzyl-3-dimethylamino-2-methyl-1-phenylpropyl acetate and (c) 4-dimethylamino-3-methyl-1,2-diphenylbutan-2-ol hydrochloride. In the chromatogram obtained with test solution (b) the ratio of the area of any peak corresponding to (b) to that of the peak due to (a) and the ratio of the area of any peak corresponding to (c) to that of the peak due to (a) are not more than the corresponding ratios in the chromatogram obtained with reference solution (0.67 per cent each).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

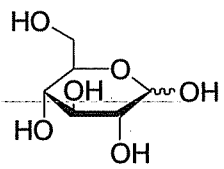
**Water** (2.3.43). 3.0 to 5.0 per cent, determined on 0.5 g.

**Assay**. Weigh accurately about 0.75 g add 50 ml of *water*, swirl to disperse, add 5 ml of *5 M sodium hydroxide* and extract with five 25 ml quantities of *dichloromethane*, washing each extract with the same 20 ml of *water*. Dry the combined extracts with *anhydrous sodium sulphate*, evaporate on water-bath in a current of air and allow to evaporate to dryness at room temperature. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.05478 g of $C_{22}H_{29}NO_2,C_{10}H_8O_3S$.

# Dextrose

Glucose; D-Glucose




$C_6H_{12}O_6$ Mol. Wt. 180.2 (anhydrous)

$C_6H_{12}O_6,H_2O$ Mol. Wt. 198.2 (monohydrate)

Dextrose is D-(+)-glucopyranose or D-(+)-glucopyranose monohydrate.

**Category**. Nutrient; fluid replenisher.

**Description**. A white crystalline powder.

## Identification

A. When heated, it melts, swells up and burns, and an odour of burnt sugar is perceptible.

B. Dissolve 0.1 g in 10 ml of *water*, add 3 ml of *potassium cupri-tartrate solution*; the solution is blue and clear. Heat to boiling; a copious red precipitate is formed.

## Tests

**Appearance and odour of solution**. Dissolve 10.0 g in 15 ml of *water*. The solution is clear (2.4.1), not more intensely coloured than reference solution BYS7 (2.4.1), and is odourless.

**Acidity or alkalinity**. Dissolve 6.0 g in 25 ml of *carbon dioxide-free water* and add 0.3 ml of *phenolphthalein solution*. The solution is colourless and not more than 0.15 ml of *0.1 M sodium hydroxide* is required to change the colour of the solution to pink.

**Specific optical rotation** (2.4.22). +52.5° to +53.3°, determined in a solution prepared by dissolving 10.0 g in 80 ml of *water,* adding 0.2 ml of *5 M ammonia*, mixing well, allowing to stand for 30 minutes and diluting to 100.0 ml with *water.*

**Arsenic** (2.3.10). Dissolve 10.0 g in 50 ml of *water* and add 10 ml of *stannated hydrochloric acid AsT*. The resulting solution complies with the limit test for arsenic (1 ppm).

**Heavy metals** (2.3.13). A solution prepared by dissolving 4.0 g in 10 ml of *water*, 2 ml of *dilute acetic acid* and sufficient *water* to produce 25 ml, complies with the limit test for heavy metals, Method A (5 ppm).

**Chlorides** (2.3.12). 20 ml of a 10.0 per cent w/v solution (solution A) complies with the limit test for chlorides (125 ppm).

**Sulphates** (2.3.17). 7.5 ml of solution A diluted to 15 ml with *distill water* complies with the limit test for sulphates (200 ppm).

**Sulphite**. Dissolve 5.0 g in 40 ml of *water*, add 2.0 ml of *0.1 M sodium hydroxide* and dilute to 50.0 ml with *water*. To 10.0 ml of the solution add 1 ml of a 31 per cent w/v solution of *hydrochloric acid*, 2.0 ml of *decolorised magenta solution* and 2.0 ml of a 0.5 per cent v/v solution of *formaldehyde solution*. Allow to stand for 30 minutes and measure the absorbance of the resulting solution at the maximum at about 583 nm (2.4.7). The absorbance is not more than that of a standard prepared in the following manner. Dissolve 76 mg of *sodium metabisulphite* in sufficient *water* to produce 50.0 ml, dilute 5.0 ml of this solution to 100.0 ml and to 3.0 ml of resulting solution add 4.0 ml of *0.1 M sodium hydroxide* and dilute to 100.0 ml with *water*. Immediately treat 10.0 ml of the resulting solution in the same manner as the test solution beginning at



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the words "add 1 ml of a 31 per cent w/v solution.....". Use as the blank for both measurements a solution prepared in the same manner using 10 ml of *water.*

**Barium**. To 10 ml of solution A add 1 ml of *1 M sulphuric acid.* Examine exactly after 1 hour; any opalescence in the solution is not more intense than that in a mixture of 10 ml of solution A and 1 ml of *water.*

**Foreign sugars, soluble starch and dextrins**. Boil 1.0 g in 30 ml of *ethanol (90 per cent)* to dissolve. The appearance of the solution does not change on cooling.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent determined by the following method. Dissolve 5.0 g in 5 ml of *water*, add 2 ml of *sulphuric acid,* evaporate to dryness and ignite to constant weight. If necessary, repeat the heating with the *sulphuric acid.*

**Water** (2.3.43). Not more than 1.0 per cent (anhydrous form) and 7.0 to 9.5 per cent (monohydrate), determined on 0.5 g.

*Dextrose intended for use in the manufacture of parenteral preparations complies with the following additional requirement.*

**Bacterial endotoxins** (2.2.3). Not more than 0.5 Endotoxin Unit per ml of a 5 per cent w/v solution in *water for injections.*

**Storage**. Store protected from moisture.

**Labelling**. The label states (1) whether the material is monohydrate or is in the anhydrous form; (2) whether or not the contents are intended for use in the manufacture of parenteral preparations.

## Dextrose Injection

Dextrose Intravenous Infusion; Glucose Intravenous Infusion

Dextrose Injection is a sterile solution of Dextrose in Water for Injections.

Dextrose Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of anhydrous dextrose, $C_6H_{12}O_6$.

**Usual strengths**. 5, 10, 25, and 50 per cent w/v.

**Description**. A clear, colourless solution. Solutions containing 20.0 per cent w/v or more of Dextrose may be not more than faintly straw-coloured.

### Identification

A. To 1 ml add 0.05 ml of *potassium cupri-tartrate solution*; the solution is blue and clear. Heat to boiling; a copious red precipitate is formed.

B. The solution prepared as directed in the Assay is dextrorotatory.

### Tests

**pH** (2.4.24). 3.5 to 6.5, determined in a solution diluted, if necessary, with *water for injections* to contain not more than the equivalent of 5 per cent w/v of Dextrose and to which 0.30 ml of a saturated solution of *potassium chloride* has been added for each 100 ml of solution.

**5-Hydroxymethylfurfural and related substances**. Dilute a volume containing 1.0 g of Dextrose to 250.0 ml with *water.* Absorbance of the resulting solution at the maximum at about 284 nm (2.4.7), not more than 0.25.

**Heavy metals** (2.3.13). A solution prepared by evaporating a volume containing 4 g of Dextrose to 10 ml and adding 2 ml of *dilute acetic acid* and sufficient *water* to produce 25 ml complies with the limit test for heavy metals, Method A (5 ppm).

**Bacterial endotoxins** (2.2.3). Not more than 0.5 Endotoxin Units per ml for preparations containing 5 per cent w/v or less of Dextrose. Dilute injections containing more than 5 per cent w/v of Dextrose with sufficient *water BET* so as to contain 5 per cent w/v of Dextose.

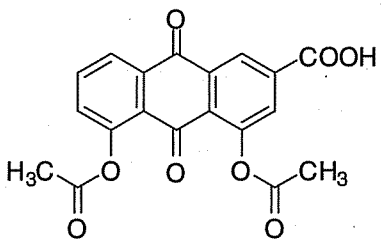
**Other tests**. Complies with the tests stated under Parenteral Preparations (Infusions).

**Assay**. To an accurately measured volume containing between 2 g and 5 g of Dextrose, add 0.2 ml of *5 M ammonia* and sufficient *water* to produce 100.0 ml. Mix well, allow to stand for 30 minutes and determine the optical rotation in a 2-dm tube (2.4.22). The observed rotation in degrees multiplied by 0.9477 represents the weight, in g, of dextrose, $C_6H_{12}O_6$, in the volume taken for assay.

**Storage**. Store in single dose containers at a temperature not exceeding 30°.

**Labelling**. The label states (1) the strength as the percentage w/v of anhydrous dextrose, $C_6H_{12}O_6$; (2) that the injection should not be used if it contains visible solid particles.

## Diacerein




$C_{19}H_{12}O_8$ Mol. Wt. 368.3

Diacerein is 9,10-dihydro-4,5-dihydroxy-9,10-dioxo-2-anthranoic acid diacetate.



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Diacerein contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{19}H_{12}O_8$, calculated on the anhydrous basis.

**Category**. Antirheumatic.

**Description**. A fine yellow powder.

## Identification

*Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *diacerein RS* or with the reference spectrum of diacerin.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel *GF254*.

*Mobile phase*. A mixture of 60 volumes of *2-propanol*, 30 volumes of *ethyl acetate* and 2 volumes of *water*.

*Test solution*. Dissolve about 10 mg of the substance under examination in 100.0 ml of *acetone*.

*Reference solution*. A 0.01 per cent w/v solution of *diacerein RS* in *acetone*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

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

## Tests

**pH** (2.4.24). 4.5 to 5.5, determined in a 1.0 per cent w/v solution.

**Related substances.** Determine by liquid chromatography (2.4.14).

*Solvent mixture*. 60 volumes of *water* and 40 volumes of *acetonitrile*.

*Test solution*. Dissolve about 25 mg of the substance under examination in 10 ml of *N,N-dimethylacetamide* and dilute to 25.0 ml with the solvent mixture.

*Reference solution (a)*. Dilute 1 ml of the test solution to 200.0 ml with the solvent mixture.

*Reference solution (b)*. Dissolve 5 mg of *triacetyl aloe-emodin RS* in 5 ml of *N,N-dimethylacetamide* and dilute to 25 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm packed with endcapped octadecylsilane bonded to porous silica (5 µm) (such as Thermo BDS C18),
- mobile phase: a mixture of 50 volumes of *methanol* and 50 volumes of 0.1 per cent v/v solution of *triethylamine* in *water*, adjusted to pH 2.5 with *trifluroacetic acid*,
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Inject reference solution (a). The test is not valid unless theoretical plates are not less than 4000, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject reference solution (a), (b) and the test solution. In the chromatogram obtained with the test solution, the area of the peak due to triacetyl aloe-emodin is not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than 3 times the area of the peak in the chromatogram obtained with reference solution (a) (1.5 per cent).

**Aloe-emodin content.** Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve about 100 mg of the substance under examination into a 250-ml conical flask. Add 25.3 ml of *0.5 M sodium hydroxide*. Stir well for atleast 10 minutes and add 74.7 ml of *glycine solution*. Adjust the pH to 9.5 with *0.5 M sodium hydroxide*. Transfer this solution in 150 ml separating funnel. Extract the solution three times with 8.5 ml of *chloroform*. Collect the extracts and wash with 10 ml of buffer pH 9.5 and then with 10 ml of *0.01 M sulphuric acid*. Filter well through *anhydrous sodium sulphate*. Evaporate at 40° to dryness on water-bath. Dissolve the residue in 1 ml of *methanol* and 1 ml of the mobile phase, filter.

*Reference solution*. Dissolve 100 mg of *aloe-emodin RS* in 20 ml of *N, N-Dimethylacetamide* and dilute to 100 ml with *methanol*. Dilute 5 ml of this solution to 100 ml with *methanol*. Further dilute 5.0 ml of this solution to 50 ml with *methanol*. Transfer 1.0 ml of this solution into a 250-ml conical flask and add 25.3 ml of *0.5 M sodium hydroxide*. Mix well for atleast 10 minutes and add 74.7 ml of *glycine solution*. Adjust the pH to 9.5 with *0.5 M sodium hydroxide* and transfer this solution to 150-ml separating funnel. Extract the solution three times with 8.5 ml of *chloroform*. Collect the extracts and wash with 10 ml of buffer pH 9.5 and then with 10 ml of *0.005 M sulphuric acid*. Filter well through *anhydrous sodium sulphate*. Evaporate at 40° to dryness on water-bath. Dissolve the residue in 1 ml of *methanol* and 1 ml of the mobile phase, filter.

Use chromatographic system as described under Related substances using injection volume. 100 µl.



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Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 20.0 per cent.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution the area of peak corresponding to aloe-emodin is not more than the area of the principal peak in the chromatogram obtained with the reference solution (50 ppm).

**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals Method B, (10 ppm).

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Chromium**. Not more than 5 ppm.

Determine by atomic absorption spectrophotometry (2.4.2), Method A, using a solution prepared by dissolving 0.5 g of the substance under examination in *1 M nitric acid*. Measure the absorbance at 357.9 nm using chromium hollow cathode lamp and an air-acetylene flame. For the standard solutions dissolve 2.82 g of dried *potassium dichromate* at 120° for 4 hours in 1000 ml of *water* and dilute suitably with *1 M nitric acid* for standard solutions.

**Water** (2.3.43). Not more than 0.5 per cent, determined on 0.5 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. 60 volumes of *water* and 40 volumes of *acetonitrile*.

*Test solution*. Dissolve about 50 mg of the substance under examination in 100 ml of *N, N dimethylacetamide*. Dilute 5.0 ml of this solution to 50.0 ml with the solvent mixture.

*Reference solution*. Dissolve 50 mg of *diacerein RS* in 100 ml of *N, N dimethylacetamide*. Dilute 5.0 ml of this solution to 50.0 ml with the solvent mixture.

Use chromatographic system as described under Related substances.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{19}H_{12}O_8$.

**Storage**. Store protected from light.

# Diacerein Capsules

Diacerein Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of diacerein, $C_{19}H_{12}O_8$.

**Usual strength**. 50 mg.

## Identification

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

## Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *citrate buffer pH 6.0*, prepared by dissolving 1.816 g of *citric acid* and 11.91 g of *trisodium citrate* in 1000 ml of *water*, adjusted to pH 6.0 with *0.1 M sodium hydroxide* or *0.1 M hydrochloric acid*,
Speed and time. 75 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter. Measure the absorbance of the filtrate, suitably diluted if necessary with dissolution medium at 340 nm (2.4.7). Calculate the content of diacerin, $C_{19}H_{12}O_8$ in the medium from the absorbance obtained from a solution prepared by dissolving 25 mg of *diacerein RS* in 10.0 ml of *acetonitrile* and dilute to 100 ml with dissolution medium. Dilute 5.0 ml of this solution to 50 ml with the dissolution medium.

D. Not less than 75 per cent of the stated amount of $C_{19}H_{12}O_8$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*NOTE — Store and inject the solutions at 4°.*

*Solvent mixture (a)*. 60 volumes of *acetonitrile* and 20 volumes of *water*.

*Solvent mixture (b)*. 20 volumes of *tetrahydrofuran*, 60 volumes of *acetonitrile* and 20 volumes of *water*.

*Test solution*. Disperse the contents of capsules containing about 25 mg of Diacerin in 10 ml of *tetrahydrofuran*, sonicate and dilute to 50.0 ml with solvent mixture (a), filter.

*Reference solution (a)*. Dissolve about 25 mg of the *diacerein RS* in 10.0 ml of *tetrahydrofuran*, sonicate and dilute to 50.0 ml with solvent mixture (a).

*Reference solution (b)*. Dilute 5.0 ml of reference solution (a) to 50.0 ml with solvent mixture (b). Further dilute 5.0 ml of this solution to 50.0 ml with the same solvent.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3.5 µm) (such as Waters X-terra RP18),
- mobile phase: a mixture of 62 volumes of a buffer solution prepared by diluting 2 ml of *orthophosphoric acid* in 1000 ml of *water* and 38 volumes of *acetonitrile*,



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- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Inject reference solution (b). The test is not valid unless the relative standard deviation for replicate injections is not more than 5.0 per cent.

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

**Other tests**. Comply with the tests stated under Capsules.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture.* 50 volumes of buffer pH 3.0 prepared by diluting 1.4 ml of *triethylamine* in 100 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid* and 50 volumes of *acetonitrile*.

*Test solution.* Disperse the contents of capsules containing about 50 mg of Diacerein in 200 ml of solvent mixture, sonicate for 10 minutes and dilute to 250.0 ml with the solvent mixture, filter. Further dilute 5.0 ml of this solution to 20.0 ml with the solvent mixture.

*Reference solution.* A 0.005 per cent w/v solution of *diacerein RS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Inertsil ODS-3),
- mobile phase: a mixture of 75 volumes of buffer solution prepared by diluting 1.4 ml of *triethylamine* in 100 ml of *water*, adjusted to pH 7.0 with *orthophosphoric acid* and 25 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

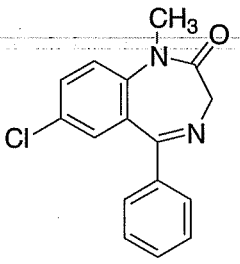
Inject the reference solution. The test is not valid unless the theoretical plates is not less than 2000, tailing factor is not more than 2.0 and the relative standard deviation of replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{19}H_{12}O_8$ in the capsules.

**Storage**. Store protected from moisture.

# Diazepam




$C_{16}H_{13}ClN_2O$ Mol. Wt. 284.7

Diazepam is 7-chloro-1,3-dihydro-1-methyl-5-phenyl-1,4-benzodiazepin-2-one.

Diazepam contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{16}H_{13}ClN_2O$, calculated on the dried basis.

**Category**. Anxiolytic; sedative; anticonvulsant.

**Dose**. In anxiety states, 2 mg thrice daily, increased if necessary to 15 to 30 mg daily, in divided doses; in insomnia associated with anxiety, 5 to 15 mg at bedtime. By intramuscular or slow intravenous injection, 10 mg, repeated if necessary after 4 hours.

**Description**. A white or almost white to pale yellow, crystalline powder; odourless or almost odourless.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

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

B. *Measure the absorbances in subdued light immediately after preparation of the solution.*

When examined in the range 230 nm to 360 nm (2.4.7), a 0.0005 per cent w/v solution in *0.05 M methanolic sulphuric acid* shows absorption maxima at about 241 nm and 284 nm; absorbance at about 241 nm, about 0.5 and at about 284 nm, about 0.23.

C. *Measure the absorbance in subdued light immediately after preparation of this solution.*

When examined in the range 325 nm to 400 nm (2.4.7), a 0.0025 per cent w/v solution in *0.05 M methanolic sulphuric acid* shows an absorption maximum only at about 366 nm; absorbance at about 366 nm, between 0.35 and 0.39.

D. Determine by the oxygen-flask method (2.3.34), using 20 mg of the substance under examination and 5 ml of *dilute*



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*sodium hydroxide solution* as the absorbing liquid. When the process is complete, acidify the solution with *dilute sulphuric acid* and boil gently for 2 minutes; the solution gives the reactions of chlorides (2.3.1).

### Tests

**Related substances and decomposition products.** Determine in subdued light by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of equal volumes of *hexane* and *ethyl acetate*.

*Prepare the following solutions freshly.*

*Test solution*. Dissolve 1 g of the substance under examination in sufficient *acetone* to produce 10 ml.

*Reference solution*. Dilute 0.1 ml of the test solution to 100 ml with *acetone*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

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

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven over *phosphorus pentoxide* at 60° at a pressure of 1.5 to 2.5 kPa for 4 hours.

**Assay**. Weigh accurately about 0.25 g, dissolve in 80 ml of *anhydrous glacial acetic acid* with the aid of heat, if necessary and cool. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02847 g of $C_{16}H_{13}ClN_2O$.

**Storage**. Store protected from light.

## Diazepam Capsules

Diazepam Capsules contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of diazepam, $C_{16}H_{13}ClN_2O$.

**Usual strengths**. 2 mg; 5 mg; 10 mg.

### Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 100 volumes of *chloroform* and 10 volumes of *methanol*.

*Test solution*. Shake a quantity of the contents of the capsules with sufficient *methanol* to produce a solution containing 0.5 per cent w/v of Diazepam, allow to settle and decant the supernatant liquid.

*Reference solution*. A 0.5 per cent w/v solution of *diazepam RS* in *methanol*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air, spray with a 10 per cent v/v solution of *sulphuric acid* in *ethanol*, heat at 105° for 10 minutes and examine in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. When examined in the range 230 nm to 360 nm (2.4.7), the solution obtained in the Assay shows two absorption maxima at about 242 nm and 284 nm.

### Tests

**Related substances and decomposition products.** Determine in subdued light by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of equal volumes of *hexane* and *ethyl acetate*.

*Prepare the following solutions freshly.*

*Test solution*. Shake a quantity of the contents of the capsules containing 50 mg of Diazepam with 5 ml of *acetone* and filter.

*Reference solution*. Dilute 1 volume of the test solution to 50 volumes with *acetone*.

Apply to the plate 20 µl of the test solution and 5 µl of the reference solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc with an average pore diameter not greater than 1.0 µm. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate with *0.1 M hydrochloric acid*. Measure the absorbance of the resulting solution at the maximum at about 242 nm (2.4.7). Calculate the content of diazepam, $C_{16}H_{13}ClN_2O$ in the medium from the absorbance obtained from a solution of known concentration of *diazepam RS*.



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D. Not less than 85 per cent of the stated amount of $C_{16}H_{13}ClN_2O$.

**Uniformity of content**. Comply with the test stated under Capsules using the following method of analysis. Weigh an intact capsule. Open the capsule without losing any part of the shell and transfer the contents as completely as possible to a 100-ml volumetric flask. Weigh the shell, remove any retained contents and reweigh the shell. To the flask add 1 ml of *water*, mix and allow to stand for 15 minutes. Add 80 ml of a 0.5 per cent w/v solution of *sulphuric acid* in *methanol*, shake for 15 minutes, add sufficient of the *methanolic sulphuric acid* to produce 100.0 ml and filter. Dilute suitably, if necessary and measure the absorbance at the maximum at about 284 nm (2.4.7). Calculate the content of $C_{16}H_{13}ClN_2O$ taking 450 as the specific absorbance at 284 nm, making an appropriate adjustment for any retained capsule content.

**Other tests**. Comply with the tests stated under Capsules.

**Assay**. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 10 mg of Diazepam, add 5 ml of *water* and complete the test as described under Uniformity of content beginning at the words "mix and allow to stand for 15 minutes....". Calculate the content of $C_{16}H_{13}ClN_2O$ taking 450 as the specific absorbance at 284 nm.

**Storage**. Store protected from light.

## Diazepam Injection

Diazepam Injection is a sterile solution of Diazepam in Water for Injections or other suitable solvent.

Diazepam Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of diazepam, $C_{16}H_{13}ClN_2O$.

**Usual strengths**. 5 mg per ml; 10 mg per ml.

**Description**. A clear, colourless or almost colourless solution.

### Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 100 volumes of *chloroform* and 10 volumes of *methanol*.

*Test solution*. Dilute if necessary, an accurately measured volume of the injection with *water* to produce a solution containing 5 mg of Diazepam per ml.

*Reference solution*. A 0.5 per cent w/v solution of *diazepam RS* in *methanol*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air, spray with a 10 per cent v/v solution of *sulphuric acid* in *ethanol*, heat at 105° for 10 minutes and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. When examined in the range 230 nm to 360 nm (2.4.7), the solution obtained in the Assay shows absorption maxima at about 242 and at about 284 nm.

### Tests

**pH** (2.4.24). 6.2 to 6.9.

**Bacterial endotoxins** (2.2.3). Not more than 11.6 Endotoxin Units per mg of diazepam.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. To an accurately measured volume containing 10 mg of Diazepam, add 20 ml of *buffer solution pH 7.0* and extract with four quantities, each of 20 ml, of *chloroform*, passing each extract through about 5 g of *anhydrous sodium sulphate* and dilute the combined chloroform extracts to 100.0 ml with *chloroform*. Evaporate 10.0 ml of this solution to dryness under nitrogen, add sufficient volume of a 0.5 per cent w/v solution of *sulphuric acid* in *methanol* to produce 100.0 ml. Measure the absorbance of the resulting solution at the maximum at about 284 nm (2.4.7). Calculate the content of $C_{16}H_{13}ClN_2O$ taking 450 as the specific absorbance at 284 nm.

**Storage**. Store in single dose or multiple dose containers protected from light.

## Diazepam Tablets

Diazepam Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of diazepam, $C_{16}H_{13}ClN_2O$.

**Usual strengths**. 2 mg; 5 mg; 10 mg.

### Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 100 volumes of *chloroform* and 10 volumes of *methanol*.

*Test solution*. Shake a quantity of the powdered tablets with sufficient *methanol* to produce a solution containing 0.5 per cent w/v of Diazepam, allow to settle and decant the supernatant liquid.

*Reference solution*. A 0.5 per cent w/v solution of *diazepam RS* in *methanol*.



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Apply to the plate 2 µl of each solution. After development, dry the plate in air, spray with a 10 per cent v/v solution of *sulphuric acid* in *ethanol*, heat at 105° for 10 minutes and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. When examined in the range 230 to 360 nm (2.4.7), the solution obtained in the Assay shows absorption maxima at about 242 and at about 284 nm.

### Tests

**Related substances and decomposition products**. Determine in subdued light by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of equal volumes of *hexane* and *ethyl acetate*.

*Test solution*. Prepare freshly by shaking a quantity of the powdered tablets containing 50 mg of Diazepam with 5 ml of *ethanol (95 per cent)* and filtering.

*Reference solution*. Dilute 1 ml of the test solution to 50 ml with *ethanol (95 per cent)*.

Apply to the plate 20 µl of the test solution and 5 µl of the reference solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc with an average pore diameter not greater than 1.0 µm. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate with *0.1 M hydrochloric acid*. Measure the absorbance of the resulting solution at the maximum at about 242 nm (2.4.7). Calculate the content of diazepam, $C_{16}H_{13}ClN_2O$ in the medium from the absorbance obtained from a solution of known concentration of *diazepam RS*.

D. Not less than 85 per cent of the stated amount of $C_{16}H_{13}ClN_2O$.

**Uniformity of content**. Comply with the test stated under Tablets.

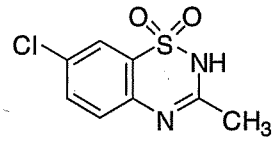
Powder one tablet, add 1 ml of *water*, mix and allow to stand for 15 minutes. Add 80 ml of a 0.5 per cent w/v solution of *sulphuric acid* in *methanol*, shake for 15 minutes, add sufficient of the *methanolic sulphuric acid* to produce 100.0 ml and filter. Dilute suitably, if necessary and measure the absorbance at the maximum at about 284 nm (2.4.7). Calculate the content of $C_{16}H_{13}ClN_2O$ in the tablet taking 450 as the specific absorbance at 284 nm.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 10 mg of Diazepam, add 5 ml of *water* and complete the test as described under Uniformity of content beginning at the words "mix and allow to stand for 15 minutes....". Calculate the content of $C_{16}H_{13}ClN_2O$ taking 450 as the specific absorbance at 284 nm.

**Storage**. Store protected from light.

## Diazoxide




$C_8H_7ClN_2O_2S$ Mol. Wt. 230.7

Diazoxide is 7-chloro-3-methyl-2*H*-1,2,4-benzothiadiazine 1,1-dioxide.

Diazoxide contains not less than 98.0 per cent and not more than 101.0 per cent of $C_8H_7ClN_2O_2S$, calculated on the dried basis.

**Category**. Antihypertensive.

**Description**. A white or almost white, fine or crystalline powder.

### Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

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

B. Dissolve 0.05 g in 5 ml of *1 M sodium hydroxide*, dilute to 50 ml with *water*. Dilute 1.0 ml of this solution to 100 ml with *0.1 M sodium hydroxide*. When examined in the range 230 nm to 350 nm (2.4.7), the resulting solution shows an absorption maximum at 280 nm and a shoulder at 304 nm. The specific absorbance is 570 to 610.

C. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to the principal spot in the chromatogram obtained with reference solution (b).



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D. Dissolve about 20 mg in a mixture of 5 ml of *hydrochloric acid* and 10 ml of *water*. Add 0.1 g of *zinc powder*. Boil for 5 minutes, cool and filter. To the filtrate add 2 ml of a 0.1 per cent w/v solution of *sodium nitrite* and mix. Allow to stand for 1 minute and add 1 ml of a 0.5 per cent solution of *naphthylethylenediamine dihydrochloride*. A red or violet-red colour develops.

### Tests

**Appearance of solution**. Dissolve 0.4 g in 2 ml of *1 M sodium hydroxide* and dilute to 20 ml with *water*. The solution is clear (2.4.1), and not more intensely coloured than reference solution YS1 (2.4.1).

**Acidity or alkalinity**. To 0.5 g of the substance under examination, add 30 ml of *carbon dioxide-free water*, shake for 2 minutes and filter. To 10 ml of the filtrate add 0.2 ml of *0.01 M sodium hydroxide* and 0.15 ml of *methyl red solution*, the solution is yellow. Not more than 0.4 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator to red.

**Related substance**. Determine by thin layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 7 volumes of *ammonia*, 25 volumes of *methanol* and 68 volumes of *chloroform*.

*Test solution (a)*. Dissolve 0.1 g of the substance under examination in a mixture of 0.5 ml of *1 M sodium hydroxide* and 1 ml of *methanol* and dilute to 5 ml with *methanol*.

*Test solution (b)*. Dilute 1 ml of test solution (a) to 5 ml with a mixture of 1 volume of *1 M sodium hydroxide* and 9 volumes of *methanol*.

*Reference solution (a)*. Dilute 0.5 ml of test solution (a) to 100 ml with a mixture of 1 volume of *1 M sodium hydroxide* and 9 volumes of *methanol*.

*Reference solution (b)*. Dissolve 20 mg of *diazoxide RS* in a mixture of 0.5 ml of *1M sodium hydroxide* and 1 ml of *methanol* and dilute to 5 ml with *methanol* .

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.2 g and dissolve with gentle heating in 50 ml of a mixture of 1 ml of *water* and 2 ml of *dimethylformamide*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.02307 g of $C_8H_7ClN_2O_2S$.

## Diazoxide Tablets

Diazoxide Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of diazoxide, $C_8H_7ClN_2O_2S$.

**Usual strength**. 50 mg.

### Identification

Shake a quantity of the powdered tablets containing 0.2 g of Diazoxide with 50 ml of *absolute ethanol*, filter and evaporate the filtrate to dryness at a pressure of 2 kPa. The residue complies with the following tests.

A. When examined in the range 230 nm to 350 nm (2.4.7), a 0.001 per cent w/v solution in *0.1 M sodium hydroxide* shows an absorbance maximum only at 280 nm.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 50 volumes of *toluene*, 30 volumes of *ether* and 20 volumes of *acetone*.

*Test solution*. A 0.02 per cent w/v solution of the residue in *methanol*.

*Reference solution*. A 0.02 per cent w/v solution of *diazoxide RS* in *methanol*.

Apply to the plate 20 µl of each solution. After development, dry the plate in air until the solvent has evaporated, examine in ultraviolet light at 254 nm and then spray the dried plate with *ethanolic sulphuric acid (20 per cent)*, heat at 105° for 30 minutes and immediately expose to nitrous fumes in a closed glass tank for 15 minutes (the nitrous fumes may be generated by adding *7 M sulphuric acid* dropwise to a solution containing 10 per cent w/v of *sodium nitrite* and 3 per cent w/v of *potassium iodide*. Place the plate in a current of warm air for 15 minutes and spray with a 0.5 per cent w/v solution of *N-(1-naphthyl)ethylenediamine dihydrochloride* in *ethanol (95 per cent)*. If necessary allow to dry and repeat the spraying. The principal spot in the chromatogram obtained with test solution corresponds to that in the chromatogram obtained with reference solution.

### Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.



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*Mobile phase*. A mixture of 7 volumes of *18 M ammonia*, 25 volumes of *methanol* and 68 volumes of *chloroform*.

*Test solution*. Shake a quantity of the powdered tablets containing 0.75 g of Diazoxide with 40 ml of *0.1 M sodium hydroxide* for 30 minutes, filter and dilute the filtrate to 50 ml with *0.1 M sodium hydroxide*.

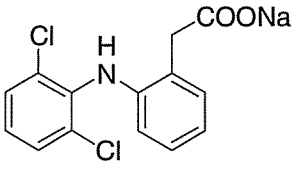
*Reference solution*. Dilute 1 ml of the test solution to 200 ml with *0.1 M sodium hydroxide*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.05 g of Diazoxide, add 70 ml of *methanol*, shake for 1 hour, add sufficient *methanol* to produce 100 ml, mix and filter. Dilute 5 ml of the filtrate to 250 ml with *0.1 M sodium hydroxide*. Measure the absorbance of the resulting solution at the maximum at about 280 nm (2.4.7). Calculate the content of $C_8H_7ClN_2O_2S$ taking 585 as the specific absorbance at 280 nm.

# Diclofenac Sodium




$C_{14}H_{10}Cl_2NNaO_2$ Mol. Wt. 318.1

Diclofenac Sodium is sodium 2-[(2,6-dichlorophenyl)-amino]phenylacetate.

Diclofenac Sodium contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{14}H_{10}Cl_2NNaO_2$, calculated on the dried basis.

**Category**. Analgesic; anti-inflammatory.

**Dose**. Orally or by intramuscular injection, 25 to 75 mg.

**Description**. A white to slightly yellowish crystalline powder; slightly hygroscopic.

## Identification

*Test A may be omitted if tests B, C, and D are carried out. Tests B and C may be omitted if tests A and D are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *diclofenac sodium RS* or with the reference spectrum of diclofenac sodium.

B. To 1 ml of a 0.4 per cent w/v solution in *methanol* add 1 ml of *nitric acid;* a dark red colour develops.

C. In the test for Related substances, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

D. A 1 per cent w/v solution gives the reaction of sodium salts (2.3.1).

## Tests

**Appearance of solution**. A 5.0 per cent w/v solution in *methanol* is clear (2.4.1), and not more intensely coloured than reference solution BYS6 (2.4.1).

**pH** (2.4.24). 6.5 to 8.5, determined on a 1.0 per cent w/v solution.

**Light absorption** (2.4.7). Absorbance of a 5.0 per cent w/v solution in *methanol* at about 440 nm, not more than 0.050.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 50 mg of the substance under examination in *methanol* and dilute to 50 ml with the same solvent.

*Reference solution*. A 0.0002 per cent w/v solution of *diclofenac sodium RS* in *methanol*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with end-capped octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 34 volumes of a solution containing 0.5 g per litre of *phosphoric acid* and 0.8 g per litre of *sodium dihydrogen phosphate* adjusted to pH 2.5 with *phosphoric acid*, and 66 volumes of *methanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject the test solution and the reference solution. In the chromatogram obtained with the test solution: the area of any peak other than the principal peak is not greater than the area of the principal peak in the chromatogram obtained with the reference solution (0.2 per cent); the sum of the areas of all peaks other than the principal peak is not greater than 2.5 times that of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent). Ignore any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with the reference solution.



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**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (10 ppm).

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

**Assay**. Weigh accurately about 0.2 g and dissolve in 50 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03181 g of $C_{14}H_{10}Cl_2NNaO_2$.

**Storage**. Store protected from light.

# Diclofenac Injection

Diclofenac Sodium Injection

Diclofenac Injection is a sterile solution of Diclofenac Sodium in Water for Injections. It may contain Propylene Glycol, Benzyl Alcohol and sufficient Sodium Hydroxide to adjust the pH of the solution.

Diclofenac Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of diclofenac sodium, $C_{14}H_{10}Cl_2NNaO_2$.

**Usual strength**. 25 mg per ml.

**Description**. A clear, colourless to yellowish liquid.

## Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 90 volumes of *chloroform*, 5 volumes of *acetone* and 5 volumes of *formic acid* in a saturated chamber.

*Test solution*. Dilute a suitable volume of the injection containing 25 mg of Diclofenac Sodium to 10 ml with *methanol*.

*Reference solution*. A 0.25 per cent w/v solution of *diclofenac sodium RS* in *methanol*.

Apply to the plate 2 µl of each solution. After development, dry the plate in a current of warm air and examine in ultraviolet light at 254 nm. Alternatively, spray with a 0.5 per cent w/v solution of *potassium dichromate* in *sulphuric acid (20 per cent)*. By both methods of visualisation, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

## Tests

**pH** (2.4.24). 8.1 to 9.0.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dilute a suitable volume of the injection containing 25 mg of Diclofenac Sodium to 10.0 ml with the mobile phase.

*Reference solution*. A 0.25 per cent w/v solution of *diclofenac sodium RS* in the mobile phase.

Chromatographic system
- a stainless steel column 12.5 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of *methanol* and 40 volumes of *0.1 M sodium acetate solution*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Inject alternately the test solution and the reference solution and record the chromatograms for 2.5 times the retention time of the principal peak. If necessary adjust the concentration of *methanol* in the mobile phase to obtain the resolution of the peak due to diclofenac sodium.

Calculate the content of $C_{14}H_{10}Cl_2NNaO_2$ in the injection.

# Diclofenac Tablets

Diclofenac Sodium Tablets

Diclofenac Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of diclofenac sodium, $C_{14}H_{10}Cl_2NNaO_2$. The tablets may be enteric-coated.

**Usual strengths**. 25 mg; 50 mg.

## Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel 60 F254* or using a precoated *silica gel 60 F254* plate.

*Mobile phase*. A mixture of 100 volumes of *toluene*, 10 volumes of *hexane* and 10 volumes of *anhydrous formic acid*.

*Test solution*. Shake a quantity of the powdered tablets containing 50 mg of Diclofenac Sodium with 5 ml of *methanol*, centrifuge and use the supernatant liquid.

*Reference solution*. A 1 per cent w/v solution of *diclofenac sodium RS* in *methanol*.



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Apply separately to the plate 1 µl of each solution. After development, dry the plate in a current of warm air and examine in ultraviolet light at 254 nm. Alternatively, spray the plate with a 0.5 per cent w/v solution of *potassium dichromate* in *sulphuric acid (20 per cent)*. By both methods of visualisation, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

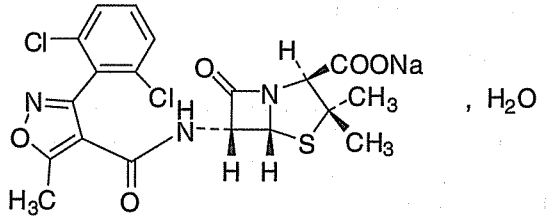
### Tests

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 50 mg of Diclofenac Sodium, shake with 60 ml of *methanol* in a 200-ml volumetric flask and dilute to volume with *methanol*. Dilute 5.0 ml of this solution to 100.0 ml with *methanol* and measure the absorbance of the resulting solution at the maximum at about 285 nm (2.4.7). Calculate the content of $C_{14}H_{10}Cl_2NNaO_2$ from the absorbance obtained by repeating the procedure using *diclofenac sodium RS* in place of the substance under examination.

**Storage**. Store protected from light.

## Dicloxacillin Sodium




$C_{19}H_{16}Cl_2N_3NaO_5S,H_2O$ Mol. Wt. 510.3

Dicloxacillin Sodium is sodium (6*R*)-6-[3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carboxamido] penicillanate monohydrate.

Dicloxacillin Sodium contains not less than 95.0 per cent and not more than 102.0 per cent of $C_{19}H_{16}Cl_2N_3NaO_5S$, calculated on the anhydrous basis.

**Category**. Antibacterial.

**Description**. A white or almost white crystalline powder, hygroscopic.

### Identification

*Tests A and D may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if tests A and D are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dicloxacillin sodium RS* or with the reference spectrum of dicloxacillin sodium.

B. Determine by thin layer chromatography (2.4.17), coating the plate with *silica gel H*.

*Mobile phase*. A mixture of 30 volumes of *acetone* and 70 volumes of a 15.4 per cent w/v solution of *ammonium acetate*, adjusted to pH 5.0 with *glacial acetic acid*.

*Test solution*. Dissolve 25 mg of the substance under examination in 5 ml of *water*.

*Reference solution (a)*. A 0.5 per cent w/v solution of *dicloxacillin sodium RS* in *water*.

*Reference solution (b)*. A solution containing 0.5 per cent w/v each of *cloxacillin sodium RS*, *dicloxacillin sodium RS* and *flucloxacillin sodium RS* in *water*.

Apply to the plate 1 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air. Expose to iodine vapour until the spots appear and examine in daylight, the chromatogram obtained with reference solution (b) shows three clearly separated spots. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

C. Place about 2 mg in a test-tube about 150 mm long and about 15 mm in diameter. Moisten with 0.05 ml of *water* and add 2 ml of *sulphuric acid-formaldehyde reagent*. Mix the contents of the tube by swirling; the colour of the solution is slightly greenish-yellow. Place the test-tube in a water-bath for 1 minute; a yellow colour develops.

D. Gives reaction A of sodium (2.3.1).

### Tests

**Appearance of solution**. A 10.0 per cent w/v solution in *carbon dioxide-free water* (solution A) is clear (2.4.1) and its absorbance at 430 nm (2.4.7) is not more than 0.04.

**pH** (2.4.24). 5.0 to 7.0, determined in solution A.

**Specific optical rotation** (2.4.22). +128° to +143°, determined in a 1 per cent w/v solution.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution (a)*. Dissolve 50 mg of the substance under examination in 50.0 ml of the mobile phase.

*Test solution (b)*. Dilute 5.0 ml of test solution (a) to 50.0 ml with the mobile phase.

*Reference solution (a)*. A 0.01 per cent w/v solution of *dicloxacillin sodium RS* in the mobile phase.



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*Reference solution (b).* Dilute 5.0 ml of test solution (b) to 50.0 ml with the mobile phase.

*Reference solution (c).* A solution containing 0.01 per cent w/v each of *flucloxacillin sodium RS* and *dicloxacillin sodium RS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 25 volumes of *acetonitrile* and 75 volumes of a 0.27 per cent w/v solution of *potassium dihydrogen phosphate*, adjusted to pH 5.0 with *dilute sodium hydroxide solution*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume. 20 µl.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to flucloxacillin and dicloxacillin is not less than 2.5.

Inject reference solution (b) and test solution (a). Run the chromatogram 5 times the retention time of the principal peak. In the chromatogram obtained with test solution (a), the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent). The sum of the areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (5 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

***N,N*-Dimethylaniline** (2.3.21). Not more than 20 ppm, determined by Method A.

**2-Ethylhexanoic acid**. Not more than 0.8 per cent.

Determine by gas chromatography (2.4.13).

*Test solution.* Prepare a 0.1 per cent w/v solution of *3-cyclopropionic acid* (Internal standard) in *cyclohexane* (solution A). To 0.3 g of the substance under examination add 4.0 ml of 33 per cent v/v solution of *hydrochloric acid* and 1.0 ml of solution (A). Shake vigorously for 1 minute, centrifuge if necessary and use the clear supernatant layer.

*Reference solution.* Dissolve 75 mg of *2-ethylhexanoic acid* in solution A and dilute to 50 ml with solution A. To 1 ml of this solution add 4.0 ml of a 33 per cent v/v solution of *hydrochloric acid*, shake vigorously for 1 minute, centrifuge if necessary and use the clear supernatant layer.

Chromatographic system
- a wide-bore fused silica column 10 m x 0.53 mm coated with *macrogol* 20,000 *2-nitrophthalate* (film thickness 1.0 µm),
- temperature:
  column 40° from 0 to 2 minutes, 40°-200° from 2 to 7.3 minutes and 200° from 7.3 to 10.3 minutes,
  inlet port 200°and detector 300°,
- flow rate. 10 ml per minute, using nitrogen as carrier gas.

Inject 1 µl of the reference solution. The test is not valid unless the resolution between the peaks of *2-ethyhexanoic acid* and *3-cyclohexylpropionic acid* is not less than 2.0.

Inject 1 µl of the reference solution and the test solution.

Calculate the content of 2-ethylhexanoic acid.

**Water** (2.3.43). 3.0 to 4.5 per cent, determined on 0.3 g.

*Dicloxacillin Sodium intended for use in the manufacture of parenteral preparations complies with the following additional tests.*

**Pyrogens**. Complies with the test for pyrogens (2.2.8), using not less than 20 mg per kg of the rabbit's weight, dissolved in 1 ml of *water for injection.*

**Assay**. Determine by liquid chromatography (2.4.14), using test solution (b), reference solution (a) and chromatographic system as described under the test for Related substances.

Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject reference solution (a) and test solution (b).

Calculate the content of $C_{19}H_{16}Cl_2N_3NaO_5S$.

**Storage**. Store protected from moisture, at a temperature not exceeding 25°. If it is intended for use in manufacture of parenteral preparation, the container should be sterile, airtight and temper-proof.

# Dicloxacillin Capsules

## Dicloxacillin Sodium Capsules

Dicloxacillin Capsules contain not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of dicloxacillin, $C_{19}H_{17}Cl_2N_3O_5S$.

**Usual strengths**. 250 mg; 500 mg.

### Identification

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



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### Tests

**Dissolution** (2.5.2).

Apparatus No. 2,
Medium. 900 ml of *water,*
Speed and time. 100 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

*Test solution.* Dilute the filtrate with buffer solution and filter.

*Reference solution.* A 0.11 per cent w/v solution of *dicloxacillin sodium RS* in the buffer solution.

Use the chromatographic system as described under Assay.

Inject the reference solution and the test solution.

Calculate the content of $C_{19}H_{17}Cl_2N_3O_5S$.

D. Not less than 75 per cent of the stated amount of $C_{19}H_{17}Cl_2N_3O_5S$.

**Uniformity of content** *(For capsules containing 10 mg or less).* Comply with the test stated under capsules.

Determine by liquid chromatography (2.4.14), as described under Assay, using the following solution as the test solution.

*Test solution.* Empty the contents of one capsule, crush, if necessary, add buffer solution, shake vigorously, centrifuge and dilute to 200.0 ml with the buffer solution, filter.

Calculate the content of $C_{19}H_{17}Cl_2N_3O_5S$ in the capsules.

**Water** (2.3.43). Not more than 5.0 per cent, determined on 0.1 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*NOTE—Use freshly prepared solutions.*

*Buffer solution.* Dissolve 5.44 g of *monobasic potassium phosphate* in 2000 ml of *water*, adjust the pH to 5.0 with *8 M potassium hydroxide.*

*Test solution.* Weigh accurately a quantity of the mixed contents of 20 capsules containing about 100 mg of dicloxacillin, disperse in 100.0 ml of buffer solution and filter.

*Reference solution.* A 0.11 per cent w/v solution of *dicloxacillin sodium RS* in the buffer solution.

Chromatographic system
- a stainless steel column 30 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 75 volumes of buffer solution and 50 volumes of *acetonitrile,*
- flow rate. 2 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{19}H_{17}Cl_2N_3O_5S$ in the capsules.

**Storage**. Store protected from moisture, at a temperature not exceeding 30°.

## Dicloxacillin Oral Suspension

Dicloxacillin Sodium Oral Suspension

Dicloxacillin Oral Suspension is a dry mixture of Dicloxacillin with buffering agents and other excipients. It contains a suitable flavouring agent. It is filled in a sealed container.

The suspension is constituted by dispersing the contents of sealed container in the specified volume of water just before use.

Dicloxacillin Oral Suspension contains not less than 90.0 per cent and not more than 120.0 per cent of the labeled amount of dicloxacillin, $C_{19}H_{17}Cl_2N_3O_5S$.

When stored at the temperature and for the period stated on the label during which the constituted suspension may be expected to be satisfactory for use, it contains not less than 80.0 per cent of the stated amount of dicloxacillin, $C_{19}H_{17}Cl_2N_3O_5S$.

**Usual strength**. 12.5 mg per ml.

### Identification

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

### Tests

**pH** (2.4.24). 4.7 to 7.5, of the constituted oral suspension.

**Water** (2.3.43). Not more than 2.0 per cent, determined on 0.1 g.

**Other tests**. Complies with the tests stated under Oral Suspension.

**Assay**. Determine by liquid chromatography (2.4.14).

*NOTE—Use freshly prepared solutions.*

*Buffer solution.* Dissolve 5.44 g of *monobasic potassium phosphate* in 2000 ml of *water*, adjusted to pH 5.0 with *8 M potassium hydroxide.*



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*Test solution.* An accurately measured volume of the constituted suspension containing about 125 mg of dicloxacillin to a 200-ml flask. Add 20.0 ml of *dimethylformamide* and 5.0 ml of *ethanol*, and stir for 15 minutes. Add another 50.0 ml of buffer, centrifuge this mixture for 15 minutes, dilute to 200.0 ml with the buffer solution and filter.

*Reference solution.* Weigh accurately a 65 mg of *dicloxacillin sodium RS*, to 100.0 ml of volumetric flask, add 20.0 ml of *dimethylformamide*, 5.0 ml of *ethanol (95 per cent)*, and 20.0 ml of buffer solution, and stir for 5.0 minutes, dilute to volume with the buffer solution and filter .

Chromatographic system

- a stainless steel column 30 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 75 volumes of buffer solution and 50 volumes of *acetonitrile,*
- flow rate. 2 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume. 10 µl.

Inject the reference solution The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injection is not more than 2.0 per cent.

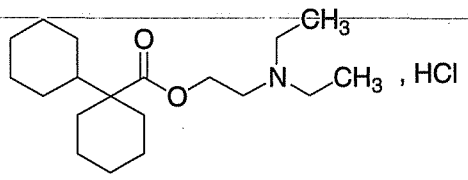
Inject the reference solution and the test solution.

Calculate the content of $C_{19}H_{17}Cl_2N_3O_5S$ in the suspension.

**Storage.** Store protected from moisture at a temperature not exceeding 30°.

# Dicyclomine Hydrochloride

Dicycloverine Hydrochloride




$C_{19}H_{35}NO_2,HCl$ Mol. Wt. 345.9

Dicyclomine Hydrochloride is 2-diethylaminoethyl bicyclohexyl-1-carboxylate hydrochloride.

Dicyclomine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{19}H_{35}NO_2,HCl$, calculated on the dried basis.

**Category.** Antispasmodic.

**Dose.** 30 to 60 mg daily, in divided doses. If used as an oral solution and the solution is required to be diluted, the diluted solution should be freshly prepared.

**Description.** A white or almost white, crystalline powder; odourless or almost odourless.

## Identification

A. Dissolve a suitable quantity in *acetone* and evaporate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dicyclomine hydrochloride RS* or with the reference spectrum of dicyclomine hydrochloride.

B. To 3 ml of a 0.1 per cent w/v solution of *sodium dodecyl sulphate,* add 5 ml of *chloroform* and 0.05 ml of a 0.25 per cent w/v solution of *methylene blue*, mix gently and allow to separate; the chloroform layer is blue. Add 20 mg of the substance under examination dissolved in 2 ml of *water,* mix gently and allow to separate; the aqueous layer is blue and the chloroform layer is colourless.

C. Dissolve 10 mg in 5 ml of *water* and add 0.2 ml of *2 M nitric acid* and 0.5 ml of *silver nitrate solution*; a white precipitate is produced.

## Tests

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

*Mobile phase.* A mixture of 50 volumes of *1-propanol*, 30 volumes of *ethyl acetate*, 15 volumes of *water* and 5 volumes of *strong ammonia solution.*

*Test solution.* Dissolve 0.5 g of the substance under examination in 10 ml of *methanol.*

*Reference solution.* Dilute 5 ml of the test solution to 50 ml with *methanol* and mix. To 2 ml of this solution add sufficient *methanol* to produce 100 ml.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with *dilute potassium iodobismuthate solution.* Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay.** Weigh accurately about 0.6 g, dissolve in 20 ml of *anhydrous glacial acetic acid* and add 10 ml of *mercuric*



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*acetate solution*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03460 g of $C_{19}H_{35}NO_2$, HCl.

## Dicyclomine Injection

Dicyclomine Hydrochloride Injection

Dicyclomine Injection is a sterile, isotonic solution of Dicyclomine Hydrochloride in Water for Injections.

Dicyclomine Injection contains not less than 93.0 per cent and not more than 107.0 per cent of the stated amount of dicyclomine hydrochloride, $C_{19}H_{35}NO_2$,HCl.

**Usual strength**. 10 mg per ml.

### Identification

A. To a volume containing 0.1 g of Dicyclomine Hydrochloride add 10 ml of *water* and 1 ml of *hydrochloric acid*, shake with 25 ml of *ether* and allow to separate. Extract the aqueous layer with 30 ml of *chloroform*, wash the extract with two quantities, each of 10 ml, of *water* and filter the chloroform solution through *anhydrous sodium sulphate*. Evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dicyclomine hydrochloride RS* treated in the same manner.

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

### Tests

**Bacterial endotoxins** (2.2.3). Not more than 17.2 Endotoxin Unit per mg of Dicyclomine Hydrochloride.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. 1 volume of *0.04 M phosphate buffer, pH 7.5* and 1 volume of *acetonitrile*.

*Test solution*. Dilute a volume containing about 20 mg of Dicyclomine Hydrochloride to 50.0 ml with the solvent mixture.

*Reference solution*. A 0.04 per cent w/v solution of *dicyclomine hydrochloride RS* in the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 30 volumes of *0.02 M phosphate buffer pH 7.5* prepared by dissolving 2.72 g of *monobasic potassium phosphate* in 900 ml of *water*, adjusting the pH to 7.5 with 10 per cent w/v solution of *sodium hydroxide*, diluting to 1000 ml with *water* and 70 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor for the analyte peak is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_{19}H_{35}NO_2$,HCl in the injection.

**Storage**. Store protected from light, in single dose or multiple-dose containers.

## Dicyclomine Oral Solution

Dicyclomine Hydrochloride Oral Solution; Dicycloverine Hydrochloride Oral Solution

Dicyclomine Oral Solution is a solution of Dicyclomine Hydrochloride in a suitable flavoured vehicle.

Dicyclomine Oral Solution contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of dicyclomine hydrochloride, $C_{19}H_{35}NO_2$,HCl.

**Usual strength**. 10 mg in 5 ml.

### Identification

A. To a volume containing 0.1 g of Dicyclomine Hydrochloride add 10 ml of *water* and 1 ml of *hydrochloric acid*, shake with 30 ml of *ether* and allow to separate. Extract the aqueous layer with 30 ml of *chloroform*, wash the extract with two quantities, each of 10 ml, of *water* and filter the chloroform solution through *anhydrous sodium sulphate*. Evaporate the filtrate to dryness, recrystallise the residue from hot *acetone* and dry at 105° for 30 minutes. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dicyclomine hydrochloride RS* or with the reference spectrum of dicyclomine hydrochloride.

B. Acidify the oral solution with *2 M nitric acid* and add *silver nitrate solution*; a white precipitate is produced.

### Tests

**Other tests**. Complies with the tests stated under Oral Liquids.



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**Assay**. Weigh accurately a quantity containing about 5 mg of Dicyclomine Hydrochloride add 5 ml of *sulphuric acid (10 per cent v/v)* and 2 ml of *0.02 M potassium permanganate*, mix, allow to stand, add 20 ml of *water* and 20 ml of *chloroform* to the decolorised solution and titrate with *0.001 M sodium dodecyl sulphate*, using 1 ml of *dimethyl yellow solution* as indicator.

1 ml of *0.001 M sodium dodecyl sulphate* is equivalent to 0.0003460 g of $C_{19}H_{35}NO_2$,HCl.

Determine the weight per ml of the oral solution (2.4.29), and calculate the content of $C_{19}H_{35}NO_2$,HCl, weight in volume.

**Storage**. Store protected from light.

## Dicyclomine Tablets

Dicyclomine Hydrochloride Tablets; Dicycloverine Hydrochloride Tablets

Dicyclomine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of dicyclomine hydrochloride, $C_{19}H_{35}NO_2$,HCl.

**Usual strength**. 20 mg.

### Identification

A. Extract a quantity of the powdered tablets containing 0.2 g of Dicyclomine Hydrochloride with 20 ml of *chloroform*, filter, evaporate the filtrate to dryness, recrystallise the residue from hot *acetone* and dry at 105° for 4 hours. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dicyclomine hydrochloride RS* or with the reference spectrum of dicyclomine hydrochloride.

B. To 3 ml of a 0.1 per cent w/v solution of *sodium dodecyl sulphate*, add 5 ml of *chloroform* and 0.05 ml of a 0.25 per cent w/v solution of *methylene blue*, mix gently and allow to separate; the chloroform layer is blue. Add a quantity of the powdered tablets containing 20 mg of Dicyclomine Hydrochloride dispersed in 2 ml of *water*, mix gently and allow to separate; the aqueous layer is blue and the chloroform layer is colourless.

C. Shake a quantity of the powdered tablets containing 10 mg of Dicyclomine Hydrochloride with 5 ml of *water* and 0.2 ml of *2 M nitric acid*, filter and add 0.5 ml of *silver nitrate solution* to the filtrate; a white precipitate is produced.

### Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 50 volumes of *1-propanol*, 30 volumes of *ethyl acetate*, 15 volumes of *water* and 5 volumes of *strong ammonia solution*.

*Test solution*. Shake a quantity of the powdered tablets containing 0.2 g of Dicyclomine Hydrochloride with 8 ml of *water* and 2 ml of *strong ammonia solution*, extract with two quantities, each of 20 ml, of *chloroform*, shake with *anhydrous sodium sulphate*, filter, evaporate the filtrate to dryness and dissolve the residue in 4 ml of *chloroform*.

*Reference solution*. Dilute 1 volume of the test solution to 500 volumes with *chloroform*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with *dilute potassium iodobismuthate solution*. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

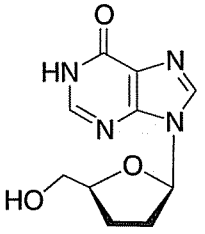
**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 30 mg of Dicyclomine Hydrochloride, add 20 ml of *water* and shake. Add 10 ml of *1 M sulphuric acid*, 1 ml of *dimethyl yellow solution* and 40 ml of *chloroform*, shake and titrate with *0.004 M sodium dodecyl sulphate*, shaking vigorously and allowing the layers to separate after each addition, until a permanent orange-pink colour is produced in the *chloroform* layer.

1 ml of *0.004 M sodium dodecyl sulphate* is equivalent to 0.001384 g of $C_{19}H_{35}NO_2$,HCl.

**Storage**. Store protected from light.

## Didanosine




$C_{10}H_{12}N_4O_3$ Mol. Wt. 236.2

Didanosine is 2′,3′–dideoxyinosine.

Didanosine contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{10}H_{12}N_4O_3$, calculated on the dried basis.

**Category**. Antiretroviral.



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**Dose**. 250 or 400 mg daily, depending on body weight of patient.

**Description**. A white or almost white crystalline powder.

### Identification

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

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

### Tests

**Specific optical rotation** (2.4.22). –24.0° to –28.0°, determined in a 1.0 per cent w/v solution in *water*.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 0.1 g of the substance under examination in 100 ml of the mobile phase.

*Reference solution*. A 0.001 per cent w/v solution of the substance under examination in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a filtered and degassed mixture of 6 volumes of *acetonitrile* and 94 volumes of *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject the test solution and the reference solution. In the chromatogram obtained with the test solution, the area of any peak other than the principal peak is not greater than half of the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of the areas of all such peaks is not greater than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent).

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

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Loss on drying** (2.4.19). Not more that 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

**Assay**. Determine by liquid chromatography (2.4.14) as given under the test for Related substances using the following solutions.

*Test solution*. Dilute 5.0 ml of a 0.1 per cent w/v solution of the substance under examination in *water* to 100.0 ml with the mobile phase.

*Reference solution*. Dilute 5.0 ml of a 0.1 per cent w/v solution of *didanosine RS* in *water* to 100.0 ml with the mobile phase.

Inject the reference solution. The test is not valid unless the column efficiency determined from the didanosine peak is not less than 5000 theoretical plates, the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{10}H_{12}N_4O_3$.

**Storage**. Store protected from light.

## Didanosine Capsules

Didanosine Capsules contain enteric-coated granules of Didanosine.

Didanosine Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of didanosine, $C_{10}H_{12}N_4O_3$.

**Usual strengths**. 125 mg; 200 mg; 250 mg; 400 mg.

### Identification

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

### Tests

**Dissolution** (2.5.2).

A. Apparatus No. 2,
Medium. 1000 ml of *0.1 M hydrochloric acid*,
Speed and time. 100 rpm and 120 minutes.

Determine by liquid chromatography (2.4.14).

*Test solution*. At the end of the test period dissolve all the granules from the basket in 750 ml of buffer solution pH 7.5 prepared by dissolving 1.41 g of *disodium hydrogen orthophosphate anhydrous* in 1000 ml of *water*, adjusting the pH to 7.5 with *orthophosphoric acid* and filtering, and dilute to 1000 ml with the buffer solution. Dilute suitably to get a solution containing about 0.005 per cent w/v of didanosine.

*Reference solution*. A 0.005 per cent w/v solution of *didanosine RS* in the buffer solution.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded porous silica (5µm),
- mobile phase: a mixture of 950 volumes of *buffer solution pH 7.5* and 50 volumes of *acetonitrile*,
- flow rate. 1.5 ml per minute,



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– spectrophotometer set at 249 nm,
– injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates the tailing factor is not more than 1.5 and the relative standard deviation of replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the percentage of $C_{10}H_{12}N_4O_3$ released in the acid medium by subtracting the content of $C_{10}H_{12}N_4O_3$ in the test solution from the total content of didanosine, $C_{10}H_{12}N_4O_3$ determined in the Assay.

Not more than 10 per cent of the stated amount of $C_{10}H_{12}N_4O_3$ is dissolved in 120 minutes.

B. Apparatus No. 2,

Medium. 1000 ml of a buffer solution prepared by mixing 250 ml of *0.2 M tribasic sodium phosphate buffer* and 750 ml of *0.1 M hydrochloric acid* and adjusting the pH to 6.8 with *2 M hydrochloric acid* or *2 M sodium hydroxide*,

Speed and time. 100 rpm and 45 minutes.

Run for 120 minutes at 100 rpm using the medium given in method A. At the end of this period discard the medium from each vessel without losing any of the granules and fill the empty vessel with the dissolution medium preheated to 37°. After running the apparatus for 45 minutes, withdraw a suitable volume of the medium and dilute to get a concentration of about 0.005 per cent w/v of didanosine in the dissolution medium.

Determine by liquid chromatography (2.4.14).

*Test solution*. The solution obtained in the manner described above.

*Reference solution*. A 0.005 per cent w/v solution of *didanosine RS* in the dissolution medium.

Use the chromatographic system described in test A.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 1.5 and the relative standard deviation of replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

D. Not less than 75 per cent of the stated amount of $C_{10}H_{12}N_4O_3$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*NOTE — Prepare the solutions immediately before use.*

*Test solution*. Weigh accurately a quantity of the contents of the capsules containing 100 mg of Didanosine, dissolve in 100 ml of mobile phase and filter.

*Reference solution (a)*. A 0.1 per cent w/v solution of *didanosine RS* in the mobile phase.

*Reference solution (b)*. Dilute 1 ml of reference solution (a) to 100 ml with the mobile phase.

Chromatographic system
– a stainless steel column 25 cm x 4.0 mm packed with octadecylsilane bonded to porous silica (5µm), (such as Lichrospher RP18e),
– mobile phase: a mixture of 95 volumes of a buffer solution prepared by dissolving 1.15 g of *ammonium dihydrogen orthophosphate* in 1000 ml of *water*, and 5 volumes of *acetonitrile*, adjust the pH to 6.8 with *triethylamine* and filter,
– flow rate. 1 ml per minute,
– spectrophotometer set at 254 nm,
– injection volume. 20 µl.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 4500 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and the reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 4 times the area of the peak in the chromatogram obtained with the reference solution (b) (4.0 per cent) and the sum of all the secondary peaks is not more than 5.5 times the area of the peak in the chromatogram obtained with the reference solution (5.5 per cent),

**Other tests**. Comply with the tests stated under Capsules.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh accurately a quantity of the contents of the capsules containing 50 mg of Didanosine, dissolve in 100.0 ml of the *buffer solution pH 7.5* and filter. Dilute 5.0 ml of the solution to 50.0 ml with the *buffer solution pH 7.5*.

*Reference solution*. A 0.005 per cent w/v solution of *didanosine RS* in *buffer solution pH 7.5*.

Chromatographic system
– a stainless steel column 15 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
– mobile phase: a mixture of 950 volumes of *buffer solution pH 7.5* and 50 volumes of *acetonitrile*,
– flow rate. 1.5 ml per minute,
– spectrophotometer set at 249 nm,
– injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates the tailing factor is not more than 1.5 and the relative standard deviation of replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{10}H_{12}N_4O_3$ in the capsules.



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**Storage**. Store protected from moisture, at a temperature not exceeding 30°.

# Didanosine Tablets

Didanosine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of didanosine, $C_{10}H_{12}N_4O_3$. The tablets may contain permitted flavouring agents.

**Usual strengths**. 25 mg; 50 mg; 100 mg; 150 mg; 200 mg.

## Identification

A. Shake a quantity of the powdered tablets containing 0.1 g of Didanosine with 80 ml of *water*, dilute to 100 ml with *water* and filter. Dilute 5 ml of the filtrate to 100 ml with *water*. When examined in the range 220 nm to 350 nm (2.4.7), the resulting solution shows an absorption maximum only at about 250 nm.

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

## Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh accurately a quantity of the powdered tablets containing about 50 mg of Didanosine and transfer to a 50-ml volumetric flask. Add about 25 ml of *buffer solution pH 7.0*, and mix with the aid of ultrasound for 5 minutes, dilute to volume with the same solvent, mix and filter.

*Reference solution*. Weigh accurately about 50 mg *didanosine RS* and transfer to a 50-ml volumetric flask. Dissolve in about 25 ml of *buffer solution pH 7.0* and dilute to volume with the same solvent. Dilute 5.0 ml of this solution to 50.0 ml with the same solvent. Dilute further 5.0 ml to 50.0 ml with the same solvent and filter through a membrane filter disc with an average pore diameter not greater than 0.45 µm.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Kromasil C18),
- mobile phase: filtered and degassed gradient mixtures of *acetonitrile* and buffer solution pH 7.0 prepared by dissolving 1.42 g of *disodium hydrogen phosphate* and 6.8 g of *tetrabutylammonium hydrogen sulphate* in 1000 ml of *water*, adjusting the pH of the solution to 7.0 ± 0.05 with *sodium hydroxide solution*,
- flow rate. 1.5 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 245 nm,
- injection volume. 5 µl.

| Time (in min.) | Buffer (pH 7.0) (per cent v/v) | Acetonitrile (per cent v/v) | Comment |
|---|---|---|---|
| 0 – 8 | 100 → 0 | 0 | isocratic |
| 20 – 25 | 70 →30 | 30 | linear gradient |
| 26 – 35 | 100 → 0 | 0 | re-equilibrium |

Inject the reference solution. The test is not valid unless the column efficiency determined from the didanosine peak is not less than 3000 theoretical plates and the tailing factor is not more than 1.5.

Inject separately the buffer and test solution. Examine the chromatogram obtained with the buffer solution for any extraneous peaks and ignore the corresponding peaks observed in the chromatogram obtained with the test solution.

Any secondary peak observed in the chromatogram obtained with the test solution should not be more than 5.0 per cent and the sum of the areas of all the secondary peaks should not be more than 6.0 per cent when calculated by percentage area normalisation.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh accurately a quantity of the powdered tablets containing about 100 mg of Didanosine and transfer to a 100-ml volumetric flask. Add about 50 ml of *buffer solution pH 7.0*, mix with the aid of ultrasound for 10 minutes, dilute to volume with the same solvent, mix and filter through a membrane filter disc with an average pore diameter not greater than 0.45 µm.

*Reference solution*. A 0.1 per cent w/v solution of *didanosine RS* in *buffer solution pH 7.0*. Filter through a membrane filter disc with an average pore diameter not greater than 0.45 µm.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Kromasil C18),
- mobile phase: a filtered and degassed mixture of 5 volumes of *acetonitrile* and 95 volumes of a buffer solution prepared by dissolving 1.42 g of *disodium hydrogen phosphate* in 1000 ml of *water*, adjusting the pH to 7.5 ± 0.05 with *dilute phosphoric acid*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 245 nm,
- injection volume. 5 µl.

Inject the reference solution and record the chromatogram for twice the retention time of didanosine. The test is not valid unless the column efficiency determined from the didanosine peak is not less than 4500 theoretical plates, the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 1.0 per cent.



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Inject alternately the test solution and the reference solution.

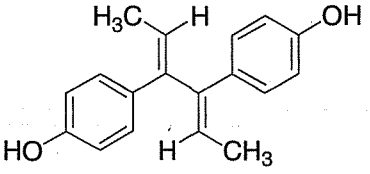
Calculate the content of $C_{10}H_{12}N_4O_3$ in the tablets.

**Storage**. Store protected from light.

**Labelling**. The label states that the tablets should be chewed before swallowing.

# Dienoestrol

Dienestrol




$C_{18}H_{18}O_2$ Mol. Wt. 266.3

Dienoestrol is *(E,E)*-4,4′-[bis(ethylidene)ethylene]diphenol.

Dienoestrol contains not less than 98.5 per cent and not more than 101.5 per cent of $C_{18}H_{18}O_2$, calculated on the dried basis.

**Category**. Oestrogen.

**Dose**. In the treatment of menopausal symptoms, 500 µg to 5 mg daily; in the treatment of prostate carcinoma and mammary carcinoma, 15 to 30 mg daily; for suppression of lactation, 15 mg thrice daily for 3 days, followed by 15 mg daily for 6 days.

**Description**. A white or almost white, crystalline powder.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dienoestrol RS*.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

C. Heat a mixture of about 1 mg in 5 ml of *glacial acetic acid* and 1 ml of a 1 per cent w/v solution of *bromine* in *glacial acetic acid* in a water-bath for 2 minutes. To 0.5 ml of the solution in a dry test tube add 0.5 ml of *ethanol*, mix and add 10 ml of *water*; a reddish-violet colour is produced. Add 5 ml of *chloroform*, shake vigorously and allow to separate; the chloroform layer is red and the aqueous layer is almost colourless.

D. Dissolve 0.5 mg in 0.2 ml of *glacial acetic acid*, add 1 ml of *phosphoric acid* and heat on a water-bath for 3 minutes; a reddish-violet colour is produced.

## Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 90 volumes of *toluene* and 10 volumes of *diethylamine*.

*Test solution (a)*. Dissolve 0.5 g of the substance under examination in 5 ml of *ethanol (95 per cent)*.

*Test solution (b)*. Dilute 5 ml of test solution (a) to 100 ml with *ethanol (95 per cent)*.

*Reference solution (a)*. A 0.5 per cent w/v solution of *dienoestrol RS* in *ethanol (95 per cent)*.

*Reference solution (b)*. Dilute 5 ml of reference solution (a) to 50 ml with *ethanol (95 per cent)*.

*Reference solution (c)*. A solution containing 0.25 per cent w/v each of *dienoestrol RS* and *stilbestrol RS* in *ethanol (95 per cent)*.

Apply to the plate 1 µl of each solution. After development, dry the plate in air, spray with *ethanolic sulphuric acid (20 per cent v/v)* and heat at 120° for 10 minutes. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b). The test is not valid unless the chromatogram obtained with reference solution (c) shows at least two clearly separated spots having approximately the same intensity.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 25 mg and dissolve in sufficient *ethanol* to produce 100.0 ml. To 5.0 ml of this solution add 10 ml of *ethanol*, dilute with *0.1 M sodium hydroxide* to 250.0 ml and measure the absorbance of the resulting solution at the maximum at about 245 nm (2.4.7). Calculate the content of $C_{18}H_{18}O_2$ from the absorbance obtained by repeating the procedure using *dienoestrol RS* in place of the substance under examination.

**Storage**. Store protected from light.

# Dienoestrol Tablets

Dienestrol Tablets

Dienoestrol Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of dienoestrol, $C_{18}H_{18}O_2$.

**Usual strength**. 1 mg.



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### Identification

A. Extract a quantity of the powdered tablets containing about 15 mg of Dienoestrol with *ether* and filter; evaporate the filtrate to dryness. Reserve a portion of the residue for test C. Heat a mixture of about 1 mg of the residue in 5 ml of *glacial acetic acid* and 1 ml of a 1 per cent w/v solution of *bromine* in *glacial acetic acid* in a water-bath for 2 minutes. To 0.5 ml of the solution in a dry test tube add 0.5 ml of *ethanol*, mix and add 10 ml of *water*; a reddish-violet colour is produced. Add 5 ml of *chloroform*, shake vigorously and allow to separate; the chloroform layer is red and the aqueous layer is almost colourless.

B. Dissolve 0.5 mg of the residue in 0.2 ml of *glacial acetic acid*, add 1 ml of *phosphoric acid* and heat on a water-bath for 3 minutes; a reddish-violet colour is produced.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 90 volumes of *toluene* and 10 volumes of *diethylamine*.

*Test solution*. Shake a quantity of the powdered tablets containing 2 mg of Dienoestrol with 4 ml of *acetone*, centrifuge and use the supernatant liquid.

*Reference solution (a)*. A 0.05 per cent w/v solution of *dienoestrol RS* in *acetone*.

*Reference solution (b)*. A solution containing 0.1 per cent w/v each of *dienoestrol RS* and *stilbestrol RS* in *acetone*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with *ethanolic sulphuric acid (20 per cent v/v)* and heat at 120° for 10 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots having approximately the same intensity.

### Tests

**Uniformity of content**. Comply with test stated under Tablets.

Powder one tablet and extract with successive quantities of *ether* until complete extraction is effected. Filter the ether solution and wash the filter paper with small quantities of *ether*. Evaporate the *ether* and dissolve the residue in 10 ml of *ethanol* and add sufficient *0.1 M sodium hydroxide* to produce a solution containing 0.0005 per cent w/v of Dienoestrol. Complete the test as described in the Assay beginning at the words "Measure the absorbance....".

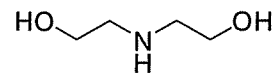
Calculate the content of $C_{18}H_{18}O_2$ in the tablet.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 10 mg of Dienoestrol and triturate with successive quantities of *ether* until complete extraction is effected. Filter the ether extracts and wash the filter with small quantities of *ether*. Combine the filtrate and washings and remove the *ether*; dissolve the residue in sufficient *ethanol* to produce 50.0 ml. To 5.0 ml of this solution add 10 ml of *ethanol* and sufficient *0.1 M sodium hydroxide* to produce 200.0 ml. Measure the absorbance of the resulting solution at the maximum at about 245 nm (2.4.7). Calculate the content of $C_{18}H_{18}O_2$ from the absorbance obtained by repeating the operation using a solution obtained by dissolving 2.5 mg, accurately weighed, of *dienoestrol RS* in 20 ml of *ethanol* and diluting with sufficient *0.1 M sodium hydroxide* to produce 500.0 ml.

**Storage**. Store protected from light.

## Diethanolamine




$C_4H_{11}NO_2$ Mol. Wt. 105.1

Diethanolamine is bis(hydroxyethyl)amine.

Diethanolamine is a mixture of ethanolamines, consisting largely of diethanolamine.

Diethanolamine contains not less than 98.5 per cent and not more than 101.0 per cent of ethanolamines, $NH(C_2H_4OH)_2$, calculated on the anhydrous basis.

**Category**. Pharmaceutical aid.

### Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *diethanolamine RS* or with the reference spectrum of diethanolamine.

### Tests

**Refractive index** (2.4.27). 1.473 to 1.476, determined at 30°.

**Water** (2.3.43). Not more than 0.15 per cent, determined on a 20.0 g of substance under examination in a mixture of 25 volumes of *glacial acetic acid* and 40 volumes of *methanol*.

**Triethanolamine**. To 100 ml of *methanol*, add 6 to 8 drops of mixed indicator of 0.15 g of *methyl orange* and 0.08 g of *xylene cyanole* in 100 ml of *water* in a 500-ml glass-stoppered conical flask and neutralize with *0.1 M ethanolic sulphuric acid* or *0.1 M ethanolic potassium hydroxide*. The neutral solution is amber when viewed by transmitted light and is red-brown when viewed by reflected light. Weigh accurately 20 g of the substance under examination, add into 500-ml glass-stoppered



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conical flask, cautiously add 75 ml of *acetic anhydride*, and swirl to effect complete solution. Allow to stand at room temperature for 30 minutes, cool. Titrate with *0.5 M ethanolic sulphuric acid solution*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

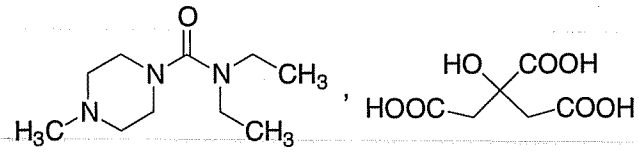
1 ml of *0.5 M ethanolic sulphuric acid* is equivalent to 0.0746 g of triethanolamine.

**Assay**. Weigh accurately about 2.0 g in a 250-ml conical flask and dissolve in sufficient *water* , using *bromocresol green solution* as indicator . Titrate with *0.5 M hydrochloric acid*, determine the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.5 M hydrochloric acid* is equivalent to 0.05257 g of $NH(C_2H_4OH)_2$.

**Storage**. Store protected from light and moisture.

# Diethylcarbamazine Citrate



$C_{10}H_{21}N_3O,C_6H_8O_7$ Mol. Wt. 391.4

Diethylcarbamazine Citrate is *N,N*-diethyl-4-methylpiperazine-1-carboxamide dihydrogen citrate.

Diethylcarbamazine Citrate contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{10}H_{21}N_3O,C_6H_8O_7$, calculated on the dried basis.

**Category**. Anthelmintic; antifilarial.

**Dose**. 150 mg to 500 mg daily.

**Description**. A white, crystalline powder; odourless; slightly hygroscopic.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *diethylcarbamazine citrate RS* or with the reference spectrum of diethylcarbamazine citrate.

B. In the test for *N,N'*-Dimethylpiperazine and *N*-methylpiperazine, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution (a).

C. A 2 per cent w/v solution gives reaction A of citrates (2.3.1).

## Tests

**Appearance of solution**. A 10.0 per cent w/v solution is not more opalescent than opalescence standard OS2 (2.4.1), and not more intensely coloured than reference solution BYS6 (2.4.1).

***N,N'*-Dimethylpiperazine and *N*-methylpiperazine**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 65 volumes of *methanol*, 30 volumes of *2-butanone* and 5 volumes of *strong ammonia solution*.

*Test solution*. Dissolve 0.5 g of the substance under examination in 10 ml of *methanol*.

*Reference solution (a)*. A 5 per cent w/v solution of *diethylcarbamazine citrate RS* in *methanol*.

*Reference solution (b)*. A 0.01 per cent w/v solution of *N,N'-dimethylpiperazine* in *methanol*.

*Reference solution (c)*. A 0.01 per cent w/v solution of *N-methylpiperazine* in *methanol*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate at 105° and expose it to iodine vapour for 30 minutes. Any spots corresponding to *N,N'*-dimethylpiperazine and *N*-methylpiperazine in the chromatogram obtained with the test solution are not more intense than the spots in the chromatogram obtained with reference solutions (b) and (c) respectively.

**Heavy metals** (2.3.13). 1.0 g dissolved in 20 ml of *water*, 0.5 ml of *0.1 M hydrochloric acid* and sufficient *water* to produce 25 ml complies with the limit test for heavy metals, Method A (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven over *phosphorus pentoxide* at 60° at a pressure of 1.5 to 2.5 kPa for 4 hours.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh accurately about 25 mg of the substance under examination, dissolve in 20 ml of a 3.124 per cent w/v solution of *potassium dihydrogen phosphate*, dilute to 25.0 ml with the same solvent, mix well and filter. Dilute 5.0 ml of the filtrate to 50.0 ml with the potassium dihydrogen phosphate solution.

*Reference solution*. A 0.01 per cent w/v solution of *diethylcarbamazine citrate RS* in a 3.124 per cent w/v solution of *potassium dihydrogen phosphate*.

Chromatographic system

– a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),



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- mobile phase: a mixture of 100 volumes of *methanol* and 900 volumes of a 1 per cent solution of *potassium dihydrogen phosphate*,
- flow rate. 2.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 20 µl.

Inject alternately suitable volumes of the test solution and reference solution. The test is not valid unless the relative standard deviation of the peak areas of diethylcarbamazine in replicate injections is not more than 2.0 per cent.

Calculate the content of $C_{10}H_{21}N_3O,C_6H_8O_7$.

**Storage**. Store protected from moisture.

# Diethylcarbamazine Tablets

Diethylcarbamazine Citrate Tablets

Diethylcarbamazine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of diethylcarbamazine citrate, $C_{10}H_{21}N_3O,C_6H_8O_7$.

**Usual strengths**. 50 mg; 100 mg.

## Identification

To a quantity of the powdered tablets containing 0.15 g of Diethylcarbamazine Citrate add 15 ml of *ethanol (95 per cent)*, shake for 5 minutes, filter and evaporate the filtrate to dryness. To the residue add 10 ml of *2 M sodium hydroxide* and extract with three quantities, each of 10 ml, of *chloroform*. Dry the combined extracts over *anhydrous sodium sulphate*, filter and evaporate the *chloroform*. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *diethylcarbamazine citrate RS* or with the reference spectrum of diethylcarbamazine citrate.

## Tests

***N,N'*-Dimethylpiperazine and *N*-methylpiperazine**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 65 volumes of *methanol*, 30 volumes of *2-butanone* and 5 volumes of *strong ammonia solution*.

*Test solution*. Dissolve 0.5 g of the substance under examination in 10 ml of *methanol*.

*Reference solution (a)*. A 5 per cent w/v solution of *diethylcarbamazine citrate RS* in *methanol*.

*Reference solution (b)*. A 0.01 per cent w/v solution of *N,N'- dimethylpiperazine* in *methanol*.

*Reference solution (c)*. A 0.01 per cent w/v solution of *N-methylpiperazine* in *methanol*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate at 105° and expose it to iodine vapour for 30 minutes. Any spots corresponding to *N,N'*-dimethylpiperazine and *N*-methylpiperazine in the chromatogram obtained with the test solution are not more intense than the spots in the chromatogram obtained with reference solutions (b) and (c) respectively.

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *water*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate. Dilute the filtrate, if necessary, with an equal volume of a 6.248 per cent w/v solution of *potassium dihydrogen phosphate*. Carry out the determination as described in the Assay. Calculate the content of $C_{10}H_{21}N_3O,C_6H_8O_7$ using a solution of known concentration of *diethylcarbamazine citrate RS* in a 3.124 per cent w/v solution of *potassium dihydrogen phosphate*.

D. Not less than 75 per cent of the stated amount of $C_{10}H_{21}N_3O,C_6H_8O_7$.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 25 mg of Diethylcarbamazine Citrate, add 20 ml of a 3.124 per cent w/v solution of *potassium dihydrogen phosphate* and place in an ultrasonic bath for 5 minutes. Cool, dilute to 25.0 ml with the same solvent and filter. Dilute 5.0 ml of the filtrate to 50.0 ml with the same solvent.

*Reference solution*. A 0.01 per cent w/v solution of *diethylcarbamazine citrate RS* in a 3.124 per cent w/v solution of *potassium dihydrogen phosphate*.

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 100 volumes of *methanol* and 900 volumes of a 1 per cent w/v solution of *potassium dihydrogen phosphate*,
- flow rate. 2.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 20 µl.



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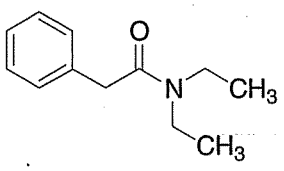


Inject alternately suitable volumes of the test solution and reference solution. The test is not valid unless the relative standard deviation of the peak areas of diethylcarbamazine in replicate injections is not more than 2.0 per cent.

Calculate the content of $C_{10}H_{21}N_3O,C_6H_8O_7$ in the tablets.

**Storage**. Store protected from moisture.

## Diethylphenylacetamide



$C_{12}H_{17}NO$ Mol. Wt. 191.3

Diethylphenylacetamide is *N,N*-diethylbenzeneacetamide

Diethylphenylacetamide contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{12}H_{17}NO$, calculated on the anhydrous basis.

**Category**. Pharmaceutical aid.

**Description**. A clear to faintly yellow liquid. It shall be free from suspended matter.

### Identification

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

### Tests

**Boiling point** (2.4.8). About 143°.

**Relative density** (2.4.29). About 1.01 at 30°.

**Refractive index** (2.4.27). 1.439 to 1.447 at 20° sodium D lines.

**Water** (2.3.43). Not more than 0.5 per cent.

**Assay**. Determine by gas chromatography (2.4.13).

*Internal standard solution*. A 3 per cent w/v solution of *diethyl sebaeate* in *acetone*.

*Test solution*. To 0.2 g of the substance under examination, add 10 ml of internal standard solution and dilute to the 100.0 ml with *acetone*.

*Reference solution*. To 50 mg of *diethylphenylacetamide RS*, add 2.5 ml of internal standard solution and dilute to the 25.0 ml with *acetone*.

Chromatographic system

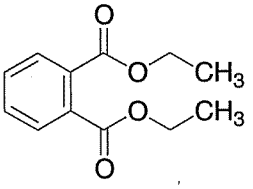
- a glass column 1.2 m x 2 mm packed with 10 per cent OV-101 on chromosorb WHP (100-120 mesh),
- temperature :
  column 150°,
  inlet port and detector at 300°,
- flow rate 30 ml per minute of the Nitrogen, 30 ml per minute of the Hydrogen, 210 ml per minute of the Air.

Inject 2 µl of the test solution and the reference solution.

Calculate the content of $C_{12}H_{17}NO$.

**Storage**. Store protected from light and moisture.

## Diethyl Phthalate



$C_{12}H_{14}O_4$ Mol. Wt. 222.2

Diethyl phthalate is diethyl benzene-1,2-dicarboxylate.

Diethyl phthalate contains not less than 99.0 per cent and not more than 101.0 per cent of diethyl phthalate, $C_{12}H_{14}O_4$.

**Category**. Pharmaceutical aid.

**Description**. A clear, oily liquid, colourless or very slightly yellow.

### Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *diethyl phthalate RS*.

B. Relative density (2.4.29). 1.117 to 1.121.

C. Determine by thin layer chromatography (2.4.17), coating the plate with *silica gel GF 254*.

*Mobile phase*. A mixture of 30 volumes of *heptane* and 70 volumes of *ether*.

*Test solution*. Dissolve 50 mg of the substance under examination in 10 ml of *ether*.

*Reference solution*. Dissolve 50 mg of *diethyl phthalate RS* in 10 ml of *ether*.

Apply to the plate 10 µl of each solution. After development dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution.

D. To about 0.1 ml, add 0.25 ml of *sulphuric acid* and 50 mg of *resorcinol*. Heat on a water-bath for 5 minutes. Allow to cool. Add 10 ml of *water* and 1 ml of *strong sodium hydroxide*



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*solution*. The solution becomes yellow or brownish-yellow and shows green fluorescence.

### Tests

**Appearance**. The substance under examination is clear (2.4.1) and not more intensely coloured than reference solution YS6 (2.4.1).

**Acidity**. Dissolve 20.0 g in 50 ml of *alcohol* previously neutralised to *phenolphthalein solution*. Add 0.2 ml of *phenolphthalein solution*. Not more than 0.1 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to pink.

**Related substances**. Determine by gas chromatography (2.4.13).

*Internal standard solution.* Dissolve 60 mg of *naphthalene* in 20 ml of *methylene chloride*.

*Test solution (a)*. Dissolve 1 g of the substance under examination in 20 ml of *methylene chloride*.

*Test solution (b)*. Dissolve 1 g of the substance under examination in *methylene chloride*, add 2.0 ml of the internal standard solution and dilute to 20 ml with *methylene chloride*.

*Reference solution.* To 1 ml of test solution (a) add 10 ml of the internal standard solution and dilute to 100 ml with *methylene chloride*.

Chromatographic system

- a glass column 2.0 m x 2 mm, packed with silanised diatomaceous earth for gas chromatography (150 µm to 180 µm) impregnated with 3 per cent m/m of *polymethylphenylsiloxane*,
- temperature :
  column 150°,
  inlet port and detector at 225°,
- flow rate. 30 ml per minute of the carrier gas.

Inject 1 µl of the reference solution. The test is not valid unless the resolution between the peaks corresponding to naphthalene and diethyl phthalate is at least 10.

Inject 1 µl of test solution (a). In the chromatogram obtained, verify that there is no peak with the same retention time as the internal standard.

Inject separately 1 µl of test solution (b) and the reference solution. Continue the chromatography for three times the retention time of diethyl phthalate. From the chromatogram obtained with the reference solution, calculate the ratio (*R*) of the area of the peak due to diethyl phthalate to the area of the peak due to the internal standard. From the chromatogram obtained with test solution (b), calculate the ratio of the sum of the areas of any peaks, other than the principal peak and the peaks due to the internal standard and the solvent, to the area of the peak due to the internal standard; this ratio is not greater than *R* (1.0 per cent).

**Water** (2.3.43). Not more than 0.2 per cent, determined on 5.0 g.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent, determined on 1.0 g.

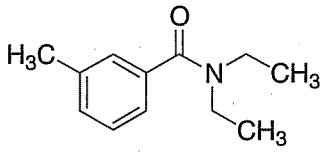
**Assay**. Weigh accurately about 0.75 g, dissolve in 25.0 ml of *0.5 M alcoholic potassium hydroxide* and add few glass beads. Boil on a water-bath under a reflux condenser for 1 hour. Add 1 ml of *phenolphthalein solution* and titrate immediately with *0.5 M hydrochloric acid*. Carry out a blank titration.

1 ml of *0.5 M alcoholic potassium hydroxide* is equivalent to 0.05556 g of $C_{12}H_{14}O_4$.

**Storage**. Store protected from moisture.

## Diethyltoluamide

Deet




$C_{12}H_{17}NO$ Mol. Wt. 191.3

Diethyltoluamide is *N,N*-diethyl-*3*-toluamide.

Diethyltoluamide contains not less than 95.0 per cent and not more than 103.0 per cent of $C_{12}H_{17}NO$, calculated on the anhydrous basis.

**Category**. Insect repellent.

**Description**. A colourless or faintly yellow liquid; odourless or almost odourless.

*CAUTION — Diethyltoluamide is irritant to the eyes and mucous membranes.*

### Identification

*Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *diethyltoluamide RS*.

B. Heat 2 ml with 25 ml of a 50 per cent v/v solution of *hydrochloric acid* under a reflux condenser for 1 hour. Make the mixture alkaline with *sodium hydroxide solution*, cool and extract with three quantities, each of 30 ml, of *ether*. Reserve the aqueous layer. Evaporate the ether, dissolve the residue in 5 ml of *dilute hydrochloric acid*, cool to 5°, add 5 ml of *sodium*



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*nitrite solution* and allow to stand for 10 minutes at 5°. Add 10 ml of *water* and extract with two quantities, each of 20 ml, of *ether*. Evaporate the ether, add 1 g of *phenol* to the residue, cool and add 1 ml of *sulphuric acid*; an intense green colour is produced, which becomes red on pouring into *water* and green on making alkaline with *dilute sodium hydroxide solution*.

C. Acidify the aqueous layer reserved in test B with *dilute hydrochloric acid*, extract with two quantities, each of 20 ml, of *ether* and evaporate the ether from the combined extracts. The residue, after drying at 60°, melts at about 108° (2.4.21).

### Tests

**Weight per ml** (2.4.29). 0.997g to 1.000 g, determined at 20°.

**Refractive index** (2.4.27). 1.520 to 1.524.

**Acidity**. A solution of 10.0 g dissolved in 50 ml of *ethanol (95 per cent)* previously neutralised to *phenolphthalein solution* requires not more than 4.0 ml of *0.01 M sodium hydroxide* to change the colour of the solution, using *phenolphthalein solution* as indicator.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

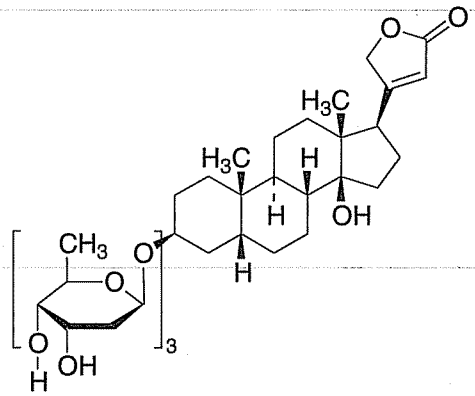
**Water** (2.3.43). Not more than 0.5 per cent, determined on 1.0 g.

**Assay**. Weigh accurately about 0.3 g, add 7 ml of *nitrogen-free sulphuric acid* and carry out the determination of nitrogen (2.3.30), using *0.05 M sulphuric acid* as the titrant.

1 ml of *0.05 M sulphuric acid* is equivalent to 0.01913 g of $C_{12}H_{17}NO$.

**Storage**. Store protected from moisture in dry containers.

## Digitoxin




$C_{41}H_{64}O_{13}$ Mol. Wt. 764.9

Digitoxin is 3β-[(*O*-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-*O*-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl)oxy]-14β-hydroxy-5β-card-20(22)-enolide.

Digitoxin contains not less than 95.0 per cent and not more than 103.0 per cent of $C_{41}H_{64}O_{13}$, calculated on the dried basis.

**Category**. Cardiotonic.

**Dose**. 50 to 200 µg daily.

**Description**. A white or almost white powder; odourless.

### Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry 2.4.6). Compare the spectrum with that obtained with *digitoxin RS*.

B. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

C. Dissolve about 1 mg in 2 ml of *glacial acetic acid* with the aid of gentle heat, cool and add 0.05 ml of *ferric chloride test solution*. Cautiously add 1 ml of *sulphuric acid* under the two liquids without mixing; a brown ring develops at the interface which gradually becomes blue and a green colour, finally passes to the upper layer.

D. Suspend about 0.5 mg in 0.2 ml of *ethanol (60 per cent)* and add 0.1 ml *dinitrobenzoic acid solution* and 0.1 ml of *2 M sodium hydroxide*; a violet colour develops.

### Tests

**Appearance of solution**. A 0.5 per cent w/v solution in a mixture of equal volumes of *chloroform* and *methanol* is clear (2.4.1), and colourless (2.4.1).

**Specific optical rotation** (2.4.22). +16.0° to +18.5°, determined at 20° in a 2.5 per cent w/v solution in *chloroform*.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 90 volumes of *chloroform*, 40 volumes of *cyclohexane* and 15 volumes of *methanol*.

*Solvent mixture*. A mixture of equal volumes of *chloroform* and *methanol*.

*Test solution*. Dissolve 0.1 g of the substance under examination in 10 ml with solvent mixture.

*Reference solution (a)*. A 1.0 per cent w/v solution of digitoxin RS in the same solvent mixture.

*Reference solution (b)*. Dilute 1 ml of reference solution (a) to 100 ml with the same solvent mixture.



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*Reference solution (c)*. A 0.02 per cent w/v solution of *gitoxin RS* in the same solvent mixture.

*Reference solution (d)*. Dilute 5 ml of reference solution (b) to 10 ml with the same solvent mixture.

*Reference solution (e)*. A solution containing 0.5 per cent w/v of *digitoxin RS* and 0.01 per cent w/v of *gitoxin RS* in the same solvent mixture.

Apply to the plate 5 µl of each solution and develop the chromatograms immediately after applying the solutions. After development, dry the plate in a current of cold air for 5 minutes. Repeat the development and again dry the plate in a current of cold air for 5 minutes. Spray with *ethanolic sulphuric acid (10 per cent)* and heat at 130° for 15 minutes. Examine the chromatograms in daylight. Any spot in the chromatogram obtained with the test solution corresponding to gitoxin is not more intense than the spot in the chromatogram obtained with reference solution (c). Any other secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b). The test is not valid unless the chromatogram obtained with reference solution (e) shows clearly separated spots corresponds to digitoxin and gitoxin and the spot in the chromatogram obtained with reference solution (d) is clearly visible.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent, determined on the residue obtained from the test for Loss on drying.

**Loss on drying** (2.4.19). Not more than 1.5 per cent, determined on 0.5 g by drying in an oven at 105° for 2 hours.

**Assay**. Weigh accurately about 40 mg, dissolve in sufficient *ethanol (95 per cent)* to produce 50.0 ml and dilute 5.0 ml of this solution to 100.0 ml with the same solvent. To 5.0 ml of this solution add 3.0 ml of *alkaline picric acid solution*, allow to stand in subdued light for 30 minutes and measure the absorbance of the resulting solution at the maximum at about 495 nm (2.4.7), using as the blank a mixture of 5.0 ml of *ethanol (95 per cent)* and 3.0 ml of *alkaline picric acid solution*. Calculate the content of $C_{41}H_{64}O_{13}$ from the absorbance obtained by repeating the operation using *digitoxin RS* in place of the substance under examination.

**Storage**. Store protected from moisture and light in a refrigerator (2° to 8°).

# Digitoxin Tablets

Digitoxin Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of digitoxin, $C_{41}H_{64}O_{13}$.

**Usual strengths**. 100 µg; 200 µg.

## Identification

To a quantity of the powdered tablets containing 250 µg of Digitoxin add 1 ml of *glacial acetic acid* containing 0.01 per cent w/v of *ferric chloride*, shake for a few minutes, filter through sintered-glass and add cautiously 1 ml of *sulphuric acid* to the filtrate without mixing; a brown ring free from red colour is produced at the interface which gradually becomes blue and finally the upper layer acquires an indigo colour.

## Tests

**Dissolution** (2.5.2).

Apparatus No. 2,
Medium. 600 ml of freshly distilled *water*,
Speed and time. 120 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter disc having an average pore diameter not greater than 0.8 µm, rejecting the first 1 ml of the filtrate. Transfer 1.0 ml to a 10-ml volumetric flask, add 3.0 ml of a 0.1 per cent w/v solution of *L-ascorbic acid* in *methanol* and 0.2 ml of a 0.009M solution of hydrogen peroxide [prepared by accurately diluting *hydrogen peroxide solution (100 vol)* that has been standardised by titration with *0.02 M potassium permanganate*], mix and dilute to volume with *hydrochloric acid*. After exactly 30 minutes measure the fluorescence of the solution (2.4.5), using an excitation wavelength of about 400 nm and an emission wavelength of about 570 nm and setting the spectrophotofluorimeter to zero with *water* and to 100 with a solution of suitable concentration of *digitoxin RS* prepared at the same time and treated in the same manner as the test solution.

D. Not less than 75 per cent of the stated amount of digitoxin, $C_{41}H_{64}O_{13}$.

**Uniformity of content**. Comply with test stated under Tablets.

*Test solution*. For tablets containing 100 µg of Digitoxin, shake 1 tablet with 15 ml of *methanol (50 per cent)* for 30 minutes and dilute to 25.0 ml with the same solvent.

For tablets containing 200 µg of Digitoxin, shake 1 tablet with 30 ml of *methanol (50 per cent)* for 30 minutes and dilute to 50.0 ml with the same solvent.

Filter through a suitable membrane filter disc having an average pore diameter not greater than 0.8 µm, rejecting the first few ml of the filtrate. Transfer 1.0 ml to a 10-ml volumetric flask, add 3.0 ml of a 0.1 per cent w/v solution of *L-absorbic acid* in *methanol* and 0.2 ml of a 0.009M solution of hydrogen peroxide [prepared by accurately diluting *hydrogen peroxide solution (100 vol)* that has been standardised by titration with *0.02 M potassium permanganate*], mix and dilute to volume with



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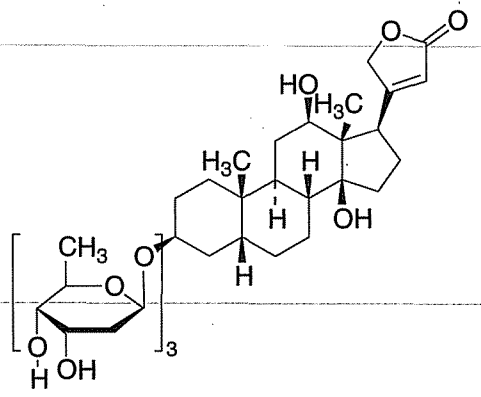
*hydrochloric acid*. After exactly 30 minutes measure the fluorescence of the solution (2.4.5), using an excitation wavelength of about 400 nm and an emission wavelength of about 570 nm and setting the spectrophotofluorimeter to zero with *water*. Calculate the content of digitoxin, $C_{41}H_{64}O_{13}$, from the *fluorescence* obtained by carrying out the operation described above at the same time using a 0.0004 per cent w/v solution of *digitoxin RS* in *methanol (50 per cent)* and beginning at the words "Transfer 1.0 ml to a 10-ml volumetric flask....".

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and finely powder 20 tablets. Weigh accurately a quantity of the powder containing about 1.25 mg of Digitoxin, add 3.0 ml of *water*, swirl to disperse the powder and allow to stand for 10 minutes, swirling occasionally. Add 25.0 ml of *glacial acetic acid*, shake for 1 hour and filter, discarding the first few ml of the filtrate. To 4.0 ml of the filtrate add 1.0 ml of *dimethyl sulphoxide, dilute* to 25.0 ml with *xanthydrol reagent*, mix well and allow to stand in the dark for 4½ hours (solution A). At the same time prepare two further solutions in the same manner but using for solution B 4.0 ml of *digitoxin standard solution* and for solution C 4.0 ml of a mixture of 25 volumes of *glacial acetic acid* and 3 volumes of *water* and beginning at the words "add 1.0 ml of *dimethyl sulphoxide*.....". Measure the absorbances of solutions A and B at the maximum at about 550 nm (2.4.7), using solution C as the blank. Calculate the content of $C_{41}H_{64}O_{13}$ from the absorbances obtained.

**Storage**. Store protected from light and moisture at a temperature not exceeding 30°.

# Digoxin



$C_{41}H_{64}O_{14}$ Mol. Wt. 780.9

Digoxin is 3β-[(*O*-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-O-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1128W1Ä4)-2, 6-dideoxy-β-D-*ribo*-hexopyranosyl)oxy]-12b,1428β-dihydroxy-5β-card-20(22)-enolide.

Digoxin contains not less than 95.0 per cent and not more than 103.0 per cent of $C_{41}H_{64}O_{14}$, calculated on the dried basis.

**Category**. Cardiotonic.

**Dose**. Orally, for rapid digitalisation, 1 to 1.5 mg in divided doses over 24 hours; for less urgent digitalisation, 250 to 500 µg daily; maintenance dose, 62.5 to 500 µg daily. By intravenous injection, 750 µg to 1 mg. For paediatric use, orally, 10 to 35 µg per kg of body weight in 3 or 4 divided doses over 24 hours followed by 4 to 10 µg per kg of body weight daily; maintenance dose, 250 µg once or twice daily.

**Description**. Colourless crystals or a white or almost white powder.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

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

B. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

C. Dissolve about 1 mg in 2 ml of *glacial acetic acid* with the aid of gentle heat, cool and add 0.05 ml of *ferric chloride test solution*. Cautiously add 1 ml of *sulphuric acid* under the two liquids without mixing; a brown ring develops at the interface which gradually becomes blue and a green colour, finally passes to the upper layer.

D. Suspend about 0.5 mg in 0.2 ml of *ethanol (60 per cent)* and add 0.1 ml *dinitrobenzoic acid solution* and 0.1 ml of *2 M sodium hydroxide*; a violet colour develops.

## Tests

**Appearance of solution**. A 0.5 per cent w/v solution in a mixture of equal volumes of *dichloromethane* and *methanol* is clear (2.4.1), and colourless (2.4.1).

**Specific optical rotation** (2.4.22). +10.0° to +13.0°, determined in a 2.0 per cent w/v solution in *anhydrous pyridine*.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *kieselguhr G*. Place the dry plate in a closed tank containing the necessary quantity of a mixture of 90 volumes of *acetone* and 10 volumes of *formamide* so that the plate dips about 5 mm into the liquid and allow the impregnating solvent to ascend at least 15 cm. Remove the plate from the tank, allow to stand for 30 minutes and then use immediately.



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*Mobile phase*. A mixture of 50 volumes of *2-butanone*, 50 volumes of *xylene* and 4 volumes of *formamide*.

*Solvent mixture*. A mixture of equal volumes of *dichloromethane* and *methanol*.

*Test solution*. Dissolve 0.1g of the substance under examination in 10 ml with solvent mixture.

*Reference solution (a)*. A 1.0 per cent w/v solution of *digoxin RS* in the same solvent mixture.

*Reference solution (b)*. Dilute 1 ml of reference solution (a) to 50 ml with the same solvent mixture.

*Reference solution (c)*. A 0.01 per cent w/v solution of *digoxin RS* in the same solvent mixture.

*Reference solution (d)*. Dilute 1 ml of reference solution (a) to 100 ml with the same solvent mixture.

*Reference solution (e)*. A 0.02 per cent w/v of *gitoxin RS* in the same solvent mixture.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of cold air until only the lower edge is still moist. Repeat the development and dry the plate at 115° for 20 minutes. Allow to cool, spray with a mixture of 15 volumes of a 25 per cent w/v solution of *trichloroacetic acid* in *ethanol (95 per cent)* and 1 volume of freshly prepared 3 per cent w/v solution of *chloramine T* and heat at 115° for 5 minutes. Examine in ultraviolet light at 365 nm. Any spots corresponding to digitoxin and gitoxin in the chromatogram obtained with the test solution are not more intense than the spots in the chromatograms obtained with reference solutions (d) and (e) respectively. Any other secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (c).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent, determined on the residue obtained from the test for Loss on drying.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 0.5 g by drying over *phosphorus pentoxide* at a pressure not exceeding 2.7 kPa.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 50 mg of the substance under examination in 200 ml of *ethanol (95 per cent)*.

*Reference solution*. A 0.025 per cent w/v solution of *digoxin RS* in *diluted ethanol (95 per cent)*.

Chromatographic system

- a stainless steel column 25 cm x 4.2 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 37 volumes of *water* and 13 volumes of *acetonitrile*,
- flow rate. 3 ml per minute,
- spectrophotometer set at 218 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the theoretical plates is not less than 1200 and tailing factor for the principal peak is not more than 2.0. The resolution between the peaks due to digoxin and digoxigenin analogue is not less than 4.0. The relative standard deviation for the replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{41}H_{64}O_{14}$.

**Storage**. Store protected from light and moisture at a temperature not exceeding 30°.

# Digoxin Injection

Digoxin Injection is a sterile solution of Digoxin in Water for Injections and Ethanol or other suitable solvents.

Digoxin Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of digoxin, $C_{41}H_{64}O_{14}$.

**Usual strength**. 250 µg per ml.

## Identification

Evaporate 2 ml to dryness, dissolve the residue in 1 ml of *glacial acetic acid* containing 0.01 per cent w/v of *ferric chloride* and cautiously add 1 ml of *sulphuric acid* without mixing; a brown ring develops at the interface which gradually becomes blue and finally the upper layer acquires a blue colour.

## Tests

**pH** (2.4.24). 6.7 to 7.3.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. Transfer 20 ml, accurately measured, to a separating funnel containing 10 ml of *water*. Make alkaline with *5 M ammonia* and extract with four quantities, each of 25 ml, of *chloroform*. Wash each extract with the same 10 ml of *water*. Evaporate the combined extracts to dryness on a water-bath, dissolve the residue in 5.0 ml of a mixture of 65 volumes of *chloroform* and 35 volumes of *methanol* and add 20.0 ml of *glacial acetic acid* (solution A). To 5.0 ml of a 0.2 per cent w/v solution of *digoxin RS* in *glacial acetic acid* add 10.0 ml of a mixture of 65 volumes of *chloroform* and 35 volumes of *methanol* and sufficient *glacial acetic acid* to produce 50.0 ml (solution B). Dilute 5.0 ml of solution A to 25.0 ml with



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*digoxin reagent,* mix, allow to stand for 1 hour and measure the absorbance of the resulting solution at about 590 nm, using *water* as the blank (2.4.7). Calculate the content of $C_{41}H_{64}O_{14}$ from the absorbance obtained by treating 5.0 ml of solution B at the same time and in the same manner.

**Storage**. Store protected from light in single dose containers.

## Digoxin Paediatric Solution

Paediatric Digoxin Elixir

Digoxin Paediatric Solution is a solution of Digoxin in a suitable flavoured vehicle.

Digoxin Paediatric Solution contains not less than 90.0 per cent and not more than 110.0 per cent w/v of the stated amount of digoxin, $C_{41}H_{64}O_{14}$.

**Usual strength**. 50 µg per ml.

### Identification

Digoxin Paediatric Solution should not be diluted before use and should be measured with a pipette.

Extract a quantity of the solution containing 250 µg of Digoxin with four quantities, each of 20 ml, of *chloroform,* washing each extract with the same 10 ml of *water*, evaporate the combined extracts to dryness and dissolve the residue in 1 ml of *glacial acetic acid* containing 0.01 per cent w/v of *ferric chloride*. Add cautiously 1 ml of *sulphuric acid* without mixing; a brown ring develops at the interface which gradually becomes blue and finally the upper layer acquires a blue colour.

### Tests

**pH** (2.4.24). 6.8 to 7.2.

**Other tests**. Complies with the tests stated under Oral Liquids.

**Assay**. Extract an accurately measured volume containing about 5 mg of Digoxin with four quantities, each of 25 ml, of *chloroform,* washing each extract with the same 5 ml of *water,* and evaporate the combined extracts to dryness. To the residue add 3 ml of *ethanol* and carefully evaporate to dryness on a water-bath with the aid of a gentle current of air. Repeat the evaporation using a further 3 ml of *ethanol* and cool. Dissolve the residue in 5.0 ml of a mixture of 65 volumes of *chloroform* and 35 volumes of *methanol*, add 20.0 ml of *glacial acetic acid* and filter if necessary. Dilute 5.0 ml of the filtrate to 25.0 ml with digoxin reagent, allow to stand for 1 hour and measure the absorbance of the resulting solution at the maximum at about 590 nm (2.4.7). Calculate the content of $C_{41}H_{64}O_{14}$ from the absorbance obtained by carrying out the operation described above at the same time but using a solution prepared by mixing 5.0 ml of a 0.2 per cent w/v solution of *digoxin RS* in *glacial acetic acid* with 10.0 ml of a mixture of 65 volumes of *chloroform* and 35 volumes of *methanol* and adding sufficient *glacial acetic acid* to produce 50.0 ml beginning at the words "Dilute 5.0 ml of the filtrate....." and using *water* as the blank.

**Storage**. Store protected from light at a temperature not exceeding 30°.

## Digoxin Tablets

Digoxin Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of digoxin, $C_{41}H_{64}O_{14}$.

**Usual strengths**. 62.5 µg; 125 µg; 250 µg.

### Identification

To a quantity of the powdered tablets containing 250 µg of Digoxin add 1 ml of *glacial acetic acid* containing 0.01 per cent w/v of *ferric chloride*, shake for a few minutes, filter through sintered-glass and add cautiously 1 ml of *sulphuric acid* to the filtrate without mixing; a brown ring free from red colour is produced at the interface which gradually becomes blue and finally the upper layer acquires an indigo colour.

### Tests

**Dissolution** (2.5.2).

Apparatus No. 2,
Medium. 600 ml of freshly distilled *water,*
Speed and time. 120 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter disc having an average pore diameter not greater than 0.8 µm, rejecting the first 1 ml of the filtrate. Transfer 1.0 ml to a 10-ml volumetric flask, add 3.0 ml of a 0.1 per cent w/v solution of L-*absorbic acid* in *methanol* and 0.2 ml of a 0.009M solution of hydrogen peroxide (prepared by accurately diluting *hydrogen peroxide solution (100 vol)* that has been standardised by titration with *0.02 M potassium permanganate*), mix and dilute to volume with *hydrochloric acid*. After exactly 2 hours measure the fluorescence of the solution (2.4.5), using an excitation wavelength of about 360 nm and an emission wavelength of about 490 nm and setting the spectrophotofluorimeter to zero with *water* and to 100 with a solution prepared at the same time as the test solution in the following manner. Dilute 2.5 ml of a 0.1 per cent w/v solution of *digoxin RS* in *ethanol (80 per cent)* to 100.0 ml with *water*, dilute the resulting solution further with *water* to



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produce a solution containing in 1 ml an amount of digoxin equal to one-hundredth of the strength of the tablets under examination, transfer 1.0 ml of the solution to a 10-ml volumetric flask and carry out the operation described above, beginning at the words "add 3.0 ml....".

D. Not less than 75 per cent of the stated amount of digoxin, $C_{41}H_{64}O_{14}$.

**Uniformity of content**. Comply with the test stated under Tablets.

*Test solution*. For tablets containing 250 µg of Digoxin, place 1 tablet with 10 ml of *water* at 37°, agitate to disintegrate, add 56 ml of *ethanol (95 per cent)*, shake for 60 minutes and add sufficient *ethanol (80 per cent)* to produce 100.0 ml.

For tablets containing 125 µg and 62.5 µg of Digoxin, repeat the above procedure by using proportionately smaller quantities of *water*, *ethanol (95 per cent)* and *ethanol (80 per cent)*.

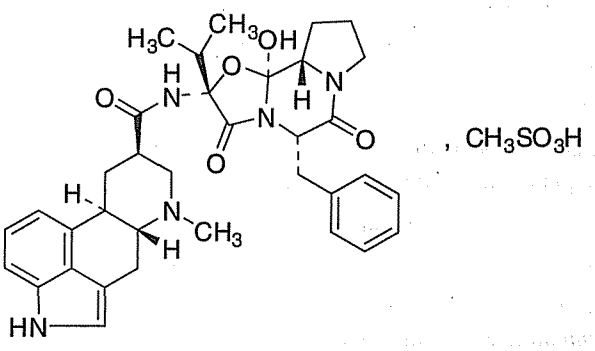
Filter through a suitable membrane filter disc having an average pore diameter not greater than 0.8 µm, rejecting the first few ml of the filtrate. Transfer 1.0 ml to a 10-ml volumetric flask, add 3.0 ml of a 0.1 per cent w/v solution of *L-absorbic acid in methanol* and 0.2 ml of a 0.009M solution of hydrogen peroxide [prepared by accurately diluting *hydrogen peroxide solution (100 vol)* that has been standardised by titration with *0.02 M potassium permanganate*], mix and dilute to volume with *hydrochloric acid*. After exactly 2 hours measure the fluorescence of the solution (2.4.5), using an excitation wavelength of about 360 nm and an emission wavelength of about 490 nm and setting the spectrophotofluorimeter to zero with *water*. Calculate the content of digoxin, $C_{41}H_{64}O_{14}$, from the fluorescence obtained by carrying out the operation described above at the same time using a 0.00025 per cent w/v solution of *digoxin RS* in *ethanol (80 per cent)* and beginning at the words "Transfer 1.0 ml to a 10-ml volumetric flask...."

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and finely powder 20 tablets. Weigh accurately a quantity of the powder containing about 1.25 mg of Digoxin, add 3.0 ml of *water*, swirl to disperse the powder and allow to stand for 10 minutes, swirling occasionally. Add 25.0 ml of *glacial acetic acid*, shake for 1 hour and filter, discarding the first few ml of the filtrate. To 4.0 ml of the filtrate add 1.0 ml of *dimethyl sulphoxide, dilute* to 25.0 ml with *xanthydrol reagent*, mix well and allow to stand in the dark for 4½ hours (solution A). At the same time prepare two further solutions in the same manner but using for solution B 4.0 ml of *digoxin standard solution* and for solution C 4.0 ml of a mixture of 25 volumes of *glacial acetic acid* and 3 volumes of *water* and beginning at the words "add 1.0 ml of *dimethyl sulphoxide*.....". Measure the absorbances of solutions A and B at the maximum at about 545 nm (2.4.7), using solution C as the blank. Calculate the content of $C_{41}H_{64}O_{14}$ from the absorbances obtained.

**Storage**. Store protected from light.

# Dihydroergocristine Mesylate



$C_{36}H_{45}N_5O_8S$ Mol. Wt. 708

Dihydroergocristine Mesylate is (5′a)-12′-hydroxy-2′-(1-methylethyl)-5′-(phenylmethyl)dihyroergotaman-3′,6′,18-trione methanesulphonate.

Dihydroergocristine Mesylate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{36}H_{45}N_5O_8S$, calculated on the dried basis.

**Category**. Antimigraine.

## Production

The production method must be evaluated to determine the potential for formation of alkyl mesilates, which is particularly likely to occur if the reaction medium contains lower alcohols. Where necessary, the production method is validated to demonstrate that alkyl mesilates are not detectable in the final product.

**Description**. A white or almost white, fine crystalline powder.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dihydroergocristine mesylate RS* or with the reference spectrum of dihydroergocristine mesylate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Solvent mixture*. 10 volumes of *methanol* and 90 volumes of *dichloromethane*.



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*Mobile phase.* A mixture of 2 volumes of *ammonia*, 15 volumes of *dimethylformamide* and 85 volumes of *ether*.

*Test solution.* Dissolve 0.1 g of the substance under examination in 5 ml of the same solvent mixture.

*Reference solution.* A 2.0 per cent w/v solution of *dihydroergocristine mesylate RS* in the solvent mixture.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 8 cm, protected from light. Dry the plate in current of cold air for 5 minutes. Spray with *dimethylamino-benzaldehyde solution* and dry in a current of hot air for 2 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Solvent mixture.* 10 volumes of *methanol* and 90 volumes of *dichloromethane*.

*Mobile phase.* A mixture of 5 volumes of *water*, 10 volumes of *ammonia*, 20 volumes of *butanol* and 65 volumes of *acetone*.

*Test solution.* Dissolve 0.2 g of the substance under examination in 5 ml of the solvent mixture.

*Reference solution.* A 0.4 per cent w/v solution of *methanesulphonic acid* in the solvent mixture.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 10 cm, protected from light. Dry the plate in current of cold air for not more than 1 minute. Spray with a 0.1 per cent w/v solution of *bromocresol purple* in *methanol*, adjusting the colour to violet-red with one drop of *dilute ammonia solution* and dry the plate in a current of hot air at 100°. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

## Tests

**Appearance of solution.** A 2.0 per cent w/v solution in *methanol* is clear (2.4.1) and not more intensely coloured than reference solution BS7 (2.4.1).

**pH** (2.4.24). 4.0 to 5.0, determined in a 0.5 per cent w/v solution in *carbon dioxide-free water*.

**Specific optical rotation** (2.4.22). –37.0° to –43.0°, determined in a 1.0 per cent w/v solution in *anhydrous pyridine*.

**Related substances.** Determine by liquid chromatography (2.4.14).

*NOTE—Carry out the test protected from light.*

*Solvent mixture.* 50 volumes of *acetonitrile* and 50 volumes of 0.1 per cent w/v solution of *orthophosphoric acid*.

*Test solution.* Dissolve 75 mg of the substance under examination in solvent mixture and dilute to 50.0 ml with *water*.

*Reference solution.* Dissolve 20 mg of *codergocrine mesilate RS* in solvent mixture and dilute to 50.0 ml with *water*. Dilute 6.0 ml of the solution to 50.0 ml with a mixture of 20 volumes of *acetonitrile*, 20 volumes of 0.1 per cent w/v solution of *orthophosphoric acid* and 60 volumes of *water*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 10 volumes of *acetonitrile*, 90 volumes of *water* and 1 volume of *triethylamine*,
  B. a mixture of 10 volumes of *water*, 90 volumes of *acetonitrile* and 1 volume of *triethylamine*,
- a linear gradient programme using the conditions given below,
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume. 10 µl.

| Time (min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0-5 | 75 | 25 |
| 5-20 | 75 → 25 | 25 → 75 |
| 20-22 | 25 → 75 | 75 → 25 |
| 22-30 | 75 | 25 |

Inject the reference solution. The relative retention time with reference to dihydroergocristine for dihydroergocornine (dihydroergocristine impurity F) is about 0.8; for α-dihydroergocryptine (dihydroergocristine impurity H) is about 0.9; for α-dihydroergocryptine or epicriptine (dihydroergocristine impurity I) is about 1.02. In the chromatogram shows 4 peaks, the resolution between the peaks corresponding to dihydroergocristine and dihydroergocristine impurity I is not less than 1.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent), the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (2.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (0.1 per cent).

**Loss on drying** (2.4.19). Not more than 3 per cent, determined on 0.5 g by drying under vacuum, in an oven at 80°.

**Assay.** Dissolve 0.3 g in 60 ml of *pyridine*. Pass a stream of nitrogen over the surface of the solution. Titrate with *0.1 M tetrabutylammonium hydroxide* to the second point of



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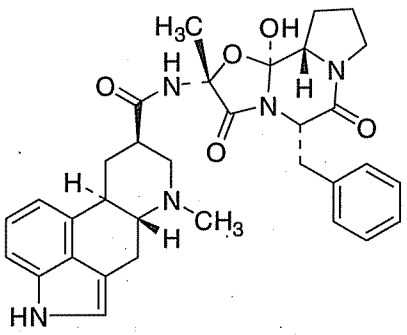


inflexion, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.03539 g of $C_{36}H_{45}N_5O_8S$.

**Storage**. Store protected from light.

# Dihydroergotamine Mesylate



, $CH_3SO_3H$

$C_{33}H_{37}N_5O_5,CH_4SO_3$ Mol. Wt. 680.0

Dihydroergotamine Mesylate is (5′a)-12′-hydroxy-2′-methyl-5′-(phenylmethyl)dihyroergotaman-3′,6′,18-trione methanesulphonate.

Dihydroergotamine Mesylate contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{33}H_{37}N_5O_5,CH_4SO_3$, calculated on the dried basis.

**Category**. Antimigraine.

## Production

The production method must be evaluated to determine the potential for formation of alkyl mesilates, which is particularly likely to occur if the reaction medium contains lower alcohols. Where necessary, the production method is validated to demonstrate that alkyl mesilates are not detectable in the final product.

**Description**. A white or almost white, crystalline powder or colourless crystals.

## Identification

*Tests A and C may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if tests A and C are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dihydroergotamine mesylate RS* or with the reference spectrum of dihydroergotamine mesylate.

B. When examined in the range 250 nm to 350 nm (2.4.7), a 0.005 per cent w/v solution in *methanol*, shows absorption maxima, at about 281 nm and 291 nm and a shoulder at 275 nm; specific absorbance at about 281 nm is 95 to 105.

C. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to the principal spot in the chromatogram obtained with reference solution (a).

D. To 0.1 g of the substance under examination, add 5 ml of *dilute hydrochloric acid,* shake for about 5 minutes and filter. Add 1 ml of *barium chloride solution*. The filtrate remains clear. Mix 0.1 g of the substance under examination with 0.4 g of powdered *sodium hydroxide*, heat to fusion and continue to heat for 1 minute. Cool, add 5 ml of *water*, boil and filter. Acidify the filtrate with *hydrochloric acid* and filter again. The filtrate gives reaction A of sulphates (2.3.1).

## Tests

**Appearance of solution**. Dissolve 0.1 g in a mixture of 0.1 ml of a 7.0 per cent w/v solution of *methanesulphonic acid* and 50 ml of *water*. The solution is clear (2.4.1) and not more intensely coloured than reference solution YS7 or BYS7 (2.4.1).

**pH** (2.4.24). 4.4 to 5.4, determined on 0.1 per cent w/v solution in *carbon dioxide- free water*.

**Specific optical rotation** (2.4.22). –42.0° to –47.0°, determined in a 1.0 per cent w/v solution in *anhydrous pyridine*.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*NOTE—Prepare the solutions immediately before use.*

*Solvent mixture*. 10 volumes of *methanol* and 90 volumes of *dichloromethane*.

*Mobile phase*. A mixture of 1 volume of *ammonia*, 6 volumes of *methanol*, 50 volumes of *ethyl acetate* and 50 volumes of *dichloromethane*.

*Test solution (a)*. Dissolve 0.1 g of the substance under examination in 5 ml of the solvent mixture.

*Test solution (b)*. Dilute 1 ml of test solution (a) to 10 ml with the solvent mixture.

*Reference solution (a)*. A 0.2 per cent w/v solution of *dihydroergotamine mesilate RS* in the solvent mixture.

*Reference solution (b)*. Dilute 2.5 ml of reference solution (a) to 50.0 ml with the solvent mixture.

*Reference solution (c)*. Dilute 2.0 ml of reference solution (b) to 5.0 ml with the solvent mixture.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 15 cm, protected from light. Dry the plate in current of cold air for not more than 1 minute and repeat the



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development, allow the freshly prepared mobile phase to rise 15 cm. Spray abundantly with *dimethylaminobenzaldehyde solution* and dry in a current of hot air for about 2 minutes. In the chromatogram obtained with test solution (a), any secondary spot is not more intense than the principal spot in the chromatogram obtained with reference solution (b) (0.5 per cent) and not more than 2 such spots are more intense than the principal spot in the chromatogram obtained with reference solution (c) (0.2 per cent).

**Loss on drying** (2.4.19). Not more than 4.0 per cent, determined on 0.5 g by drying in an oven at 105° at a pressure not exceeding 0.1 kPa for 5 hours.

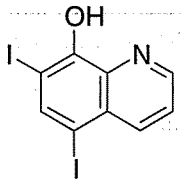
**Assay**. Dissolve 0.5 g in a mixture of 10 ml of *anhydrous acetic acid* and 70 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid is* equivalent to 0.068 g of $C_{34}H_{41}N_5O_8S$.

**Storage**. Store protected from light.

# Diiodohydroxyquinoline

Iodoquinol




$C_9H_5I_2NO$ Mol. Wt. 396.9

Diiodohydroxyquinoline is 5,7-diiodoquinolin-8-ol.

Diiodohydroxyquinoline contains not less than 97.0 per cent and not more than 100.5 per cent of $C_9H_5I_2NO$, calculated on the dried basis.

**Category**. Antiamoebic.

**Dose**. 1 to 2 g daily, in divided doses.

**Description**. A light yellowish to yellowish-brown, microcrystalline powder; odourless or with a faint odour.

## Identification

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

B. Dissolve 10 mg in 100 ml of *dioxan* and dilute 5 ml to 100 ml with *ethanol*. When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum at about 258 nm; absorbance at about 258 nm, about 0.53.

C. Heat a few crystals with about 1 ml of *sulphuric acid;* violet vapours of iodine are evolved.

## Tests

**Acidity or alkalinity**. Shake 0.5 g with 10 ml of *water* previously neutralised to *phenolphthalein solution*. The solution is colourless and not more than 0.1 ml of *0.1 M sodium hydroxide* is required to change the colour of the solution to pink.

**Free iodine and iodide**. Shake 1.0 g with 20 ml of *water* for 30 seconds, allow to stand for 5 minutes and filter. To 10 ml of the filtrate add 1 ml of *1 M sulphuric acid* and 2 ml of *chloroform* and shake; the chloroform layer does not become violet. To the mixture add 5 ml of *1 M sulphuric acid* and 1 ml of *potassium dichromate solution* and shake for 15 seconds; the colour of the chloroform layer does not become more intense than that produced by diluting 2 ml of a 0.016 per cent w/v solution of *potassium iodide* to 10 ml with *water*, adding 6 ml of *1 M sulphuric acid*, 1 ml of *potassium dichromate solution* and 2 ml of *chloroform* and shaking for 15 seconds.

**Related substances**. Determine by gas chromatography (2.4.13).

*Test solution*. Add 0.5 ml of N,O-*bis (trimethylsilyl)acetamide* to 0.5 ml of a solution in *pyridine* containing 0.4 per cent w/v of each of *5-chloro-8- hydroxyquinoline, 5,7-dichloro-8-hydroxy- quinoline* and *5-chloro-7-iodo-8-hydroxyquinoline* and 0.04 per cent w/v of the substance under examination, mix, allow to stand for 15 minutes and add 5 ml of a 0.05 per cent w/v solution of *dibutylphthalate* (internal standard) in *hexane*.

*Reference solution (a)*. Add 0.5 ml of N,O-*bis (trimethylsilyl) acetamide* to a mixture of 0.1 g of the substance under examination and 0.5 ml of *pyridine,* mix, allow to stand for 15 minutes and add 5 ml of *hexane*.

*Reference solution (b)*. Treat a mixture of 0.1 g of the substance under examination and 0.5 ml of *pyridine* as described for the test solution.

Chromatographic system

- a glass column 1.5m x 4 mm, packed with silanised diatomaceous support (100 to 120 mesh) coated with 3 per cent w/w of methyl silicone gum,
- temperature:
  column.190°,
  inlet port and detector. 240°,
- flame ionisation detector,
- nitrogen as carrier gas.



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In the chromatogram obtained with the test solution the peaks following the solvent peak, in order of emergence, are due to (a) 5-chloro-8-hydroxyquinoline, (b) 5,7-dichloro-8-hydroxyquinoline, (c) the internal standard, (d) 5-chloro-7-iodo-8-hydroxyquinoline and (e) diiodohydroxyquinoline. In the chromatogram obtained with reference solution (b) calculate the content of 5-chloro-8-hydroxy-quinoline, 5,7-dichloro-8-hydroxyquinoline and 5-chloro-7-iodo-8-hydroxyquinoline by reference to the corresponding peaks in the chromatogram obtained with the test solution. The total content of the named impurities and any other impurities does not exceed 4.0 per cent w/w.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 4 hours.

**Assay**. Weigh accurately about 0.3 g and dissolve in 50 ml of *anhydrous pyridine*. Titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.03969 g of $C_9H_5I_2NO$.

**Storage**. Store protected from light.

## Diiodohydroxyquinoline Tablets

Iodoquinol Tablets

Diiodohydroxyquinoline Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of diiodohydroxyquinoline, $C_9H_5I_2NO$.

**Usual strengths**. 300 mg; 600 mg.

### Identification

A. Triturate a quantity of the powdered tablets containing about 50 mg of Diiodohydroxyquinoline with 10 ml of *carbon disulphide*, filter and evaporate the solvent. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *diiodohydroxyquinoline RS* or with the reference spectrum of diiodohydroxyquinoline.

B. Shake a quantity of the powdered tablets containing about 10 mg of Diiodohydroxyquinoline with 100 ml of *dioxan*, filter and dilute 5 ml of the filtrate to 100 ml with *ethanol*. When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum at about 258 nm; absorbance at about 258 nm, about 0.53 (2.4.7).

### Tests

**Soluble iodides**. Digest a quantity of the powdered tablets containing 0.1 g of Diiodohydroxyquinoline with 5 ml of *water* for 10 minutes, cool and filter. To the filtrate add 1 ml of *3 M hydrochloric acid*, 0.1 ml of *ferric chloride test solution* and 2 ml of *chloroform*, shake gently and allow to separate; any violet colour in the chloroform is not more intense than that in a blank to which 1 ml of a 0.02 per cent w/v solution of *potassium iodide* has been added.

**Disintegration** (2.5.1). 30 minutes.

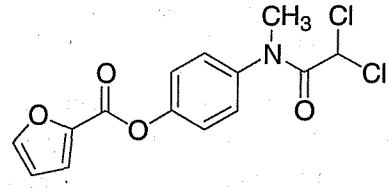
**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 12 mg of Diiodohydroxyquinoline and determine by the oxygen-flask method (2.3.34), using a mixture of 10 ml of *water* and 2 ml of *1 M sodium hydroxide* as the absorbing liquid. When the process is complete, add to the flask an excess (5 ml to 10 ml) of *acetic bromine solution* and allow to stand for 2 minutes. Remove the excess of bromine by the addition of *formic acid* (about 0.5 ml to 1 ml). Rinse the sides of the flask with *water* and sweep out any bromine vapour above the liquid with a current of air. Add 1 g of *potassium iodide* and titrate with *0.02 M sodium thiosulphate* using *starch solution*, added towards the end of the titration, as the indicator.

1 ml of *0.02 M sodium thiosulphate* is equivalent to 0.0006616 g of $C_9H_5I_2NO$.

**Storage**. Store protected from light.

## Diloxanide Furoate




$C_{14}H_{11}Cl_2NO_4$ Mol. Wt. 328.2

Diloxanide Furoate is 4-(*N*-methyl-2,2-dichloroacetamido) phenyl 2-furoate.

Diloxanide Furoate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{14}H_{11}Cl_2NO_4$, calculated on the dried basis.

**Category**. Antiamoebic.

**Dose**. 1.5 g daily, in divided doses.



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**Description**. A white or almost white, crystalline powder; odourless or almost odourless

### Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *diloxanide furoate RS* or with the reference spectrum of diloxanide furoate.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *ethanol (95 per cent)* shows an absorption maximum only at about 258 nm; absorbance at about 258 nm, about 0.70.

C. On 20 mg determine by the oxygen-flask method (2.3.34), using 10 ml of *1 M sodium hydroxide* as the absorbing liquid. When the process is complete, acidify the liquid with *nitric acid* and add *silver nitrate solution*; a white precipitate is produced.

### Tests

**Free acidity**. Shake 3.0 g with 50 ml of *water*, filter and wash the residue with three quantities, each of 20 ml, of *water*. Titrate the combined filtrate and washings with *0.1 M sodium hydroxide* using *phenolphthalein solution* as indicator; not more than 1.3 ml is required.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254*.

*Mobile phase*. A mixture of 96 volumes of *dichloromethane* and 4 volumes of *methanol*.

*Test solution*. Dissolve 0.5 g of the substance under examination in 5 ml of *chloroform*.

*Reference solution*. Dilute 1 ml of the test solution to 100 ml with *chloroform* and mix. Dilute 5 ml of the resulting solution to 20 ml with *chloroform*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.3.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.3 g and dissolve in 50 ml of *anhydrous pyridine*. Titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.03282 g of $C_{14}H_{11}Cl_2NO_4$.

**Storage**. Store protected from light.

## Diloxanide Tablets

Diloxanide Furoate Tablets

Diloxanide Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of diloxanide furoate, $C_{14}H_{11}Cl_2NO_4$.

**Usual strength**. 500 mg.

### Identification

A. Extract a quantity of the powdered tablets containing 0.2 g of Diloxanide Furoate with 20 ml of *chloroform*, filter and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *diloxanide furoate RS* or with the reference spectrum of diloxanide furoate.

B. On 20 mg of the residue obtained in test A determine by the oxygen-flask method (2.3.34), using 10 ml of *1 M sodium hydroxide* as the absorbing liquid. When the process is complete, acidify the liquid with *nitric acid* and add *silver nitrate solution*; a white precipitate is produced.

C. The residue obtained in test A melts at 114° to 116° (2.4.21).

### Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254*.

*Mobile phase*. A mixture of 96 volumes of *dichloromethane* and 4 volumes of *methanol*.

*Test solution*. Shake a quantity of the powdered tablets containing 0.5 g of Diloxanide Furoate with 5 ml of *chloroform*, centrifuge and use the supernatant liquid.

*Reference solution*. Dilute 1 ml of the test solution to 100 ml with *chloroform* and mix. Dilute 5 ml of the resulting solution to 20 ml with *chloroform*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 40 mg of Diloxanide Furoate, shake with 150 ml of *ethanol (95 per cent)* for 30 minutes, add sufficient *ethanol (95 per cent)* to produce 200.0 ml, mix and filter. Dilute 10.0 ml of the filtrate to 250.0 ml with *ethanol (95 per cent)* and measure the absorbance of the resulting solution at the maximum at about 258 nm (2.4.7).



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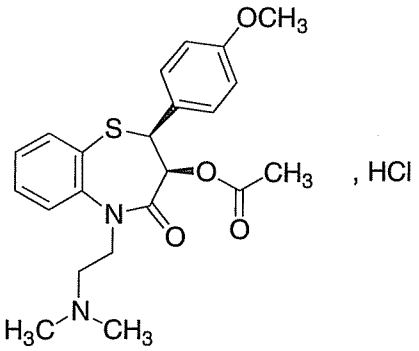
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Calculate the content of $C_{14}H_{11}Cl_2NO_4$ taking 705 as the specific absorbance at 258 nm.

**Storage**. Store protected from light.

## Diltiazem Hydrochloride




$C_{22}H_{26}N_2O_4S,HCl$ Mol. Wt. 451.0

Diltiazem Hydrochloride is (*2S,3S*)-2,3,4,5-tetrahydro-5-(2- dimethylaminoethyl)-2-(4-methoxyphenyl)-4-oxobenzo[*b*]thiazepin-3-yl acetate hydrochloride.

Diltiazem Hydrochloride contains not less than 98.5 per cent and not more than 101.5 per cent of $C_{22}H_{26}N_2O_4S,HCl$, calculated on the dried basis.

**Category**. Antianginal; (calcium-channel blocker).

**Dose**. Initially, 30 to 60 mg thrice daily; maximum, 480 mg daily.

**Description**. A white, crystalline powder or small crystals.

### Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *diltiazem hydrochloride RS*.

B. In the test for Related substances, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to diltiazem hydrochloride in the chromatogram obtained with the reference solution.

C. A 5 per cent w/v solution gives the reactions of chlorides (2.3.1).

### Tests

**Specific optical rotation** (2.4.22). +110° to +116°, determined in a 1.0 per cent w/v solution.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve 0.12 g of the substance under examination in 100 ml of *methanol.*

*Reference solution.* A solution containing 0.0012 per cent w/v each of *diltiazem hydrochloride RS* and *desacetyl diltiazem hydrochloride RS* in *methanol.*

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of a buffer solution containing 0.116 per cent w/v of d-*10-camphorsulphonic acid* in *0.1 M sodium acetate*, with the pH adjusted to 6.2 by the addition of *0.1 M sodium hydroxide*, 25 volumes of *acetonitrile* and 25 volumes of *methanol,* filtered and degassed,
- flow rate. 1.6 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume. 10 µl.

Inject the reference solution and measure the peak responses of all the peaks. The relative retention times for desacetyl diltiazem and diltiazem are about 0.65 and 1.0 respectively. The resolution between desacetyl diltiazem and diltiazem is not less than 3, and the theoretical plates for the diltiazem peak is not less than 1200. The relative standard deviation of the peak response for replicate injections due to diltiazem hydrochloride and desacetyl diltiazem is not more than 2.0 per cent.

Inject the test solution and the reference solution and measure the responses of all the peaks.

Calculate the percentage content of desacetyl diltiazem hydrochloride in the substance under examination by comparing the area of the peaks due to desacetyl diltiazem in the chromatograms of the test solution and the reference solution and from the content of desacetyl diltiazem hydrochloride in the reference solution. Similarly, calculate the percentage content of each impurity peak other than the peaks due to diltiazem and desacetyl diltiazem with that of the peak due to desacetyl diltiazem in the chromatogram obtained with the test solution and from the content of desacetyl diltiazem hydrochloride in the reference solution. Desacetyl diltiazem hydrochloride content is not more than 0.5 per cent w/v, the total impurities including desacetyl diltiazem hydrochloride content is not more than 1 per cent w/v with no individual impurity more than 0.5 per cent w/v.

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

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 2 hours.

**Assay**. Determine by liquid chromatography (2.4.14) as given under the test for Related substances using the following solutions.



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*Test solution*. Dissolve 0.12 g of the substance under examination in 100 ml of *methanol*.

*Reference solution (a)*. A solution containing 0.0012 per cent w/v each of *diltiazem hydrochloride RS* and *desacetyl diltiazem hydrochloride RS* in *methanol*.

*Reference solution (b)*. Dissolve 60 mg of *diltiazem hydrochloride RS* in 50 ml of *methanol*.

Inject reference solution (a) and check the system suitability parameters like the relative retention times, the resolution and the column efficiency in terms of theoretical plates.

Inject reference solution (b). The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and reference solution (b).

Calculate the content of $C_{22}H_{26}N_2O_4S$,HCl.

**Storage**. Store protected from light.

# Diltiazem Tablets

Diltiazem Hydrochloride Tablets

Diltiazem Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of diltiazem hydrochloride, $C_{22}H_{26}N_2O_4S$, HCl. They may be Modified-release Tablets.

**Usual strengths**. 30 mg; 60 mg.

## Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to diltiazem hydrochloride in the chromatogram obtained with the reference solution (a).

## Tests

**Other tests**. Comply with the tests stated under Tablets.

**Dissolution** (2.5.2).

A. *For Modified-release Tablets*
Apparatus No. 1,
Medium. 900 ml of freshly distilled *water,*
Speed and time. 100 rpm and 30 minutes and 3 hours.

Withdraw a suitable volume of the medium after 30 minutes and 3 hours. Filter promptly through a membrane filter disc with an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate. Dilute a suitable volume of the filtrate with the same solvent and measure the absorbance of the resulting solution at the maximum at about 240 nm (2.4.7). Calculate the content of $C_{22}H_{26}N_2O_4S$,HCl from the absorbance obtained from a solution of known concentration of *diltiazem hydrochloride RS*.

Use the following acceptance criteria for the 30-minute time interval. At $S_1$: no unit is more than D; at $S_2$, the average value is equal to or less than D, and no unit is greater than D + 10 per cent; at $S_3$. the average value is equal to or less than D, not more than 2 units are more than D + 10 per cent and no unit is more than D + 25 per cent. Use the acceptance criteria in Acceptance Table 1(2.5.2) for the 3-hour time interval.

D. Not more than 60 per cent of the stated amount of $C_{22}H_{26}N_2O_4S$,HCl is dissolved in 30 minutes and not less than 80 per cent is dissolved in 3 hours.

B. *For Conventional-release Tablets*

Apparatus No. 1,
Medium. 900 ml of freshly distilled *water,*
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium. Filter promptly, rejecting the first few ml of the filtrate. Dilute a suitable volume of the filtrate with the same solvent and measure the absorbance of the resulting solution at the maximum at about 240 nm (2.4.7). Calculate the content of $C_{22}H_{26}N_2O_4S$,HCl from the absorbance obtained from a solution of known concentration of *diltiazem hydrochloride RS*.

D. Not less than 75 per cent of the stated amount of $C_{22}H_{26}N_2O_4S$,HCl.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.6 g of Diltiazem Hydrochloride, add 200 ml of *methanol*, mix with the aid of ultrasound for 1 hour, cool and dilute to 500.0 ml with *methanol*; centrifuge 25 ml at 3500 rpm for 15 minutes and use the clear, supernatant liquid.

*Reference solution (a)*. A solution containing 0.0012 per cent w/v each of *diltiazem hydrochloride RS* and *desacetyl diltiazem hydrochloride RS* in *methanol*.

*Reference solution (b)*. Dissolve 60 mg of *diltiazem hydrochloride RS* in 50 ml of *methanol*.

Chromatographic system
- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of a buffer solution containing 0.116 per cent w/v of d-*10-camphorsulphonic acid* in *0.1 M sodium acetate*, with the pH adjusted to 6.2 by the addition of *0.1 M sodium hydroxide*, 25 volumes of *acetonitrile* and 25 volumes of *methanol,* filtered and degassed,
- flow rate. 1.6 ml per minute,



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- spectrophotometer set at 240 nm,
- injection volume. 10 µl.

Inject reference solution (a) and measure the peak responses of all the peaks. The relative retention times for desacetyl diltiazem and diltiazem are about 0.65 and 1.0 respectively. The resolution between desacetyl diltiazem and diltiazem is not less than 3, and the theoretical plates for the diltiazem peak is not less than 1200.

Inject reference solution (b).The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

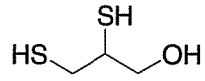
Inject alternately the test solution and reference solution (b).

Calculate the content of $C_{22}H_{26}N_2O_4S$,HCl in the tablets..

**Storage**. Store protected from light.

# Dimercaprol

B.A.L.




$C_3H_8OS_2$ Mol. Wt. 124.2

Dimercaprol is (*RS*)-2,3-dimercaptopropanol.

Dimercaprol contains not less than 98.5 per cent w/w and not more than 101.5 per cent w/w of $C_3H_8OS_2$.

**Category**. Antidote in heavy metal poisoning; metal complexing agent.

**Dose**. By intramuscular injection, 2 to 3 mg per kg of body weight every 4 hours during the first day and subsequently, in accordance with the needs of the patient.

**Description**. A clear, colourless or slightly yellow liquid; odour, strong, characteristic and alliaceous.

## Identification

A. Dissolve 0.1 ml in 4 ml of *water* and to 2 ml of the solution add *lead acetate solution*; a yellow precipitate is obtained.

B. To 2 ml of the solution prepared for test A add 1 ml of *0.05 M iodine*; the colour of iodine is immediately discharged.

C. In a ground-glass-stoppered tube suspend 0.6 g of *sodium bismuthate*, previously heated to 200° for 2 hours, in a mixture of 6 ml of *water* and 2.8 ml of a 10 per cent w/w solution of *phosphoric acid*. Add 0.2 ml of the substance under examination, mix and allow to stand for 10 minutes shaking frequently. To 1 ml of the supernatant liquid add 5 ml of a 0.4 per cent w/v solution of *chromotropic acid sodium salt* in *sulphuric acid*, mix and heat for 15 minutes in a water-bath; a violet-red colour is produced.

## Tests

**Appearance of solution**. The substance under examination is clear (2.4.1), and not more intensely coloured than reference solution BS6 or BYS6 (2.4.1).

**pH** (2.4.24). 5.0 to 6.5, determined in a saturated solution.

**Refractive index** (2.4.27). 1.568 to 1.574, determined at 20°.

**Weight per ml** (2.4.29). 1.238 g to 1.240 g.

**Iron** (2.3.14). Ignite 2.0 g with 1 g of *anhydrous sodium carbonate*, cool, dissolve the residue in 15 ml of *dilute hydrochloric acid* and dilute to 45 ml with *water*; the resulting solution complies with the limit test for iron (20 ppm).

**Halides**. To 2.0 g add 25 ml of *0.5 M ethanolic potassium hydroxide* and heat under a reflux condenser for 2 hours. Remove the ethanol by evaporation in a current of warm air, add 20 ml of *water* and cool. Add a mixture of 10 ml of *strong hydrogen peroxide solution* and 40 ml of *water*. Boil gently for 10 minutes; cool and filter rapidly. Add 10 ml of *dilute nitric acid* and 5 ml of *0.1 M silver nitrate* and titrate with *0.1 M ammonium thiocyanate* using *ferric ammonium sulphate solution* as indicator. Repeat the operation without the substance under examination. The difference in the volumes of *0.1 M ammonium thiocyanate* used in the two titrations is not more than 1.0 ml.

**Assay**. Weigh accurately about 0.1 g, dissolve in 40 ml of *methanol* and add 20 ml of *0.1 M hydrochloric acid* and 50.0 ml of *0.05 M iodine*. Allow to stand for 10 minutes and titrate with *0.1 M sodium thiosulphate*. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of iodine required.

1 ml of *0.05 M iodine* is equivalent to 0.00621 g of $C_3H_8OS_2$.

**Storage**. Store protected from light in well-filled containers in a refrigerator (2° to 8°).

# Dimercaprol Injection

B.A.L. Injection

Dimercaprol injection is a sterile solution of Dimercaprol in a mixture of Benzyl Benzoate and Arachis Oil.

Dimercaprol Injection contains not less than 90.0 per cent and more than 110.0 per cent of the stated amount of dimercaprol, $C_3H_8OS_2$.

**Usual strength**. 50 mg per ml.



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**Description**. A clear, yellow, viscous solution, having a pungent, disagreeable odour.

### Tests

**Acidity**. Shake with an equal volume of *water* for 2 minutes and set aside for separation; pH of the aqueous layer after filtration through a neutral filter is 4.5 to 6.5 (2.4.24).

**Refractive index** (2.4.27). 1.481 to 1.486, determined at 20°.

**Weight per ml** (2.4.29). About 0.95 g.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. Weigh accurately about 1.0 g, add 20 ml of *0.1 M hydrochloric acid* and titrate with *0.05 M iodine*.

1 ml of *0.05 M iodine* is equivalent to 0.00621 g of $C_3H_8OS_2$.

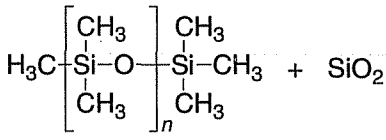
Determine the weight per ml of the injection (2.4.29), and calculate the percentage w/v of $C_3H_8OS_2$.

**Storage**. Store protected from light.

**Labelling**. The label states (1) the nature of the solvent; (2) that the injection is meant for intramuscular use only.

## Activated Dimethicone

Simethicone; Activated Polydimethylsiloxane



$(CH_3)_3Si[OSi(CH_3)_2]_nCH_3+SiO_2$

Activated Dimethicone is a mixture of fully methylated linear siloxane polymers containing repeating $-(CH_3)_2SiO-$ units stabilised with trimethylsiloxy, $(CH_3)_3SiO-$, end-blocking units and finely divided silicon dioxide.

Activated Dimethicone contains not less than 90.0 per cent and not more than 99.0 per cent of polydimethylsiloxane, $[-(CH_3)_2SiO-]_n$ and not less than 4.0 per cent and not more than 7.0 per cent of silicon dioxide, $SiO_2$.

**Category**. Defoaming agent.

**Dose**. 40 to 100 mg, four times daily.

**Description**. A translucent, grey viscous liquid; almost odourless.

### Identification

A. To 50 mg add 25 ml of *carbon tetrachloride* and swirl to disperse. Add 50 ml of *dilute hydrochloric acid* and shake for 5 minutes. Transfer to a separating funnel and remove about 5 ml of the lower layer to a stoppered tube containing 0.5 g of *anhydrous sodium sulphate*. Shake vigorously and centrifuge the mixture until a clear supernatant liquid is obtained. The resulting liquid complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dimethicone RS*. Ignore the region of the spectrum from 850 to 750 $cm^{-1}$ since slight differences may be observed depending on the degree of polymerisation.

B. Heat 0.5 g in a test-tube over a small flame until white fumes are evolved. Invert the test-tube over a second tube containing 1 ml of a 0.1 per cent w/v solution of *chromotropic acid sodium salt* in *sulphuric acid* so that the fumes reach the solution. Shake the second tube for about 10 seconds and heat on a water-bath for 5 minutes; the solution is violet.

C. To 50 mg in a platinum crucible add 0.15 ml of *sulphuric acid* and ignite until a white residue is obtained; the residue gives the reaction of silicates (2.3.1).

### Tests

**Acidity**. To 2.0 g add 25 ml of a mixture of equal volumes of *ethanol* and *ether* previously neutralised to 0.2 ml of *bromothymol blue solution* and shake; not more than 0.15 ml of *0.01 M sodium hydroxide* is required to change the colour of the solution to blue.

**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

**Defoaming activity**. Weigh accurately about 0.2 g, transfer to a 100-ml bottle, add 50 ml of *2-methylpropanol* and shake vigorously, warming, if necessary, to effect solution. Add dropwise, 0.5 ml of this solution to a clean, unused, cylindrical 250-ml glass jar, fitted with a 50-mm cap, containing 100 ml of a 1.0 per cent w/v solution of *octoxinol*. Cap the jar and clamp it in an upright position in a wrist-action shaker capable of moving the jar through a radius of 13.3 ± 0.4 cm (measured from the centre of the shaft to the centre of the jar) and an arc of 10 degrees at a frequency of 300 ± 30 strokes per minute. Shake for 10 seconds and record the time required in seconds for the foam to collapse. The time for foam collapse is determined at the instant the first portion of foam-free liquid surface appears, measured from the end of the shaking period. The defoaming activity time is not more than 15 seconds.

**Assay**. *For polydimethylsiloxane* - Weigh accurately about 50 mg, transfer to a narrow-mouthed glass bottle and add 25 ml of *carbon tetrachloride*. Swirl to disperse, add 50 ml of *dilute hydrochloric acid*, close the bottle securely with a cap having an inert liner and shake for exactly 5 minutes. Transfer the mixture to a 125-ml separating funnel and remove about 5 ml of the lower layer to a stoppered test-tube containing



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0.5 g of *anhydrous sodium sulphate*. Close the test-tube, agitate vigorously and centrifuge the mixture until a clear supernatant liquid is obtained. Prepare a blank by mixing 10 ml of *carbon tetrachloride* with 0.5 g of *anhydrous sodium sulphate* and centrifuging to obtain a clear supernatant liquid. Determine the absorbance of a 0.5-mm layer of the solution at the maximum at about 7.9 µm in a suitable infra-red spectrophotometer (2.4.6), using the blank to set the instrument. Calculate the content of $[-(CH_3)_2SiO-]_n$ from the absorbance obtained by repeating the Assay on a 0.2 per cent w/v solution of *dimethicone RS* in place of the substance under examination.

*For silicon dioxide* - Mix thoroughly and weigh accurately about 1.0 g; transfer to a tared, sintered-glass filtering crucible (porosity No. 4) and pass through the filter, with suction, 200 ml of *carbon tetrachloride*, added with stirring in small portions, followed by similar washing of the material on the filter with 200 ml of *n-hexane*, and discard the filtrates. Place the filtering crucible in a muffle furnace at room temperature, raise the temperature of the furnace to 550°. Heat at 550° ± 25° for 2 hours. Cool the filtering crucible with its contents in a desiccator, weigh and calculate the content of silicon dioxide, $SiO_2$, in the sample taken.

## Dimethicone

Dimethicone is α-(Trimethylsilyl)-ω-methylpoly[oxy(dimethylsilylene).

Dimethicone is a mixture of fully methylated linear siloxane polymers containing repeating units of the formula; $[-(CH_3)_2SiO-]_n$ stabilized with trimethylsiloxy end-blocking units of the formula; $[(CH_3)_3SiO-]$ where in *n* has an average value such that the corresponding nominal viscosity is in a discrete range between 20 and 30,000 centistokes.

Dimethicone contains not less than 97.0 per cent and not more than 103.0 per cent of polydimethylsiloxane, $([-(CH_3)_2SiO-]_n)$.

### Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *polydimethylsiloxane RS* or with the reference spectrum of polydimethylsiloxane.

### Tests

**Specific gravity**. Within the limits specified in the accompanying table.

**Viscosity** (2.4.28). Determine the viscosity of Dimethicone having a nominal viscosity of less than 1000 centistokes at 25 ± 0.1°, using a capillary viscosimeter. Determine the viscosity of Dimethicone having a nominal viscosity of 1000 centistokes or more at 25 ± 0.1°, using a rotational viscosimeter. The viscosity is within the limits specified in the accompanying table.

**Refractive Index** (2.4.27). Within the limits specified in the accompanying table.

**Acidity**. Dissolve 15.0 g in a mixture of 15 ml of *toluene* and 15 ml of *butyl alcohol*, previously neutralized to *bromophenol blue*, and titrate with *0.050 M alcoholic potassium hydroxide* to a bromophenol blue endpoint. Not more than 0.1 ml is required.

**Loss on heating**. Preheat an open aluminum vessel (60 mm in diameter and 10 mm high) at 150° for 30 minutes, and allow to cool to room temperature. Tare the vessel, transfer to it about 1 g of Dimethicone, accurately weighed, heat at 150° in a circulating air oven for 2 hours, and allow to come to room temperature in a desiccator before weighing: it loses not more than the maximum percentage of its weight specified in the accompanying table.

**Heavy metals** (2.3.13). Mix 1.0 g of Dimethicone with 20 ml of *chloroform*. Add 1.0 ml of a freshly prepared 0.002 per cent w/v solution of *dithizone* in *chloroform*, 0.5 ml of *water*, and 0.5 ml of a mixture of 1 ml of *ammonia* and 9 ml of a 0.2 per cent solution of *hydroxylamine hydrochloride*. Prepare reference solution by adding 1.0 ml of a freshly prepared 0.002 per cent w/v solution of dithizone in *chloroform* to 20 ml of *chloroform*; add 0.5 ml of reference lead Solution and 0.5 ml of a mixture of 1 ml of *ammonia* and 9 ml of a 0.2 per cent solution of *hydroxylamine hydrochloride*. Immediately shake both solutions vigorously for 1 minute. Any red color in the test solution is not more intense than that in the reference solution (5 µg per g).

**Assay.** Determine by infra red spectrophotometery (2.4.6), with a resolution of 4 $cm^{-1}$ and fitted with an accessory for attenuated total reflectance and a germanium sample trough (45° or 60°). Fill the trough of the accessory with Dimethicone, and record the spectrum between 4000 $cm^{-1}$ and 700 $cm^{-1}$. Clean the trough, fill it with *polydimethylsiloxane RS* and record the spectrum as above. Clean the trough, and record the spectrum as above to obtain a background spectrum. Examine the spectra in the range between 1300 $cm^{-1}$ and 1200 $cm^{-1}$, and calculate the absorbance of the peak in each spectrum at about 1259 $cm^{-1}$.

Calculate the content of $[-(CH_3)_2SiO-]_n$ in the Dimethicone taken by the formula:

$$100(A_U / A_S)(D_S / D_U)$$

in which $A_U$ is the absorbance of the Dimethicone; $A_S$ is the absorbance of *polydimethylsiloxane RS*; and $D_S$ and $D_U$ are



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the specific gravities of *polydimethylsiloxane RS* and Dimethicone, respectively.

| Nominal Viscosity (centistokes) | Viscosity (centistokes) | | Specific gravity | | Refractive index | | Loss on heating |
|---|---|---|---|---|---|---|---|
| | Min. | Max. | Min. | Max. | Min. | Max. | Max. |
| 20 | 18 | 22 | 0.946 | 0.954 | 1.3980 | 1.4020 | 20.0 |
| 50 | 47.5 | 52.5 | 0.955 | 0.965 | 1.4005 | 1.4045 | 2.0 |
| 100 | 95 | 105 | 0.962 | 0.970 | 1.4005 | 1.4045 | 0.3 |
| 200 | 190 | 220 | 0.964 | 0.972 | 1.4013 | 1.4053 | 0.3 |
| 350 | 332.5 | 367.5 | 0.965 | 0.973 | 1.4013 | 1.4053 | 0.3 |
| 500 | 475 | 525 | 0.967 | 0.975 | 1.4013 | 1.4053 | 0.3 |
| 1000 | 950 | 1050 | 0.967 | 0.975 | 1.4013 | 1.4053 | 0.3 |
| 12500 | 11875 | 13125 | — | — | 1.4015 | 1.4055 | 2.0 |
| 30000 | 27000 | 33000 | 0.969 | 0.977 | 1.4010 | 1.4100 | 2.0 |

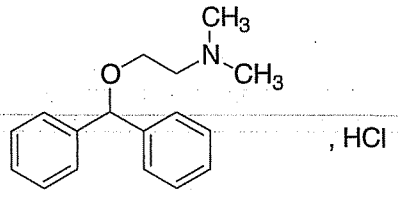
*Dimethicone intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.*

**Bacterial Endotoxin** (2.2.3). Not more than 10 Endotoxin Units per ml of the Dimethicone.

**Storage**. Store protected from moisture.

**Labeling**. Label it to indicate its nominal viscosity value. Dimethicone intended for use in coating containers that come in contact with articles for parenteral use is so labelled.

# Diphenhydramine Hydrochloride




$C_{17}H_{21}NO,HCl$ Mol. Wt. 291.8

Diphenhydramine Hydrochloride is 2-benzhydryloxyethyldimethylamine hydrochloride.

Diphenhydramine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{17}H_{21}NO,HCl$, calculated on the dried basis.

**Category**. Histamine $H_1$-receptor antagonist.

**Dose**. 50 to 200 mg daily, in divided doses.

**Description**. A white or almost white, crystalline powder; odourless or almost odourless.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *diphenhydramine hydrochloride RS* or with the reference spectrum of diphenhydramine hydrochloride. Examine the substances as discs prepared using *potassium chloride IR*.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.025 per cent w/v solution in *ethanol (95 per cent)* shows absorption maxima at about 253 nm, 258 nm and 264 nm; absorbance at about 253 nm, about 0.31, at about 258 nm, about 0.38 and at about 264 nm, about 0.3.

C. To 0.05 ml of a 5 per cent w/v solution add 2 ml of *sulphuric acid*; an intense yellow colour develops which changes to red on the addition of 0.5 ml of *nitric acid*. Add 15 ml of *water*, cool, add 5 ml of *chloroform* and shake; an intense violet colour develops in the chloroform layer.

D. Gives the reactions of chlorides (2.3.1).

## Tests

**Appearance of solution**. A 5.0 per cent w/v solution in *carbon dioxide-free water*, and a 5-fold dilution thereof, are both clear (2.4.1). The 5.0 per cent solution is not more intensely coloured than reference solution BYS6 (2.4.1).

**pH** (2.4.24). 4.0 to 6.0, determined in a 5.0 per cent w/v solution.

**Related substances.** Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 70 mg of the substance under examination in 20.0 ml of the mobile phase. Dilute 2.0 ml of this solution to 10.0 ml with the mobile phase.

*Reference solution*. Dilute 1.0 ml of the test solution to 10.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 20.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with endcapped octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 35 volumes of *acetonitrile* and 65 volumes of 0.54 per cent w/v solution of *potassium dihydrogen phosphate* previously adjusted to pH 3.0 with *orthophosphoric acid*,
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to diphenhydramine and diphenhydramine impurity A is not less than 2.0. The relative retention time with reference to diphenhydramine for diphenhydramine impurity A is about 0.9, for diphenhydramine



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impurity B is about 1.5, diphenhydramine impurity C is about 1.8, for diphenhydramine impurity D is about 2.6 and diphenhydramine impurity E is about 5.1.

Inject the test solution and the reference solution. Run the chromatogram seven times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent). The sum of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.250 g and dissolve in 50 ml of *ethanol*, add 5.0 ml of *0.01 M hydrochloric acid*. Titrate with *0.1 M sodium hydroxide*, determining end point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.02918 g of $C_{17}H_{22}ClNO$.

**Storage**. Store protected from light.

# Diphenhydramine Capsules

Diphenhydramine Hydrochloride Capsules

Diphenhydramine Capsules contain not less than 93.0 per cent and not more than 107.0 per cent of the stated amount of diphenhydramine hydrochloride, $C_{17}H_{21}NO,HCl$.

**Usual strengths**. 25 mg; 50 mg.

## Identification

Extract a quantity of the contents of the capsules containing 0.1 g of Diphenhydramine Hydrochloride with two quantities, each of 15 ml, of *chloroform*. Evaporate the combined extracts to dryness on a water-bath and dry the residue at 80° for 1 hour. The residue melts at about 168° (2.4.21), and complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *diphenhydramine hydrochloride RS* or with the reference spectrum of diphenhydramine hydrochloride. Examine the substances as discs prepared using *potassium chloride IR*.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.025 per cent w/v solution in *ethanol (95 per cent)* shows absorption maxima at about 253 nm, 258 nm and 264 nm; absorbance at about 253 nm, about 0.31, at about 258 nm, about 0.38 and at about 264 nm, about 0.3.

C. To 0.05 ml of a 5 per cent w/v solution add 2 ml of *sulphuric acid*; an intense yellow colour develops which changes to red on the addition of 0.5 ml of *nitric acid*. Add 15 ml of *water*, cool, add 5 ml of *chloroform* and shake; an intense violet colour develops in the chloroform layer.

## Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H*.

*Mobile phase*. A mixture of 80 volumes of *chloroform*, 20 volumes of *methanol* and 1 volume of *diethylamine*.

*Test solution*. Shake a quantity of the contents of the capsules containing 100 mg of Diphenhydramine Hydrochloride with three quantities, each of 10 ml, of *chloroform*, filter and evaporate the combined filtrate almost to dryness; dissolve the residue in 5 ml of *chloroform*.

*Reference solution*. Dilute 1 ml of the test solution to 100 ml with *chloroform*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in air for 5 minutes, spray with *sulphuric acid* and heat at 120° for 10 minutes until spots appear. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Other tests**. Comply with the tests stated under Capsules.

**Assay**. Weigh accurately a quantity of the mixed contents of 20 capsules and transfer to a 100-ml volumetric flask, add sufficient *water* to produce 100.0 ml, shake well to dissolve and filter. To an accurately measured volume of the filtrate containing 0.3 g of Diphenhydramine Hydrochloride add 5 g of *sodium chloride* and 5 ml of *sodium hydroxide solution* and extract with successive quantities, each of 20 ml, of *ether* until complete extraction is effected. Wash the combined extracts with two quantities, each of 5 ml, of *water*, extract the combined washings with two quantities, each of 10 ml, of *ether*, add the ether to the combined ether extracts and evaporate to about 10 ml. Add 25.0 ml of *0.1 M hydrochloric acid*, warm gently to complete the removal of ether, cool and titrate the excess of acid with *0.1 M sodium hydroxide* using *methyl red solution* as indicator.

1 ml of *0.1 M hydrochloric acid* is equivalent to 0.02918 g of $C_{17}H_{21}NO,HCl$.

**Storage**. Store protected from moisture.

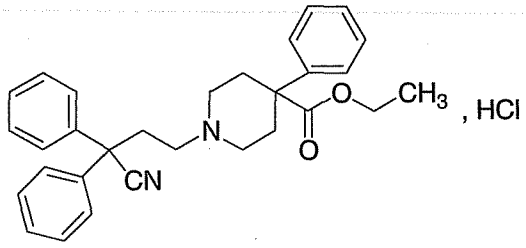


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## Diphenoxylate Hydrochloride




$C_{30}H_{32}N_2O_2,HCl$ Mol. Wt. 489.1

Diphenoxylate Hydrochloride is ethyl 1-(3-cyano-3,3-diphenylpropyl)-4-phenylpiperidine-4-carboxylate hydrochloride.

Diphenoxylate Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{30}H_{32}N_2O_2,HCl$, calculated on the dried basis.

**Category**. Antidiarrhoeal.

**Dose**. 5 to 30 mg daily, in divided doses.

**Description**. A white or almost white, crystalline powder; odourless or almost odourless.

### Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *diphenoxylate hydrochloride RS* or with the reference spectrum of diphenoxylate hydrochloride.

B. Dissolve about 30 mg in 5 ml of *methanol*, add 0.25 ml of *nitric acid* and 0.4 ml of *silver nitrate solution*. Shake and allow to stand; a curdled precipitate is formed. Centrifuge and rinse the precipitate with three quantities, each of 2 ml, of *methanol*. Carry out this operation rapidly in subdued light. Suspend the precipitate in 2 ml of *water* and add 1.5 ml of *10 M ammonia*; the precipitate dissolves easily.

C. Gives reaction A of chlorides (2.3.1).

### Tests

**Appearance of solution**. A 10.0 per cent w/v solution in *dichloromethane* is clear (2.4.1), and not more intensely coloured than reference solution YS6 (2.4.1).

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with a suitable octadecylsilyl silica gel (5 µm) containing a fluorescent indicator with a maximum intensity at 254 nm.

*Mobile phase*. A mixture of 60 volumes of *dioxan*, 30 volumes of a 5.9 per cent w/v solution of *sodium chloride* and 10 volumes of *methanol*.

*Test solution*. Dissolve 1 g of the substance under examination in a mixture of 1 volume of *methanol* and 2 volumes of *dichloromethane* and dilute to 20 ml with the same solvent mixture.

*Reference solution (a)*. Dilute 0.5 ml of the test solution to 100 ml with the same solvent mixture.

*Reference solution (b)*. Dissolve 0.5 g of the substance under examination in 25 ml of a 1.5 per cent w/v solution of *potassium hydroxide* in *methanol* and add 1 ml of *water*. Heat on a water-bath under a reflux condenser for 4 hours. Cool and add 25 ml of *0.5 M hydrochloric acid* and shake with 100 ml of *dichloromethane*. Evaporate the organic layer to dryness on a water-bath. Dissolve the residue in 10 ml of a mixture of 1 volume of *methanol* and 2 volumes of *dichloromethane*, add 10 ml of test solution and dilute to 25 ml with a mixture of 1 volume of *methanol* and 2 volumes of *dichloromethane*.

Apply to the plate 1 µl of each solution. Allow the mobile phase to rise 7 cm in an unsaturated tank. Dry the plate in an oven at 160° for 15 minutes and place the plate while hot in a closed tank containing 20 ml of *fuming nitric acid* for 30 minutes. Remove the plate and heat it again at 160° for 15 minutes. Allow to cool and examine immediately in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated principal spots.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

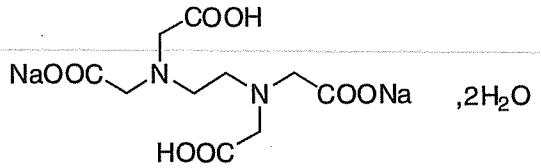
**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.4 g, dissolve in 40 ml of *ethanol (95 per cent)* and add 5 ml of *0.01 M hydrochloric acid*. Titrate with *0.1 M ethanolic sodium hydroxide*, determining the end-point potentiometrically (2.4.25). Read the volume added between the two points of inflection.

1 ml of *0.1 M ethanolic sodium hydroxide* is equivalent to 0.04891 g of $C_{30}H_{32}N_2O_2,HCl$.

**Storage**. Store protected from light.

## Disodium Edetate




$C_{10}H_{14}N_2Na_2O_8,2H_2O$ Mol. Wt. 372.2

Disodium Edetate is disodium ethylenediaminetetraacetate dihydrate.



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Disodium Edetate contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{10}H_{14}N_2Na_2O_8,2H_2O$.

**Category**. Pharmaceutical aid; chelating agent in metal poisoning.

**Dose**. By intravenous injection, 50 mg per kg of body weight, upto a maximum of 3 g per day.

**Description**. A white, crystalline powder; odourless.

### Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *disodium edetate RS*.

B. Dissolve 2 g in 25 ml of water, add 6 ml of *lead nitrate solution*, shake and add 3 ml of *potassium iodide solution*; no yellow precipitate is produced. Make alkaline to *red litmus paper* with *2 M ammonia* and add 5 ml of *ammonium oxalate solution*; no precipitate is produced.

C. Dissolve 0.5 g in 10 ml of *water*, add 0.5 ml of a 10 per cent w/v solution of *calcium chloride*, make alkaline to *red litmus paper* with *2 M ammonia* and add 3 ml of *ammonium oxalate solution*; no precipitate is produced.

D. Gives the reactions of sodium salts (2.3.1).

### Tests

**Appearance of solution**. A 5.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and colourless (2.4.1).

**pH** (2.4.24). 4.0 to 5.5, determined in a 5.0 per cent w/v solution.

**Impurity A**. Determine by liquid chromatography (2.4.14).

*Note—Carry out the test protected from light.*

*Solvent mixture*. Dissolve 10 g of *ferric sulphate pentahydrate* in 20 ml of *0.5 M sulphuric acid,* add 780 ml of *water*, adjust the pH to 2.0 with *1 M sodium hydroxide* and dilute to 1000 ml with *water*,

*Test solution*. Dissolve 0.1 g of the substance under examination in 25.0 ml of the solvent mixture.

*Reference solution*. Dissolve 40 mg of *nitrilotriacetic acid* in 100.0 ml of the solvent mixture. To 1.0 ml of this solution, add 0.1 ml of the test solution and dilute to 100.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with spherical graphitized carbon R1 (5 µm),
- mobile phase: dissolve 50 mg of *ferric sulphate pentahydrate* in 50 ml of *0.1 M sulphuric acid,* adjust the pH to 1.5 with *0.5 M sulphuric acid* or *1 M sodium hydroxide*. Add 20.0 ml of *ethylene glycol* and dilute to 1000 ml with *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 273 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to iron complex of impurity A and the iron complex of edetic acid is not less than 7.0. The signal- to- noise ratio is not more than 50.

Inject the test solution and the reference solution. Run the chromatogram 4 times the retention time of the iron complex of impurity A. In the chromatogram obtained with the test solution the area of secondary peak corresponding to disodium edetate impurity A is not is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent).

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

**Iron** (2.3.14). 20 ml of a 2.5 per cent w/v solution complies with the limit test for iron (80 ppm). Add 0.25 g of *calcium chloride* to each solution before adding mercaptoacetic acid.

**Assay**. Weigh accurately about 0.5 g, dissolve in sufficient *water* to produce 300 ml and add 2 g of *hexamine* and 2 ml of *2 M hydrochloric acid*. Titrate with *0.1 M lead nitrate* using about 50 mg of *xylenol orange triturate* as indicator.

1 ml of *0.1 M lead nitrate* is equivalent to 0.03722 g of $C_{10}H_{14}N_2Na_2O_8,2H_2O$.

## Disodium Edetate Injection

Disodium Edetate Injection is a sterile solution of Disodium Edetate in Water for Injections, containing varying amounts of the disodium and trisodium salts as a result of pH adjustment.

Disodium Edetate Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of disodium edetate, $C_{10}H_{14}N_2Na_2O_8$.

**Usual strengths**. 3 g per 15 ml; 3 g per 20 ml.

### Identification

To a volume containing about 3 g of Disodium Edetate add *3 M hydrochloric acid* to adjust the pH to 5.0 and evaporate to dryness on a steam-bath to dryness. The residue so obtained complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *disodium edetate RS*.

B. Dissolve 2 g in 25 ml of *water*, add 6 ml of *lead nitrate solution*, shake and add 3 ml of *potassium iodide solution*;



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no yellow precipitate is produced. Make alkaline to *red litmus paper* with *2 M ammonia* and add 5 ml of *ammonium oxalate solution*; no precipitate is produced.

C. Dissolve 0.5 g in 10 ml of *water*, add 0.5 ml of a 10 per cent w/v solution of *calcium chloride*, make alkaline to *red litmus paper* with *2 M ammonia* and add 3 ml of *ammonium oxalate solution*; no precipitate is produced.

### Tests

**pH** (2.4.24). 6.5 to 7.5.

**Bacterial endotoxins** (2.2.3). Not more than 0.2 Endotoxin Unit per mg of disodium edetate.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

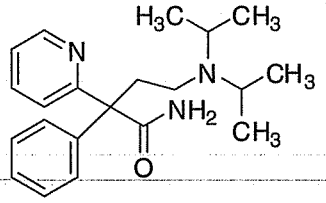
**Assay**. Dilute an accurately measured volume containing about 0.6 g of Disodium Edetate with *water* to produce 100 ml, mix and add 2 g of *hexamine* and 2 ml of *2 M hydrochloric acid*. Titrate with *0.1 M lead nitrate* using about 50 mg of *xylenol orange triturate* as indicator.

1 ml of *0.1 M lead nitrate* is equivalent to 0.03722 g of $C_{10}H_{14}N_2Na_2O_8,2H_2O$.

**Storage**. Store in single dose containers.

**Labelling**. The label states the strength in terms of anhydrous disodium edetate contained in a suitable dose-volume.

## Disopyramide



$C_{21}H_{29}N_3O$ Mol. Wt. 339.5

Disopyramide is (*RS*)-4-(diisopropylamino)-2-phenyl-2-(pyridin-2-yl)butyramide.

Disopyramide contains not less than 98.5 per cent and not more than 101.5 per cent of $C_{21}H_{29}N_3O$, calculated on the dried basis.

**Category**. Antiarrhythmic.

**Description**. A white or almost white powder.

### Identification

*Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *disopyramide RS* or with the reference spectrum of disopyramide. Examine the substances as discs prepared by placing 50 µl of a 5 per cent solution in *dichloromethane* on a disc of *potassium bromide*. Dry the discs at 60° for 1 hour before use.

B. When examined in the range 240 nm to 350 nm (2.4.7), a 0.004 per cent w/v solution in 0.5 per cent *methanolic sulphuric acid* shows an absorption maximum at about 269 nm and a shoulder at 263 nm ; specific absorbance at about 269 nm is 190 to 210.

C. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

### Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 1 volume of *ammonia*, 30 volumes of *acetone* and 30 volumes of *cyclohexane*.

*Test solution (a)*. Dissolve 0.2 g of the substance under examination in *methanol* and dilute to 10 ml with the same solvent.

*Test solution (b)*. Dilute 1 ml of test solution (a) to 10 ml with *methanol*.

*Reference solution (a)*. A 0.2 per cent w/v solution of *disopyramide RS* in *methanol*.

*Reference solution (b)*. Dilute 0.5 ml of test solution (b) to 20 ml with *methanol*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in a current of warm air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.25 per cent).

**Heavy metals** (2.3.13). 2.0 g complies with limit test for heavy metals, Method B (10 ppm).

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying at 80° over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 2 hours.

**Assay**. Dissolve 0.13 g in 30 ml of *anhydrous acetic acid*, add 0.2 ml of *naphtholbenzein solution*. Titrate with *0.1 M perchloric acid* until the colour changes from yellow to green.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01697 g of $C_{21}H_{29}N_3O$.

**Storage**. Store protected from light.



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# Disopyramide Capsules

Disopyramide Capsules contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of disopyramide, $C_{21}H_{29}N_3O$.

**Usual strength.** 200 mg.

## Identification

A. Shake a quantity of the contents of the capsules containing about 0.2 g of Disopyramide with 50 ml of *chloroform* for 15 minutes, filter, evaporate the filtrate to dryness using a rotary evaporator and dissolve the residue in 2 ml of *chloroform*. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *disopyramide RS* or with the reference spectrum of disopyramide.

B. When examined in the range 230 nm to 350 nm (2.4.7), the solution obtained in the assay shows an absorption maximum at about 269 nm.

## Tests

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

*Mobile phase.* A mixture of 1 volume of *18 M ammonia*, 30 volumes of *acetone* and 30 volumes of *cyclohexane*.

*Test solution.* Shake a quantity of the contents of the capsules containing about 0.2 g of Disopyramide with 20.0 ml of *methanol* for 30 minutes and filter.

*Reference solution.* Dilute 1.0 ml of test solution to 400 ml with *methanol.*

Apply to the plate 10 µl of each solution. After development, dry the plate in current of air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (0.25 per cent).

**Assay.** Weigh accurately a quantity of the mixed contents of 20 capsules containing about 0.04 g of Disopyramide, add 40 ml of *0.05 M methanolic sulphuric acid*, shake for 15 minutes, dilute to 100 ml with the same solvent and filter. Dilute 5 ml of the filtrate to 100 ml with *0.05 M methanolic sulphuric acid*. Measure the absorbance of the resulting solution at the maximum at about 269 nm (2.4.7). Calculate the content of $C_{21}H_{29}N_3O$ taking 198.5 as the specific absorbance at 269 nm.

# Disopyramide Phosphate Capsules

Disopyramide Phosphate Capsules contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of disopyramide, $C_{21}H_{29}N_3O$.

**Usual strength.** 150 mg.

## Identification

Suspend a quantity of the contents of the capsules containing about 0.2 g of disopyramide in 50 ml of *chloroform*, add 2 ml of *13.5 M ammonia*, shake and filter through *anhydrous sodium sulphate*. Evaporate the filtrate to dryness using a rotary evaporator. The residue complies with the following tests.

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

B. When examined in the range 230 nm to 350 nm (2.4.7), the solution obtained in the Assay shows absorption maximum only at about 269 nm and a shoulder at 263 nm.

C. Shake a quantity of the contents of the capsules containing about 0.4 g of disopyramide with 20 ml of *water* and filter. The filtrate gives the reactions of phosphates (2.3.1).

## Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *water,*
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter. Measure the absorbance of the filtrate, suitably diluted if necessary, at the maximum at about 262 nm (2.4.7). Calculate the content of $C_{21}H_{29}N_3O$ in the medium taking 87 as the specific absorbance at 262 nm.

D. Not less than 70 per cent of the stated amount of $C_{21}H_{29}N_3O$.

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

*Mobile phase.* A mixture of 1 volume of *18 M ammonia*, 30 volumes of *acetone* and 30 volumes of *cyclohexane.*

*Test solution.* Shake a quantity of the contents of the capsules containing 0.2 g of disopyramide with 20.0 ml of *methanol* for 30 minutes and filter.

*Reference solution.* Dilute 1.0 ml of test solution to 200.0 ml with *methanol.*

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

**Other tests.** Comply with the tests stated under Capsules.

**Assay.** Weigh accurately a quantity of the mixed contents of 20 capsules containing about 0.04 g of Disopyramide, add



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40 ml of *0.05 M methanolic sulphuric acid*, shake for 15 minutes, dilute to 100 ml with the same solvent and filter. Dilute 5 ml of the filtrate to 100 ml with *0.05 M methanolic sulphuric acid*. Measure the absorbance of the resulting solution at the maximum at 269 nm (2.4.7). Calculate the content of $C_{21}H_{29}N_3O$ taking 198.5 as the specific absorbance at 269 nm.

**Labelling.** The quantity of active ingredient is stated in terms of the equivalent amount of disopyramide.

## Disopyramide Phosphate Sustained-release Capsules

Disopyramide Phosphate Sustained-release Capsules contain an amount of Disopyramide Phosphate.

Disopyramide Phosphate Sustained-release Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of disopyramide, $C_{21}H_{29}N_3O$.

### Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 85 volumes of *toluene*, 14 volumes of *absolute ethanol* and 1 volume of *ammonium hydroxide*.

*Test solution*. Transfer a quantity of the contents of the capsules containing about 195 mg of Disopyramide Phosphate to a 25-ml volumetric flask, add 20 ml of *methanol*, and shake for 20 minutes. Dilute with *methanol* to volume, mix, and filter.

*Reference solution*. A 0.77 per cent w/v solution of *disopyramide phosphate RS* in *methanol*.

Apply to the plate 20 µl of each solution. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with reference solution.

### Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 1000 ml of a buffer solution prepared by dissolving 13.6 g of *monobasic potassium phosphate* in 1000 ml of *water*, adjusted to pH 2.5 with *hydrochloric acid*,
Speed and time. 100 rpm and 1 hour, 2 hours, 5 hours, 12 hours.

Withdraw a suitable volume of the medium and filter through a membrane filter. Measure the absorbance (2.4.7) of the filterate, suitably diluted with the dissolution medium if necessary at 261 nm. Calculate the content of disopyramide phosphate, $C_{21}H_{29}N_3O$ in the medium from the absorbance obtained from a solution of known concentration of *disopyramide phosphate RS*.

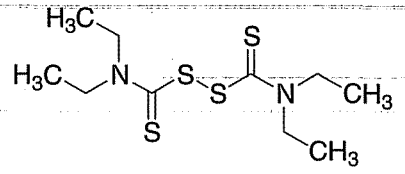
D. Not less than 5 per cent and not more than 25 per cent in 1 hour, not less than 17 per cent and not more than 43 per cent in 2 hours, not less than 50 per cent and not more than 80 per cent in 5 hours, and not less than 85 per cent in 12 hours, of the stated amount of $C_{21}H_{29}N_3O$.

**Other tests**. Comply with the tests stated under Capsules.

**Assay**. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 650 mg of Disopyramide Phosphate to a 500-ml volumetric flask, add about 400 ml of *0.1 M sulphuric acid*, and shake for 30 minutes. Dilute with *0.1 M sulphuric acid* to volume, mix, and filter. Dilute further with *0.1 M sulphuric acid* to get a final concentration of about 40 µg per ml and measure the absorbance of the resulting solution at the maximum at about 261 nm (2.4.7).

Calculate the content of $C_{21}H_{29}N_3O$ from the absorbance of a solution of known concentration of *disopyramide phosphate RS*.

## Disulfiram



$C_{10}H_{20}N_2S_4$ Mol. Wt. 296.5

Disulfiram is tetraethyldisulphanedicarbothioamide.

Disulfiram contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{10}H_{20}N_2S_4$, calculated on the dried basis.

**Category**. Used in the treatment of alcoholism.

**Dose**. 500 mg as a single dose for 1 to 2 weeks; maintenance dose, 125 to 500 mg daily.

**Description**. A white or almost white, crystalline powder.

### Identification

*Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.*

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

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to



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that in the chromatogram obtained with reference solution (b).

C. Dissolve about 10 mg in 10 ml of *methanol* and add 2 ml of 0.05 per cent w/v solution of *cupric chloride* in *methanol;* a yellow colour is produced which changes to greenish-yellow.

### Tests

**Diethyldithiocarbamate**. Dissolve 0.2 g in 10 ml of *ether*, add 5 ml of *phosphate buffer pH 8.0*, shake vigorously, reject the ether layer and wash the aqueous layer with 10 ml of *ether.* To the aqueous layer add 0.2 ml of a 0.4 per cent w/v solution of *cupric sulphate* and 5 ml of *carbon tetrachloride* and shake well. Any yellow colour in the carbon tetrachloride layer is not more intense than that of a standard prepared at the same time and in the same manner using 0.2 ml of a freshly prepared 0.015 per cent w/v solution of *sodium diethyldithiocarbamate* in place of the substance under examination (150 ppm).

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

*Mobile phase*. A mixture of 70 volumes of *n-hexane* and 30 volumes of *butyl acetate.*

*Test solution (a)*. Dissolve 0.2 g of the substance under examination in 10 ml of *ethyl acetate.*

*Test solution (b)*. Dilute 1 ml of test solution (a) to 10 ml with *ethyl acetate.*

*Reference solution (a)*. Dilute 1 ml of test solution (b) to 20 ml with *ethyl acetate.*

*Reference solution (b)*. A 0.2 per cent w/v solution of *disulfiram RS* in *ethyl acetate.*

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a).

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

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven over *phosphorus pentoxide* at 50° at a pressure of 1.5 to 2.5 kPa.

**Assay**. Weigh accurately about 0.45 g and dissolve in 80 ml of *acetone*. Add 20 ml of a 2 per cent w/v solution of *potassium nitrate*. Titrate with *0.1 M silver nitrate* determining the end-point potentiometrically (2.4.25), using a silver indicator electrode and a silver-silver chloride double-junction electrode saturated with potassium nitrate.

1 ml of *0.1 M silver nitrate* is equivalent to 0.05930 g of $C_{10}H_{20}N_2S_4$.

**Storage**. Store protected from light.

## Disulfiram Tablets

Disulfiram Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of disulfiram, $C_{10}H_{20}N_2S_4$.

**Usual strength**. 200 mg.

### Identification

A. Extract a quantity of the powdered tablets containing 0.2 g of Disulfiram by boiling with 5 ml of *carbon tetrachloride*, filter and evaporate the filtrate to dryness. The residue, after drying at 40° at a pressure not exceeding 0.7 kPa, complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *disulfiram RS* or with the reference spectrum of disulfiram.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with the reference solution.

C. Extract a quantity of the powdered tablets containing 0.3 g of Disulfiram with *ethanol (95 per cent)*, filter and evaporate the filtrate to dryness. Dissolve the residue in 5 ml of *ethanol (95 per cent)*, add 1 ml of *potassium cyanide solution*; a yellow colour is produced which changes to green and then darkens to bluish-green.

### Tests

**Diethyldithiocarbamate**. Shake a quantity of the powdered tablets containing 0.1 g of Disulfiram with 10 ml of *chloroform* and filter. To the filtrate add 10 ml of *0.1 M sodium hydroxide*, shake, reject the chloroform layer and wash the aqueous layer with three quantities, each of 10 ml, of *chloroform*. To the aqueous layer add 0.25 ml of a 0.4 per cent w/v solution of *cupric sulphate* and 2 ml of *carbon tetrachloride*, shake and allow to separate. The lower layer is not more intensely coloured than reference solution BYS4 (2.4.1).

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

*Mobile phase*. A mixture of 70 volumes of *n-hexane* and 30 volumes of *butyl acetate.*

*Test solution (a)*. Extract a quantity of the powdered tablets containing 0.5 g of Disulfiram with 20 ml of *ethyl acetate* and filter.

*Test solution (b)*. Dilute 1 ml of test solution (a) to 100 ml with *ethyl acetate.*



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*Reference solution.* A 0.025 per cent w/v solution of *disulfiram RS* in *ethyl acetate.*

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with the reference solution.

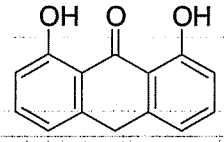
**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.4 g of Disulfiram and shake with 75 ml of *methanol* for 30 minutes. Add sufficient *methanol* to produce 100.0 ml, mix, filter and dilute 5.0 ml of the filtrate to 100.0 ml with *methanol.* To 5.0 ml add sufficient of a 0.1 per cent w/v solution of *cupric chloride* in *methanol* to produce 25.0 ml, mix and allow to stand for 1 hour. Measure the absorbance of the resulting solution at the maximum at about 400 nm (2.4.7), using as the blank a solution prepared by diluting 5.0 ml of *methanol* to 25.0 ml with the cupric chloride solution. Calculate the content of $C_{10}H_{20}N_2S_4$ from the absorbance obtained by repeating the operation using 5.0 ml of 0.02 per cent w/v solution of *disulfiram RS* in *methanol* beginning at the words "add sufficient of a 0.1 per cent w/v solution of cupric *chloride....*".

**Storage**. Store protected from light.

# Dithranol

Anthralin; Dioxyanthranol




$C_{14}H_{10}O_3$ Mol. Wt. 226.2

Dithranol is 1,8-dihydroxyanthrone.

Dithranol contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{14}H_{10}O_3$, calculated on the dried basis.

**Category**. Topical antipsoriatic.

**Description**. A yellow or orange-yellow, microcrystalline powder; odourless or almost odourless.

## Identification

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

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *chloroform* shows absorption maxima at about 255 nm, 287 nm and 354 nm; absorbances at the maxima, about 0.55, 0.5 and 0.45 respectively.

C. Melts at about 178° (2.4.21).

## Tests

**Dihydroxyanthracene**. Dissolve 0.1 g in 5 ml of hot *benzene*; a clear yellow or orange solution is produced.

**Dihydroxyanthraquinone**. Dissolve 1 mg in a few drops of *sulphuric acid*; a clear orange solution with no trace of violet colour is produced.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.2 g and dissolve in 50 ml of *anhydrous pyridine*. Titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.02262 g of $C_{14}H_{10}O_3$.

**Storage**. Store protected from light.

# Dithranol Ointment

Dithranol Ointment contains Dithranol, in fine powder, in a suitable base.

Dithranol Ointment contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of dithranol, $C_{14}H_{10}O_3$.

**Usual strengths**. 0.1 to 2 per cent w/w.

## Identification

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

B. Heat a quantity containing 0.5 mg of Dithranol with 5 ml of *1 M sodium hydroxide* on a water-bath with constant stirring; a pink colour is produced in the aqueous layer.

## Tests

**Dihydroxyanthracene**. Dissolve a quantity containing 0.1 g of Dithranol in 5 ml of hot *benzene*; a yellow or orange solution is produced.

**Dihydroxyanthraquinone**. Dissolve a quantity containing 1 mg of Dithranol in a few drops of *sulphuric acid*; an orange solution with no trace of violet colour is produced.



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**Other tests**. Complies with the tests stated under Ointments.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh accurately a quantity of the ointment containing about 5 mg of Dithranol, disperse in 20 ml of *dichloromethane,* add 1.0 ml of *glacial acetic acid*, dilute to 100.0 ml with *hexane* and filter.

*Reference solution.* Add 1.0 ml of *glacial acetic acid* to 20.0 ml of a 0.025 per cent w/v solution of *dithranol RS* in *dichloromethane* and add sufficient *hexane* to produce 100.0 ml.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with porous silica particles (5 µm),
- mobile phase: a mixture of 82 volumes of *hexane,* 5 volumes of *dichloromethane* and 1 volume of *glacial acetic acid,*
- flow rate. 2 ml per minute,
- spectrophotometer set at 354 nm,
- injection volume. 20 µl.

Inject the test solution and the reference solution.

Calculate the content of $C_{14}H_{10}O_3$ in the ointment.

**Storage**. Store protected from light.

# Divalproex Sustained-release Tablets

Divalproex Sodium Sustained-release Tablets; Sodium Valproate Sustained-release Tablets

Divalproex Sustained-release Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of valproic acid, $C_8H_{16}O_2$.

**Usual strengths**. 125 mg; 250 mg; 500 mg.

## Identification

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

## Tests

**Dissolution** (2.5.2).

A. Apparatus No. 1,
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 50 rpm and 2 hours.

Withdraw the dissolution medium completely and transfer the tablets in 900 ml of *phosphate buffer 7.5* for test B.

B. Apparatus No. 1,

Medium. 900 ml of *phosphate buffer 7.5,*
Speed and time. 50 rpm and 1 hour.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

*Test solution.* Dilute the filtrate to obtain a solution of 0.012 per cent w/v in the dissolution medium.

*Reference solution.* A 0.012 per cent w/v solution of *valproic acid RS* in the dissolution medium.

*NOTE—A volume of acetonitrile not exceeding 10 per cent may be used to dissolve.*

Chromatographic system
- a stainless steel column 15 cm x 3.9 mm packed with phenyl group bonded to porous silica (4 µm),
- mobile phase: a mixture of 35 volumes of *citrate buffer,* 35 volumes of *potassium phosphate buffer and* 30 volumes of *acetonitrile*, adjusted to pH 3.0 with *orthophosphoric acid,*
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 50 µl.

Inject the reference solution. The test is not valid unless the theoretical plates is not less than 1000, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_8H_{16}O_2$ in the tablets.

D. Not less than 80 per cent of the stated amount of $C_8H_{16}O_2$.

**Uniformity of content** (*For tablets containing 10 mg or less*). Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay using following test solution.

*Test solution.* Disperse 1 tablet in 50 ml of mobile phase and sonicate for 10 minutes and dilute to 100.0 ml with the mobile phase and filter.

Calculate the content of $C_8H_{16}O_2$ in the tablet.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh accurately a quantity of the powdered tablets containing about 50 mg of valproic acid, disperse in 100.0 ml of the mobile phase.

*Reference solution.* A 0.05 per cent w/v solution of *valproic acid RS* in the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 3.9 mm packed with phenyl group bonded to porous silica (4 µm),
- mobile phase: a mixture of 70 volumes of *citrate buffer and* 30 volumes of *acetonitrile*, adjusted to pH 3.0 with *orthophosphoric acid,*



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- flow rate. 0.9 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 10 µl.

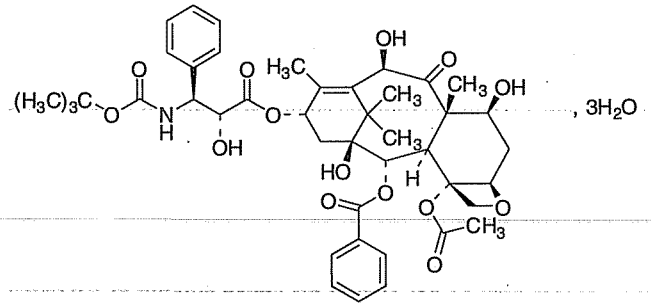
Inject the reference solution. The test is not valid unless the theoretical plates is not less than 1000, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_8H_{16}O_2$ in the tablets.

**Storage**. Store protected from moisture, at a temperature not exceeding 30°.

# Docetaxel Trihydrate



$C_{43}H_{53}NO_{14}, 3H_2O$ Mol. Wt. 861.9

Docetaxel Trihydrate is *N*-debenzoyl-*N*-(*tert*-butoxycarbonyl)-10-deacetyltaxol trihydrate.

Docetaxel Trihydrate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{43}H_{53}NO_{14}$, calculated on the anhydrous basis.

**Category**. Anticancer.

**Description**. A white to off-white powder.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *docetaxel trihydrate RS* or with the reference spectrum of docetaxel trihydrate.

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

## Tests

**Specific optical rotation** (2.4.22). – 40° to – 47°, determined in 0.75 per cent w/v solution in *absolute ethanol.*

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve about 25 mg of the substance under examination in 1.0 ml of *acetonitrile* and dilute to 25 ml with the mobile phase.

*Reference solution.* Dissolve 25 mg of *docetaxel trihydrate RS* in 1.0 ml of *acetonitrile* and dilute to 25.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 100.0 ml with the mobile phase. Further dilute 10.0 ml of this solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm) (such as Hypersil MOS-1),
- column temperature.30°,
- mobile phase: a mixture of 40 volumes of *acetonitrile* and 60 volumes of *0.02 M ammonium acetate*, adjusted to pH 4.5 with *orthophosphoric acid,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation of the replicate injections is not more than 5.0 per cent.

Inject the reference solution and the test solution. Run the chromatogram 4 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent).

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

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Water** (2.3.43). 6.0 per cent to 8.0 per cent, determined on 0.1 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve about 20 mg of the substance under examination in *acetonitrile* and dilute to 50 ml with the mobile phase. Dilute 10.0 ml of this solution to 100.0 ml with the mobile phase.

*Reference solution.* Dissolve 20 mg of *docetaxel trihydrate RS* in *acetonitrile* and dilute to 50.0 ml with the mobile phase.



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Dilute 10.0 ml of this solution to 100.0 ml with the mobile phase.

Chromatographic system as described under Related substances.

Inject the reference solution. The test is not valid unless the theoretical plates is not less than 2000, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{43}H_{53}NO_{14}$.

**Storage.** Store protected from light and moisture, at a temperature not exceeding 8°.

## Docetaxel Injection

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections in accordance with the manufacturer's instructions, immediately before use.

*The constituted solution complies with the requirements for clarity of solution and particulate matter stated under Parenteral Preparations (Infusions).*

**Storage.** The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

**Usual strengths.** 20 mg; 80 mg; 120 mg.

## Docetaxel Concentrate

Docetaxel Concentrate is a sterile solution of Docetaxel in a suitable vehicle.

*The concentrate complies with the requirements stated under Parenteral Preparations (Concentrated solutions for injections) and with the following requirements.*

Docetaxel Concentrate contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of docetaxel, $C_{43}H_{53}NO_{14}$.

### Identification

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

### Tests

**pH** (2.4.24). 2.5 to 3.5, determined in a solution constituted as directed in the label, in 15.3 per cent v/v solution of *absolute ethanol.*

**Related substances.** Determine by liquid chromatography (2.4.14).

*NOTE—Use freshly prepared solution.*

*Solvent mixture.* 55 volumes of mobile phase A and 45 volumes of mobile phase B.

*Test solution.* Reconstitute 1 vial of sample with 1 vial of solvent. Weigh accurately 1.25 g of the reconstituted solution and dilute to 25.0 ml with the solvent mixture.

*Reference solution.* A 0.0005 per cent w/v solution of *docetaxel trihydrate RS* in the solvent mixture.

Chromatographic system

- a stainless steel column of 50 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Inertsil ODS-3),
- mobile phase: A. dissolve 0.771 g of *ammonium acetate* in 1000 ml of *water*, adjusted to pH 4.0 with *orthophosphoric acid,*
  B. *acetonitrile,*
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 20 µl.

| Time (in min) | Mobile Phase A (Per cent v/v) | Mobile phase B (Per cent v/v) |
|---|---|---|
| 0 - 25 | 60 | 40 |
| 25 - 35 | 60 → 40 | 40 → 60 |
| 35 - 50 | 40 | 60 |
| 50 - 60 | 40 → 60 | 60 → 40 |
| 60 - 75 | 60 | 40 |

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 5.0 per cent.

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

**Other tests.** Complies with the tests stated under Parenteral Preparations (Injections).

**Assay.** Determine by liquid chromatography (2.4.14).

*NOTE—Use the solution within 24 hours when stored at 25°.*



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*Test solution.* Reconstitute 1 vial of sample with 1 vial of solvent. Weigh accurately 1.25 g of reconstituted solution and dilute to 50 ml with the mobile phase.

*Reference solution.* A 0.02 per cent w/v solution of *docetaxel trihydrate RS* in the mobile phase.

Chromatographic system

- a stainless steel column 50 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Inertsil ODS-3),
- mobile phase: a mixture of 45 volumes of *acetonitrile* and 55 volumes of buffer solution prepared by dissolving 0.771 g of *ammonium acetate* in 1000 ml of *water* and adjust the pH to 4.0 with *orthophosphoric acid,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the theoretical plates is not less than 2000, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

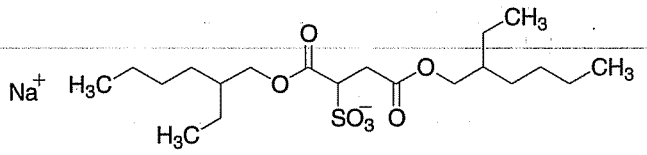
Calculate the content of $C_{43}H_{53}NO_{14}$ in the injection.

**Storage**. Store at a temperature between 2° to 8°.

**Labelling**. The label states the strength in terms of the equivalent amount of Docetaxel anhydrous.

# Docusate Sodium

Dioctyl Sodium Sulphosuccinate



$C_{20}H_{37}NaO_7S$ Mol. Wt. 444.6

Docusate Sodium is sodium 1,4-bis[(2-ethylhexyl)oxy]-1,4-dioxobutane-2-sulphonate.

Docusate Sodium contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{20}H_{37}NaO_7S$, calculated on the anhydrous basis.

**Category**. Emollient laxative or purgative.

**Description**. White or almost white, waxy masses or flakes, hygroscopic.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *docusate sodium RS*.

B. Ignite 0.75 g in the presence of *dilute sulphuric acid*, until an almost white residue is obtained. Cool and add 5 ml of *water*, filter. 2 ml of the filtrate gives reaction (a) of sodium salts (2.3.1).

## Tests

**Alkalinity**. Dissolve 1.0 g in 100 ml of a mixture of equal volumes of *methanol* and *water*, previously neutralised to *methyl red solution*. Add 0.1 ml of *methyl red solution*. Not more than 0.2 ml of *0.1 M hydrochloric acid* is required to change the colour of the indicator to red.

**Related non-ionic substances**. Determine by gas chromatography (2.3.13).

*Internal standard solution.* Dissolve 10 mg of *methyl behenate* in 50 ml of *hexane*.

*Test solution (a).* Dissolve 0.1 g of the substance under examination in 2.0 ml of the internal standard solution and dilute to 5.0 ml with *hexane*. Pass the solution, at a rate of about 1.5 ml per minute, through a column 10 mm in internal diameter, packed with 5 g of *basic aluminium oxide* and previously washed with 25 ml of *hexane*. Elute with 5 ml of *hexane* and discard the eluate. Elute with 20 ml of a mixture of equal volumes of *ether* and *hexane*. Evaporate the eluate to dryness and dissolve the residue in 2.0 ml of *hexane*.

*Test solution (b).* Prepare as described for test solution (a) but dissolving 0.1 g of the substance under examination in 5.0 ml of *hexane* and using a new column.

*Reference solution.* Dilute 2.0 ml of the internal standard solution to 5.0 ml with *hexane*.

Chromatographic system

- a glass column 2 m x 2 mm, packed with silanised diatomaceous earth for gas chromatography (150 µm to 180 µm) impregnated with 3 per cent m/m of *polymethylphenylsiloxane*,
- temperature :
  column 230°,
  Inlet port and detector at 280°,
- flow rate. 30 ml per minute of the nitrogen carrier gas.

Inject 1 µl of test solution (a), (b) and the reference solution. There is no peak with the same retention time as the internal standard in the chromatogram obtained with test solution (b). The area of any impurity peak is not more than the area of the peak due to the internal standard (0.4 per cent).



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**Chlorides** (2.3.12). Dissolve 5.0 g in 50 ml of *alcohol (50 per cent v/v)* and add 0.1 ml of *potassium dichromate solution*. Not more than 0.5 ml of *0.1 M silver nitrate* is required to change the colour of the indicator from yellow to orange (350 ppm).

**Sodium sulphate**. Not more than 2 per cent.

Dissolve 0.25 g in 40 ml of a mixture of 20 volumes of *water* and 80 volumes of *2-propanol*. Adjust to pH between 2.5 and 4.0 using *perchloric acid solution*. Add 0.4 ml of *naphtharson solution* and 0.1 ml of 0.0125 per cent w/v solution of *methylene blue*. Not more than 1.5 ml of *0.025 M barium perchlorate* is required to change the colour of the indicator from yellowish-green to yellowish-pink.

**Heavy metals** (2.3.13). Dissolve 4.0 g in 20 ml of *alcohol (80 per cent v/v)*. 12 ml of the solution complies with the limit test for heavy metals, Method B (10 ppm ).

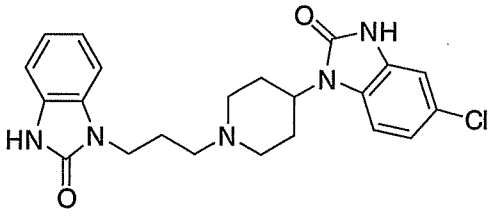
**Water** (2.3.43). Not more than 3.0 per cent, determined on 0.25 g.

**Assay**. Weigh accurately about 1.0 g, dissolve in 25.0 ml of *0.5 M alcoholic potassium hydroxide* and heat on a water-bath under reflux for 45 minutes. Cool, add 0.25 ml of *phenolphthalein solution* and titrate with *0.5 M hydrochloric acid* until the red colour disappears. Carry out a blank titration.

1 ml of *0.5 M hydrochloric acid* is equivalent to 0.1112 g of $C_{20}H_{37}NaO_7S$.

**Storage**. Store protected from moisture.

# Domperidone




$C_{22}H_{24}ClN_5O_2$ Mol. Wt. 425.9

Domperidone is 5-chloro-1-[1-[3-(2-oxo-2,3-dihydro-1*H*-benzimidazol-1-ylpropyl]piperidin-4-yl]-1,3-dihydro-2*H*-benzimidazol-2-one.

Domperidone contains not than 99.0 per cent and more than 101.0 per cent of $C_{22}H_{24}ClN_5O_2$, calculated on the dried basis.

**Category**. Antiemetic.

**Description**. A white or almost white powder.

## Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *domperidone RS* or with the reference spectrum of domperidone.

## Tests

**Appearance of solution**. A 1.0 per cent w/v solution in *dimethylformamide* is clear (2.4.1) and not more intensely coloured than reference solution YS6 (2.4.1).

**Related substances**. Determine by liquid chromatography (2.4.14).

*NOTE—Prepare the solutions immediately before use.*

*Test solution*. Dissolve 0.1 g of the substance under examination in *dimethylformamide* and dilute to 10.0 ml with the same solvent.

*Reference solution (a)*. A solution containing 0.01 per cent w/v of *domperidone RS* and 0.015 per cent w/v of *droperidol RS* in *dimethylformamide*.

*Reference solution (b)*. Dilute 1.0 ml of the test solution to 100.0 ml with *dimethylformamide*. Dilute 5.0 ml of the solution 20.0 ml with *dimethylformamide*.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with base-deactivated octadecylsilane bonded to porous silica (3 µm),
- mobile phase: A. 0.5 per cent w/v solution of *ammonium acetate*,
  B. *methanol*,
- a linear gradient programme using the conditions given below,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume. 10 µl.

| Time (min) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0 – 10 | 70 → 0 | 30 → 100 |
| 10 – 12 | 0 | 100 |

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (b) is at least 50 per cent of the full scale of the recorder.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to domperidone and droperidol is not less than 2.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with test solution, the area of any secondary peak is not more than the area of the principal peak



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in the chromatogram obtained with reference solution (b) (0.25 per cent); the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.2 times that of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

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

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

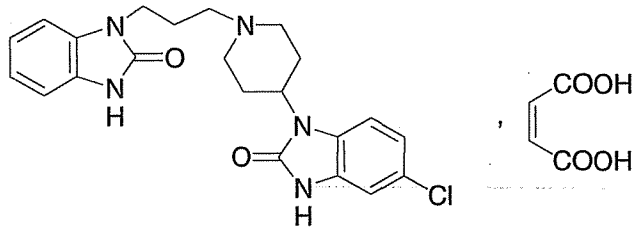
**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Dissolve 0.3 g in 50 ml of a mixture of 1 volume of *anhydrous acetic acid* and 7 volumes of *methyl ethyl ketone*. Titrate with *0.1 M perchloric acid* until the colour changes from orange-yellow to green using 0.2 ml of *naphtholbenzein solution* as indicator.

1 ml of *0.1 M perchloric* acid is equivalent to 0.04259 g of $C_{22}H_{24}ClN_5O_2$.

**Storage**. Store protected from light.

# Domperidone Maleate



$C_{22}H_{24}ClN_5O_2,C_4H_4O_4$ Mol. Wt. 542.0

Domperidone Maleate is 5-chloro-1-[1-[3-(2,3-dihydro-2-oxo-1*H*-benzimidazol-1-yl)propyl]-4-piperidinyl]-1,3-dihydro-2*H*-benzimidazol-2-one maleate.

Domperidone Maleate contains not less than 99.0 per cent and not more than 101.0 per cent of domperidone maleate, $C_{22}H_{24}ClN_5O_2,C_4H_4O_4$, calculated on the dried basis.

**Category**. Antiemetic.

**Description**. A white or almost white powder.

## Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *domperidone maleate RS* or with the reference spectrum of domperidone maleate. If the spectra obtained show differences, dissolve the substance under examination and the reference substance separately in the minimum volume of *2-propanol*, evaporate to dryness on a water-bath and record new spectra using the residues.

## Tests

**Appearance of solution**. Dissolve 0.2 g in *dimethylformamide* and dilute to 20.0 ml with the same solvent. The solution is clear (2.4.1) and not more intensely coloured than reference solution YS6 (2.4.1).

**Related substances**. Determine by liquid chromatography (2.4.14).

*NOTE - Prepare the solutions immediately before use.*

*Test solution.* Dissolve 0.1 g of the substance under examination in *dimethylformamide* and dilute to 10 ml with the same solvent.

*Reference solution (a).* Dissolve 10 mg of *domperidone maleate RS* and 15 mg of *droperidol RS* in *dimethylformamide* and dilute to 100 ml with the same solvent.

*Reference solution (b).* Dilute 1 ml of the test solution to 100 ml with *dimethylformamide*. Dilute 5 ml of the solution to 20 ml with *dimethylformamide*.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with base-deactivated octadecylsilane bonded to porous silica (3 µm),
- mobile phase: a mixture of 30 volumes of *methanol* and 70 volumes of a 0.5 per cent w/v solution of *ammonium acetate*,
- flow rate. 1.5 ml per minute,
- a linear gradient programme with the mobile phase changing to *methanol* over 10 minutes, followed by elution with *methanol* for 2 minutes,
- spectrophotometer set at 280 nm,
- injection volume. 10 µl.

Equilibrate the column for at least 30 minutes with *methanol* and then equilibrate with the initial mobile phase.

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (b) is at least 50 per cent of the full scale of the recorder.

Inject reference solution (a). The retention times are: domperidone maleate, about 6.5 minutes and droperidol, about 7 minutes. The test is not valid unless the resolution between the peaks due to domperidone maleate and droperidol is at least 2.0. If necessary adjust the concentration of *methanol* in the mobile phase or adjust the time programme for the linear gradient.



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Inject *dimethylformamide* as a blank, the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any peak, other than the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent); the sum of the areas of all peaks, other than the principal peak, is not greater than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak in the chromatogram obtained with the blank run, any peak due to maleic acid at the beginning of the chromatogram and any peak with an area less than 0.2 times that of the principal peak in the chromatogram obtained with reference solution (b).

**Heavy metals** (2.3.13.). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb)*.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 100° to 105°.

**Assay**. Dissolve 0.4 g in 50 ml of *anhydrous acetic acid*. Titrate with *0.1 M perchloric acid* using 0.2 ml of *naphtholbenzein solution* as indicator, until the colour changes from orange-yellow to green.

1 ml of *0.1 M perchloric acid* is equivalent to 0.0542 g of $C_{26}H_{28}ClN_5O_6$.

**Storage**. Store protected from light.

# Domperidone Tablets

Domperidone Maleate Tablets

Domperidone Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of domperidone, $C_{22}H_{24}ClN_5O_2$.

**Usual strength**. 10 mg.

## Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel F254* or using a precoated plate (such as Merck silica gel 60 F254 plates).

*Mobile phase*. A mixture of 5 volumes of a solution prepared by dissolving 1.36 g of *sodium acetate* in 50 ml of *water*, adjusting the pH to 4.7 with *dilute acetic acid* and adding sufficient *water* to produce 100 ml, 18 volumes of *methanol*, 23 volumes of *dichloromethane* and 54 volumes of *ethyl acetate*.

*Test solution*. Shake a quantity of the powdered tablets containing 10 mg of domperidone with 10 ml of a mixture of equal volumes of *dichloromethane* and *methanol* and filter through a glass microfibre filter (such as Whatman GF/C).

*Reference solution*. A 0.127 per cent w/v solution of *domperidone maleate RS* in a mixture of equal volumes of *dichloromethane* and *methanol*.

Apply to the plate 10 µl of each solution. After development, allow the plate to dry in air and examine in ultraviolet light at 254 nm. Spray the plate with *potassium iodobismuthate solution* and examine again. With each method of visualisation, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

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

## Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, diluted with the dissolution medium if necessary, at 286 nm (2.4.7), using the dissolution medium as the blank. Calculate the content of $C_{22}H_{24}ClN_5O_2$ in the medium from the absorbance obtained from a solution of known concentration *domperidone maleate RS*.

D. Not less than 70 per cent of the stated amount of $C_{22}H_{24}ClN_5O_2$.

**Related substances.** Determine by liquid chromatography (2.4.14).

*NOTE - Prepare the following solutions immediately before use.*

*Test solution*. To a quantity of the powdered tablets containing 50 mg of domperidone add 10 ml of a mixture of equal volumes of *0.01 M hydrochloric acid* and *methanol*, mix with the aid of ultrasound for 20 minutes and filter through a glass microfibre filter (such as Whatman GF/C).

*Reference solution (a)*. Dilute 1 volume of the test solution to 200 volumes with a mixture of equal volumes of *0.01 M hydrochloric acid* and *methanol*. Dilute 1 volume of the resulting solution to 2 volumes with a mixture of equal volumes of *0.01 M hydrochloric acid* and *methanol*.

*Reference solution (b)*. A solution containing 0.01 per cent w/v of *domperidone maleate RS* and 0.015 per cent w/v of *droperidol RS* in a mixture of equal volumes of *0.01 M hydrochloric acid* and *methanol*.



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Chromatographic system
- a stainless steel column 10 cm x 4.6 mm, packed with base-deactivated, end-capped octadecylsilyl silica gel (3 µm) (such as Hypersil BDS),
- mobile phase: A. *methanol*,
  B. a 0.5 per cent w/v solution of *ammonium acetate*,
- flow rate. 1.5 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 280 nm,
- injection volume. 10 µl.

| Time (in min.) | mobile phase A (per cent v/v) | mobile phase B (per cent v/v) |
|---|---|---|
| 0 | 30 | 70 |
| 10 | 100 | 0 |
| 12 | 100 | 0 |

Equilibrate the column for at least 30 minutes with *methanol* and equilibrate with the initial mobile phase for at least 5 minutes. Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (a) is at least 50 per cent of the full scale of the recorder.

Inject a mixture of equal volumes of *0.01 M hydrochloric acid* and *methanol* as a blank, the test solution and reference solutions (a) and (b). The test is not valid unless, in the chromatogram obtained with reference solution (b), the resolution between the two principal peaks is at least 2. If necessary adjust the concentration of *methanol* in the mobile phase or adjust the time programme for the linear gradient.

In the chromatogram obtained with the test solution the area of any secondary peak is not greater than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.25 per cent) and the sum of the areas of any secondary peaks is not greater than twice the area of the principal peak in the chromatogram obtained with the reference solution (a) (0.5 per cent). Ignore any peak in the chromatogram obtained with the blank solution and any peak with an area less than 0.2 times the area of the peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

**Other tests**. Comply with the tests stated under Tablets.

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

*Test solution.* Add sufficient *methanol* to 10 whole tablets to produce a solution containing 0.02 per cent w/v of domperidone, mix with the aid of ultrasound for 20 minutes and filter through a glass microfibre filter (such as Whatman GF/C). To 50.0 ml of the filtrate add 1 ml of *0.1 M hydrochloric acid* and sufficient *water* to produce 100.0 ml.

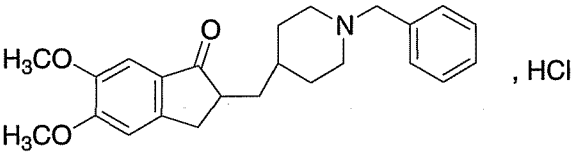
*Reference solution.* A solution containing 0.0127 per cent w/v of *domperidone maleate RS* in a mixture of equal volumes of *0.002 M hydrochloric acid* and *methanol.*

Calculate the content of $C_{22}H_{24}ClN_5O_2$ in the tablets.

**Storage**. Store protected from moisture.

**Labelling**. The label states the amount of domperidone.

# Donepezil Hydrochloride




$C_{24}H_{29}NO_3,HCl$ Mol. Wt. 415.5

Donepezil Hydrochloride is (*RS*)-2-[(1-benzyl-4-piperidyl) methyl]-5,6-dimethoxy-1-indanone hydrochloride.

Donepezil Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{24}H_{29}NO_3,HCl$, calculated on the anhydrous basis.

**Category.** Anti-Alzheimer.

**Description**. A white to off-white powder.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *donepezil hydrochloride RS* or with the reference spectrum of donepezil hydrochloride.

B. When examined in the range 200 nm to 400 nm ( 2.4.7), a 0.001 per cent w/v solution in *methanol* shows absorption maximum at about 230 nm, 268 nm and 313 nm.

## Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve 50 mg of the substance under examination in 50 ml of mobile phase.

*Reference solution (a).* A 0.1 per cent w/v solution of *donepezil hydrochloride RS* in mobile phase.



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*Reference solution (b).* Dilute 1 ml of reference solution (a) to 100 ml with mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 600 volumes of *water*, 400 volumes of *methanol* and 1 volume of *triethylamine*, adjust the pH to 3.0 with *orthophosphoric acid* and filter,
- flow rate. 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 20 µl.

Inject reference solution (a). The test is not valid unless the column effciency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with the reference solution (b) (2.0 per cent).

**Heavy metals** (2.3.13). The residue obtained from residue on ignition complies with limit test for heavy metals, Method B (20 ppm).

**Sulphated ash** (2.3.19). Not more than 0.2 per cent.

**Water** (2.3.43). Not more than 7.0 per cent, determined on 1.0 g.

**Assay**. Weigh accurately about 0.4 g, dissolve in a mixture of 40 ml of *glacial acetic acid* and 10 ml of 5 per cent of *mercuric acetate* in *glacial acetic acid*. Titrate with *0.1 M perchloric acid*. Determine the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04155 g of $C_{24}H_{29}NO_3$,HCl.

**Storage**. Store protected from moisture.

# Donepezil Tablets

Donepezil Hydrochloride Tablets

Donepezil Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of donepezil hydrochloride, $C_{24}H_{29}NO_3$,HCl.

**Usual strengths**. 5 mg; 10 mg.

## Identification

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

## Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 230 nm (2.4.7). Calculate the content of $C_{24}H_{29}NO_3$,HCl in the medium from the absorbance obtained from a solution of known concentration of *donepezil hydrochloride RS* in the same medium.

D. Not less than 75 per cent of the stated amount of $C_{24}H_{29}NO_3$,HCl.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. 25 volumes of *acetonitrile* and 75 volumes of *water*.

*Test solution.* Weigh accurately a quantity of powdered tablets containing about 50 mg of Donepezil Hydrochloride, add 25 ml of solvent mixture, sonicate for 15 minutes and make up the volume to 50 ml with solvent mixture and filter.

*Reference solution (a).* A 0.1 per cent w/v solution of *donepezil hydrochloride RS* in solvent mixture.

*Reference solution (b).* Dilute 1 ml of reference solution (a) to 100 ml with solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed octadecylsilane bonded to porous silica (5 µm),
- column temperature. 50°,
- mobile phase: A. a buffer solution pH 6.5 prepared by adding 1 ml of *orthophosphoric acid* in 1000 ml of *water*, adjust the pH to 6.5 with *triethylamine* and filter,
  B. *acetonitrile*,
- a linear gradient programme using the conditions given below,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 286 nm,
- injection volume. 20 µl.



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| Time (mins.) | Mobile phase A (per cent w/v) | Mobile phase B (per cent w/v) |
|---|---|---|
| 0 | 75 | 25 |
| 10 | 40 | 60 |
| 40 | 40 | 60 |
| 41 | 75 | 25 |
| 50 | 75 | 25 |

Inject reference solution (b). The test is not valid unless the column effciency is not less than 20000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 1.5 times the area of the peak in the chromatogram obtained with reference solution (b) (1.5 per cent) and the sum of areas of all the secondary peaks is not more than 3 times the area of the peak in the chromatogram obtained with the reference solution (b) (3.0 per cent).

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Transfer intact tablets and sonicate to disperse the tablets completely, add mobile phase and sonicate again for 30 minutes and cool and make up the volume with mobile phase. Allow the excipients to settle down completely and dilute finally with mobile phase to obtain a solution of final concentration of 0.01 per cent w/v and filter.

*Reference solution.* A 0.01 per cent w/v solution of *donepezil hydrochloride RS* in mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 40°,
- mobile phase: a mixture of 60 volumes of buffer pH 2.2 prepared by dissolving about 6.8 g of *potassium dihydrogen phosphate* in 1000 ml of *water*. Add 5 ml of *triethylamine* and adjust the pH to 2.2 with *orthophosphoric acid*, filter and 40 volumes of *methanol*,
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 268 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 7000 theoretical plates. The tailing factor is not more than 1.5. The relative standard deviation of replicate injections is not more than 2.0 per cent.

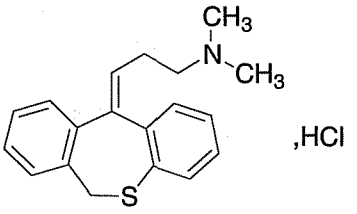
Inject the test solution and the reference solution

Calculate the content of $C_{24}H_{29}NO_3$,HCl in the tablets.

**Storage**. Store protected from moisture, at a temperature not exceeding 25°.

# Dothiepin Hydrochloride

Dosulepin Hydrochloride




$C_{19}H_{21}NS$,HCl Mol. Wt. 331.9

Dothiepin Hydrochloride is 3-(6*H*-dibenzo[*b,e*]thiepin-11-ylidene)propyldimethylamine hydrochloride, consisting predominantly of the *E*-isomer.

Dothiepin Hydrochloride contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{19}H_{21}NS$,HCl, calculated on the dried basis.

**Category**. Antidepressant.

**Dose**. Initially 75 mg daily, in divided doses, increased gradually, as necessary, to 150 mg daily.

**Description**. A white to faintly yellow, crystalline powder; odourless or almost odourless.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dothiepin hydrochloride RS* or with the reference spectrum of dothiepin hydrochloride.

B. Dissolve 1 mg in 5 ml of *sulphuric acid*; a dark red colour is produced.

C. On 20 mg determine by the oxygen-flask method (2.3.34), using a mixture of 15 ml of *water* and 1 ml of *hydrogen peroxide solution (20 volume)* as the absorbing liquid. The solution gives the reactions of sulphates (2.3.1).

D. Gives reaction A of chlorides (2.3.1).

## Tests

**pH** (2.4.24). 3.0 to 4.5, determined in a 5.0 per cent w/v solution.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254*.

*Mobile phase.* A mixture of 90 volumes of *1,2-dichloroethane*, 10 volumes of *2-propanol* and 1 volume of *strong ammonia solution.*

*NOTE —Prepare the following solutions freshly before use.*

*Test solution (a).* Dissolve 1.0 g of the substance under examination in 10 ml of *chloroform.*



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*Test solution (b).* Dissolve 0.4 g of the substance under examination in 10 ml of *chloroform.*

*Reference solution.* A solution containing 0.02 per cent w/v each of *11-(3-dimethylamino-propylidene)-6H-dibenzo[b,e]thiepin- 5-oxide RS* and *6H-dibenzo-[b,e]thiepin-11-one RS* in *chloroform.*

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. In the chromatogram obtained with the reference solution the spot with the lower $R_f$ value is more intense than any corresponding spot in the chromatogram obtained with test solution (b). In the chromatogram obtained with test solution (a) any secondary spot other than any spot corresponding to the spot with the lower $R_f$ value in the chromatogram obtained with the reference solution is not more intense than the proximate spot in the chromatogram obtained with the reference solution.

**Z-Isomer**. Determine by gas chromatography (2.4.13).

*Test solution.* Dissolve 0.5 g of the substance under examination in 100 ml of *methanol.*

*Reference solution.* A 0.5 per cent w/v solution of *dothiepin hydrochloride RS* in *methanol.*

Chromatographic system

- a glass column 1.8 m x 3 mm, packed with acid-washed, silanised diatomaceous support (100 to 120 mesh) coated with 3 per cent w/w of cyanopropylmethyl phenyl methyl silicone fluid (such as OV-225),
- temperature:
  column.200°,
  inlet port. 260°,
- flame ionisation detector,
- nitrogen as carrier gas.

In the chromatogram obtained with the reference solution a peak due to Z-dothiepin is present with a retention time of approximately 0.83 relative to the retention time of the principal peak which is due to *E*-dothiepin. In the chromatogram obtained with the test solution the area of any peak corresponding to Z-dothiepin is not greater than 7.5 per cent of the sum of the areas of the peaks due to Z-dothiepin and *E*-dothiepin.

**Heavy metals** (2.3.13). Dissolve the residue obtained in the test for Sulphated ash in 0.5 ml of *hydrochloric acid,* evaporate to dryness, dissolve the residue in 2 ml of *water*, neutralise to *phenolphthalein solution* with *dilute sodium hydroxide solution* and dilute to 15 ml with *water*, 12 ml of the resulting solution complies with the limit test for heavy metals, Method D (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent, determined on 1.5 g.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.6 g, dissolve in 100 ml of *acetone* and add 15 ml *mercuric acetate solution.* Titrate with *0.1 M perchloric acid,* using 3 ml of a saturated solution of *methyl orange* in *acetone* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03319 g of $C_{19}H_{21}NS,HCl$.

**Storage**. Store protected from light.

## Dothiepin Capsules

Dothiepin Hydrochloride Capsules; Dosulepin Capsules; Dosulepin Hydrochloride Capsules.

Dothiepin Capsules contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of dothiepin hydrochloride, $C_{19}H_{21}NS,HCl$.

**Usual strength**. 25 mg.

### Identification

Extract a quantity of the contents of the capsules containing 0.1 g of Dothiepin Hydrochloride with 20 ml of *ethanol*, filter and remove the ethanol from the filtrate by evaporation. The residue complies with the following tests.

A. Dissolve 1 mg in 5 ml of *sulphuric acid*; a dark red colour is produced.

B. On 20 mg determine by the oxygen-flask method (2.3.34), using a mixture of 15 ml of *water* and 1 ml of *hydrogen peroxide solution (20 volume)* as the absorbing liquid. The solution gives the reactions of sulphates (2.3.1).

C. Gives reaction A of chlorides (2.3.1).

### Tests

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254.*

*Mobile phase.* A mixture of 90 volumes of *1,2-dichloroethane*, 10 volumes of *2-propanol* and 1 volume of *strong ammonia solution.*

*Test solution (a).* Extract a quantity of the contents of the capsules containing about 0.25 g of Dothiepin Hydrochloride by shaking for 2 minutes with 5 ml of *chloroform*, centrifuge and use the supernatant liquid.

*Test solution (b).* Dilute 2 ml of test solution (a) to 5 ml with *chloroform.*

*Reference solution.* A freshly prepared solution containing 0.02 per cent w/v each of *11-(3-dimethylamino-propylidene)-6H-dibenzo[b,e]thiepin-5-oxide RS* and *6H-dibenzo-[b,e]thiepin-11-one RS* in *chloroform.*



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Apply to the plate 5 µl of the reference solution and 10 µl of the test solutions. After development, dry the plate in air and examine in ultraviolet light at 254 nm. In the chromatogram obtained with the reference solution the spot with the lower $R_f$ value is more intense than any corresponding spot in the chromatogram obtained with test solution (b). In the chromatogram obtained with test solution (a) any secondary spot other than any spot corresponding to the spot with the lower $R_f$ value in the chromatogram obtained with the reference solution is not more intense than the proximate spot in the chromatogram obtained with the reference solution.

**Z-Isomer**. Determine by gas chromatography (2.4.13).

*Test solution.* Use the supernatant liquid obtained by extracting a quantity of the mixed contents of 20 capsules containing 25 mg of Dothiepin Hydrochloride with 5 ml of *methanol* and centrifuging.

*Reference solution.* A 0.5 per cent w/v solution of *dothiepin hydrochloride RS* in *methanol.*

Chromatographic system
- a glass column 1.8 m x 3 mm, packed with acid-washed, silanised diatomaceous support (100 to 120 mesh) coated with 3 per cent w/w of cyanopropylmethyl phenyl methyl silicone fluid (such as OV-225),
- temperature:
  column 200°,
  inlet port. 260°,
- flame ionisation detector,
- nitrogen as carrier gas.

In the chromatogram obtained with the reference solution a peak due to Z-dothiepin is present with a retention time of approximately 0.83 relative to the retention time of the principal peak which is due to *E*-dothiepin. In the chromatogram obtained with the test solution the area of any peak corresponding to Z-dothiepin is not greater than 7.5 per cent of the sum of the areas of the peaks due to Z-dothiepin and *E*-dothiepin.

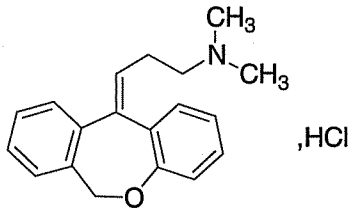
**Other tests.** Comply with the tests stated under Capsules.

**Assay**. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 0.5 g of Dothiepin Hydrochloride and extract with 20 ml followed by four quantities, each of 10 ml, of *chloroform*, filtering each extract through the same filter. Evaporate the combined extracts to dryness, dissolve the residue in 100 ml of *acetone* and add 15 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid,* using 3 ml of a saturated solution of *methyl orange* in *acetone* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03319 g of $C_{19}H_{21}NS,HCl$.

**Storage**. Store protected from moisture.

# Doxepin Hydrochloride



$C_{19}H_{21}NO,HCl$ Mol. Wt. 315.8

Doxepin Hydrochloride is 3-(6*H*-dibenz[*b,e*]oxepin-11-ylidene)propyldimethylamine hydrochloride. It consists of a mixture of *Z* and *E* isomers.

Doxepin Hydrochloride contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{19}H_{21}NO,HCl$, calculated on the dried basis.

**Category**. Antidepressant.

**Dose**. Initially, the equivalent of 75 mg of doxepin daily, in divided doses, increased gradually to a maximum of 300 mg daily, in divided doses.

**Description**. A white, crystalline powder; odour, slight and amine-like.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *doxepin hydrochloride RS* or with the reference spectrum of doxepin hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.004 per cent w/v solution in *0.01 M methanolic hydrochloric acid* shows an absorption maximum only at about 297 nm; absorbance at about 297 nm, about 0.60.

C. Dissolve 5 mg in 2 ml of *nitric acid*; a red colour is produced.

D. Gives reaction A of chlorides (2.3.1).

## Tests

**Z-isomer**. 13.0 per cent to 18.5 per cent, determined by the following method.

Determine by gas chromatography (2.4.13).

*Test solution.* Dissolve 0.5 g of the substance under examination in 100 ml of *methanol.*

*Reference solution.* A 0.5 per cent w/v solution of *doxepin hydrochloride RS* in *methanol.*

Chromatographic system
- a glass column 1.5 m x 4 mm, packed with acid-washed, silanised diatomaceous support (100 to 120 mesh) coated



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with 3 per cent w/w of cyanopropylmethyl phenyl methyl silicone fluid (such as OV-225),
- temperature:
  column.200°,
  inlet port. 260 °,
- flame ionisation detector,
- nitrogen as carrier gas.

In the chromatogram obtained with the reference solution a peak due to Z-doxepin immediately precedes and is adequately separated from the principal peak which is due to *E*-doxepin. Measure the areas or heights of the peaks due to Z-doxepin and *E*-doxepin in the chromatograms obtained with the test solution and the reference solution. Calculate the content of the Z-isomer in the substance under examination.

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

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.6 g, dissolve in 100 ml of *acetone* and add 15 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, using 3 ml of a saturated solution of *methyl orange* in *acetone* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03158 g of $C_{19}H_{21}NO,HCl$.

**Storage**. Store protected from light.

# Doxepin Capsules

## Doxepin Hydrochloride Capsules

Doxepin Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of doxepin, $C_{19}H_{21}NO$.

**Usual strengths**. The equivalent of 25 mg, 50 mg and 75 mg of doxepin.

### Identification

Wash a quantity of the contents of the capsules containing 0.1 g of doxepin with 3 quantities, each of 5 ml, of *light petroleum (40° to 60°)*. Dry the residue in air and extract with 3 quantities, each of 10 ml, of *chloroform*, evaporate the combined extracts to dryness and dry the residue at 105°. The dried residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *doxepin hydrochloride RS* or with the reference spectrum of doxepin hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.004 per cent w/v solution in *0.01 M methanolic hydrochloric acid* shows an absorption maximum only at about 297 nm; absorbance at about 297 nm, about 0.60.

C. Dissolve 5 mg in 2 ml of *nitric acid*; a red colour is produced.

D. Gives reaction A of chlorides (2.3.1).

### Tests

**Z-isomer**. 13.0 per cent to 18.5 per cent, determined by the following method.

Determine by gas chromatography (2.4.13).

*Test solution*. Use the supernatant liquid obtained by extracting a quantity of the mixed contents of 20 capsules containing 25 mg of doxepin with 5 ml of *methanol* and centrifuging.

*Reference solution*. A 0.5 per cent w/v solution of *doxepin hydrochloride RS* in *methanol*.

Chromatographic system
- a glass column 1.5 m x 4 mm, packed with acid-washed, silanised diatomaceous support (100 to 120 mesh) coated with 3 per cent w/w of cyanopropylmethyl phenyl methyl silicone fluid (such as OV-225),
- temperature:
  column.200°,
  inlet port. 260 °,
- flame ionisation detector,
- nitrogen as carrier gas.

In the chromatogram obtained with the reference solution a peak due to Z-doxepin immediately precedes and is adequately separated from the principal peak which is due to E-doxepin. Measure the areas or heights of the peaks due to Z-doxepin and E-doxepin in the chromatograms obtained with the test solution and the reference solution. Calculate the content of the Z-isomer in the capsules.

**Other tests**. Comply with the tests stated under Capsules.

**Assay**. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 30 mg of doxepin, add 50 ml of *0.1 M methanolic hydrochloric acid*, shake for 30 minutes and add sufficient *0.01 M methanolic hydrochloric acid* to produce 100.0 ml. Centrifuge 40 ml of this solution and dilute 10.0 ml of the clear supernatant liquid to 100.0 ml with *0.01 M methanolic hydrochloric acid*. Measure the absorbance of the resulting solution at the maximum at about 297 nm (2.4.7). Calculate the content of $C_{19}H_{21}NO$ taking 150 as the specific absorbance at 297 nm.

**Storage**. Store protected from light and moisture.

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

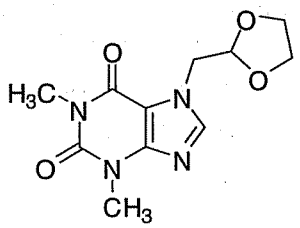


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# Doxofylline



$C_{11}H_{14}N_4O_4$ Mol. Wt. 266.3

Doxofylline is 7-(1,3-dioxolan-2-ylmethyl)-3,7-dihydro-1,3-dimethyl-1*H*-purine-2,6-dione.

Doxofylline contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{11}H_{14}N_4O_4$, calculated on the dried basis.

**Category**. Bronchodilator.

**Description**. A white to off white crystalline powder.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum obtained with *doxofylline RS* or with the reference spectrum of doxofylline.

B. When examined in the range of 200 nm to 400 nm (2.4.7), a 0.001 per cent w/v solution in *water* shows an absorption maximum at about 274 nm.

## Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 0.1 g of the substance under examination in 100.0 ml of the mobile phase.

*Reference solution (a)*. A solution containing 0.05 per cent w/v each of *doxofylline RS* and *theophylline RS* in the mobile phase.

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

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Cosmosil C18),
- mobile phase: a mixture of 80 volumes of *water* and 20 volumes of *acetonitrile*,
- flow rate. 0.8 ml per minute,
- spectrophotometer set at 274 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to theophylline and doxofylline is not less than 5.0 and the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of the peak due to theophylline is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the area of any other secondary peak is not more than the area of the peak in the chromatogram obtained reference solution (b) (0.5 per cent). The sum of areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained reference solution (b) (1.0 per cent).

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

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in oven at 105° for 3 hours.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve about 40 mg of substance under examination in 100.0 ml of the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

*Reference solution*. A 0.004 per cent w/v solution of *doxofylline RS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Cosmosil C18),
- mobile phase: a mixture of 80 volumes of a buffer solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, and 20 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 274 nm,
- injection volume. 10 µl.

Inject reference solution (b). The test is not valid unless the theoretical plates is not less than 2000, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0.

Inject reference solution (b) and the test solution.

Calculate the content of $C_{11}H_{14}N_4O_4$.

**Storage**. Store protected from light and moisture, at a temperature not exceeding 30°.

# Doxofylline Tablets

Doxofylline Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of doxofylline, $C_{11}H_{14}N_4O_4$.



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**Usual strength**. 400 mg.

## Identification

A. Extract a quantity of the powdered tablets containing about 0.1 g of Doxofylline with 40 ml of *chloroform*, filter and evaporate the filtrate to dryness. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained from *doxofylline RS* or with the reference spectrum of doxofylline.

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

## Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.01 M hydrochloric acid,*
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter. Measure the absorbance of the filtered solution, suitably diluted if necessary with the medium, at the maximum at about 275 nm (2.4.7). Calculate the content of $C_{11}H_{14}N_4O_4$ in the medium from the absorbance obtained from a solution of known concentration of *doxofylline RS* in the same medium.

D. Not less than 75 per cent of the stated amount of $C_{11}H_{14}N_4O_4$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution.* Disperse a quantity of powdered tablets containing about 25 mg of Doxofylline in 25.0 ml in the mobile phase and filter.

*Reference solution (a).* A 0.1 per cent w/v solution of *doxofylline RS* in the mobile phase.

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

Use chromatographic system as described under Assay.

Inject reference solution (a). The test is not valid unless the tailing factor is not more than 2.0, theoretical plates is not less than 1500 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.2 times the area of the peak obtained with reference solution (b) (0.2 per cent) and the sum of areas of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with reference solution (b) (1.0 per cent).

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution (a).* Weigh and powder 20 tablets. Disperse a quantity of powder containing about 50 mg of Doxofylline in 70 ml of *methanol*, sonicate for 15 minutes and dilute to 100 ml with *methanol,* filter.

*Test solution (b).* Dilute 2.0 ml of test solution (a) to 50.0 ml with the mobile phase.

*Reference solution (a).* A 0.05 per cent w/v solution of *doxofylline RS* in *methanol.*

*Reference solution (b).* Dilute 2.0 ml of reference solution (a) to 50.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 30 volumes of *water* and 70 volumes of *methanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 274 nm,
- injection volume. 20 µl.

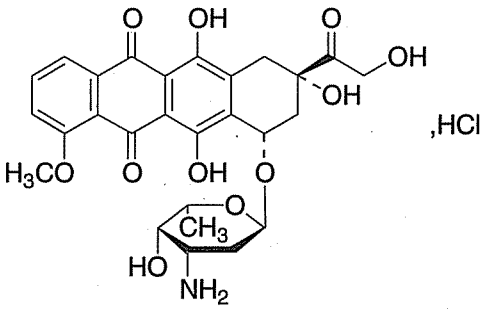
Inject reference solution (b). The test is not valid unless the tailing factor is not more than 2.0, theoretical plates is not less than 1500 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (b) and test solution (b).

Calculate the content of $C_{11}H_{14}N_4O_4$ in the tablets.

**Storage**. Store protected from light and moisture.

# Doxorubicin Hydrochloride




$C_{27}H_{29}NO_{11},HCl$ Mol. Wt. 580.0

Doxorubicin Hydrochloride is (8*S*,10*S*)-10-[(3-amino-2,3, 6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-6,8,11-trihydroxy- 8-hydroxyacetyl-1-methoxy-7,8,9,10-tetrahydronaphthacene-5,12-dione hydrochloride, a substance produced by the growth of certain strains of *Streptomyces coeruleorubidus* or *S. peucetius* or obtained by any other means.



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Doxorubicin Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{27}H_{29}NO_{11}$,HCl, calculated on the anhydrous and solvent-free basis.

**Category**. Cytotoxic.

**Dose**. To be determined by the physician in accordance with the needs of the patient.

**Description**. An orange-red, crystalline powder; hygroscopic.

*CAUTION - Doxorubicin Hydrochloride is poisonous. It must be handled with care avoiding contact with skin and inhalation of airborne particles.*

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *doxorubicin hydrochloride RS*.

B. When examined in the range 220 nm to 550 nm (2.4.7), a 0.001 per cent w/v solution in *ethanol (95 per cent)* exhibits maxima at about 234 nm, 252 nm, 288 nm, 475 nm, 495 nm and 530 nm.

C. In the test for Related substances, the principal peak in the chromatogram obtained with test solution (b) corresponds to the peak in the chromatogram obtained with reference solution (c).

D. Dissolve 10 mg in 0.5 ml of *nitric acid*, add 0.5 ml of *water* and heat over a flame for 2 minutes. Allow to cool and add 0.5 ml of *silver nitrate solution*; a white precipitate is produced.

## Tests

**pH** (2.4.24). 4.0 to 5.5, determined in a 0.5 per cent w/v solution.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution (a)*. Dissolve 50 mg of the substance under examination in sufficient of the mobile phase to produce 50 ml.

*Test solution (b)*. Dilute 10 ml of test solution (a) to 100 ml with the mobile phase.

*Reference solution (a)*. Dissolve 10 mg of *doxorubicin hydrochloride RS* and 10 mg of *epirubicin hydrochloride RS* in the mobile phase and dilute to 50 ml with the mobile phase. Dilute 10 ml of this solution to 100 ml with the mobile phase.

*Reference solution (b)*. Dilute 5 ml of reference solution (a) to 20 ml with the mobile phase.

*Reference solution (c)*. Dissolve 50 mg of *doxorubicin hydrochloride RS* in the mobile phase and dilute to 50 ml with the mobile phase. Dilute 10 ml of this solution to 100 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of equal volumes of *acetonitrile* and a solution containing 2.88 g per litre of *sodium dodecyl sulphate* and 2.25 g per litre of *phosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject test solution (a) and reference solutions (a) and (b). Continue the chromatography for 3.5 times the retention time of doxorubicin of about 8 minutes. The test is not valid unless in the chromatogram obtained with reference solution (a) the resolution factor between the peaks due to doxorubicin and epirubicin is at least 2.0.

In the chromatogram obtained with the test solution the area of any peak other than the principal peak is not more than the area of the peak corresponding to doxorubicin in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.1 times the area of the peak corresponding to doxorubicin in the chromatogram obtained with reference solution (b) (0.05 per cent).

**Acetone and ethanol**. Not more than 2.0 per cent w/w together of which not more than 0.5 per cent w/w is acetone, determined by gas chromatography (2.4.13), injecting 1 µl of each of two solutions

*Test solution*. A 5.0 per cent w/v solution of the substance under examination and 0.1 per cent w/v of *dioxon* in *water*.

Reference solution. 0.05 per cent w/v of *acetone*, 0.05 per cent w/v of *ethanol* and 0.1 per cent w/v of the internal standard.

Chromatographic system
- a glass column 2 m x 3 mm, packed with acid-washed diatomaceous support (180 to 250 mesh) impregnated with 10 per cent w/w of *polyethylene glycol 20,000* (such as Carbowax 20M or Chromosorb E/AW),
- temperature:
  column.70°,
  inlet port and detector. 125°,
- flow rate. 30 ml per minute of the carrier gas.

**Water** (2.3.43). Not more than 4.0 per cent, determined on 0.1 g.

**Assay**. Determine by liquid chromatography (2.4.14) by the procedure described under the test for Related substances.

Inject test solution (b) and reference solution (c).

Calculate the content of $C_{27}H_{29}NO_{11}$,HCl.

*Doxorubicin Hydrochloride intended for use in the manufacture of parenteral preparations without a further*



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*appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.*

**Bacterial endotoxins** (2.2.3). Not more than 2.2 Endotoxin Units per mg.

*Doxorubicin Hydrochloride intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.*

**Sterility** (2.2.11). Complies with the test for sterility.

**Storage**. Store protected from moisture. If the material is sterile, it should be stored in sterile, tamper-evident containers and sealed so as to exclude micro-organisms.

**Labelling**. The label states whether or not the material is intended for use in the manufacture of parenteral preparations.

## Doxorubicin Injection

Doxorubicin Hydrochloride Injection

Doxorubicin Injection is a sterile solution of Doxorubicin Hydrochloride in Water for Injections made isotonic with Sodium Chloride, Dextrose or other suitable added substances. It is either supplied as preformed solution or it is prepared by dissolving the contents of a sealed container containing Doxorubicin Hydrochloride with or without auxiliary substances in the requisite amount of Water for Injections or Sodium Chloride Injection as directed on the label.

Doxorubicin Injection contains not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of doxorubicin hydrochloride, $C_{27}H_{29}NO_{11}$,HCl.

**Usual strength**. 2 mg per ml.

### Identification

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

### Tests

**pH** (2.4.24). 2.5 to 4.5 for the preformed solution and 4.5 to 6.5, determined in the injection prepared in accordance with the directions on the label.

**Bacterial endotoxins** (2.2.3). Not more than 2.2 Endotoxin Units per mg of doxorubicin hydrochloride, determined in a solution prepared by diluting the injection, if necessary, with *water BET* to obtain a concentration of 2.0 mg of doxorubicin hydrochloride per ml.

**Sterility**. Complies with the test for sterility, Method A, (2.2.11), using the entire contents of all the containers collected aseptically.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. A solution containing 0.05 per cent w/v of doxorubicin hydrochloride prepared by diluting an accurately measured volume of the injection containing not less than 2 mg of Doxorubicin Hydrochloride with the mobile phase or by dissolving the contents of the sealed container in sufficient mobile phase to give a solution of the same strength.

*Reference solution (a)*. A 0.05 per cent w/v solution of *doxorubicin hydrochloride RS* in the mobile phase.

*Reference solution (b)*. A solution containing 0.002 per cent w/v each of *doxorubicin hydrochloride RS* and *epirubicin hydrochloride RS* in the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of a solution containing 0.288 per cent w/v of *sodium dodecyl sulphate* and 0.23 per cent w/v of *phosphoric acid*, 45 volumes of *acetonitrile* and 5 volumes of *methanol*,
- flow rate. 0.8 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

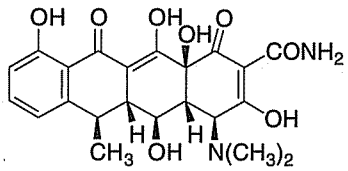
Inject reference solution (b). The test is not valid unless the resolution between the peaks due to doxorubicin and epirubicin is not less than 2.0 and the relative standard deviation for replicate injections is not more than 1.0 per cent.

Calculate the content of $C_{27}H_{29}NO_{11}$,HCl in the injection.

**Storage**. Store the sealed container at a temperature not exceeding 30°. Store the preformed solution protected from light in a refrigerator. Use the solution prepared in the liquid stated on the label immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the instructions of the manufacturer.

## Doxycycline Hydrochloride

Doxycycline Hyclate




,HCl, $^1/_2$ $C_2H_5OH$, $^1/_2$ $H_2O$

$C_{22}H_{24}N_2O_8$,HCl,½$C_2H_6O$,½$H_2O$ Mol. Wt. 513.0

Doxycycline Hydrochloride is (4*S*,4a*R*,5*S*,5a*R*,6*R*,12a*S*)-4-dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12, 12a-



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pentahydroxy-6-methyl-1,11-dioxonaphthacene-2-carboxamide hydrochloride hemiethanolate hemihydrate, an antimicrobial substance obtained from oxytetracycline or methacycline or by any other means.

Doxocycline Hydrochloride is not less than 95.0 per cent and not more than 102.0 per cent of $C_{22}H_{25}ClN_2O_8$, calculated on anhydrous basis.

**Category**. Antibacterial.

**Dose**. The equivalent of 200 mg of doxycycline on the first day followed by 100 mg daily.

**Description**. A yellow, crystalline powder; odour, slightly ethanolic; hygroscopic.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *doxycycline hydrochloride RS* or with the reference spectrum of doxycycline hydrochloride.

B. Determine by thin-layer chromatography (2.4.7), coating the plate with *silica gel H*.

*Mobile phase*. A mixture of 59 volumes of *dichloromethane*, 35 volumes of *methanol* and 6 volumes of *water*.

*Test solution*. Dissolve 50 mg of the substance under examination in 100 ml of *methanol*.

*Reference solution (a)*. A 0.05 per cent w/v solution of *doxycycline hydrochloride RS* in *methanol*.

*Reference solution (b)*. A solution containing 0.05 per cent w/v each of *doxycycline hydrochloride RS* and *tetracycline hydrochloride RS* in *methanol*.

Spray the plate evenly with a 10 per cent w/v solution of *disodium edetate* the pH of which has been adjusted to 9.0 with *10 M sodium hydroxide*. Allow the plate to dry in a horizontal position for at least 1 hour. Immediately before use dry it at 110° for 1 hour. Apply to the plate 1 µl of each solution. After development, dry the plate in a current of air and examine in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

C. To about 2 mg add 5 ml of *sulphuric acid*; a yellow colour is produced.

D. A 5 per cent w/v solution gives the reactions of chlorides (2.3.1).

## Tests

**pH** (2.4.24). 2.0 to 3.0, determined in a 1.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). -105° to -120°, determined within 5 minutes of preparing, in a 1.0 per cent w/v solution in a mixture of 1 volume of *1 M hydrochloric acid* and 99 volumes of *methanol*.

**Light absorption** (2.4.7). Absorbance of a 0.001 per cent w/v solution in a mixture of 1 volume of *1 M hydrochloric acid* and 99 volumes of *methanol*, measured within 1 hour of preparing the solution, at the maximum at about 349 nm, 0.300 to 0.335.

**Light-absorbing impurities**. Dissolve 0.1 g in sufficient of a mixture of 1 volume of *1 M hydrochloric acid* and 99 volumes of *methanol* to produce 10 ml. Absorbance of the solution at about 490 nm, when measured within 1 hour of preparing the solution, not more than 0.07 (2.4.7).

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 80 mg of the substance under examination in 100 ml of *0.01 M hydrochloric acid*.

*Reference solution (a)*. A 0.08 per cent w/v solution of *doxycycline hydrochloride RS* in *0.01 M hydrochloric acid*.

*Reference solution (b)*. A 0.08 per cent w/v solution of *6-epidoxycycline hydrochloride RS* in *0.01 M hydrochloric acid*.

*Reference solution (c)*. A 0.08 per cent w/v solution of *methacycline hydrochloride RS* in *0.01 M hydrochloric acid*.

*Reference solution (d)*. A solution containing 0.0016 per cent w/v each of *6-epidoxyccline hydrochloride RS* and *methacycline hydrochloride RS* in *0.01 M hydrochloric acid*.

*Reference solution (e)*. Dilute a mixture of 4 volumes of reference solution (a), 1.5 volumes of reference solution (b) and 1 volume of reference solution (c) to 25 volumes with *0.01 M hydrochloric acid*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with styrene-divinylbenzene co-polymer (8 to10 µm),
- mobile phase: a solution prepared by adding 60 g of *2-methyl-2-propanol* to a volumetric flask with the aid of 200 ml of *water*, adding 400 ml of *phosphate buffer pH 8.0*, 50 ml of a 1 per cent w/v solution of *tetrabutyl ammonium hydrogen sulphate* previously adjusted to pH 8.0 with *2 M sodium hydroxide* and 10 ml of a 4 per cent w/v solution of *disodium edetate* previously adjusted to pH 8.0 with *2 M sodium hydroxide* and diluting to 1000 ml with *water*,
- column temperature. 60°,



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– flow rate. 1 ml per minute,
– spectrophotometer set at 254 nm,
– injection volume. 20 µl.

Using reference solution (e), adjust the attenuation to obtain peaks with a height corresponding to at least 50 per cent of full-scale deflection on the chart paper. The test is not valid unless (a) the resolution between the first peak (methacycline) and the second peak (6-epidoxycycline) is at least 1.25, (b) the resolution between the second peak and the third peak (doxycycline) is at least 2.0 (adjust the content of 2-methyl-2-propanol in the mobile phase if necessary) and (c) the tailing factor for the third peak is at most 1.25.

Inject reference solution (a). The test is not valid unless the relative standard deviation of the area of the peak due to doxycycline is not more than1.0 per cent.

Inject the test solution and reference solution (d). In the chromatogram obtained with the test solution the area of any peak corresponding to methacycline or 6-epidoxycycline is not greater than the area of the corresponding peak in the chromatogram obtained with reference solution (d); the area of any peak appearing between the solvent peak and the peak corresponding to methacycline and the area of any peak appearing on the tail of the main peak is not greater than 25 per cent of that of the peak corresponding to 6-epidoxycycline in the chromatogram obtained with reference solution (d).

**Ethanol**. 4.3 to 6.0 per cent w/w of $C_2H_6O$.

Determine by gas chromatography (2.4.13).

*Test solution (a)*. A 1 per cent w/v solution of the substance under examination in a 0.05 per cent v/v solution of *1-propanol* (internal standard) in *water* (solution A).

*Test solution (b)*. A 1 per cent w/v solution of the substance under examination in *water*.

*Reference solution*. A 0.05 per cent v/v solution of *ethanol* in solution A.

Chromatographic system
– a column 1.5m x 4 mm, packed with porous polymer beads (80 to 100 mesh) (such as Porapak Q),
– temperature:
column.135°,
inlet port and detector. 150°,

Calculate the content of $C_2H_6O$ taking 0.790 g as its weight per ml (2.4.29) at 20°.

**Heavy metals** (2.3.13). 0.4 g complies with the limit test for heavy metals, Method B (50 ppm).

**Sulphated ash** (2.3.18). Not more than 0.4 per cent.

**Water** (2.3.43). 1.4 to 2.8 per cent, determined on 1.2 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh accurately 80 mg of the substance under examination and dissolve in 100 ml of *0.01 M hydrochloric acid*.

*Reference solution*. A 0.08 per cent w/v solution of *doxycycline hydrochloride RS* in *0.01 M hydrochloric acid*.

Chromatographic system
– a stainless steel column 25 cm x 4.6 mm, packed with styrene-divinylbenzene co-polymer (8 to 10 µm),
– column temperature: 60°
– mobile phase: a solution prepared by adding 60 g of *2-methyl-2-propanol* to a volumetric flask with the aid of 200 ml of *water*, adding 400 ml of *phosphate buffer pH 8.0*, 50 ml of a 1 per cent w/v solution of *tetrabutyl ammonium hydrogen sulphate* previously adjusted to pH 8.0 with *2 M sodium hydroxide* and 10 ml of a 4 per cent w/v solution of *disodium edetate* previously adjusted to pH 8.0 with *2 M sodium hydroxide* and diluting to 1000 ml with *water*,
– flow rate. 1 ml per minute,
– spectrophotometer set at 254 nm,
– injection volume. 20 µl.

Inject the reference solution six times. The test is not valid unless the relative standard deviation of the area of the peak due to doxycycline is not more than 1.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_{22}H_{24}N_2O_8$.

*Doxycycline Hydrochloride intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.*

**Bacterial endotoxins** (2.2.3). Not more than 1.14 Endotoxin Units per mg.

*Doxycycline Hydrochloride intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.*

**Sterility** (2.2.11). Complies with the test for sterility.

**Storage**. Store protected from light and moisture at a temperature not exceeding 30°. If the substance is intended for use in the manufacture of parenteral preparations, the container should be sterile, tamper-evident and sealed so as to exclude micro-organisms.

**Labelling**. The label states, where applicable, that the material is sterile.



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# Doxycycline Capsules

Doxycycline Hydrochloride Capsules

Doxycycline Capsules contain not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of doxycycline, $C_{22}H_{24}N_2O_8$.

**Usual strengths**. The equivalent of 50 mg; 100 mg; 200 mg of doxycycline.

## Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H*.

*Mobile phase*. A mixture of 59 volumes of *dichloromethane*, 35 volumes of *methanol* and 6 volumes of *water*.

*Test solution*. Shake a quantity of the contents of the capsules containing 50 mg of *anhydrous doxycycline* with 100 ml of *methanol* for 1 to 2 minutes, centrifuge and use the supernatant liquid. Prepare freshly.

*Reference solution (a)*. A 0.05 per cent w/v solution of *doxycycline hydrochloride RS* in *methanol*.

*Reference solution (b)*. A solution containing 0.05 per cent w/v each of *doxycycline hydrochloride RS* and *tetracycline hydrochloride RS* in *methanol*.

Spray the plate evenly with a 10 per cent w/v solution of *disodium edetate* the pH of which has been adjusted to 9.0 with *10 M sodium hydroxide*. Allow the plate to dry in a horizontal position for at least 1 hour. Immediately before use dry it at 110° for 1 hour. Apply to the plate 1 µl of each solution. After development, dry the plate in a current of air and examine it in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

B. To 0.5 mg of the contents of the capsules add 2 ml of *sulphuric acid*; a yellow colour is produced.

C. A 5 per cent w/v solution of the contents of the capsules gives the reactions of chlorides (2.3.1).

## Tests

**Light-absorbing impurities**. Dissolve the contents of 5 capsules as completely as possible in sufficient of a mixture of 1 volume of *1 M hydrochloric acid* and 99 volumes of *methanol* to produce a solution containing the equivalent of 1.0 per cent w/v of anhydrous doxycycline and filter. Absorbance of the filtrate at about 490 nm, not greater than 0.2 (2.4.7), calculated with reference to the dried contents of the capsules.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve a quantity of the contents of the capsules containing 7 mg of anhydrous doxycycline in 10 ml of *0.01 M hydrochloric acid*, filter and use the filtrate.

*Reference solution (a)*. A 0.08 per cent w/v solution of *doxycycline hydrochloride RS* in *0.01 M hydrochloric acid*.

*Reference solution (b)*. A 0.08 per cent w/v solution of *6-epidoxycycline hydrochloride RS* in *0.01 M hydrochloric acid*.

*Reference solution (c)*. A 0.08 per cent w/v solution of *methacycline hydrochloride RS* in *0.01 M hydrochloric acid*.

*Reference solution (d)*. A solution containing 0.0016 per cent w/v each of *6-epidoxyccline hydrochiloride RS* and *methacycline hydrochloride RS* in *0.01 M hydrochloric acid*.

*Reference solution (e)*. Dilute a mixture of 4 volumes of reference solution (a), 1.5 volumes of reference solution (a) and 1 volume of reference solution (c) to 25 volumes with *0.01 M hydrochloric acid*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with styrene-divinylbenzene co-polymer (8 to 10 µm),
- column temperature. 60°,
- mobile phase: a solution prepared by adding 60 g of *2-methyl-2-propanol* to a volumetric flask with the aid of 200 ml of *water*, adding 400 ml of *phosphate buffer pH 8.0*, 50 ml of a 1 per cent w/v solution of *tetrabutyl ammonium hydrogen sulphate* previously adjusted to pH 8.0 with *2 M sodium hydroxide* and 10 ml of a 4 per cent w/v solution of *disodium edetate* previously adjusted to pH 8.0 with *2 M sodium hydroxide* and diluting to 1000 ml with *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Using reference solution (e) adjust the attenuation to obtain peaks with a height corresponding to at least 50 per cent of full-scale deflection of the recorder. The test is not valid unless (a) the resolution factor between the first peak (methacycline) and the second peak (6-epidoxycycline) is at least 1.25, (b) the resolution factor between the second peak and the third peak (doxycycline) is at least 2.0 (adjust the content of 2-methylpropan-2-ol in the mobile phase if necessary).

Inject reference solution (a).The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and reference solution (d) and record the chromatograms. In the chromatogram obtained with the



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test solution the area of any peak corresponding to methacycline or 6- epidoxycycline is not greater than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (2 per cent, with reference to doxycycline hydrochloride), the area of any peak appearing between the solvent peak and the peak corresponding to methacycline and the area of any peak appearing on the tail of the main peak is not greater than 25 per cent of that of the peak corresponding to 6-epidoxycycline in the chromatogram obtained with reference solution (d) (0.5 per cent, with reference to doxycycline hydrochloride).

**Loss on drying** (2.4.19). Not more than 8.5 per cent, determined on 0.5 g of the contents of the capsules by drying in an oven at 105° for 2 hours.

**Other tests**. Comply with the tests stated under Capsules.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve the mixed contents of 20 capsules containing about 17.5 mg of anhydrous doxycycline in sufficient *0.01 M hydrochloric acid* to produce 25.0 ml and dilute 4.0 ml of this solution to 25.0 ml with the same solvent.

*Reference solution*. A 0.0128 per cent w/v solution of *doxycycline hydrochloride RS* in *0.01 M hydrochloric acid*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with styrene-divinylbenzene co-polymer (8 to 10 µm),
- column temperature. 60°,
- mobile phase: a solution prepared by adding 60 g of *2-methyl-2-propanol* to a volumetric flask with the aid of 200 ml of *water*, adding 400 ml of *phosphate buffer pH 8.0*, 50 ml of a 1 per cent w/v solution of *tetrabutyl ammonium hydrogen sulphate* previously adjusted to pH 8.0 with *2 M sodium hydroxide* and 10 ml of a 4 per cent w/v solution of *disodium edetate* previously adjusted to pH 8.0 with *2 M sodium hydroxide* and diluting to 1000 ml with *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation of the area of the peak due to doxycycline is not more than1.0 per cent.

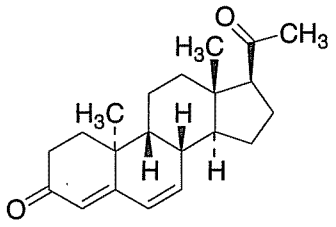
Inject alternately the test solution and the reference solution.

Calculate the content of $C_{22}H_{24}N_2O_8$ in the capsules.

**Storage**. Store protected from light and moisture at a temperature not exceeding 30°.

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

# Dydrogesterone




$C_{21}H_{28}O_2$ Mol. Wt. 312.5

Dydrogesterone is 9β,10α-pregna-4,6-diene-3,20-dione.

Dydrogesterone contains not less than 97.0 per cent and not more than 103.0 per cent of $C_{21}H_{28}O_2$, calculated on the dried basis.

**Category**. Progestogen.

**Dose**. 10 mg twice daily.

**Description**. A white or almost white, crystalline powder; odourless or almost odourless.

## Identification

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

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

## Tests

**Specific optical rotation** (2.4.22). –446° to –464°, determined in a 1.0 per cent w/v solution in *dioxan*.

**Light absorption** (2.4.7). When examined in the range 230 nm to 360 nm, a 0.0015 per cent w/v solution in *methanol* shows an absorption maximum only at about 286 nm. The ratio of the absorbance at about 240 nm to that at about 286 nm is not more than 0.12.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 20 mg of the substance under examination in the mobile phase and dilute to 100.0 ml with the mobile phase.

*Reference solution (a)*. Dissolve 20 mg of *dydrogesterone RS* in the mobile phase and dilute to 100 ml with the mobile phase.

*Reference solution (b)*. Dilute 1 ml of the test solution to 500 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),



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- column. temperature. 40°,
- mobile phase: a mixture of 53 volumes of *water*, 26 volumes of *ethanol (95 per cent)* and 21 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 280 nm and 385 nm,
- injection volume. 10 µl.

The relative retention time with respect to dydrogesterone (retention time, about 10.5 minutes): impurity A (6-dehydroprogesterone), about 1.16, impurity B (17α-dydrogesterone) about 1.32 and impurity C (Δ 8-14 triene dydrogesterone), about 0.97.

The response factors relative to that of dydrogestrone: impurity A (6-dehydroprogesterone) 1.0, impurity B (17α–dydrogesterone), 1.0 and impurity C (Δ 8-14 triene dydrogesterone), 0.899.

The test is not valid unless the column efficiency is not less than 10000 theoretical plates in the chromatogram obtained with the test solution.

For impurity A and impurity B, spectrophotometer set at 280 nm.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution the area of any peak corresponding to impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent) and the area of any peak corresponding to impurity B is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent).

For impurity C, spectrophotometer set at 385 nm.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution the area of any peak corresponding to impurity C, using the response factor, is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). The area of any other individual impurity is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The sum of the areas of all the impurities is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent, determined on 2.0 g.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

**Assay**. Determine by liquid chromatography (2.4.14) as described under Related substances.

Inject alternately the test solution and reference solution (a).

Calculate the content of $C_{21}H_{28}O_2$.

**Storage**. Store protected from light and moisture.

## Dydrogesterone Tablets

Dydrogesterone Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of dydrogesterone, $C_{21}H_{28}O_2$.

**Usual strength**. 10 mg.

### Identification

Extract a quantity of the powdered tablets containing 60 mg of Dydrogesterone with 20 ml of *methanol*, filter and evaporate the filtrate to dryness. The residue complies with the following tests.

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

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

### Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Determine the average weight of 20 tablets. Do not powder the tablets. Accurately weigh 20 tablets. Add 25 ml of *water* and mix with aid of ultrasound until complete disintegration has occurred. Add 200 ml of *acetonitrile* and mix with aid of ultrasound for 15 minutes, dilute to 500.0 ml with *water* and mix. Centrifuge a part of this solution in a closed tube for about 5 minutes. Dilute a suitable volume of the supernatant liquid with the mobile phase to obtain a solution containing about 0.1 mg of dydrogesterone per ml and mix. Filter a part of this solution, discarding the first few ml of the filtrate.

*Reference solution (a)*. Weigh accurately about 10 mg of *dydrogesterone RS*, add 40 ml of *acetonitrile* and mix with aid of ultrasound and dilute to 100.0 with *water*,

*Reference solution (b)*. Dilute 1 ml of the test solution to 200.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- column temperature. 40°,
- mobile phase: a mixture of 600 volumes of *water* and 425 volumes of *acetonitrile*,



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- flow rate. 1 ml per minute,
- spectrophotometer set at 310 nm,
- injection volume. 20 µl.

The relative retention time with respect to dydrogesterone (retention time, about 10.5 minutes); impurity A (6-dehydroprogesterone), about 1.16 and impurity B (17α–dydrogesterone), about 1.32.

The response factors relative to dydrogestrone are 0.74 for impurity A and 1.0 for impurity B.

The test is not valid unless the column efficiency is not less than 10000 theoretical plates in the chromatogram obtained with test solution.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution the area of any peaks corresponding to 6-dehydroprogesterone and 17α-dydrogesterone are not more than the area of the principal peak obtained with resference solution (b) (0.5 per cent each) and the sum of all impurities found is not more than twice the area of the principal peak obtained with reference solution (b) (1.0 per cent).

**Uniformity of content**. Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14) as given under Related substances using the following test solution.

*Test solution.* To one tablet add 5 ml of *water* and mix with the aid of ultrasound. Add 40 ml of *acetonitrile* and mix with the aid of ultrasound for 15 minutes. Add about 50 ml of *water* and swirl for 15 minutes. Dilute with sufficient *water to* produce 100.0 ml.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14) as described under Related substances.

Inject alternately the test solution and reference solution (a).

Calculate the content of $C_{21}H_{28}O_2$ in the tablets.

**Storage**. Store protected from light.



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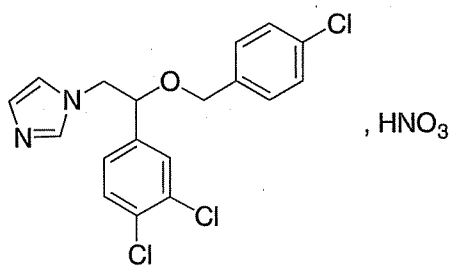


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# Econazole Nitrate




$C_{18}H_{15}Cl_3N_2O,HNO_3$ Mol. Wt. 444.7

Econazole Nitrate is (*RS*)-1-[2-[(4-chlorophenyl)methoxy]-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole nitrate.

Econazole Nitrate contains not less than 98.5 per cent and not more than 101.5 per cent of $C_{18}H_{15}Cl_3N_2O,HNO_3$, calculated on the dried basis.

**Category**. Antifungal.

**Dose**. As pessary, 150 mg.

**Description**. A white or almost white, crystalline powder.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *econazole nitrate RS* or with the reference spectrum of econazole nitrate.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.04 per cent w/v solution in a mixture of 1 volume of *0.1 M hydrochloric acid* and 9 volumes of *2-propanol* shows absorption maxima at about 265 nm, 271 nm and 280 nm; the ratio of the absorbance at the maximum at about 271 nm to that at the maximum at about 280 nm is 1.55 to 1.70.

C. In the test for Related substances examine the chromatograms obtained in ultraviolet light at 254 nm before spraying. The principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

D. Gives reaction A of nitrates (2.3.1).

## Tests

**Appearance of solution**. A 1.0 per cent w/v solution in *methanol* is clear (2.4.1), and not more intensely coloured than reference solution YS7 (2.4.1).

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 60 volumes of *dioxan*, 40 volumes of *toluene* and 1 volume of *strong ammonia solution*.

*Solvent mixture*. A mixture of 9 volumes of *methanol* and 1 volume of *strong ammonia solution*.

*Test solution (a)*. Dissolve 0.5 g of the substance under examination in 10 ml of the solvent mixture.

*Test solution (b)*. Dilute 5 ml of test solution (a) to 50 ml with the solvent mixture.

*Reference solution (a)*. Dilute 5 ml of test solution (b) to 200 ml with the solvent mixture.

*Reference solution (b)*. A 0.5 per cent w/v solution of *econazole nitrate RS* in the solvent mixture.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in a current of air for 15 minutes and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a). Spray the plate with *modified potassium iodobismuthate solution* and examine in daylight. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a). The test is not valid unless a spot is clearly visible in the chromatogram obtained with reference solution (a).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 2 hours.

**Assay**. Weigh accurately about 0.4 g and dissolve in 50 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04447 g of $C_{18}H_{15}Cl_3N_2O,HNO_3$.

**Storage**. Store protected from light.

# Econazole Cream

Econazole Nitrate Cream

Econazole Cream contains Econazole Nitrate in a suitable basis.

Econazole Cream contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of econazole nitrate, $C_{18}H_{15}Cl_3N_2O,HNO_3$.

**Usual strength**. 1 per cent w/w.

## Identification

A. Mix a quantity of the cream containing 40 mg of Econazole Nitrate with 20 ml of a mixture of 1 volume of *1 M sulphuric*



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*acid* and 4 volumes of *methanol* and shake with two quantities, each of 50 ml, of *carbon tetrachloride*, discarding the organic layers. Make the aqueous phase alkaline with *2 M ammonia* and extract with two quantities, each of 40 ml, of *chloroform*. Combine the chloroform extracts, shake with 5 g of *anhydrous sodium sulphate*, filter and dilute the filtrate to 100 ml with *chloroform*. Evaporate 50 ml to dryness and dissolve the residue in 50 ml of a mixture of 1 volume of *0.1 M hydrochloric acid* and 9 volumes of *2-propanol*.

When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows absorption maxima at about 265 nm, 271 nm and 280 nm. The ratio of the absorbance at about 271 nm to that at about 280 nm is 1.55 to 1.70.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to econazole in the chromatogram obtained with the reference solution (a).

### Tests

**Other tests**. Complies with the tests stated under Creams.

**Assay**. Determine by gas chromatography (2.4.13).

*Test solution*. Mix a quantity of the cream containing about 40 mg of Econazole Nitrate with 20 ml of a mixture of 1 volume of *0.5 M sulphuric acid* and 4 volumes of *methanol* and shake with two quantities, each of 50 ml, of *carbon tetrachloride*. Wash each organic layer in turn with the same 10-ml quantity of a mixture of 1 volume of *0.5 M sulphuric acid* and 4 volumes of *methanol*. Combine the aqueous phase and the washings, make alkaline with *2 M ammonia* and extract with two quantities, each of 50 ml, of *chloroform*. To the combined extracts add 10.0 ml of a 0.3 per cent w/v solution of *1,2,3,4-tetraphenylcyclopenta-1,3-diene* (internal standard) in *chloroform* and 5 g of *anhydrous sodium sulphate*, shake, filter, evaporate the filtrate to a low volume and add sufficient *chloroform* to produce 10.0 ml.

*Reference solution (a)*. Shake 40 mg of *econazole nitrate RS* with 10.0 ml of a 0.3 per cent w/v solution of the internal standard in *chloroform* and 0.2 ml of *strong ammonia solution*, add 1 g of *anhydrous sodium sulphate*, shake again and filter.

*Reference solution (b)*. Prepare in the same manner as reference solution (a) but omit the addition of the internal standard solution.

Chromatographic system
- a glass column 1.5 m x 2 mm, packed with acid-washed, silanised diatomaceous support (80 to 100 mesh) coated with 3 per cent w/w of phenyl methyl silicone fluid (50 per cent phenyl) (such as OV-17),
- temperature:
  column. 270°,
  inlet port and detector. 300°,
- flow rate. 30 ml per minute of the carrier gas.

Calculate the content of $C_{18}H_{15}Cl_3N_2O,HNO_3$ in the cream.

**Storage**. Store protected from light at a temperature not exceeding 30°. If it is packed in aluminium tubes the inner surfaces of the tubes should be coated with a suitable lacquer.

## Econazole Pessaries

Econazole Nitrate Pessaries; Econazole Vaginal Tablets

Econazole Pessaries contain Econazole Nitrate in a suitable base.

Econazole Pessaries contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of econazole nitrate, $C_{18}H_{15}Cl_3N_2O,HNO_3$.

**Usual strength**. 150 mg.

### Identification

A. Mix a quantity of the crushed pessaries containing 40 mg of Econazole Nitrate with 20 ml of a mixture of 1 volume of *1 M sulphuric acid* and 4 volumes of *methanol* and shake with two quantities, each of 50 ml, of *carbon tetrachloride*, discarding the organic layers. Make the aqueous phase alkaline with *2 M ammonia* and extract with two quantities, each of 40 ml, of *chloroform*. Combine the chloroform extracts, shake with 5 g of *anhydrous sodium sulphate*, filter and dilute the filtrate to 100 ml with *chloroform*. Evaporate 50 ml to dryness and dissolve the residue in 50 ml of a mixture of 1 volume of *0.1 M hydrochloric acid* and 9 volumes of *2-propanol*.

When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows absorption maxima at about 265 nm, 271 nm and 280 nm. The ratio of the absorbance at about 271 nm to that at about 280 nm is 1.55 to 1.70.

B. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (b).

### Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), using a precoated silica gel plate (such as Merck silica gel 60 plates).

*Mobile phase*. A mixture of 70 volumes of *chloroform*, 20 volumes of *methanol* and 10 volumes of an 85 per cent w/v solution of *formic acid*.

*Test solution*. Mix a quantity of the crushed pessaries containing 40 mg of Econazole Nitrate with 40 ml of *methanol*



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and heat under a reflux condenser for 15 minutes. Allow to cool, filter, wash the filter paper with *methanol* and evaporate the filtrate and washings to a volume of about 5 ml. Filter through a filter paper (such as Whatman No. 50 paper), wash the paper with *methanol*, evaporate the filtrate and washings to dryness and dissolve the residue in 2 ml of *methanol*.

*Reference solution (a)*. Dilute 0.5 ml of the test solution to 100 ml with *methanol*.

*Reference solution (b)*. A 2.0 per cent w/v solution of *econazole nitrate RS* in *methanol*.

Apply to the plate 20 µl of each solution. After development, dry the plate in air and expose to iodine vapour for 1 hour. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a). Ignore any spot with an $R_f$ value higher than 0.9.

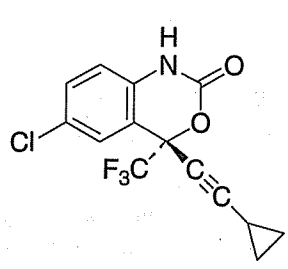
**Other tests**. Complies with the tests stated under Pessaries.

**Assay**. Dissolve 5 pessaries in 250.0 ml of *anhydrous glacial acetic acid* with the aid of gentle heat and allow to cool. Titrate 100.0 ml of the solution with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04447 g of $C_{18}H_{15}Cl_3N_2O,HNO_3$.

**Storage**. Store protected from light.

# Efavirenz




$C_{14}H_9ClF_3NO_2$ Mol. Wt. 315.7

Efavirenz is (4*S*)-6-chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2*H*-3,1-benzoxazin-2-one.

Efavirenz contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{14}H_9ClF_3NO_2$, calculated on the dried basis.

**Category**. Antiretroviral.

**Dose**. 600 mg daily in divided doses.

**Description**. A white or almost white powder.

## Identification

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

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

## Tests

**Specific optical rotation** (2.4.22). –90.0° to –100.0°, determined in a 0.3 per cent w/v solution in *methanol*.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 0.1 g of the substance under examination in 10 ml of *methanol*.

*Reference solution*. Dilute 1 ml of the test solution to 10 ml with *methanol*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with base deactivated octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a filtered and degassed mixture of 50 volumes of *acetonitrile* and 50 volumes of a 0.86 per cent w/v solution of *ammonium dihydrogen phosphate*, the pH of which is adjusted to 3.0 ± 0.05 with *phosphoric acid*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency determined from the efavirenz peak is not less than 6000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution. Determine the amount of related substances by area normalisation method. The content of any individual impurity is not more than 0.5 per cent and the sum of all the impurities is not more than 1.0 per cent.

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

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying at 105° in an oven for 3 hours.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 0.12 g of the substance under examination in 100 ml of *methanol*.

*Reference solution*. A 0.12 per cent w/v solution of *efavirenz RS* in *methanol*.



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Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a filtered and degassed mixture of 50 volumes of *acetonitrile* and 50 volumes of a 0.86 per cent w/v solution of *ammonium dihydrogen phosphate*, the pH of which is adjusted to 3.0 ± 0.05 with *phosphoric acid*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency determined from the efavirenz peak is not less than 6000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_{14}H_9ClF_3NO_2$.

**Storage**. Store protected from light.

## Efavirenz Capsules

Efavirenz Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of efavirenz, $C_{14}H_9ClF_3NO_2$.

**Usual strengths**. 50 mg; 100 mg; 200 mg.

### Identification

A. When examined in the range 220 nm to 350 nm (2.4.7), a 0.001 per cent w/v solution in *methanol* shows an absorption maximum as obtained with *efavirenz RS* of the same concentration.

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

### Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Mix well the contents of 20 capsules and shake a quantity of the mixed contents containing about 5 mg of Efavirenz with sufficient *methanol* to obtain a solution containing 1 mg per ml of Efavirenz.

*Reference solution*. A 0.1 per cent w/v solution of *efavirenz RS* in *methanol*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with base deactivated octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a filtered and degassed mixture of 50 volumes of *acetonitrile* and 50 volumes of a 0.86 per cent w/v solution of *ammonium dihydrogen phosphate*, the pH of which is adjusted to 3.0 ± 0.05 with *phosphoric acid*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 252 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency determined from the efavirenz peak is not less than 6000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution. Determine the amount of related substances by the area normalisation method. The content of any individual impurity is not more than 1.0 per cent and the sum of all impurities is not more than 2.0 per cent.

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of a 1 per cent w/v solution of *sodium lauryl sulphate*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter disc with an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate and dilute a suitable volume of the filtrate if necessary, with the same solvent. Measure the absorbance of the resulting solution at the maximum at about 252 nm (2.4.7). Calculate the content of efavirenz, $C_{14}H_9ClF_3NO_2$ in the medium from the absorbance obtained from a solution of known concentration of *efavirenz RS* in the same solvent.

D. Not less than 70 per cent of the stated amount of $C_{14}H_9ClF_3NO_2$.

**Other tests**. Comply with the tests stated under Capsules.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Mix well the contents of 20 capsules and shake a quantity of the mixed contents containing about 60 mg of Efavirenz with sufficient *methanol* to obtain a solution containing 6 mg of Efavirenz per ml. Disperse the mixture with the aid of ultrasound for 20 minutes, filter through a membrane filter disc with an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate, and dilute 10.0 ml of the filtrate to 50.0 ml with *methanol*.

*Reference solution*. A 0.12 per cent w/v solution of *efavirenz RS* in *methanol*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with base deactivated octadecylsilyl silica gel (5 µm),
- mobile phase: a filtered and degassed mixture of 50 volumes of *acetonitrile* and 50 volumes of a 0.86 per



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cent w/v solution of *ammonium dihydrogen phosphate*, the pH of which is adjusted to 3.0 ± 0.05 with *phosphoric acid*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 252 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency determined from the efavirenz peak is not less than 6000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_{14}H_9ClF_3NO_2$.

## Efavirenz Tablets

Efavirenz Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of efavirenz, $C_{14}H_9ClF_3NO_2$.

**Usual strength**. 600 mg.

### Identification

A. When examined in the range 220 nm to 350 nm (2.4.7), a 0.001 per cent w/v solution in *methanol* shows an absorption maximum as obtained with *efavirenz RS* of the same concentration.

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

### Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Shake a quantity of the powdered tablets with a suitable quantity of *methanol* to obtain a mixture containing 0.1 per cent w/v of Efavirenz and filter through a membrane filter disc with an average pore diameter not exceeding 1.0 µm, rejecting the first few ml of the filtrate.

*Reference solution*. A 0.1 per cent w/v solution of *efavirenz RS* in *methanol*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with base deactivated octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a filtered and degassed mixture of 50 volumes of *acetonitrile* and 50 volumes of a 0.86 per cent w/v solution of *ammonium dihydrogen phosphate*, the pH of which is adjusted to 3.0 ± 0.05 with *phosphoric acid*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 252 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency determined from the efavirenz peak is not less than 6000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution. Determine the amount of related substances by the area normalisation method. The content of any individual impurity is not more than 1.0 per cent and the sum of all impurities is not more than 2.0 per cent.

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 1000 ml of a 2 per cent w/v solution of *sodium lauryl sulphate*,
Speed and time. 50 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter disc with an average pore diameter not greater than 1.0 µm, reject the first few ml of the filtrate and dilute a suitable volume of the filtrate if necessary, with the same solvent. Measure the absorbance of the resulting solution at the maximum at about 252 nm (2.4.7). Calculate the content of efavirenz, $C_{14}H_9ClF_3NO_2$ in the medium from the absorbance obtained from a solution of known concentration of *efavirenz RS* in the same solvent.

D. Not less than 70 per cent of the stated amount of $C_{14}H_9ClF_3NO_2$.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh and powder 20 tablets. Weigh accurately a quantity of the powdered tablets containing about 100 mg of Efavirenz and shake with sufficient *methanol* to obtain a mixture containing 6 mg of Efavirenz per ml. Disperse the mixture with the aid of ultrasound for 20 minutes, filter through a membrane filter disc with an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate, and dilute 10.0 ml of the filtrate to 50.0 ml with *methanol*.

*Reference solution*. A 0.12 per cent w/v solution of *efavirenz RS* in *methanol*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with base deactivated octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a filtered and degassed mixture of 50 volumes of *acetonitrile* and 50 volumes of a 0.86 per cent w/v solution of *ammonium dihydrogen phosphate*, the pH of which is adjusted to 3.0 ± 0.05 with *phosphoric acid*,



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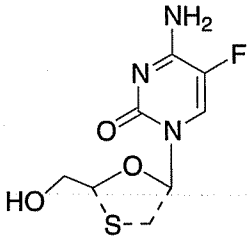
– flow rate. 1.5 ml per minute,
– spectrophotometer set at 252 nm,
– injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency determined from the efavirenz peak is not less than 6000 theoretical plates and the tailing factor is not more than 2.0 and the relative standard deviation is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_{14}H_9ClF_3NO_2$.

# Emtricitabine




$C_8H_{10}FN_3O_3S$ Mol. Wt. 247.3

Emtricitabine is 4-amino-5-fluoro-1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-2-(1*H*)-pyrimidone.

Emtricitabine contain not less than 98.0 per cent and not more than 102.0 per cent of $C_8H_{10}FN_3O_3S$, calculated on the dried basis.

**Category**. Antiretroviral.

**Description**. A white to off-white, crystalline powder.

## Identification

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

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

## Tests

**Specific optical rotation** (2.4.22). - 125.0° to - 150.0°, determined in a 0.5 per cent w/v solution in *methanol*.

**Enantiomeric purity**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve about 25 mg of the substance under examination in 50 ml of the mobile phase.

*Reference solution*. Dissolve 25 mg of *racemic emtricitabine RS* in 25 ml of the mobile phase.

Chromatographic system
– a stainless steel column 25 cm x 4.6 mm, packed with a chiral stationary phase (5 µm) (such as Chirobiotic V),
– mobile phase: a mixture of 1000 volumes of *methanol*, 2 volumes of *diethyl amine* and 1volume of *glacial acetic acid*,
– flow rate. 0.5 ml per minute,
– spectrophotometer set at 277 nm,
– injection volume. 10 µl.

Inject the reference solution. The elution order is, the 5-fluoro-1-(2*R*,5*S*)-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine isomer followed by the other isomer. The resolution between the two isomers should not be less than 2.0.

Inject the test solution and measure the areas of the two isomers.

Calculate the content of the 5-fluoro-1-(2*R*,5*S*)-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine isomer by area normalization, not more than 1.0 per cent.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve about 25 mg of the substance under examination in 25 ml of the mobile phase.

*Reference solution (a)*. A 0.1 per cent w/v solution of *emtricitabine RS* in the mobile phase.

*Reference solution (b)*. Dilute 1 ml of reference solution (a) to 100 ml with the mobile phase.

Chromatographic system
– a stainless steel column 25 cm x 4.6 mm, packed with pentaflurophenyl bonded to silica (5 µm) (such as F-5 Supelco discovery)
– mobile phase: a mixture of 99 volumes of *0.025 M ammonium acetate solution* with the pH adjusted to 5.0 with *glacial acetic acid*, and 1 volume of *methanol*,
– flow rate. 1.5 ml per minute,
– spectrophotometer set at 277 nm,
– injection volume. 20 µl.

Inject reference solution (a). The test is not valid unless the tailing factor is not more than 2.0, the column efficiency in not less than 2000 theoretical plates.

Inject the test solution and the reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak due to the reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak due to the reference solution (b) (2.0 per cent).



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**Heavy metals** (2.3.13). 1 g complies with the limit test for heavy metals, Method B (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1 g by drying in an oven at 105°.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve about 25 mg of the substance under examination in 25.0 ml of the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

*Reference solution*. A 0.1 per cent w/v solution of *emtricitabine RS* in the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with pentaflurophenyl bonded to silica (5 µm) (such as F-5 Supelco discovery),
- mobile phase: a mixture of 95 volumes of 0.025 *M ammonium acetate solution* adjusted the pH to 5.0 and 5 volumes of *methanol,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 277 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, the column efficiency in not less than 2000 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_8H_{10}FN_3O_3S$.

**Storage**. Store protected from light and moisture.

# Emtricitabine Capsules

Emtricitabine Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of emtricitabine, $C_8H_{10}FN_3O_3S$.

**Usual strength**. 200 mg.

## Identification

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

B. Disperse the quantity of contents of the capsules containing 1 mg of Emtricitabine in 100 ml with *methanol* and filter. When examined in the range 200 nm to 400 nm (2.4.7), the filtrate shows absorption maxima at the same wavelengths as 0.001 per cent w/v solution of *emtricitabine RS* in *methanol.*

## Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not more than 0.5 µm, rejecting the first 2 ml of the filtrate.

Determine by liquid chromatography (2.4.14).

*Test solution*. Use the filtrate.

*Reference solution*. Dissolve 27.5 mg of *emtricitabine RS* in 15 ml of *methanol*, dilute to 25 ml with the mobile phase. Dilute 2 ml of the solution to 10 ml with the dissolution medium.

Use the chromatographic system described under Assay.

Inject the test solution and the reference solution.

D. Not less than 75 per cent of the stated amount of $C_8H_{10}FN_3O_3S$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh a quantity of the contents of the capsules containing 100 mg of Emtricitabine, disperse in 100 ml of the mobile phase and filter.

*Reference solution (a)*. A 0.1 per cent w/v solution of *emtricitabine RS* in the mobile phase.

*Reference solution (b)*. Dilute 1 ml of reference solution (a) to 100 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with pentaflurophenyl bonded to silica (5 µm) (such as F-5 Supelco discovery),
- mobile phase: a mixture of 99 volumes of a buffer solution prepared by dissolving 1.9 g of *ammonium acetate* in 1000 ml of *water* and adjusting the pH to 5.0 with *glacial acetic acid*, and 1 volume of *methanol,*
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 277 nm,
- injection volume. 10 µl.

Inject reference solution (a). The test is not valid unless the tailing factor is not more than 2.0 and the column efficiency in not less than 2000 theoretical plates.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any



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secondary peak is not more than the area of the peak due to the reference solution (b) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than 3 times the area of the peak due to the reference solution (b) (3.0 per cent).

**Other tests**. Comply with the tests stated under Capsules.

**Water** (2.3.43). Not more than 5.0 per cent, determined on 0.5 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 100 mg of Emtricitabine, disperse in 100.0 ml of *methanol* and filter. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

*Reference solution*. A 0.1 percent w/v solution of *emtricitabine RS* in *methanol*. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Intersil ODS 3V),
- mobile phase: a mixture of 80 volumes of a buffer solution prepared by dissolving 1.9 g of *ammonium acetate* in 1000 ml of *water* and adjusting the pH to 3.8 with *glacial acetic acid*, and 20 volumes of *methanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 277 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, the column efficiency in not less than 2000 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_8H_{10}FN_3O_3S$ in the capsules.

**Storage**. Store protected from moisture.

# Emulsifying Wax

## Anionic Emulsifying Wax

Emulsifying Wax is a waxy solid containing 90 parts of Cetostearyl Alcohol, 10 parts of Sodium Lauryl Sulphate or sodium salts of similar sulphated higher primary aliphatic alcohols, and 4 parts of Purified Water.

**Category**. Pharmaceutical aid (emulsifying agent).

**Description**. An almost white or pale yellow, waxy solid or flakes; odour, faint and characteristic. It becomes soft on warming.

### Identification

The residue obtained in the test for Unsaponifiable matter melts at about 52° (2.4.21).

### Tests

**Acidity**. Weigh accurately about 20.0 g, add a mixture of 40 ml of *ether* and 75 ml of *ethanol (95 per cent)*, previously neutralised to *phenolphthalein solution*, and warm gently until solution is effected. Titrate with *0.1 M sodium hydroxide* using *phenolphthalein solution* as indicator until a pink colour which persists for at least 15 seconds is obtained. Not more than 1.0 ml of *0.1 M sodium hydroxide* is required.

**Alkalinity**. 25 ml of a 20 per cent w/v dispersion in warm *ethanol (95 per cent)*, previously neutralised to *phenolphthalein solution* and cooled, exhibits no colour on the addition of 0.5 ml of *phenolphthalein solution*.

**Saponification value** (2.3.37). Not more than 2.0, determined on 20.0 g.

**Unsaponifiable matter** (2.3.39). Not less than 86.0 per cent, calculated on the anhydrous basis, determined on 5 g and omitting the titration of the residue.

**Iodine value** (2.3.28). Not more than 3.0, determined by the iodine monochloride method.

**Alcohols**. To 3.5 g of the residue obtained in the test for Unsaponifiable matter add 12 g of *stearic anhydride* and 10 ml of *xylene* and heat gently under a reflux condenser for 30 minutes. Cool, add a mixture of 40 ml of *pyridine* and 4 ml of *water*, reflux for a further 30 minutes and titrate the hot solution with *1 M sodium hydroxide* using *phenolphthalein solution* as indicator. Repeat the operation omitting the residue. The difference between the titrations is not less than 12.8 ml and not more than 14.2 ml.

**Sodium alkyl sulphates**. Not less than 8.7 per cent, calculated as $C_{12}H_{25}O_4SNa$, on the anhydrous basis, determined by the following method. Weigh accurately about 0.25 g, dissolve as completely as possible in 15 ml of *chloroform*, add 30 ml of *water*, 10 ml of *1 M sulphuric acid* and 1 ml of *dimethyl yellow-oracet blue B solution* and titrate with *0.004 M benzethonium chloride*, shaking vigorously and allowing the layers to separate after each addition, until the chloroform layer acquires a permanent clear green colour.

1 ml of *0.004 M benzethonium chloride* is equivalent to 0.001154 g of $C_{12}H_{25}O_4SNa$.

**Water** (2.3.43). Not more than 4.0 per cent, determined on 0.6 g.

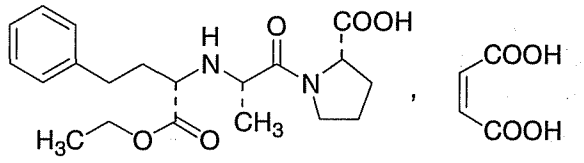


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## Enalapril Maleate



$C_{20}H_{28}N_2O_5,C_4H_4O_4$ Mol. Wt. 492.5

Enalapril Maleate is (2*S*)-1-[(2*S*)-2-[[(1*S*)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]propanoyl]pyrrolidin-2-carboxylic acid hydrogen maleate.

Enalapril Maleate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{20}H_{28}N_2O_5,C_4H_4O_4$, calculated on the dried basis.

**Category**. Antihypertensive.

**Dose**. Initial, 2.5 to 5 mg daily; maintenance dose, 10 to 20 mg daily; maximum 40 mg daily.

**Description**. An off-white, crystalline powder.

### Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *enalapril maleate RS* or with the reference spectrum of enalapril maleate.

B. Melts at about 144° (2.4.21).

### Tests

**Specific optical rotation** (2.4.22). –41.0° to –43.5°, determined in a 1.0 per cent w/v solution in *methanol.*

**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa for 2 hours.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh accurately about 30 mg of the substance under examination and dissolve in 100.0 ml of the mobile phase.

*Reference solution.* A freshly prepared 0.03 per cent w/v solution of *enalapril maleate RS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with a rigid spherical styrene divinylbenzene copolymer (5 to 10 µm),
- mobile phase: a mixture of 4 volumes of *mixed phosphate buffer pH 6.8* and 1 volume of *acetonitrile*,
- column temperature 70°,
- flow rate. 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 300 theoretical plates and the relative standard deviation for the area of the peak due to enalapril maleate is not more than 1.0 per cent.

Inject the test solution and reference solution.

Calculate the content of $C_{20}H_{28}N_2O_5,C_4H_4O_4$.

**Storage**. Store protected from light.

## Enalapril Maleate Tablets

Enalapril Maleate Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of enalapril maleate, $C_{20}H_{28}N_2O_5,C_4H_4O_4$.

**Usual strengths**. 2.5 mg; 5 mg; 10 mg; 20 mg.

### Identification

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

### Tests

**Uniformity of content** (*For tablets containing 10 mg or less).* Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14).

*Test solution.* Finely crush one tablet, transfer to a 50-ml volumetric flask, add about 30 ml of *mixed phosphate buffer pH 2.0,* disperse with the aid of ultrasound for 15 minutes, shake for another 30 minutes, dilute to volume with the buffer solution, mix and filter. Dilute a portion of the filtrate with the buffer solution to obtain a solution containing 0.01 per cent w/v of Enalapril Maleate.

*Reference solution.* A 0.01 per cent w/v solution of *enalapril maleate RS* in the same buffer solution.

Chromatographic system
- a stainless steel column 20 cm x 4.6 mm, packed with octylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a filtered and degassed mixture of 75 volumes of *phosphate buffer pH 2.0* and 25 volumes of *acetonitrile*,
- column. temperature 50°,
- flow rate. 2 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume. 50 µl.



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Inject alternately the test solution and the reference solution.

Calculate the content of $C_{20}H_{28}N_2O_5,C_4H_4O_4$ in the tablet.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

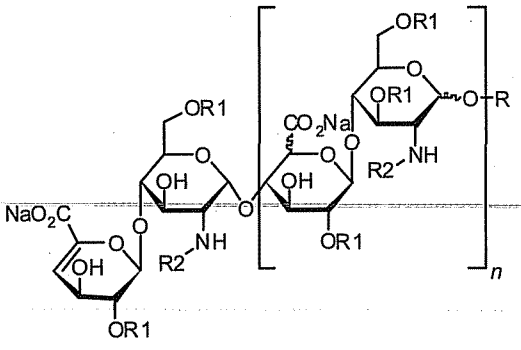
*Test solution*. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 50 mg of Enalapril Maleate, add 150 ml of *phosphate buffer pH 2.0*, disperse with the aid of ultrasound for 15 minutes, shake for another 30 minutes and dilute with the buffer to 250.0 ml, mix and filter.

*Reference solution*. A 0.02 per cent w/v solution of *enalapril maleate RS* in *phosphate buffer pH 2.0*.

Follow the chromatographic procedure described under Uniformity of content.

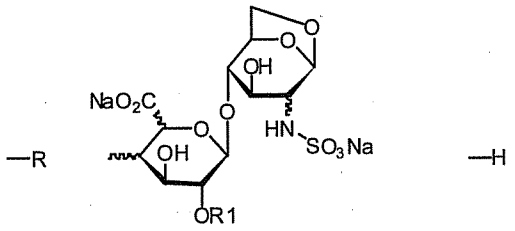
Calculate the content of $C_{20}H_{28}N_2O_5,C_4H_4O_4$ in the tablets.

## Enoxaparin Sodium




Structure at the 'reducing end'

| | 1,6-anhydro | non 1,6-anhydro |
|---|---|---|
| *n* | 0 to 20 | 1 to 21 |




R1 = H or $SO_3Na$ R2 = $SO_3Na$ or CO-$CH_3$

Enoxaparin Sodium is the sodium salt of a low-molecular-mass heparin that is obtained by alkaline depolymerisation of the benzyl ester derivative of heparin from porcine intestinal mucosa. Heparin Sodium used for the manufacture of Enoxaparin complexes with the tests under Heparin Sodium IP. Enoxaparin consists of a complex set of oligosaccharides that have not yet been completely characterised. Based on current knowledge, the majority of the components have a 4-enopyranose uronate structure at the non- reducing end of their chain. 15 per cent to 25 per cent of the components have a 1, 6- anhydro structure at the reducing end of their chain.

The mass-average relative molecular mass ranges is not less than 3800 and not more than 5000, with a characteristic value of about 4500.

The degree of sulphation is about 2 per disaccharide unit.

The potency is not less than 90 IU and not more than 125 IU of anti-factor Xa activity per milligram, calculated with reference to the dried substance. The anti-factor IIa activity is not less than 20.0 IU and not more than 35.0 IU per milligram, calculated with reference to the dried substance. The ratio of anti-factor Xa activity to anti-factor IIa activity is between 3.3 and 5.3.

The mass-average relative molecular mass ranges is not less than 3800 and not more than 5000. The mass percentage of chains lower than 2000 ranges is not less than 12.0 per cent and not more than 20.0 per cent. The mass percentage of chains is not less than 2000 and not more 8000 ranges is not less than 68.0 per cent and not more than 82.0 per cent.

**Category**. Anticogulant; antithrombotic.

### Identification

A. Determined by size-exclusion chromatography (2.4.16).

*Test solution*. Dissolve about 20 mg of the substance under examination in 2 ml of the mobile phase.

*Reference solution*. A 1.0 per cent w/v solution of *heparin low-molecular-mass for calibration RS* in the mobile phase.

Chromatographic system

- a stainless steel column 30 cm x 7.5 mm, packed porous silica beads (5 µm) with a fractionation range for proteins of approximately 15000 to 100000,
- mobile phase. a 2.84 per cent w/v solution of *anhydrous sodium sulphate*, adjusted to pH 5.0 with 10 per cent *sulphuric acid*,
- flow rate. 0.5 ml per minute,
- refractive index detector,
- injection volume. 25 µl.

Inject the reference solution. The test is not valid unless the theoretical plates are not less than 10000.

For detection, use a differential refractive index (RI) detector connected in series to a ultraviolet spectrophotometer (UV) set at 234 nm such that the UV monitor is connected to the column outlet, and the RI detector to the UV-monitor outlet.

The normalisation factor used to calculate the relative molecular mass from the RI/UV ratio is obtained as follows:



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calculate the total area under the $UV_{234}$ ($SUV_{234}$) and the RI (SRI) curves by numerical integration over the range of interest (i.e. excluding salt and solvent peaks at the end of the chromatogram). Calculate the ratio r using the following expression:

$$\frac{\Sigma RI}{\Sigma UV_{234}}$$

Calculate the factor *f* using the following expression:

$$\frac{M_{na}}{r}$$

$M_{na}$ is assigned number-average relative molecular mass of the *Heparin low-molecular-mass for calibration RS* found in the leaflet supplied with the CRS.

Provided the $UV_{234}$ and the RI responses are aligned, the relative molecular mass *M* at any point is calculated using the following expression:

$$\int \frac{RI}{UV_{234}}$$

The resulting table of retention times and relative molecular masses may be used to derive a calibration for the chromatographic system by fitting a suitable mathematical relationship to the data. A polynomial of the 3rd degree is recommended. *It must be stressed that the extrapolation of this fitted calibration curve to higher molecular masses is not valid.*

Inject 25 µl of the test solution and record the chromatogram for a period of time, ensuring complete elution of sample and solvent peaks.

The mass-average relative molecular mass is defined by the following expression:

$$\frac{\Sigma(RI_i M_i}{\Sigma RI_i}$$

$RI_i$ is mass of substance eluting in the fraction *i*;

$M_i$ is relative molecular mass corresponding to fraction *i*.

B. The ratio of anti-factor Xa activity to anti-factor IIa activity, determined as described under Assay, is between 3.3 and 5.3.

## Tests

**Appearance of solution.** A 10.0 per cent w/v solution in *water* is clear (2.4.1) and not more intensely coloured than reference solution BYS6 (2.4.1).

**pH** (2.4.24). 6.2 to 7.7, determined in a 10 per cent w/v solution in *carbon dioxide-free water.*

**Light absorbance.** A 0.05 per cent w/v solution in *0.01 M hydrochloric acid*, determined at 231 nm (2.4.7), shows specific absorbance from 14.0 to 20.0.

**Heavy metals** (2.3.13). 0.5 g complies with the limit test for heavy metals, Method B (30ppm).

**Sodium.** 11.3 per cent to 13.5 per cent, complies the test for sodium, Method I (2.3.1).

**Nirogen** (2.3.30). 1.8 to 2.5 per cent, calculated on dried basis.

**Loss on drying** (2.4.19). Not more than 10.0 per cent, determined on 1.0 g by drying in an oven over phosphorous pentaoxide at 60° at a pressure not exceeding 0.7 kPa for 3 hours.

**Bacterial endotoxins** (2.2.3). Not more than 0.01 Endotoxin Unit per IU of anti-factor Xa activity of enoxaparin sodium.

**Benzyl alcohol.** Determine by liquid chromatography (2.4.14).

*Internal standard solution.* A 10.0 per cent w/v solution of *3,4-dimethylphenol* in *methanol.*

*Test solution.* Dissolve about 0.5 g of substance under examination in 5.0 ml of *1 M sodium hydroxide*. Allow to stand for 1 hour. Add 1.0 ml of *glacial acetic acid* and 1.0 ml of the internal standard solution and dilute to 10.0 ml with the *water.*

*Reference solution.* A 0.025 per cent w/v solution of *benzyl alcohol* in *water.* Mix 0.5 ml of this solution with 1.0 ml of the internal standard solution and dilute to 10.0 ml with the *water.*

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase. a mixture of 5 volumes of *methanol,* 15 volumes of *acetonitrile* and 80 volumes of *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 256 nm,
- injection volume. 20 µl.

Inject the reference solution. In the chromatogram obtained with the reference solution, calculate the ratio of the height of the peak due to benzyl alcohol to the height of the peak due to the internal standard. In the chromatogram obtained with the test solution, calculate the ratio of the height of the peak due to benzyl alcohol to the height of the peak due to the internal standard.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of the peak due to benzyl alcohol is not more than the area of principal peak in the chromatogram obtained with the reference solution (0.1 per cent).

**Anti-factor $II_a$ activity.** Not less than 20.0 and not more than 35.0 anti-Factor $II_a$ IU per mg.

*Acetic acid solution, Polyethylene glycol 6000 buffer pH 7.4, Buffer pH 7.4, Buffer pH 8.4,* and *Human antithrombin III*



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*solution.* Proceed as directed under *Assay (anti-factor $X_a$ activity),* except that the concentration of the *Human antithrombin III solution* is 0.5 Antithrombin III Unit per ml.

*Thrombin human solution.* Reconstitute thrombin human in *water*, and dilute in Polyethylene glycol 6000 buffer pH 7.4 to obtain a solution having a concentration of 5 Thrombin Units per ml.

*Chromogenic substrate solution.* Prepare a solution of a suitable chromogenic substrate for an amidolytic test for thrombin in *water* to obtain a concentration of about 3 mM. Immediately before use, dilute with buffer pH 8.4 to 0.5 mM.

*Reference solutions.* Dilute *Enoxaparin Sodium Solution for Bioassays RS* with buffer pH 7.4 to obtain four dilutions having concentrations in the range between 0.015 and 0.075 IU of anti-factor $II_a$ activity per ml.

*Test solutions.* Proceed as directed under Reference solutions to obtain concentrations of Enoxaparin Sodium similar to those obtained for the Reference solutions.

Proceed as directed under Assay (anti-factor $X_a$ activity), except to use *Thrombin human solution* instead of *Factor $X_a$ solution* and to use the *Human antithrombin III solution* as described above.

For each series, calculate the regression of the absorbance against log concentrations of the test solutions and of the reference solutions, and calculate the potency of the enoxaparin sodium in IU of anti-factor $II_a$ activity per mg using statistical methods for parallel-line assays. The four independent dilution estimates are then combined to obtain the final weighted mean. Then calculate the confidence limits. Express the anti-factor $II_a$ activity of Enoxaparin Sodium per mg, calculated on the dried basis.

**Assay (anti-factor $X_a$ activity).** Not less than 90 and not more than 125 Anti-Factor $X_a$ IU per mg.

*Acetic acid solution.* Transfer 42 ml of *glacial acetic acid* to a 100-ml volumetric flask, dilute with *water* to volume, and mix.

*Polyethylene glycol 6000 buffer pH 7.4.* Dissolve 6.08 g of *tris(hydroxymethyl)aminomethane* and 8.77 g of *sodium chloride* in 500 ml of *water.* Add 1.0 g of *polyethylene glycol 6000*, adjust with *hydrochloric acid* to a pH of 7.4, and dilute with *water* to 1000 ml.

*Buffer pH 7.4.* Dissolve 6.08 g of *tris(hydroxymethyl) aminomethane* and 8.77 g of *sodium chloride* in 500 ml of *water.* Adjust with *hydrochloric acid* to a pH of 7.4, and dilute to 1000 ml with *water.*

*Buffer pH 8.4.* Dissolve 3.03 g of *tris(hydroxymethyl) aminomethane*, 5.12 g of *sodium chloride* and 1.40 g of *edetate sodium* in 250 ml of *water.* Adjust with *hydrochloric acid* to a pH of 8.4, and dilute to 500 ml with *water.*

*Human antithrombin III solution.* Reconstitute a vial of antithrombin III in *water* to obtain a solution containing 5 Antithrombin III Units per ml. Dilute this solution with *Polyethylene glycol 6000* buffer pH 7.4 to obtain a solution having a concentration of 1.0 Antithrombin III Unit per ml.

*Factor $X_a$ solution.* Reconstitute an accurately weighed quantity of bovine factor $X_a$ in *Polyethylene glycol 6000* buffer pH 7.4 to obtain a solution that gives an increase in absorbance value at 405 nm of not more than 0.20 absorbance units per minute when assayed as described below but using as an appropriate volume (*V*, in µl) of Buffer pH 7.4 instead of *V* µl of the enoxaparin solution.

*Chromogenic substrate solution.* Prepare a solution of a suitable chromogenic substrate for amidolytic test for factor $X_a$ in water to obtain a concentration of about 3 mM. Dilute with buffer pH 8.4 to obtain a solution having a concentration of 0.5 mM.

*Reference solutions.* Dilute *Enoxaparin Sodium Solution for Bioassays RS* with Buffer pH 7.4 to obtain four dilutions in the concentration range between 0.025 and 0.2 USP Anti-Factor $X_a$ IU per ml.

*Test solutions.* Proceed as directed for reference solutions to obtain concentrations of Enoxaparin Sodium similar to those obtained for the reference solutions.

Label 18 suitable tubes: B1 and B2 for blanks; T1, T2, T3, and T4 each in duplicate for the dilutions of the test solutions; and S1, S2, S3, and S4 each in duplicate for the dilutions of the reference solutions. [NOTE—Treat the tubes in the order B1, S1, S2, S3, S4, T1, T2, T3, T4, T1, T2, T3, T4, S1, S2, S3, S4, B2.] To each tube add the same volume, *V*, (20 to 50 µl) of *Human antithrombin III solution* and an equal volume, *V*, of either the blank, buffer pH 7.4, or an appropriate dilution of the test solutions and reference solutions. Mix, but do not allow bubbles to form. Incubate at 37° for 1.0 minute. Add to each tube volume 2*V* (40 to100 µl) of *Factor* $X_a$ solution, and incubate for 1.0 minute. Add 5*V* (100 to 250 µl) volume of *chromogenic substrate solution.* Stop the reaction after 4.0 minutes with 5*V* (100 to 250 µl) volume of *acetic acid solution.* Measure the absorbance of each solution at 405 nm against blank B1.

For each series, calculate the regression of the absorbance against log concentrations of the test solutions and reference solutions, and calculate the potency of the enoxaparin sodium in IU of anti-factor $X_a$ activity per mL using statistical methods for parallel-line assays. The four independent log relative potency estimates are then combined to obtain the final geometric mean. Its confidence limits are calculated. Express the anti-factor $X_a$ activity of Enoxaparin Sodium per mg, calculated on the dried basis.



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**Anti-factor $X_a$ to anti-factor $II_a$ ratio.** The ratio of the numerical value of the anti-factor $X_a$ activity in Anti-Factor $X_a$ IU per mg to the numerical value of the anti-factor $II_a$ activity in Anti-Factor $II_a$ IU per mg, as determined by the Assay (anti-factor $X_a$ activity) and the Anti-factor $II_a$ activity, respectively, is not less than 3.3 and not more than 5.3.

**Storage.** Store at a temperature not exceeding 30°.

**Labelling.** The label states (a) the number of International Units of anti-factor Xa activity per miligram; (b) the number of International Units of anti-factor IIa activity per miligram; (c) the mass-average molecular mass and the percentage of molecules within defined molecular mass ranges; (d) where applicable, that the contents are the sodium salt.

# Enoxaparin Injection

Enoxaparin Sodium Injection

Enoxaparin Injection is a sterile solution of Enoxaparin Sodium in Water for Injections.

The potency is not less than 90.0 per cent and not more than 110.0 per cent stated in terms of International Anti-factor $X_a$ Units (IU). It may contain, in multiple-dose containers, a suitable antimicrobial preservative, such as benzyl alcohol.

**Usual strengths.** 20 mg (2000 Anti-factor Xa units); 40 mg (4000 Anti-factor Xa units); 60 mg (6000 Anti-factor Xa units); 80 mg (8000 Anti-factor Xa units).

## Identification

A. Add 2 ml of *water* to the total content of a single-dose container or to 0.4 ml from a multiple-dose container, and 1 ml of 2 per cent w/v solution of *protamine sulphate* in a glass test tube, and mix. A creamy white precipitate is formed.

B. Transfer the total content of a single-dose container, or 0.4 ml from a multiple-dose container, dilute to 100 ml with *0.01 M hydrochloric acid*. A 0.05 per cent w/v of reference solution shows absorption maxima at 231 nm (2.4.7).

C. Complies with the test for sodium (2.3.1).

## Tests

**pH** (2.4.24). 5.5 to 7.5.

**Benzyl alcohol** *(if present).* 1.35 per cent to 1.65 per cent.

*Test solution.* Dilute 5.0 ml of the Injection to 50 ml with the mobile phase.

*Reference solution.* Dissolve about 75 mg of *benzyl alcohol RS* in 50 ml of the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 80 volumes of *water*, 15 volumes of *acetonitrile* and 5 volumes of *methanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 256 nm,
- injection volume. 20 µl.

Inject the reference solution and the test solution.

Calculate the percentage of benzyl alcohol.

**Free sulphate.** Not more than 0.12 per cent.

Determine by liquid chromatography (2.4.14).

*Test solution.* Transfer about 200 mg of a 100 mg per ml Injection, accurately weighed, to a suitable previously tared sulfate-free vial. Add mobile phase to obtain a total mass of about 20 g.

*Sulphate stock solution.* A 0.1 per cent w/v solution of *sodium sulphate* in the mobile phase. Dilute 5 ml of this solution to 25 ml with the mobile phase.

*Reference solution.* Prepare solutions of 0.1 µg per ml, 0.5 µg per ml, 1 µg per ml, 2 µg per ml, 4 µg per ml, and 5 µg per ml by appropriate dilution of the sulphate stock solution in the mobile phase.

*System suitability solution.* Prepare a solution containing 3 µg per ml of sulphate anion and 5 µg per ml of oxalate anion.

Chromatographic system
- a stainless steel column 25 cm x 4 mm, packed with anion-exchange resin, consisting of ethylvinylbenzene cross linked with 55 per cent divinylbenzene with latex coating of microbeads bonded with alkanol quaternary ammonium ions ( 6 per cent),
- mobile phase: a *3.0 mM sodium carbonate solution*,
- flow rate. 2.0 ml per minute,
- spectrophotometer set at 256 nm,
- injection volume. 25 µl.

Inject system suitability solution. The test is not valid unless the resolution between the peak due to sulphate and oxalate is not less than 1.

Inject the test solution and the reference solution.

**Other tests.** Complies with the tests stated under Parenteral Peparations (Injections).

**Baterial Endotoxin test** (2.2.3). Not more than 0.01 Endotoxin Unit per unit of anti-factor $X_a$ activity in Anti-factor $X_a$ IU.

**Sterlity** (2.2.11). Complies with the test for sterility.

**Anti-factor $II_a$ activity.** Not less than 20.0 and not more than 35.0 anti-Factor $II_a$ IU per mg.

*Acetic acid solution, Polyethylene glycol 6000 buffer pH 7.4, Buffer pH 7.4, Buffer pH 8.4,* and *Human antithrombin III solution.* Proceed as directed under *Assay (anti-factor $X_a$ activity),* except that the concentration of the *Human*



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*antithrombin III solution* is 0.5 Antithrombin III Unit per ml.

*Thrombin human solution.* Reconstitute thrombin human in *water*, and dilute in Polyethylene glycol 6000 buffer pH 7.4 to obtain a solution having a concentration of 5 Thrombin Units per ml.

*Chromogenic substrate solution.* Prepare a solution of a suitable chromogenic substrate for an amidolytic test for thrombin in *water* to obtain a concentration of about 3 mM. Immediately before use, dilute with buffer pH 8.4 to 0.5 mM.

*Reference solutions.* Dilute *Enoxaparin Sodium Solution for Bioassays RS* with buffer pH 7.4 to obtain four dilutions having concentrations in the range between 0.015 and 0.075 IU of anti-factor $II_a$ activity per ml.

*Test solutions.* Proceed as directed under Reference solutions to obtain concentrations of Enoxaparin Sodium similar to those obtained for the Reference solutions.

Proceed as directed under Assay (anti-factor $X_a$ activity), except to use *Thrombin human solution* instead of *Factor $X_a$ solution* and to use the *Human antithrombin III solution* as described above.

For each series, calculate the regression of the absorbance against log concentrations of the test solutions and of the reference solutions, and calculate the potency of the enoxaparin sodium in IU of anti-factor $II_a$ activity per ml using statistical methods for parallel-line assays. The four independent dilution estimates are then combined to obtain the final weighted mean. Then calculate the confidence limits. Express the anti-factor $II_a$ activity of Enoxaparin Sodium per ml.

**Assay (anti-factor $X_a$ activity).** Not less than 9000 (equivalent to 90 mg) and not more than 11000 (equivalent to 110 mg) anti-factor $X_a$ units per ml.

*Acetic acid solution.* Transfer 42 ml of *glacial acetic acid* to a 100-ml volumetric flask, dilute with *water* to volume, and mix.

*Polyethylene glycol 6000 buffer pH 7.4.* Dissolve 6.08 g of *tris(hydroxymethyl)aminomethane* and 8.77 g of *sodium chloride* in 500 ml of *water.* Add 1.0 g of *polyethylene glycol 6000*, adjust with *hydrochloric acid* to a pH of 7.4, and dilute with *water* to 1000 ml.

*Buffer pH 7.4.* Dissolve 6.08 g of *tris(hydroxymethyl) aminomethane* and 8.77 g of *sodium chloride* in 500 ml of *water*. Adjust with *hydrochloric acid* to a pH of 7.4, and dilute to 1000 ml with *water*.

*Buffer pH 8.4.* Dissolve 3.03 g of *tris(hydroxymethyl) aminomethane*, 5.12 g of *sodium chloride* and 1.40 g of *edetate sodium* in 250 ml of *water*. Adjust with *hydrochloric acid* to a pH of 8.4, and dilute to 500 ml with *water*.

*Human antithrombin III solution.* Reconstitute a vial of antithrombin III in *water* to obtain a solution containing 5 Antithrombin III Units per ml. Dilute this solution with *Polyethylene glycol 6000* buffer pH 7.4 to obtain a solution having a concentration of 1.0 Antithrombin III Unit per ml.

*Factor $X_a$ solution.* Reconstitute an accurately weighed quantity of bovine factor $X_a$ in *Polyethylene glycol 6000* buffer pH 7.4 to obtain a solution that gives an increase in absorbance value at 405 nm of not more than 0.20 absorbance units per minute when assayed as described below but using as an appropriate volume (*V*, in µl) of Buffer pH 7.4 instead of *V* µl of the enoxaparin solution.

*Chromogenic substrate solution.* Prepare a solution of a suitable chromogenic substrate for amidolytic test for factor $X_a$ in water to obtain a concentration of about 3 mM. Dilute with buffer pH 8.4 to obtain a solution having a concentration of 0.5 mM.

*Reference solutions.* Dilute *Enoxaparin Sodium Solution for Bioassays RS* with Buffer pH 7.4 to obtain four dilutions in the concentration range between 0.025 and 0.2 USP Anti-Factor $X_a$ IU per ml.

*Test solutions.* Proceed as directed for reference solutions to obtain concentrations of Enoxaparin Sodium similar to those obtained for the reference solutions.

Label 18 suitable tubes: B1 and B2 for blanks; T1, T2, T3, and T4 each in duplicate for the dilutions of the test solutions; and S1, S2, S3, and S4 each in duplicate for the dilutions of the reference solutions. [NOTE—Treat the tubes in the order B1, S1, S2, S3, S4, T1, T2, T3, T4, T1, T2, T3, T4, S1, S2, S3, S4, B2.] To each tube add the same volume, *V*, (20 to 50 µl) of *Human antithrombin III solution* and an equal volume, *V*, of either the blank, buffer pH 7.4, or an appropriate dilution of the test solutions and reference solutions. Mix, but do not allow bubbles to form. Incubate at 37° for 1.0 minute. Add to each tube volume 2*V* (40 to100 µl) of *Factor* $X_a$ solution, and incubate for 1.0 minute. Add 5*V* (100 to 250 µl) volume of *chromogenic substrate solution*. Stop the reaction after 4.0 minutes with 5*V* (100 to 250 µl) volume of *acetic acid solution*. Measure the absorbance of each solution at 405 nm against blank B1.

For each series, calculate the regression of the absorbance against log concentrations of the test solutions and reference solutions, and calculate the potency of the enoxaparin sodium in IU of anti-factor $X_a$ activity per mL using statistical methods for parallel-line assays. The four independent log relative potency estimates are then combined to obtain the final geometric mean. Its confidence limits are calculated. Express the anti-factor $X_a$ activity of Enoxaparin Sodium per ml.

**Anti-factor $X_a$ to anti-factor $II_a$ ratio.** The ratio of the numerical value of the anti-factor $X_a$ activity in Anti-Factor $X_a$ IU per mg to the numerical value of the anti-factor $II_a$ activity in Anti-



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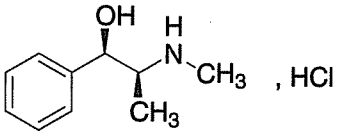
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Factor $II_a$ IU per mg, as determined by the Assay (anti-factor $X_a$ activity) and the Anti-factor $II_a$ activity, respectively, is not less than 3.3 and not more than 5.3.

**Labelling.** It indicates the amount (mg) of Enoxaparin Sodium in the total volume of contents. The label states also that the Enoxaparin Sodium starting material is porcine derived.

## Ephedrine Hydrochloride




$C_{10}H_{15}NO,HCl$ Mol. Wt. 201.7

Ephedrine Hydrochloride is (1*R*,2*S*)-2-methylamino-1-phenylpropan-1-ol hydrochloride.

Ephedrine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{10}H_{15}NO,HCl$ calculated on the dried basis.

**Category.** Sympathomimetic; bronchodilator.

**Dose.** 15 to 60 mg.

**Description.** Colourless crystals or a white, crystalline powder; odourless. It is affected by light.

### Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ephedrine hydrochloride RS* or with the reference spectrum of ephedrine hydrochloride.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

C. Dissolve 10 mg in 1 ml of *water* and add 0.1 ml of *cupric sulphate solution* and 1 ml of *sodium hydroxide solution*; a violet colour is produced. Add 2 ml of *ether* and shake; the ether layer is purple and the aqueous layer is blue.

D. A 5 per cent w/v solution gives reaction A of chlorides (2.3.1).

### Tests

**Appearance of solution.** A 10.0 per cent w/v solution (Solution A) is clear (2.4.1), and colourless (2.4.1).

**Acidity or alkalinity.** To 10 ml of Solution A add 0.1 ml of *methyl red solution* and 0.2 ml of *0.01 M sodium hydroxide*; the solution is yellow. Add 0.4 ml of *0.01 M hydrochloric acid*; the solution is red.

**Specific optical rotation** (2.4.22). –33.5° to –35.5°, determined in a 5.0 per cent w/v solution.

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

*Mobile phase.* A mixture of 80 volumes of *2-propanol*, 15 volumes of *strong ammonia solution* and 5 volumes of *chloroform.*

*Test solution (a).* Dissolve 0.2 g of the substance under examination in 10 ml of *methanol.*

*Test solution (b).* Dilute 5 ml of test solution (a) to 50 ml with *methanol.*

*Reference solution (a).* Dilute 5 ml of test solution (b) to 100 ml with *methanol.*

*Reference solution (b).* A 0.2 per cent w/v solution of *ephedrine hydrochloride RS* in *methanol.*

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with *ninhydrin solution* and heat at 110° for 5 minutes. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a). Ignore any spot of lighter colour than the background.

**Sulphates** (2.3.17). 15 ml of solution A complies with the limit test for sulphates (100 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay.** Weigh accurately about 0.17 g, dissolve in 10 ml of *mercuric acetate solution*, warming gently, add 50 ml of *acetone* and mix. Titrate with *0.1 M perchloric acid*, using 1 ml of a saturated solution of *methyl orange* in *acetone* as indicator, until a red colour is obtained. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02017 g of $C_{10}H_{15}NO,HCl$.

**Storage.** Store protected from light.

## Ephedrine Oral Solution

Ephedrine Hydrochloride Oral Solution; Ephedrine Hydrochloride Elixir; Ephedrine Elixir

Ephedrine Oral Solution is a solution containing 0.3 per cent w/v of Ephedrine Hydrochloride in a suitable flavoured vehicle containing a sufficient volume of Ethanol (95 per cent) or of an appropriate dilute ethanol to give a final concentration of not more than 3 per cent v/v of ethanol.



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Ephedrine Oral Solution contains not less than 0.27 per cent and not more than 0.33 per cent w/v of ephedrine hydrochloride, $C_{10}H_{15}NO,HCl$.

## Identification

A. To 30 ml add 2 ml of *2 M hydrochloric acid*, extract with two quantities, each of 20 ml, of *ether* and discard the ether. Add sufficient *dilute ammonia solution* to the aqueous phase to make it alkaline, extract with two quantities, each of 30 ml, of *ether*, wash the combined ether extracts with three quantities, each of 15 ml, of *water*, dry over *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ephedrine hydrochloride RS* treated in the same manner or with the reference spectrum of ephedrine.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

## Tests

**Ethanol content**. Not more than 3 per cent v/v, determined by gas chromatography (2.4.13).

*Test solution.* Use the preparation under examination.

*Reference solution (a).* Add sufficient of *1-propanol* (internal standard) to the test solution to produce a solution containing 5.0 per cent v/v of *1-propanol.*

*Reference solution (b).* A 5.0 per cent v/v solution of the internal standard in *water.*

Chromatographic system

- a column 1.5 m x 4 mm, packed with porous polymer beads (100 to 200 mesh) (such as Porapak Q and Chromosorb 101),
- temperature:
  column.150°,
  inlet port and detector. 170°,
- flow rate. 30 ml per minute of the carrier gas.

Calculate the percentage content of ethanol from the areas of the peaks due to ethanol in the chromatograms obtained with reference solutions (a) and (b).

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

*Mobile phase.* A mixture of 80 volumes of *2-propanol,* 15 volumes of *strong ammonia solution* and 5 volumes of *chloroform.*

*Test solution (a).* Add sufficient *5 M ammonia* to 50 ml of the oral solution to make it alkaline, extract with two quantities, each of 100 ml, of *ether,* wash the combined extracts with 10 ml of *water*, dry with *anhydrous sodium sulphate,* filter and evaporate the filtrate to dryness. Dissolve the oily residue in sufficient *methanol* to produce 5 ml.

*Test solution (b).* Dilute 1 ml of test solution (a) to 10 ml with *methanol.*

*Reference solution (a).* Dilute 1 ml of test solution (a) to 200 ml with *methanol.*

*Reference solution (b).* A 0.3 per cent w/v solution of *ephedrine hydrochloride RS* in *methanol.*

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with *ninhydrin solution* and heat at 110° for 5 minutes. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a). Ignore any spot of lighter colour than the background.

**Other tests**. Complies with the tests stated under Oral Liquids.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Dilute an accurately weighed quantity of the oral solution containing about 60 mg of Ephedrine Hydrochloride to 50 ml with *methanol.*

*Reference solution.* A 0.12 per cent w/v solution of *ephedrine hydrochloride RS* in *methanol.*

Chromatographic system

- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: *0.005 M dioctyl sodium sulphosuccinate* in a mixture of 65 volumes of *methanol*, 35 volumes of *water* and 1 volume of *glacial acetic acid*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 263 nm,
- injection volume. 20 µl.

Determine the weight per ml of the oral solution (2.4.29), and calculate the content of $C_{10}H_{15}NO,HCl$, weight in volume.

**Storage**. Store protected from light.

# Ephedrine Tablets

Ephedrine Hydrochloride Tablets

Ephedrine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of ephedrine hydrochloride, $C_{10}H_{15}NO,HCl$.

**Usual strengths**. 15 mg; 30 mg; 60 mg.

## Identification

A. Shake a quantity of the powdered tablets containing about 0.1 g of Ephedrine Hydrochloride with 20 ml of *0.1 M hydrochloric acid*, filter, wash the filtrate with two quantities, each of 20 ml, of *chloroform* and discard the chloroform. Make



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the aqueous layer alkaline with *5 M ammonia* and extract with two quantities, each of 30 ml, of a mixture of 3 volumes of *chloroform* and 1 volume of *ethanol (95 per cent)*. Dry the combined extracts over *anhydrous sodium sulphate*, filter and evaporate to a low volume at a pressure of 2 kPa. Prepare a disc using 0.3 g of *potassium bromide IR*, apply dropwise to the disc 0.1 ml of the *chloroform* solution, allowing the solvent to evaporate between applications, and dry the disc at 50° for 2 minutes. The disc so obtained complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ephedrine hydrochloride RS* treated in the same manner or with the reference spectrum of ephedrine.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

C. Triturate a quantity of the powdered tablets containing about 0.4 g of Ephedrine Hydrochloride with 10 ml of *chloroform* and discard the chloroform. Repeat trituration with a further 10 ml of *chloroform* and again discard the chloroform. Shake the residue with 30 ml of warm *ethanol (95 per cent)* for 20 minutes, filter, evaporate the filtrate to dryness on a water-bath and dry the residue at 80° (residue R). Dissolve 10 mg of residue R in 1 ml of *water* and add 0.1 ml of *cupric sulphate solution* followed by 1 ml of *sodium hydroxide solution*; a violet colour is produced. Add 1 ml of *ether* and shake; the ether layer is purple and the aqueous layer is blue.

D. A 5 per cent w/v solution of residue R gives reaction A of chlorides (2.3.1).

### Tests

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 80 volumes of *2-propanol*,15 volumes of *strong ammonia solution* and 5 volumes of *chloroform*.

*Test solution (a)*. Extract a quantity of the powdered tablets containing 0.1 g of Ephedrine Hydrochloride with 5 ml of *methanol* and filter.

*Test solution (b)*. Dilute 1 ml of test solution (a) to 10 ml with *methanol*.

*Reference solution (a)*. Dilute 1 ml of test solution (a) to 200 ml with *methanol*.

*Reference solution (b)*. A 0.2 per cent w/v solution of *ephedrine hydrochloride RS* in *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with *ninhydrin solution* and heat at 110° for 5 minutes. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a). Ignore any spot of lighter colour than the background.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 50 mg of Ephedrine Hydrochloride, shake with 30 ml *methanol* for 10 minutes, add sufficient *water* to produce 50.0 ml, filter through glass fibre (Whatman GF/C is suitable) and use the filtrate.

*Reference solution*. A 0.1 per cent w/v solution of *ephedrine hydrochloride RS* in *methanol*.

Chromatographic system

- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a 0.005 M solution of *dioctyl sodium sulphosuccinate* in a mixture of 65 volumes of *methanol*, 35 volumes of *water* and 1 volume of *glacial acetic acid*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 263 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

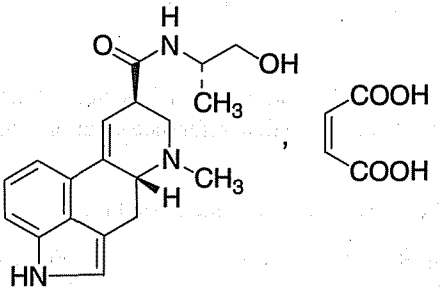
Inject the test solution and the reference solution.

Calculate the content of $C_{10}H_{15}NO,HCl$ in the tablets.

**Storage**. Store protected from light.

## Ergometrine Maleate

Ergonovine Maleate



$C_{19}H_{23}N_3O_2,C_4H_4O_4$ Mol.Wt.441.5

Ergometrine Maleate is 9,10-didehydro-*N*-[(*S*)-2-hydroxy-1-methylethyl]-6-methylergoline-8β-carboxamide hydrogen maleate.



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Ergometrine Maleate contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{19}H_{23}N_3O_2,C_4H_4O_4$, calculated on the dried basis.

**Category**. Uterine stimulant.

**Dose**. Orally, 250 µg to 1 mg by intramuscular injection, 200 to 500 µg; by intravenous injection, 100 to 500 µg.

**Description**. A white or faintly yellow, crystalline powder; odourless. It is affected by light.

### Identification

*Test A may be omitted if tests B, C, D and E are carried out. Tests B, D and E may be omitted if tests A and C are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ergometrine maleate RS* or with the reference spectrum of ergometrine maleate.

B. Dissolve 30 mg in sufficient *0.01 M hydrochloric acid* to produce 100 ml and dilute 10 ml of the solution to 100 ml with the same solvent. When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum at about 311 nm and a minimum at 265 nm to 272 nm; absorbance at about 311 nm, 0.52 to 0.58.

C. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

D. Dissolve 0.1 g, without heating and protected from light, in sufficient *carbon dioxide-free water* to produce 10 ml (solution A). To 0.1 ml of solution A add 1 ml of *glacial acetic acid*, 1 drop of *ferric chloride solution* and 1 ml of *phosphoric acid* and heat on a water-bath at 80°; a blue or violet colour is produced after about 10 minutes.

E. To 1 ml of a 0.01 per cent w/v solution, add 2 ml of *4-dimethylaminobenzaldehyde reagent*; a deep blue colour is produced after about 10 minutes.

### Tests

**Appearance of solution**. Solution A is clear (2.4.1), and not more intensely coloured than reference solution YS5 or BYS5 (2.4.1).

**pH** (2.4.24). 3.6 to 4.4, determined in solution A.

**Specific optical rotation** (2.4.22). +50.0° to +56.0°, determined in solution A.

**Related substances**. *Carry out the following operations as rapidly as possible, protected from light.*

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 75 volumes of *chloroform*, 25 volumes of *methanol* and 3 volumes of *water*.

*Prepare the following solutions freshly.*

*Solvent mixture*. A mixture of 1 volume of *strong ammonia solution* and 9 volumes of *ethanol (80 per cent)*.

*Test solution (a)*. Dissolve 0.1 g of the substance under examination in 10 ml with solvent mixture.

*Test solution (b)*. Dilute 1 ml of test solution (a) to 10 ml with the same solvent mixture.

*Reference solution (a)*. A 0.1 per cent w/v solution of *ergometrine maleate RS* in the same solvent mixture.

*Reference solution (b)*. A 0.01 per cent w/v solution of *ergometrine maleate RS* in the same solvent mixture.

*Reference solution (c)*. A 0.005 per cent w/v solution of *ergometrine maleate RS* in the same solvent mixture.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 14 cm. Dry the plate in a current of cold air and spray with *4-dimethylaminobenzaldehyde reagent*. Dry in a current of warm air for about 2 minutes. Any secondary spot in the chromatogram obtained with the test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (c).

**Loss on drying** (2.4.19). Not more than 2.0 per cent, determined on 0.2 g by drying in an oven at 80° at a pressure not exceeding 2.7 kPa for 2 hours.

**Assay**. Weigh accurately about 0.15 g and dissolve in 40 ml of *anhydrous glacial acetic acid*. Titrate with *0.05 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.05 M perchloric acid* is equivalent to 0.02207 g of $C_{19}H_{23}N_3O_2,C_4H_4O_4$.

**Storage**. Store protected from light in a refrigerator (2° to 8°).

## Ergometrine Injection

Ergometrine Maleate Injection; Ergonovine Injection, Ergonovine Maleate Injection

Ergometrine Injection is a sterile solution of Ergometrine Maleate in Water for Injections containing suitable stabilising agents.

Ergometrine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ergometrine maleate, $C_{19}H_{23}N_3O_2,C_4H_4O_4$.

**Usual strength**. 500 µg per ml.



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**Description**. A clear, colourless or faintly yellow solution.

### Identification

A. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution (a).

B. Exhibits a blue fluorescence.

C. To a volume containing 0.1 mg of Ergometrine Maleate, add 0.5 ml of *water* and 2 ml of *4-dimethylaminobenzaldehyde reagent*; a deep blue colour is produced after 10 minutes.

### Tests

**pH** (2.4.24). 2.7 to 3.5.

**Related substances**. *Carry out the following procedure in subdued light and protect from light any solutions not used immediately.*

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G* slurried with *0.1 M sodium hydroxide*.

*Mobile phase*. A mixture of 90 volumes of *chloroform* and 10 volumes of *methanol*.

*Test solution*. Evaporate a volume of the injection containing 1 mg of Ergometrine Maleate to dryness at 20° at a pressure not exceeding 2 kPa and dissolve the residue in 0.25 ml of methanol.

*Reference solution (a)*. A 0.4 per cent w/v solution of *ergometrine maleate RS* in *methanol*.

*Reference solution (b)*. Dilute 5 ml of reference solution (a) to 50 ml with *methanol*.

*Reference solution (c)*. Dilute 5 ml of reference solution (b) to 10 ml with *methanol*.

*Reference solution (d)*. Dilute 5 ml of reference solution (c) to 10 ml with *methanol*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 365 nm. Assess the intensities of any secondary spots in the chromatogram obtained with the test solution by reference to the spots in the chromatograms obtained with reference solutions (b), (c) and (d). The total of the intensities so assessed does not exceed 10 per cent of the intensity of the principal spot.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. *Carry out the following procedure protected from light.*

Dilute a suitable volume, accurately measured, of the injection with sufficient *water* to produce a solution containing 0.004 per cent w/v of Ergometrine Maleate. To 3.0 ml add 6.0 ml of *4-dimethylaminobenzaldehyde reagent*, mix, cool to room temperature and allow to stand for 30 minutes (solution A). At the same time prepare solution B in the same manner but using 3.0 ml of a 0.004 per cent w/v solution of *ergometrine maleate RS* and beginning at the words "add 6.0 ml.....". Measure the absorbance of solution B at the maximum at about 545 nm (2.4.7), using as the blank a solution prepared by mixing 6.0 ml of *4-dimethylaminobenzaldehyde solution* and 3.0 ml of *water*. Without delay replace solution B with solution A, using the same cell, and measure the absorbance of solution A at the same wavelength. Calculate the content of $C_{19}H_{23}N_3O_2,C_4H_4O_4$.

**Storage**. Store protected from light in single dose containers in a refrigerator (2° to 8°).

## Ergometrine Tablets

Ergometrine Maleate Tablets; Ergonovine Tablets; Ergonovine Maleate Tablets

Ergometrine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ergometrine maleate, $C_{19}H_{23}N_3O_2,C_4H_4O_4$.

**Usual strengths**. 250 µg; 500 µg.

### Identification

A. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution (a).

B. Extract a quantity of the powdered tablets containing 2 mg of Ergometrine Maleate with 20 ml of *water*, filter and wash the residue with sufficient *water* to produce 20 ml. The solution exhibits a blue fluorescence.

C. To 2 ml of the solution obtained in test B add 4 ml of *4-dimethylaminobenzaldehyde reagent*; a deep blue colour is produced after about 10 minutes.

### Tests

**Related substances**. *Carry out the following procedure in subdued light and protect from light any solutions not used immediately.*

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G* slurried with *0.1 M sodium hydroxide*.

*Mobile phase*. A mixture of 90 volumes of *chloroform* and 10 volumes of *methanol*.

*Test solution*. Triturate a quantity of the powdered tablets containing about 1 mg of Ergometrine Maleate with 0.2 ml of a



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1 per cent w/v solution of *domiphen bromide*, add 2 ml of *methanol*, centrifuge and remove the supernatant liquid. Extract the residue with two quantities, each of 1 ml, of *methanol*, evaporate the combined extracts to dryness at 20° at a pressure not exceeding 2 kPa and dissolve the residue in 0.25 ml of *methanol*, centrifuge if necessary.

*Reference solution (a)*. A 0.4 per cent w/v solution of *ergometrine maleate RS* in *methanol*.

*Reference solution (b)*. Dilute 5 ml of reference solution (a) to 50 ml with *methanol*.

*Reference solution (c)*. Dilute 5 ml of reference solution (b) to 10 ml with *methanol*.

*Reference solution (d)*. Dilute 5 ml of reference solution (c) to 10 ml with *methanol*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 365 nm. Assess the intensities of any secondary spots in the chromatogram obtained with the test solution by reference to the spots in the chromatograms obtained with reference solutions (b), (c) and (d). The total of the intensities so assessed does not exceed 10 per cent of the intensity of the principal spot.

**Uniformity of content**. Comply with the test stated under Tablets.

*Protect the solutions from light throughout the test.*

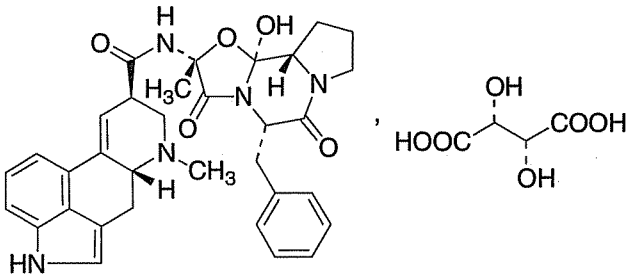
To one tablet add 10.0 ml of a 1 per cent w/v solution of *tartaric acid*, shake for 30 minutes, centrifuge and use the supernatant liquid. Dilute a suitable volume, accurately measured, with sufficient *water* to produce a solution containing 0.004 per cent w/v of Ergometrine Maleate. To 3.0 ml add 6.0 ml of *4-dimethylaminobenzaldehyde solution*, mix, cool to room temperature and allow to stand for 30 minutes (solution A). At the same time prepare solution B in the same manner but using 3.0 ml of a 0.004 per cent w/v solution of *ergometrine maleate RS* and beginning at the words "add 6.0 ml...." Measure the absorbance of solution B at the maximum at about 545 nm (2.4.7), using as the blank a solution prepared by mixing 6.0 ml of *4-dimethylaminobenzaldehyde reagent* and 3.0 ml of *water*. Without delay replace solution B with solution A, using the same cell, and measure the absorbance of solution A at the same wavelength. Calculate the content of $C_{19}H_{23}N_3O_2,C_4H_4O_4$ in the tablet.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 2 mg of Ergometrine Maleate, shake with 50.0 ml of a 1 per cent w/v solution of *tartaric acid* for 30 minutes, centrifuge and use the supernatant liquid. Carry out the procedure described under Uniformity of content beginning at the words "To 3.0 ml add 6 ml.....".

**Storage**. Store protected from light.

# Ergotamine Tartrate



$(C_{33}H_{35}N_5O_5)_2,C_4H_6O_6$ Mol. Wt. 1313.4

Ergotamine Tartrate is (5'*S*)-12'-hydroxy-2'-methyl-3',6',18-trioxo-5-benzylergotaman tartrate.

Ergotamine Tartrate contains not less than 98.0 per cent and not more than 101.0 per cent of $(C_{33}H_{35}N_5O_5)_2,C_4H_6O_6$, calculated on the dried basis.

**Category**. Sympatholytic ; antimigraine drug.

**Dose**. 1 mg to 2 mg ; by subcutaneous or intramuscular injection, 250 µg to 500 µg.

**Description**. Colourless crystals, or a white or almost white, crystalline powder; odourless; slightly hygroscopic. It may contain two molecular equivalents of methanol of crystallisation.

## Identification

*Test A may be omitted if tests B, C, D and E are carried out. Tests B, C and D may be omitted if tests A and E are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Before triturating with *potassium bromide IR* during preparation of the disc, triturate first with 0.2 ml of *methanol*. Compare the spectrum with that obtained with *ergotamine tartrate RS* or with the reference spectrum of ergotamine tartrate.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.005 per cent solution in *0.01 M hydrochloric acid* shows an absorption maximum at 311 nm to 321 nm and a minimum at 265 nm to 275 nm; absorbance at the maximum, 0.59 to 0.64, calculated on the dried basis.

C. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a) when examined for not more than 1 minute in ultraviolet light



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at 365 nm or when examined in daylight after spraying with *ethanolic 4-dimethylaminobenzaldehyde solution.*

D. Dissolve 1 mg in a mixture of 5 ml of *glacial acetic acid* and 5 ml of *ethyl acetate*. To 1 ml of the solution add 1 ml of *sulphuric acid,* with continuous shaking and cooling; a blue colour with a red tinge develops. Add 0.1 ml of *ferric chloride test solution* previously diluted with an equal volume of *water;* the red tinge becomes less apparent and the blue colour more pronounced.

E. Dissolve 1 mg in 5 ml of a 1 per cent w/v solution of *tartaric acid*. To 1 ml of this solution add slowly 3 ml of *4-dimethylaminobenzaldehyde solution* and mix; a deep blue colour is produced.

### Tests

*Carry out the following tests as rapidly as possible, protected from light.*

**Appearance of solution**. Mix 50 mg with 25 mg of *tartaric acid* and dissolve in 20 ml of *water*. The solution is clear (2.4.1), and not more intensely coloured than reference solution YS6 (2.4.1).

**pH** (2.4.24). 4.0 to 6.0, determined in a 0.25 per cent w/v suspension.

**Specific optical rotation** (2.4.22). The specific optical rotation of the ergotamine base, checked for purity by the method given below, is –154° to –165°, determined by the following method. Dissolve 0.4 g in 40 ml of a 1 per cent w/v solution of *tartaric acid*, cautiously add 0.5 g of *sodium bicarbonate* in small portions and mix well. Wash 100 ml of *chloroform* by shaking with 5 quantities, each of 50 ml, of *water* and extract the solution of the substance under examination with 4 quantities, each of 10 ml, of the washed chloroform. Filter the combined chloroform extracts through a small filter moistened with the washed chloroform, dilute to 50 ml with the same solvent and measure the optical rotation.

To 25 ml of the chloroform solution add 50 ml of *anhydrous glacial acetic acid*. Titrate with *0.05 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.05 M perchloric acid* is equivalent to 0.02908 g of ergotamine base, $C_{33}H_{35}N_5O_5$.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 70 volumes of *ether*, 15 volumes of *dimethylformamide*, 10 volumes of *chloroform* and 5 volumes of *ethanol*.

*Prepare the following solutions immediately before use in the order stated.*

*Solvent mixture*. A mixture of 9 volumes of *chloroform* and 1 volume of *methanol*.

*Test solution (a)*. Dissolve 0.1 g of the substance under examination in 10 ml with solvent mixture.

*Test solution (b)*. Dilute 5 ml of test solution (a) to 50 ml with the same solvent mixture.

*Reference solution (a)*. A 0.1 per cent w/v solution of *ergotamine tartrate RS* in the same solvent mixture.

Reference solution (b). *A 0.015 per cent w/v solution of* ergotamine tartrate RS *in the same solvent mixture.*

Reference solution (c). *A 0.005 per cent w/v solution of* ergotamine tartrate RS *in the same solvent mixture.*

Apply to the plate 5 µl of each solution. Immediately after application expose the plate to an atmosphere saturated with ammonia vapour for exactly 20 seconds, dry the plate at the line of application in a current of cold air and immediately start developing the chromatogram, allowing the mobile phase to rise 17 cm. Dry the plate in a current of dry air for 2 minutes and examine in ultraviolet light at 365 nm for not more than 1 minute. Spray abundantly with *ethanolic 4-dimethylaminobenzaldehyde solution* and dry in a current of warm air for about 2 minutes. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (c).

**Loss on drying** (2.4.19). Not more than 6.0 per cent, determined on 0.1 g by drying over *phosphorus pentoxide* at a pressure of 1.5 to 2.5 kPa for 6 hours.

**Assay**. Weigh accurately about 0.2 g and dissolve in 40 ml of *anhydrous glacial acetic acid* Titrate with *0.05 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.05 M perchloric acid* is equivalent to 0.03284 g of $(C_{33}H_{35}N_5O_5)_2,C_4H_6O_6$.

**Storage**. Store protected from light in sealed glass containers, in a refrigerator (2° to 8°).

## Ergotamine Injection

### Ergotamine Tartrate Injection

Ergotamine Injection is a sterile solution of Ergotamine Tartrate in Water for Injection containing Ethanol (95 per cent), Glycerin and sufficient Tartaric Acid to adjust the pH of the solution to 3.3.

Ergotamine Injection contains a quantity of total alkaloids, calculated as $(C_{33}H_{35}N_5O_5)_2,C_4H_6O_6$, equivalent to not less than



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90.0 per cent and not more than 110.0 per cent of the stated amount of ergotamine tartrate, of which 50 to 70 per cent is present as ergotamine tartrate.

**Usual strength**. 500 µg per ml.

**Description**. A clear, colourless or almost colourless solution.

### Identification

A. In the test for Ergot alkaloids and related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that due to ergotamine in the chromatogram obtained with the reference solution.

B. To a volume containing 0.2 mg of Ergotamine Tartrate add 1 ml of *4-dimethylaminobenzaldehyde solution*; a deep blue colour is produced.

C. Mix a volume containing about 2 mg of Ergotamine Tartrate with 2 ml of *dilute sulphuric acid*, dissolve a few mg of *magnesium powder* in the solution and add 25 mg of *resorcinol*. Shake to dissolve, carefully add 2 ml of *sulphuric acid* down the inside of the tube and warm gently; a red ring forms at the interface of the two liquid layers and spreads throughout the lower layer.

### Tests

*Carry out the following tests as rapidly as possible, protected from light.*

**pH** (2.4.24). 2.8 to 3.8.

**Ergot alkaloids and related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G* slurried with *0.1 M sodium hydroxide*.

*Mobile phase*. A mixture of 90 volumes of *chloroform* and 10 volumes of *methanol*.

*Test solution*. Add sufficient of a 10 per cent w/v solution of *sodium bicarbonate* to a volume of the injection containing about 5 mg of Ergotamine Tartrate to make it distinctly alkaline to litmus paper. Extract with five quantities, each of 10 ml, of *chloroform*, filter the extracts through a small double filter paper, wash the filter with *chloroform*, evaporate the combined filtrates and washings to dryness at 20° at a pressure of about 1.5 kPa and dissolve the residue in 1 ml of a mixture of equal volumes of *chloroform* and *methanol*.

*Reference solution*. Dissolve 5 mg of *ergotamine tartrate RS* in 10 ml of a 1 per cent w/v solution of *tartaric acid* and complete the preparation described for the test solution beginning at the words "Extract with five quantities...".

Apply without delay, to the plate 20 µl of the test solution and 14 µl, 10 µl, 7 µl and 2 µl of the reference solution. After development, dry the plate in air and examine in ultraviolet light at 365 nm. The chromatogram obtained with the test solution shows two principal spots, corresponding to ergotamine and, of higher $R_f$ value, ergotaminine; a spot between the two principal spots and a number of spots of lower $R_f$ values may also be seen. Compare the chromatogram obtained with the test solution with the chromatograms obtained with the reference solution. The spot corresponding to ergotaminine is not larger or more intense than the spot corresponding to ergotamine obtained with 7 µl of the reference solution. The spot corresponding to ergotamine is not smaller or less intense than the spot corresponding to ergotamine obtained with 10 µl of the reference solution and is not larger or more intense than the spot corresponding to ergotamine obtained with 14 µl of the reference solution, corresponding to not less than 50 per cent and not more than 70 per cent of ergotamine tartrate. Any other spots are not larger or more intense than the spot corresponding to ergotamine obtained with 2 µl of the reference solution.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. To an accurately measured volume add sufficient of a 0.25 per cent w/v solution of *tartaric acid* to produce a solution containing about 0.005 per cent w/v of Ergotamine Tartrate. Mix 3.0 ml of this solution with 6.0 ml of *4-dimethylaminobenzaldehyde solution*, cool to room temperature and allow to stand for 30 minutes (solution A). At the same time, mix 3.0 ml of a 0.003 per cent w/v solution of *ergometrine maleate RS* in a 0.25 per cent w/v solution of *tartaric acid* with 6.0 ml of *4-dimethylaminobenzaldehyde solution*, cool to room temperature and allow to stand for 30 minutes (solution B). Prepare solution C by mixing 3.0 ml of a 0.25 per cent w/v solution of *tartaric acid* with 6.0 ml of *4-dimethylaminobenzaldehyde solution*. Measure the absorbance of solution B at 545 nm (2.4.7), using solution C as the blank. Without delay replace solution B with solution A, using the same cell, and measure the absorbance of solution A at the same wavelength. Calculate the content of total alkaloids as $(C_{33}H_{35}N_5O_5)_2,C_4H_6O_6$ from the absorbances obtained.

1 mg of *ergometrine maleate RS* is equivalent to 1.488 mg of $(C_{33}H_{35}N_5O_5)_2,C_4H_6O_6$.

**Storage**. Store protected from light in single dose glass containers.

## Ergotamine Tablets

Ergotamine Tartrate Tablets

Ergotamine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ergotamine tartrate, $(C_{33}H_{35}N_5O_5)_2,C_4H_6O_6$.

**Usual strength**. 1 mg.



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## Identification

A. Triturate a quantity of the powdered tablets containing about 5 mg of Ergotamine Tartrate with 10 ml of *light petroleum (40° to 60°)*, allow to settle and discard the petroleum extract. To the residue add 10 ml of *chloroform* saturated with *strong ammonia solution*, triturate, filter and evaporate the filtrate to dryness on a water-bath. The residue so obtained complies with the following tests.

Dissolve 1 mg in a mixture of 5 ml of *glacial acetic acid* and 5 ml of *ethyl acetate*. To 1 ml of the solution add 1 ml of *sulphuric acid,* with continuous shaking and cooling; a blue colour with a red tinge develops. Add 0.1 ml of *ferric chloride test solution* previously diluted with an equal volume of *water;* the red tinge becomes less apparent and the blue colour more pronounced.

B. Dissolve 1 mg in 5 ml of a 1 per cent w/v solution of *tartaric acid*. To 1 ml of this solution add slowly 3 ml of *4-dimethylaminobenzaldehyde solution* and mix; a deep blue colour is produced.

C. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

## Tests

*Carry out the following tests as rapidly as possible, protected from light.*

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase.* A mixture of 70 volumes of *ether*, 15 volumes of *dimethylformamide*, 10 volumes of *chloroform* and 5 volumes of *ethanol*.

*Prepare the following solutions immediately before use in the order stated.*

*Test solution.* Extract a quantity of the powdered tablets containing 1 mg of Ergotamine Tartrate with 2 ml of a mixture of equal volumes of *chloroform* and *methanol* and centrifuge. Remove the supernatant liquid, extract the residue with two quantities, each of 1 ml, of the solvent mixture, evaporate the combined extracts to dryness at 20° at a pressure of 2 kPa and dissolve the residue in 0.25 ml of a mixture of equal volumes of *chloroform* and *methanol*; centrifuge if necessary.

*Reference solution (a).* A 0.4 per cent w/v solution of *ergotamine tartrate RS* in the same solvent mixture.

*Reference solution (b).* A 0.04 per cent w/v solution of *ergotamine tartrate RS* in the same solvent mixture.

*Reference solution (c).* A 0.02 per cent w/v solution of *ergotamine tartrate RS* in the same solvent mixture.

*Reference solution (d).* A 0.01 per cent w/v solution of *ergotamine tartrate RS* in the same solvent mixture.

Apply to the plate 5 µl of each solution. Immediately after application expose the plate to an atmosphere saturated with ammonia vapour for exactly 20 seconds, dry the plate at the line of application in a current of cold air and immediately start developing the chromatogram, allowing the mobile phase to rise 17 cm. Dry the plate in air and examine in ultraviolet light at 365 nm. Assess the intensity of any secondary spots in the chromatogram obtained with the test solution by reference to the spots in the chromatograms obtained with reference solutions (a), (b) and (c). The sum of the intensities so assessed in the chromatogram obtained with the test solution should not exceed 10 per cent of the intensity of the principal spot in the chromatogram obtained with the test solution. In addition, any single secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (d).

**Uniformity of content.** Comply with the test stated under Tablets.

To one tablet add sufficient quantity of a 1 per cent w/v solution of *tartaric acid* to produce a solution containing 0.05 mg of Ergotamine Tartrate per ml. Shake for 30 minutes and centrifuge. Mix 3.0 ml of this solution with 6.0 ml of *4-dimethylaminobenzaldehyde solution*, cool to room temperature and allow to stand for 30 minutes (solution A). At the same time mix 3.0 ml of a 0.003 per cent w/v solution of *ergometrine maleate RS* in a 0.25 per cent w/v solution of *tartaric acid* with 6.0 ml of *4-dimethylaminobenzaldehyde solution*, cool to room temperature and allow to stand for 30 minutes (solution B). Prepare solution C by mixing 3.0 ml of a 0.25 per cent w/v solution of *tartaric acid* with 6.0 ml of *4-dimethylaminobenzaldehyde solution*. Measure the absorbance of solution B at 545 nm (2.4.7), using solution C as the blank. Without delay replace solution B with solution A, using the same cell, and measure the absorbance of solution A at the same wavelength. Calculate the content of total alkaloids as $(C_{33}H_{35}N_5O_5)_2,C_4H_6O_6$ from the absorbances obtained.

1 mg of *ergometrine maleate RS* is equivalent to 1.488 mg of $(C_{33}H_{35}N_5O_5)_2,C_4H_6O_6$.

Calculate the content of $(C_{33}H_{35}N_5O_5)_2,C_4H_6O_6$ in the tablet.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 5 mg of Ergotamine Tartrate and dissolve in 50 ml of a 1 per cent w/v solution of *tartaric acid*, allow to stand for 30 minutes with frequent shaking and dilute to 100.0 ml with *water*. Using 3.0 ml of the clear supernatant liquid, carry out the procedure described under Uniformity of content beginning at the words "Mix 3.0 ml of this solution with 6.0 ml of *4-dimethylaminobenzaldehyde solution*....".

**Storage.** Store protected from light at a temperature not exceeding 30°.

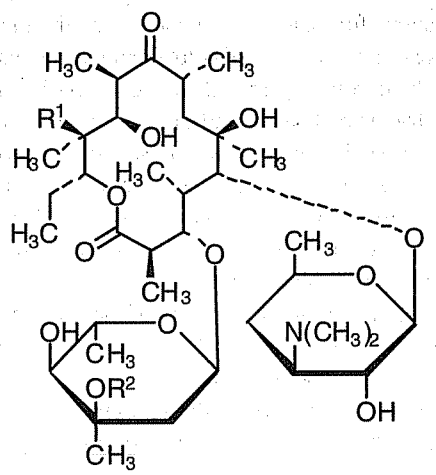


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# Erythromycin



$C_{37}H_{67}NO_{13}$ Mol. Wt. 733.9

Erythromycin is a mixture of macrolide antibiotics consisting largely of erythromycin A, (2*R*,3*S*,4*S*,5*R*,6*R*,8*R*,10*R*,11*R*,12*S*,13*R*)-5-(3-amino-3,4,6-trideoxy-*N*,*N*-dimethyl-α-D-*xylo*-hexopyranosyloxy)-3-(2,6-dideoxy-3-*C*,3-*O*- dimethyl-α-L-*ribo*-hexopyranosyloxy)-13-ethyl-6,11,12- trihydroxy-2,4,6,8,10,12-hexamethyl-9-oxotridecan-13-olide, it is produced by the growth of certain strains of *Streptomyces erythreus.*

Erythromycin has a potency not less than 920 Units per mg, calculated on the anhydrous basis.

**Category**. Antibacterial.

**Dose**. 1 to 2 g daily, in divided doses.

**Description**. Colourless or slightly yellow crystals or a white or slightly yellow powder; slightly hygroscopic.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B and D may be omitted if tests A and C are carried out.*

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

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

*Mobile phase*. The upper layer obtained by shaking together 45 volumes of *ethyl acetate*, 40 volumes of a 15 per cent w/v solution of *ammonium acetate* previously adjusted to pH 9.6 with *10 M ammonia* and 20 volumes of *2-propanol* and allowing to separate.

*Test solution*. Dissolve 0.1 g of the substance under examination in 100 ml of *methanol.*

*Reference solution (a)*. A 0.1 per cent w/v solution of *erythromycin RS* in *methanol.*

*Reference solution (b)*. A 0.2 per cent w/v solution of *spiramycin RS* in *methanol.*

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with *ethanolic anisaldehyde solution*, heat at 110° for 5 minutes and allow to cool. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a) and is different in position and colour from the spots in the chromatogram obtained with reference solution (b).

C. To about 5 mg add 5 ml of a 0.02 per cent w/v solution of *xanthydrol* in a mixture of 1 volume of *hydrochloric acid* and 99 volumes of *5 M acetic acid* and heat on a water-bath; a red colour is produced.

D. Dissolve about 10 mg in 5 ml of *7 M hydrochloric acid* and allow to stand for about 20 minutes; a yellow colour develops.

## Tests

**pH** (2.4.24). 8.0 to 10.5, determined in a 0.066 per cent w/v solution in *carbon dioxide-free water.*

**Specific optical rotation** (2.4.22). –71.0° to –78.0°, determined in a 2.0 per cent w/v solution in *ethanol.* Measure the optical rotation at least 30 minutes after preparing the solution.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silanised silica gel H.*

*Mobile phase*. A mixture of 75 volumes of *methanol* and 45 volumes of a 5 per cent w/v solution of *ammonium acetate.*

*Test solution*. Dissolve 0.2 g of the substance under examination in 100 ml of *methanol.*

*Reference solution (a)*. A 0.2 per cent w/v solution of *erythromycin RS* in *methanol.*

*Reference solution (b)*. A 0.01 per cent w/v solution of *erythromycin RS* in *methanol.*

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with *ethanolic anisaldehyde solution*, heat at 110° for 5 minutes and allow to cool. Any secondary spot with an $R_f$ value lower than that of the principal spot in the chromatogram obtained with the test solution is not more intense than the principal spot in the chromatogram obtained with reference solution (b).

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

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.



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**Water** (2.3.43). Not more than 6.5 per cent, determined on 0.2 g using a 10 per cent w/v solution of *imidazole* in *anhydrous methanol* as the solvent.

**Assay**. Determine by the microbiological assay of antibiotics, Method A (2.2.10), using a solution prepared by dissolving about 25 mg, accurately weighed, in 10 ml of *methanol* and adding sufficient *water* to produce 100.0 ml. Express the results as units per mg.

**Storage**. Store protected from light at a temperature not exceeding 30°.

## Erythromycin Tablets

Erythromycin Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of erythromycin, $C_{37}H_{67}NO_{13}$. The tablets are enteric-coated.

**Usual strength**. 250 mg.

### Identification

A. Shake a quantity of the powdered tablets containing about 0.1 g of Erythromycin with 5 ml of *chloroform*, decolorise if necessary, with *decolorising charcoal*, filter and evaporate the filtrate to dryness. The residue after drying at a pressure not exceeding 0.7 kPa complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *erythromycin RS* or with the reference spectrum of erythromycin.

B. Dissolve a quantity of the powdered tablets containing about 3 mg of Erythromycin as completely as possible in 2 ml of *acetone* and add 2 ml of *hydrochloric acid*; an orange colour is produced which changes to red and then to deep purplish red. Add 2 ml of *chloroform* and shake; the chloroform layer becomes purple.

### Tests

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by the microbiological assay of antibiotics, Method A (2.2.10) on a solution prepared in the following manner.

Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.4 g of Erythromycin and triturate with 10 ml of sterile *phosphate buffer pH 8.0* and add sufficient sterile *phosphate buffer pH 8.0* to produce 100.0 ml. Calculate the content of erythromycin in the tablets, taking each 1000 Units found to be equivalent to 1 mg of erythromycin.

**Storage**. Store protected from light at a temperature not exceeding 30°.

## Erythromycin Stearate

$C_{37}H_{67}NO_{13},C_{18}H_{36}O_2$ Mol. Wt. 1018.4

Erythromycin Stearate is a mixture of the stearate of Erythromycin with an excess of stearic acid.

Erythromycin Stearate has a potency not less than 600 Units per mg, calculated on the anhydrous basis.

**Category**. Antibacterial.

**Dose**. 1 to 2 g daily, in divided doses.

**Description**. Colourless or slightly yellow crystals or a white or slightly yellow, crystalline powder.

### Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. The upper layer of a mixture of 45 volumes of *ethyl acetate*, 40 volumes of a 15 per cent w/v solution of *ammonium acetate*, previously adjusted to pH 9.6 with *9 M ammonia*, and 20 volumes of *2-propanol*.

*Test solution*. Dissolve 0.28 g of the substance under examination in 100 ml of *methanol*.

*Reference solution (a)*. A 0.2 per cent w/v solution of *erythromycin RS* in *methanol*.

*Reference solution (b)*. A 0.1 per cent w/v solution of *stearic acid* in *methanol*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air, spray with a solution containing 0.02 per cent w/v of *2,7-dichlorofluorescein* and 0.01 per cent w/v of *rhodamine B* in *ethanol (95 per cent)*, allow the plate to stand for a few seconds in the vapour above a water-bath and examine in ultraviolet light at 365 nm. The chromatogram obtained with the test solution exhibits two spots, one of which corresponds in position to the principal spot in the chromatogram obtained with reference solution (a) and the other to the principal spot in the chromatogram obtained with reference solution (b). Spray the plate with *ethanolic anisaldehyde solution*, heat at 110° for 5 minutes and examine in daylight. The coloured spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with reference solution (a).

B. To about 5 mg add 5 ml of a 0.02 per cent w/v solution of *xanthydrol* in a mixture of 1 volume of *hydrochloric acid* and 99 volumes of *5 M acetic acid* and heat on a water-bath; a red colour is produced.

C. Dissolve about 10 mg in 5 ml of *7 M hydrochloric acid* and allow to stand for about 20 minutes; a yellow colour develops.

### Tests

**pH** (2.4.24). 7.0 to 10.5, determined in a 1.0 per cent w/v suspension.



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**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silanised silica gel H*.

*Mobile phase*. A mixture of 100 volumes of *methanol* and 60 volumes of a 15 per cent w/v solution of *ammonium acetate*.

*Test solution*. Dissolve 0.28 g of the substance under examination in 100 ml of *methanol*.

*Reference solution (a)*. A 0.2 per cent w/v solution *erythromycin RS* in *methanol*.

Reference solution (b). *A 0.01 per cent w/v solution* of *erythromycin RS* in *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with *ethanolic anisaldehyde solution*, heat at 110° for 5 minutes and allow to cool. Any spot with an $R_f$ value lower than that of the principal spot in the chromatogram obtained with the test solution is not more intense than the corresponding spot in the chromatogram obtained with reference solution (a) and any spot with an $R_f$ value higher than that of the principal spot is not more intense than the principal spot in the chromatogram obtained with reference solution (b).

**Erythromycin stearate**. Not less than 84.0 per cent of $C_{37}H_{67}NO_{13}$,$C_{18}H_{36}O_2$, calculated on the anhydrous basis and determined by the following method. Weigh accurately about 0.5 g and dissolve in 30 ml of *chloroform*. If the solution is opalescent, filter and shake the residue with three quantities, each of 25 ml, of *chloroform*. Filter, if necessary, and wash the filter with *chloroform*. Evaporate the combined filtrate and washings on a water-bath to about 30 ml, add 50 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.1018 g of $C_{37}H_{67}NO_{13}$,$C_{18}H_{36}O_2$.

**Free stearic acid**. Not more than 14.0 per cent of $C_{18}H_{36}O_2$, calculated on the anhydrous basis and determined by the following method. Weigh accurately about 0.4 g and dissolve in 50 ml of *methanol*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25). Calculate the volume of *0.1 M sodium hydroxide* required for each g of the substance and subtract the volume of *0.1 M perchloric acid* required for each g of the substance in the test for Erythromycin stearate.

1 ml of the difference is equivalent to 0.02845 g of $C_{18}H_{36}O_2$.

**Erythromycin stearate and free stearic acid**. 98.0 to 103.0 per cent, calculated by adding together the percentages of erythromycin stearate and free stearic acid determined as described above.

**Sulphated ash** (2.3.18). Not more than 0.5 per cent.

**Water** (2.3.43). Not more than 4.0 per cent, determined on 0.3 g using a 10 per cent w/v solution of *imidazole* in *anhydrous methanol* as the solvent.

**Assay**. Determine by the microbiological assay of antibiotics, Method A (2.2.10) using a solution prepared by dissolving about 50 mg accurately weighed in sufficient *methanol* to produce 100.0 ml. Express the results as units per mg.

**Storage**. Store protected from light at a temperature not exceeding 30°.

## Erythromycin Stearate Tablets

Erythromycin Stearate Tablets contain not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of erythromycin, $C_{37}H_{67}NO_{13}$.

**Usual strengths**. The equivalent of 125 mg; 250 mg of erythromycin.

### Identification

A. To a quantity of the powdered tablets containing 0.1 g of erythromycin add 10 ml of *water* and shake well. Decant the supernatant liquid and discard. Extract the residue by shaking with 10 ml of *methanol*, filter the extract and evaporate to dryness. The residue after drying at a pressure not exceeding 0.7 kPa complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *erythromycin stearate RS* or with the reference spectrum of erythromycin stearate.

B. Dissolve a quantity of the powdered tablets containing 3 mg of erythromycin as completely as possible in 2 ml of *acetone* and add 2 ml of *hydrochloric acid*; an orange colour is produced which changes to red and then to deep purplish red. Add 2 ml of *chloroform* and shake; the chloroform layer becomes purple.

C. Extract a quantity of the powdered tablets containing 50 mg of erythromycin with 10 ml of *chloroform*, filter and evaporate to dryness. Heat 0.1 g of the residue gently with 5 ml of *2 M hydrochloric acid* and 10 ml of *water* until the solution boils; oily globules rise to the surface. Cool, remove the fatty layer, heat it with 3 ml of *0.1 M sodium hydroxide* and allow to cool; the solution sets to a gel. Add 10 ml of hot *water* and shake; the solution froths. To 1 ml add a 10 per cent w/v solution of *calcium chloride;* a granular precipitate is produced which is insoluble in *hydrochloric acid.*

### Tests

**Disintegration** (2.5.1). 90 minutes.

**Other tests**. Comply with the tests stated under Tablets.



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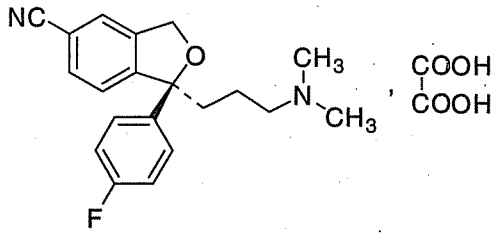
**Assay**. Determine by the microbiological assay of antibiotics, Method A (2.2.10) on a solution prepared in the following manner.

Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 25 mg of erythromycin and dissolve as completely as possible in sufficient *methanol* to produce 100.0 ml. Calculate the content of erythromycin in the tablets, taking each 1000 Units found to be equivalent to 1 mg of erythromycin.

**Storage**. Store protected from light.

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

# Escitalopram Oxalate




$C_{20}H_{21}FN_2O,C_2H_2O_4$ Mol. Wt. 414.4

Escitalopram Oxalate is (*S*)-1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydro-5-isobenzofurancarbonitrile oxalate.

Escitalopram Oxalate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{20}H_{21}FN_2O.C_2H_2O_4$, calculated on the anhydrous basis.

**Category**. Antidepressant.

**Description**. A white to slightly yellow powder.

## Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *escitalopram oxalate RS* or with the reference spectrum of escitalopram oxalate.

## Tests

**Specific optical rotation** (2.4.22). +10.0° to +13.0°, determined in 1.0 per cent w/v solution in the *methanol*.

**Enantiomeric Purity**. Not more than 2.0 per cent of R- isomer.

Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 25 mg of the substance under examination in 100.0 ml of the mobile phase.

*Reference solution*. Dissolve 25 mg of *citalopram hydrombromide RS* in 2.5 ml of *methanol* and dilute to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with OD-H (5 µm) (such as Chirelcel),
- column temperature. 30°,
- mobile phase: a mixture of 90 volumes of *n-hexane*, 10 volumes of *ethanol* and 0.4 volume of *trifluoroacetic acid,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume.20 µl.

Inject the reference solution. The test is not valid unless the resoluiton between the peaks due to S-isomer and R-isomer is not less than 1.5. The relative retention time with reference to S- isomer for R- isomer is about 1.2.

Inject the reference solution and the test solution.

Calculate the content of R- isomer.

**Related substances**. Determine by liquid chromatography (2.4.14).

*NOTE- Prepare the solutions immediately before use.*

*Test solution.* Dissolve about 50 mg of the substance under examination in 50.0 ml of the mobile phase.

*Reference solution (a).* A 0.0005 per cent w/v solution of *escitalopram oxalate RS* in the mobile phase.

*Reference solution (b).* A 0.01 per cent w/v solution of *oxalic acid* in the mobile phase.

Use chromatographic system as described under Assay.

Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0.

Inject reference solution (a), (b) and the test solution. In the chromatogram obtained with test solution the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent), the sum of the area of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore the peak due to oxalic acid.

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

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 1.0 per cent, determined on 0.1 g.



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**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 50 mg of the substance under examination in 100.0 ml of the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase. Further dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

*Reference solution*. A 0.005 per cent w/v solution of *escitalopram oxalate RS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm) (such as Waters-Xterra),
- column temperature. 40°,
- mobile phase: a mixture of 50 volumes of buffer solution prepared by dissolving 4.45 g of *disodium hydrogen phosphate dihydrate* in *0.1 per cent triethylamine,* adjust the pH to 8.5 with *orthophosphoric acid* and 50 volumes of *methanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume.20 µl.

Inject the reference solution. The test is not valid unless the theoretical plates is not less than 2000, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{21}FN_2O.C_2H_2O_4$.

**Storage** Store protected from moisture.

# Escitalopram Tablets

## Escitalopram Oxalate Tablets

Escitalopram Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of escitalopram, $C_{20}H_{21}FN_2O$.

**Usual strengths**. 5 mg; 10 mg; 20 mg.

### Identification

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

### Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *water*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

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

*Test solution.* The filtrate obtained as given above, diluted if necessary with the dissolution medium.

*Reference solution.* A solution of *escitalopram oxalate RS* containing about 0.00055 per cent w/v of escitalopram in the dissolution medium.

D. Not less than 70 per cent of the stated amount of $C_{20}H_{21}FN_2O$.

**Uniformity of content**. Comply with the test stated under tablets.

Determine by liquid chromatography (2.4.14), as described under Assay using the following solution as the test solution.

*Test solution.* Disperse 1 tablet in the mobile phase, sonicate and dilute, if necessary to obtain a solution containing 0.001 per cent w/v of escitalopram in the mobile phase.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh and powder 20 tablets. Disperse a quantity of powder containing about 25 mg of escitalopram with 30 ml of mobile phase, sonicate to dissolve and dilute to 50.0 ml with the mobile phase. Further dilute to obtain a 0.001 per cent w/v solution of escitalopram.

*Reference solution.* A solution of *escitalopram oxalate RS* containing about 0.001 per cent w/v of escitalopram in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 45 volumes of buffer solution prepared by dissolving 3.7 g of *disodium hydrogen phosphate dihydrate* in 1000 ml of *water*, adjusted to pH 7.0 with *orthophosphoric acid*, 27.5 volumes of *methanol* and 27.5 volumes of *acetonitrile*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 226 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the theoretical plates is not less than 4000, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{21}FN_2O$ in the tablets.

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

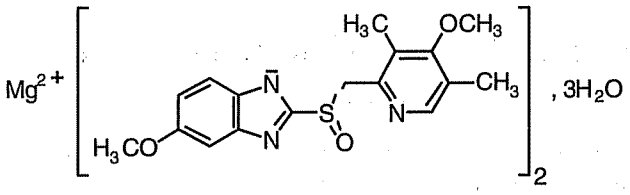


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# Esomeprazole Magnesium Trihydrate



$(C_{17}H_{18}N_3O_3S)_2,Mg.3H_2O$ Mol. Wt. 767.2

Esomeprazole Magnesium Trihydrate is 5-methoxy-2-{(*S*)-[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl}-1*H*-benzimidazole magnesium trihydrate.

Esomeprazole Magnesium Trihydrate contains not less than 98.0 per cent and not more than 102.0 per cent of $(C_{17}H_{18}N_3O_3S)_2,Mg$, calculated on the anhydrous basis.

**Description.** A white to off-white powder.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *esomeprazole magnesium RS* or with the reference spectrum esomeprazole magnesium.

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

## Tests

**Magnesium.** 3.0 per cent to 3.7 per cent, calculated on the anhydrous basis and determined in the following manner.

Weigh accurately about 1 g of the substance under examination and ignite in a silica crucible until no fumes are evolved. Cool and moisten the residue with 0.5 ml of *sulphuric acid*. Evaporate the acid until no white fumes are noticed. Heat the residue at 800° for 2 hours. Cool the residue to room temperature and dissolve in 5 ml of *dilute hydrochloric acid* with the aid of ultrasound for 5 minutes. Transfer the residue to a flask, wash with about 15 ml of *water* and transfer the washing to the flask. Repeat the washing four to five times and add the washings to the extract in the flask. Add 5 ml of a mixture of 180 g of *ammonium chloride* and 750 ml of *strong ammonia solution* and sufficient *water* to produce 1000 ml and 200 mg of *eriochrome black T mixture*. Titrate with *0.05 M disodium edetate*.

1 ml of *0.05 M disodium edetate* is equivalent to 0.0012155 g of magnesium.

**Specific optical rotation** (2.4.22). -135° to -155°, determined in a 1.0 per cent w/v solution in *methanol*.

**Related substances.** Determine by liquid chromatography (2.4.14).

*Test solution.* Disperse about 25 mg of the substance under examination and dissolve in 25.0 ml of *methanol*.

*Reference solution (a).* A 0.1 per cent w/v solution of *esomeprazole magnesium RS* in *methanol*.

*Reference solution (b).* Dilute 1.0 ml of reference solution (a) to 100.0 ml with *methanol*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 30 volumes of *acetonitrile* and 70 volumes of a buffer prepared by dissolving 1.2 g of *ammonium dihydrogen orthophosphate* in 1000 ml of *water* adding 5 ml of *triethylamine* and adjusting the pH to 7.0 with *orthophosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 302 nm,
- injection volume. 10 µl.

Inject reference solution (a). The test is not valid unless the tailing factor of esomeprazole peak is not more than 2.0 and the theoretical plates is not less than 5000.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent), and the sum of the areas of all the secondary peaks is not more than the area of the principal peak obtained in the chromatogram obtained with reference solution (b) (1.0 per cent).

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

**Water** (2.3.43). 6.0 per cent to 11.0 per cent, determined on 0.1 g.

**Assay.** Determine by liquid chromatography (2.4.14).

*Solvent mixture.* A mixture of 75 volumes of *0.01 M sodium borate* and 25 volumes of *acetonitrile*.

*Test solution.* Disperse about 20 mg of the substance under examination in 10 ml of *methanol* and dilute to 100.0 ml with the solvent mixture.

*Reference solution.* Disperse about 20 mg of the *esomeprazole magnesium RS* in 10 ml of *methanol* and dilute to 100.0 ml with the solvent mixture.

Use the chromatographic system described under the test for Related substances.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent, the tailing factor is not more than 2.0 and the theoretical plates is not less than 5000.

Inject the test solution and the reference solution.



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**Storage**. Store protected from moisture.

## Esomeprazole Tablets

Esomeprazole Magnesium Tablets

Esomeprazole Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of esomeprazole, $C_{34}H_{36}N_6O_6S_2$. The tablets are enteric coated.

### Identification

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

### Tests

*Note — Perform all the tests in subdued light using low actinic glassware.*

**Dissolution** (2.5.2). Complies with Method A or B.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.24 g of esomeprazole, add about 150 ml of *methanol* and mix with the aid of ultrasound for 15 minutes. Mix and add sufficient *methanol* to produce 250.0 ml. Mix with the aid of ultrasound for a further 5 minutes and centrifuge. Dilute 5.0 ml of the clear supernatant liquid to 25.0 ml with the mobile phase.

*Reference solution*. Weigh accurately about 0.055 g of *esomeprazole magnesium RS*, dissolve in 50 ml of *methanol*, add sufficient mobile phase to produce 250.0 ml and mix well.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of equal volumes of *methanol* and a buffer prepared by dissolving 6.8 g of *potassium dihydrogen phosphate* and about 1 g of *sodium hydroxide* in 1000 ml of *water*, and adjusting the pH to 7.0 with *orthophosphoric acid*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 300 nm,
- injection volume. 50 µl.

Inject the reference solution. Repeat the procedure at least four times. The relative standard deviation of the replicate injections is not more than 2.0 per cent, the number of theoretical plates is not less than 2000 and the tailing factor is not more than 2.0.

Calculate the content of $C_{34}H_{36}N_6O_6S_2$ in the tablets.

**Storage**. Store protected from moisture.

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

## Estradiol and Norethisterone Tablets

Estradiol and Norethisterone Acetate Tablets

Estradiol and Norethisterone Tablets contain Estradiol Hemihydrate and Norethisterone Acetate.

Estradiol and Norethisterone Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amounts of estradiol, $C_{18}H_{24}O_2$ and norethisterone acetate, $C_{22}H_{28}O_3$.

### Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 10 volumes of *acetone* and 90 volumes of *dichloromethane*.

*Test solution*. Add 0.2 ml of *water* to two tablets and shake to disperse. Add sufficient *ethanol (95 per cent)* to produce a solution containing 0.035 per cent w/v of Norethisterone Acetate, centrifuge and use the clear supernatant liquid.

*Reference solution*. A suitable concentration of *estradiol hemihydrate RS* and *norethisterone acetate RS* in *ethanol (95 per cent)*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. Spray with *ethanolic sulphuric acid (5 per cent)* and heat the plate at 105° for 15 minutes. The chromatogram obtained with test solution shows two clearly separated spots with $R_f$ values corresponding to those observed in the chromatogram obtained with the reference solution.

B. In the test for Uniformity of content, the chromatogram obtained with the test solution shows two peaks with the same retention times as the peaks due to estradiol and norethisterone acetate in the chromatogram obtained with the reference solution.

### Tests

**Dissolution** (2.5.2).

Apparatus No. 1,

Medium. 500 ml of a 0.3 per cent w/v solution of *sodium lauryl sulphate* in *water*,

Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter, discarding the first 5 ml of filtrate.

Determine by liquid chromatography (2.4.14).



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*Test solution.* Use the filtrate and, if necessary, dilute with the dissolution medium.

*Reference solution (a).* Dissolve a sufficient quantity of *estradiol hemihydrate RS* and *norethisterone acetate RS* in *methanol (80 per cent)* and dilute with dissolution medium; the concentration of the final solution should be the same as that expected for test solution.

*Reference solution (b).* A solution containing 0.0017 per cent w/v of *estradiol hemihydrate RS*, 0.00084 per cent w/v of *norethisterone acetate RS*, 0.00066 per cent w/v of *estrone RS* and 0.00034 per cent w/v of *norethisterone RS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Spherisorb ODS 2),
- mobile phase: a mixture of 45 volumes of *water* and 55 volumes of *acetonitrile*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume. 200 µl.

Inject reference solution (b). The test is not valid unless the resolution between each pair of peaks (estradiol and norethisterone, estrone and norethisterone acetate) is not less than 1.0.

Inject reference solution (a) and the test solution.

Calculate the total content of $C_{18}H_{24}O_2$ and $C_{22}H_{28}O_3$ in the medium.

D. Not less than 70 per cent of the stated amount of $C_{18}H_{24}O_2$ and $C_{22}H_{28}O_3$ in the medium.

**Estrone and norethisterone.** Determine by liquid chromatography (2.4.14).

*Test solution (a).* Weigh and powder 20 tablets. Add 20 ml of the mobile phase to a quantity of the powdered tablets containing 5 mg of estradiol, mix with the aid of ultrasound and add sufficient mobile phase to produce 25 ml. Centrifuge and use the clear supernatant liquid.

*Test solution (b).* Add 20 ml of the mobile phase to a quantity of the powdered tablets containing 2.5 mg of Norethisterone Acetate, mix with the aid of ultrasound and add sufficient mobile phase to produce 25 ml. Centrifuge and use the clear supernatant liquid.

*Reference solution (a).* A 0.0001 per cent w/v solution of *estrone RS* in the mobile phase.

*Reference solution (b).* A 0.00005 per cent w/v solution of *norethisterone RS* in the mobile phase.

*Reference solution (c).* A solution containing 0.0017 per cent w/v of *estradiol hemihydrate RS*, 0.00084 per cent w/v of *norethisterone acetate RS*, 0.00066 per cent w/v of *estrone RS* and 0.00034 per cent w/v of *norethisterone RS* in the mobile phase.

Use the chromatographic condition as described under Dissolution.

Inject 20 µl of reference solution (c). The test is not valid unless the resolution between each pair of peaks (estradiol and norethisterone, estrone and norethisterone acetate) is not less than 1.0.

Inject 20 µl of reference solution (a), (b), test solution (a) and (b). In the chromatogram obtained with test solution (a), the area of any peak corresponding to estrone is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). In the chromatogram obtained with test solution (b) the area of any peak corresponding to norethisterone is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent).

**Uniformity of content.** Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14).

*Test solution.* Powder one tablet and add 20 ml of the mobile phase, mix with the aid of ultrasound, cool, add sufficient of the mobile phase to produce 25 ml and centrifuge. Dilute the supernatant liquid if necessary, with the mobile phase to produce a solution containing 0.002 per cent w/v of estradiol.

*Reference solution (a).* Dissolve sufficient quantities of *estradiol hemihydrate RS* and *norethisterone acetate RS* in the mobile phase and dilute an aliquot with the mobile phase; the concentrations in the final solution are the same as those expected for test solution.

*Reference solution (b).* A solution containing 0.0017 per cent w/v of *estradiol hemihydrate RS*, 0.00084 per cent w/v of *norethisterone acetate RS*, 0.00066 per cent w/v of *estrone RS* and 0.00034 per cent w/v of *norethisterone RS* in the mobile phase.

Use chromatographic system as described under Dissolution.

Inject 20 µl of reference solution (a), (b) and the test solution. The test is not valid unless the resolution between each pair of peaks (estradiol and norethisterone, estrone and norethisterone acetate) is not less than 1.0.

Calculate the contents of $C_{18}H_{24}O_2$ and $C_{22}H_{28}O_3$ in the tablet.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Determine by liquid chromatography (2.4.14), as described under Uniformity of content using the following test solution.

*Test solution.* Weigh and powder 20 tablets. Weigh accurately a quantity of powder containing 10 mg of estradiol, add 20 ml of the mobile phase, mix with the aid of ultrasound, cool, add sufficient of the mobile phase to produce 25 ml and centrifuge.



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Dilute the supernatant liquid if necessary, with the mobile phase to produce a solution containing 0.002 per cent w/v of estradiol.

Inject 20 µl of reference solution (a) and the test solution.

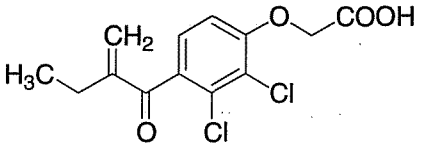
Calculate the contents of $C_{18}H_{24}O_2$ and $C_{22}H_{28}O_3$ in the tablets.

**Storage**. Store protected from light.

**Labelling**. The label states the quantity of estradiol hemihydrate in terms of the equivalent amount of estradiol.

# Ethacrynic Acid

Etacrynic Acid



$C_{13}H_{12}Cl_2O_4$ Mol. Wt. 303.1

Ethacrynic Acid is 2-[2,3-dichloro-4-(2-ethylacryloyl) phenoxy]acetic acid

Ethacrynic Acid contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{13}H_{12}Cl_2O_4$, calculated on the dried basis.

**Category**. Diuretic.

**Dose**. 50 to 200 mg daily, in divided doses.

**Description**. A white or almost white, crystalline powder.

*CAUTION - As Ethacrynic Acid irritates the skin, eyes and the mucous membranes it should be handled with care.*

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ethacrynic acid RS* or with the reference spectrum of ethacrynic acid.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.005 per cent w/v solution in a mixture of 99 volumes of *methanol* and 1 volume of *1 M hydrochloric acid* shows a well defined absorption maximum at about 270 nm and a shoulder at about 285 nm; absorbance at about 270 nm, 0.55 to 0.60.

C. To 25 mg add 2 ml of *1 M sodium hydroxide* and heat in a water-bath for 5 minutes, cool, add 0.25 ml of *sulphuric acid (50 per cent v/v)* and 0.5 ml of a 10 per cent w/v solution of *chromotropic acid sodium salt* and add cautiously 2 ml of *sulphuric acid*; a deep violet colour is produced.

D. On 20 mg determine by the oxygen-flask method (2.3.34), using 5 ml of *dilute sodium hydroxide solution* as the absorbing liquid. When the process is complete, acidify with *dilute sulphuric acid* and boil gently for 2 minutes; the solution gives the reactions of chlorides (2.3.1).

## Tests

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 60 volumes of *chloroform*, 50 volumes of *ethyl acetate* and 20 volumes of *glacial acetic acid*.

*Test solution*. Dissolve 0.2 g of the substance under examination in 10 ml of *ethanol (95 per cent)*.

*Reference solution (a)*. A 0.03 per cent w/v solution of the substance under examination in *ethanol (95 per cent)*.

*Reference solution (b)*. A 0.01 per cent w/v solution of the substance under examination in *ethanol (95 per cent)*.

Apply to the plate 10 µl of each solution. After development. dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.3.19). Not more than 0.5 per cent, determined on 2.0 g by drying in an oven over *phosphorus pentoxide* at 60° at a pressure of 0.1 to 0.5 kPa.

**Assay**. Weigh accurately about 0.25 g, dissolve in 100 ml of *methanol* and add 5 ml of *water*. Titrate with *0.1 M sodium hydroxide solution*, determining the end-point potentiometrically (2.4.25).

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.03031 g of $C_{13}H_{12}Cl_2O_4$.

# Ethacrynic Acid Tablets

Etacrynic Acid Tablets

Ethacrynic Acid Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ethacrynic acid, $C_{13}H_{12}Cl_2O_4$.

**Usual strength**. 50 mg.

## Identification

Mix a quantity of the powdered tablets containing 50 mg of Ethacrynic Acid with *0.1 M hydrochloric acid* and extract with two quantities, each of 40 ml, of *dichloromethane*. Dry



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the combined extracts with *anhrydrous sodium sulphate*, filter and evaporate to dryness with the aid of gentle heat. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ethacrynic acid RS* or with the reference spectrum of ethacrynic acid.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.005 per cent w/v solution in a mixture of 99 volumes of *methanol* and 1 volume of *1 M hydrochloric acid* shows a well defined absorption maximum at about 270 nm and a shoulder at about 285 nm; absorbance at about 270 nm, 0.55 to 0.60.

C. To 25 mg add 2 ml of *1 M sodium hydroxide* and heat in a water-bath for 5 minutes, cool, add 0.25 ml of *sulphuric acid (50 per cent v/v)* and 0.5 ml of a 10 per cent w/v solution of chromotropic acid sodium salt and add cautiously 2 ml of *sulphuric acid*; a deep violet colour is produced.

### Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 60 volumes of *chloroform*, 50 volumes of *ethyl acetate* and 20 volumes of *glacial acetic acid*.

*Test solution*. Shake a quantity of the powdered tablets containing 0.2 g of Ethacrynic Acid with 10 ml of *ethanol (95 per cent)* and filter.

*Reference solution (a)*. Dilute 3 volumes of the test solution to 200 volumes with *ethanol (95 per cent)*.

*Reference solution (b)*. Dilute 1 volume of the test solution to 200 volumes with *ethanol (95 per cent)*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution (a)*. Weigh and powder 20 tablets. Shake a quantity of the powdered tablets containing about 50 mg of Ethacrynic Acid with 0.5 ml of *glacial acetic acid* and 50.0 ml of *acetonitrile* for 15 minutes and filter.

*Test solution (b)*. Prepare in the same manner as test solution (a) but using 0.5 ml of *glacial acetic acid*, 45 ml of *acetonitrile* and 5.0 ml of a 0.15 per cent w/v solution of *propyl hydroxybenzoate* (internal standard) in *acetonitrile*.

*Reference solution*. Dissolve 50 mg of *ethacrynic acid RS* in 5.0 ml of the internal standard solution and dilute to 50.0 ml with a mixture of 70 volumes of *acetonitrile* and 30 volumes of *water*.

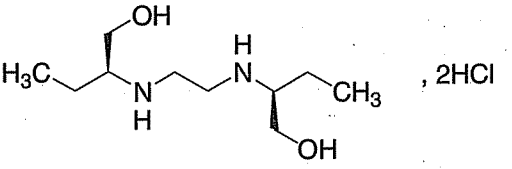
Chromatographic system

- a stainless steel column 20 cm x 4 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 60 volumes of *water*, 40 volumes of *acetonitrile* and 1 volume of *glacial acetic acid*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 270 nm,
- injection volume. 20 µl.

Inject the test and reference solution.

Calculate the content of $C_{13}H_{12}Cl_2O_4$ in the tablets.

## Ethambutol Hydrochloride




$C_{10}H_{24}N_2O_2,2HCl$ Mol. Wt. 277.2

Ethambutol Hydrochloride is (*S*,*S*)-*N*,*N*′-ethylenebis(2-aminobutan-1-ol) dihydrochloride.

Ethambutol Hydrochloride contains not less than 97.0 per cent and not more than 101.0 per cent of $C_{10}H_{24}N_2O_2,2HCl$, calculated on the dried basis.

**Category**. Antitubercular.

**Dose**. 15 to 25 mg per kg of body weight daily, for two months, followed by 15 mg per kg body weight daily.

**Description**. A white, crystalline powder; almost odourless.

### Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ethambutol hydrochloride RS* or with the reference spectrum of ethambutol hydrochloride.

B. In the test for 2-Aminobutanol, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

C. A 5 per cent w/v solution gives reaction A of chlorides (2.3.1).



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## Tests

**pH** (2.4.24). 3.4 to 4.0, determined in a 2.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). +6.0° to +6.6°, determined in a 10.0 per cent w/v solution.

**2-Aminobutanol**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 75 volumes of *methanol*, 15 volumes of *water* and 10 volumes of *strong ammonia solution*.

*Test solution (a)*. Dissolve 0.5 g of the substance under examination in 10 ml of *methanol*.

*Test solution (b)*. Dilute 1 ml of test solution (a) to 10 ml with *methanol*.

*Reference solution (a)*. A 0.05 per cent w/v solution of *2-aminobutanol RS* in *methanol*.

*Reference solution (b)*. A 0.5 per cent w/v solution of *ethambutol hydrochloride RS* in *methanol*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air, heat at 110° for 10 minutes, cool, spray with *ninhydrin solution* and heat at 110° for 5 minutes. Any spot corresponding to 2-aminobutanol in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a).

**Meso ethambutol (RS isomer)**. Determine by either of the following methods.

**Method A**. Determine by differential scanning calorimetry (DSC) (2.4.31).

Test preparation: Weigh between 4 and 6 mg of the sample in the 40 µl aluminium DSC crucible. Carry out the test by heating at a rate of 10° per minute from 25° to 250°, under nitrogen purging (20 ml/min) and record the thermogram. Observe the endotherms at 42° ± 2° and 77° ± 2° corresponding to the transitions of the RS isomer and SS isomer, respectively. There should not be any endothermic peak at 42° ± 2° in the thermogram.

**Method B**. Determine by liquid chromatography (2.4.14).

*NOTE—Use freshly prepared solution.*

*Test solution*. Suspend 4.0 mg of the substance under examination in 4.0 ml of *acetonitrile* and 100 µl of *triethylamine*. Stir the mixture with the aid of ultrasound for 5 minutes. Add 15 µl of *R-(+)-phenyl isocyanate* and heat the mixture for 20 minutes at 70° in a water-bath.

*Reference solution (a)*. Dilute 1.0 ml of the test solution to 100.0 ml with *acetonitrile*.

*Reference solution (b)*. Suspend 4.0 mg of *ethambutol for system suitability RS* (containing *RS* isomer) 4.0 ml of *acetonitrile* and 100 µl of *triethylamine*. Mix the mixture with the aid of ultrasound for 5 minutes. Add 15 µl of *R-(+)-phenyl isocyanate* and heat the mixture for 20 minutes at 70° in a water-bath.

Chromatographic system

- a column 10 cm x 4.6 mm, packed with octadecylsilane bonded to silica (3 µm),
- column temperature. 40°,
- mobile phase: A. a mixture of equal volumes of *methanol* and *water*,
  B. *methanol*,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 215 nm,
- injection volume. 10 µl.

| Time (in min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0-30 | 82 | 18 |
| 31-35 | 0 | 100 |
| 35-37 | 0 | 100 |
| 37-38 | 82 | 18 |

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to ethambutol and the *RS* isomer is not less than 4.0 and the relative retention of RS isomer with reference to ethambutol (retention time about 14 min) is about 1.3.

Inject reference solution (a) and the test solution.

In the chromatogram obtained with the test solution, the area of the peak due to the *RS* isomer is not more than the area of the peak in the chromatogram obtained with reference solution (a) (1.0 per cent).

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

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

**Assay**. Determine by liquid chromatography (2.4.14).

*Diluent*. Dissolve 1.4 g of *disodium hydrogen orthophosphate anhydrous* in 1000 ml of *water* and adjust the pH to 6.8 ± 0.05 with *orthophosphoric acid*.

*Test solution*. Dissolve 30.0 mg of the substance under examination in 100.0 ml of the diluent.

*Reference solution*. A 0.03 per cent w/v solution of *ethambutol hydrochloride RS* in the diluent.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with nitrile groups chemically bonded to porous silica particles (5 µm) ( Such as Zorbax SB-CN),



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- mobile phase: a mixture of equal volumes of a buffer consisting of 1 ml of *triethylamine* in sufficient *water* to produce 1000 ml adjusted to pH 7.0 with *orthophosphoric acid,* and *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 200 nm,
- injection volume. 100 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 3.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_{10}H_{24}N_2O_2,2HCl$.

**Storage**. Store protected from moisture.

# Ethambutol Tablets

Ethambutol Hydrochloride Tablets

Ethambutol Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of ethambutol hydrochloride, $C_{10}H_{24}N_2O_2,2HCl$.

**Usual strengths**. 200 mg; 400 mg.

## Identification

A. Extract a quantity of the powdered tablets containing 50 mg of Ethambutol Hydrochloride with 5 ml of *methanol*, filter and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ethambutol hydrochloride RS* or with the reference spectrum of ethambutol hydrochloride.

B. Shake a quantity of the powdered tablets containing 0.1 g of Ethambutol Hydrochloride with 10 ml of *water*, filter, and to the filtrate add 2 ml of a 1 per cent w/v solution of *copper sulphate* and 1 ml of *1 M sodium hydroxide*; a distinct blue colour is produced.

## Tests

**2-Aminobutanol**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 75 volumes of *methanol*, 15 volumes of water and 10 volumes of *strong ammonia solution*.

*Test solution*. Shake a quantity of the powdered tablets containing 0.5 g of Ethambutol Hydrochloride for 5 minutes with sufficient *methanol* to produce 10 ml and filter.

*Reference solution*. A 0.05 per cent w/v solution of *2-aminobutanol RS* in *methanol*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air, heat at 110° for 5 minutes, cool, spray with *ninhydrin solution* and heat at 110° for 5 minutes. Any spot corresponding to 2-aminobutanol in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of freshly distilled *water*,
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first 1 ml of the filtrate. Dilute suitably with *water* to produce a solution containing about 0.030 per cent w/v of ethambutol hydrochloride. Using the resulting solution as the test solution carry out the procedure described under Assay.

Calculate the content of $C_{10}H_{24}N_2O_2,2HCl$.

D. Not less than 75 per cent of the stated amount of $C_{10}H_{24}N_2O_2,2HCl$.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Prepare the following solutions freshly.*

*Test solution*. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 30 mg of Ethambutol Hydrochloride, add 50 ml of *water* and shake for about 15 minutes and add sufficient *water* to produce 100.0 ml. Filter and discard the first 10 ml of the filtrate. Use the clear filtrate.

*Standard solution*. A 0.03 per cent w/v solution of *ethambutol hydrochloride RS* in *water*.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with nitrile groups chemically bonded to porous silica particles (5 µm) ( such as Zorbax SB-CN),
- column. temperature. 30°,
- mobile phase: a mixture of equal volumes of a buffer consisting of 1 ml of *triethylamine* in sufficient *water* to produce 1000 ml adjusted to pH 7.0 with *orthophosphoric acid,* and *acetonitrile*,
- spectrophotometer set at 200 nm,
- injection volume. 50 µl.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_{10}H_{24}N_2O_2,2HCl$ in the tablets.



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# Ethambutol and Isoniazid Tablets

Ethambutol Hydrochloride and Isoniazid Tablets

Ethambutol and Isoniazid Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of ethambutol hydrochloride, $C_{10}H_{24}N_2O_2.2HCl$ and isoniazid, $C_6H_7N_3O$.

## Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to *ethambutol hydrochloride RS* in the chromatogram obtained with the reference solution.

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

## Tests

**2-Aminobutanol**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel* G.

*Mobile phase*. A mixture of 75 volumes of *methanol*, 15 volumes of *water* and 10 volumes of *strong ammonia solution*.

*Test solution*. Shake a quantity of the powdered tablets containing 0.5 g of ethambutol hydrochloride for 5 minutes with sufficient *methanol* to produce 10 ml and filter.

*Reference solution*. A 0.05 per cent w/v solution of *2-aminobutanol RS* in *methanol*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air, heat at 110° for 5 minutes, cool, spray with *ninhydrin solution* and heat at 110° for 5 minutes. Any spot corresponding to 2-aminobutanol in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *water*,
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first 10 ml of the filtrate.

On the filtrate determine by liquid chromatography (2.4.14).

*For Ethambutol Hydrochloride* —

*Test solution*. Dilute the filtrate to obtain 0.044 per cent w/v solution in the dissolution medium.

*Reference solution*. A 0.044 per cent w/v solution of *ethambutol hydrochloride RS* in the dissolution medium.

Determine the content of $C_{10}H_{24}N_2O_2.2HCl$ by the procedure given under Assay of Ethambutol hydrochloride.

Calculate the content of $C_{10}H_{24}N_2O_2.2HCl$ in the medium.

*For Isoniazid* — Determine the amount of $C_6H_7N_3O$ dissolved by measuring the absorbance of the filtrate, suitably diluted with the dissolution medium to obtain a solution containing about 0.015 mg of isoniazid per ml, at the maximum at about 263 nm (2.4.7). Calculate the content of $C_6H_7N_3O$ in the medium from the absorbance obtained by repeating the determination using a 0.0015 per cent w/v solution of *isoniazid RS* in place of the filtrate.

D. Not less than 75 per cent of the stated amount of $C_{10}H_{24}N_2O_2.2HCl$ and $C_6H_7N_3O$.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. *For isoniazid* - Determine by liquid chromatography (2.4.14).

*Diluent*. Dissolve 1.4 g of *disodium hydrogen orthophosphate anhydrous* in *water*, adjust the pH to 6.8 ± 0.05 with *dilute phosphoric acid* and add sufficient *water* to produce 1000 ml.

*Test solution*. Weigh and powder 20 tablets. Weigh accurately a quantity of powdered tablets containing about 40 mg of Isoniazid, dissolve in 50.0 ml of *methanol* and dilute to 500.0 ml with the diluent.

*Reference solution*. Weigh accurately about 40 mg of *isoniazid RS*, dissolve in 50.0 ml of *methanol* and dilute to 500.0 ml with the diluent.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Intersil ODS-3),
- column temperature 30°,
- mobile phase: 96 volumes of buffer solution pH 6.8 prepared by dissolving 1.4 g *disodium hydrogen orthophsophate anhydrous* in 1000 ml of *water*, the pH of which is adjusted to 6.8 ± 0.05 with dilute *phosphoric acid* and 4 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, the column efficiency determined from the isoniazid peak is not more than 1500 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_6H_7N_3O$ in the tablets.

*For ethambutol hydrochloride* — Determine by liquid chromatography (2.4.14).



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*Test solution.* Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 60 mg of Ethambutol Hydrochloride and dissolve in 100.0 ml of the diluent.

*Reference solution.* A 0.06 per cent w/v solution of *ethambutol hydrochloride RS* in. the diluent.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with nitrile groups chemically bonded to porous silica particles (such as Zorbax SB CN 5 µm),
- mobile phase: a mixture of 50 volumes of buffer pH 7.0 prepared by mixing 1 ml of *triethylamine* in 1000 ml of *water* the pH of which is adjusted to 7.0 ± 0.05 with *phosphoric acid* and 50 volumes of *acetonitrile.*
- flow rate. 1 ml per minute,
- spectrophotometer set at 200 nm,
- injection volume. 50 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 3.0, the column efficiency determined from ethambutol peak is not more than 1500 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and reference solution.

Calculate the content of $C_{10}H_{24}N_2O_2.2HCl$ the tablets.

**Storage.** Store protected from moisture.

# Ethanol

Absolute Alcohol; Dehydrated Alcohol

$CH_3CH_2OH$ Mol. Wt. 46.1

Ethanol contains not less than 99.0 per cent w/w and not more than 100.0 per cent w/w, corresponding to not less than 99.4 per cent v/v and not more than 100.0 per cent v/v, at 15.56°, of $C_2H_6O$.

**Category.** Pharmaceutical aid (solvent).

**Description.** A clear, colourless, mobile and volatile liquid; odour, characteristic and spirituous; hygroscopic. Readily volatilises even at low temperature; boils at 78°; flammable, burning with a blue, smokeless flame.

## Identification

A. Mix 0.25 ml in a small beaker with 1 ml of *potassium permanganate solution* and 0.25 ml of *dilute sulphuric acid* and cover the beaker immediately with a filter paper moistened with a solution freshly prepared by dissolving 0.1 g of *sodium nitroprusside* and 0.5 g of *piperazine hydrate* in 5 ml of *water*; an intense blue colour is produced on the filter paper, the colour becoming lighter after a few minutes.

B. To 5 ml of a 0.5 per cent v/v solution add 1 ml of *1 M sodium hydroxide* followed by slow addition of 2 ml of *iodine solution*; the odour of iodoform develops and a yellow precipitate is produced.

## Tests

**Relative density** (2.4.29). 0.7871 to 0.7902, determined at 25°.

**Appearance of solution.** Dilute 5.0 ml to 100.0 ml with *water*. The solution is clear (2.4.1). Cool to 10° for 30 minutes; the solution remains clear.

**Acidity or alkalinity.** To 20 ml add 0.25 ml of *phenolphthalein solution*; the solution remains colourless and requires not more than 0.2 ml of *0.1 M sodium hydroxide* to produce a pink colour.

**Methanol.** To 1 drop, add 1 drop of water, 1 drop of *dilute phosphoric acid* and 1 drop of *potassium permanganate solution*. Mix, allow to stand for 1 minute and add *sodium bisulphite solution* dropwise until the permanganate colour is discharged. If a brown colour remains, add 1 drop of *dilute phosphoric acid.* To the colourless solution add 5 ml of freshly prepared *chromotropic acid solution* and heat on a water-bath at 60° for 10 minutes; no violet colour is produced.

**Foreign organic substances.** Clean a glass-stoppered cylinder thoroughly with *hydrochloric acid*, rinse with *water* and finally rinse with the substance under examination. Put 20 ml in the cylinder, cool to about 15° and then add from a carefully cleaned pipette 0.1 ml of *0.1 M potassium permanganate*. Mix at once by inverting the stoppered cylinder and allow to stand at 15° for 5 minutes; the pink colour does not entirely disappear.

**2-Propanol and 2-methyl-2-propanol.** To 1 ml add 3 ml of *water* and 10 ml of *mercuric sulphate solution* and heat in a boiling water-bath; no precipitate is formed within 3 minutes.

**Aldehydes.** Not more than 10 ppm, determined by the following method. To 5.0 ml add 5 ml of *water* and 1 ml of *decolorised magenta solution* and allow to stand for 30 minutes. Any colour produced is not more intense than that produced by treating in the same manner 5.0 ml of a 0.001 per cent w/v solution of redistilled *acetaldehyde* in *aldehyde-free ethanol (95 per cent).*

**Benzene and related substances.** Determine by gas chromatography (2.4.13).

*Test solution.* The substance under examination.

*Reference solution (a).* A 0.1 per cent v/v solution of *2-butanol reagent* in the test solution.

*Reference solution (b).* A solution containing 0.1 per cent v/v each of *2-butanol reagent* and *1-propanol* in the test solution.



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*Reference solution (c)*. A 0.0002 per cent v/v solution of *benzene* in the test solution.

Chromatographic system
- a glass column 1.8 m x 2 mm, packed with acid-washed diatomaceous support (80 to 100 mesh) coated with 15 per cent w/w of *polyethyelene glycol 400*,
- temperature:
  column. 50°,
  inlet port. 150°,
- flame ionisation detector at 250°,
- flow rate. 30 ml per minute of the carrier gas (nitrogen).

Inject separately 2 µl of each of the test solution and reference solution (a). The chromatogram obtained with the test solution shows no peak with a retention time similar to the peak due to 2-butanol (retention time relative to isopropyl alcohol, about 1.5) obtained with reference solution (a). Inject 2 µl of reference solution (b) and adjust the sensitivity of the system so that the heights of the peaks due to 2-butanol and propanol in the chromatogram obtained with reference solution (b) are not less than 50 per cent of the full scale of the recorder.

The test is not valid unless the resolution between the peaks due to 2-butanol and propanol in the chromatogram obtained with reference solution (b) is at least 1.2.

Inject alternately 2 µl each of the test solution and reference solution (c). The area of any peak due to benzene in the chromatogram obtained with the test solution is not greater than the difference between the area of the peak due to benzene in the chromatogram obtained with reference solution (c) and that of the peak due to benzene in the chromatogram obtained with the test solution.

In the chromatogram obtained with reference solution (a) the sum of the areas of any peaks other than the principal peak and the peaks due to 2-butanol is not greater than 3 times the area of the peak due to 2-butanol (0.3 per cent).

**Fusel oil constituents**. Place 25 ml in a porcelain dish protected from dust and allow the liquid to evaporate on a water-bath until a little of the liquid remains. Remove the dish from the water-bath and allow the liquid to evaporate at room temperature till the dish is almost dry. No foreign odour is perceptible. Add 1 ml of *sulphuric acid*; no red or brown colour is produced.

**Non-volatile matter**. Evaporate 100.0 ml in a tared dish on a water-bath and dry the residue at 105°; the residue weighs not more than 5 mg.

**Assay**. Determine at 25° the relative density (2.4.29) and calculate from the Alcohol Table the content of $C_2H_6O$ at 15.56°.

**Storage**. Store in tightly-closed containers at a temperature not exceeding 30°, away from fire and protected from moisture.

**Labelling**. The label states that it is flammable.

# Ethanol (95 Per Cent)

Alcohol (95 per cent)

Ethanol (95 per cent) is a mixture of Ethanol and Water.

Ethanol (95 per cent) contains not less than 92.0 per cent w/w and not more than 92.7 per cent w/w, corresponding to not less than 94.7 per cent v/v and not more than 95.2 per cent v/v, at 15.56°, of $C_2H_6O$.

**Category**. Pharmaceutical aid (solvent); topical anti-infective.

**Description**. A clear, colourless, mobile and volatile liquid; odour, characteristic and spirituous. It is readily volatilised even at low temperatures; boils at about 78°; flammable, burning with a blue, smokeless flame.

## Identification

A. Mix 0.25 ml in a small beaker with 1 ml of *potassium permanganate solution* and 0.25 ml of *dilute sulphuric acid* and cover the beaker immediately with a filter paper moistened with a solution freshly prepared by dissolving 0.1 g of *sodium nitroprusside* and 0.5 g of *piperazine hydrate* in 5 ml of *water*; an intense blue colour is produced on the filter paper, the colour becoming lighter after a few minutes.

B. To 5 ml of a 0.5 per cent v/v solution add 1 ml of *1 M sodium hydroxide* followed by slow addition of 2 ml of *iodine solution*; the odour of iodoform develops and a yellow precipitate is produced.

## Tests

**Relative density** (2.4.29). 0.8084 to 0.8104, determined at 25°.

**Appearance of solution**. Dilute 5.0 ml to 100.0 ml with *water*. The solution is clear (2.4.1). Cool to 10° for 30 minutes; the solution remains clear.

**Acidity or alkalinity**. To 20 ml add 0.25 ml of *phenolphthalein solution*; the solution remains colourless and requires not more than 0.2 ml of *0.1 M sodium hydroxide* to produce a pink colour.

**Methanol**. To 1 drop add 1 drop of *water*, 1 drop of *dilute phosphoric acid* and 1 drop of *potassium permanganate solution*. Mix, allow to stand for 1 minute and add *sodium bisulphite solution* dropwise until the permanganate colour is discharged. If a brown colour remains, add 1 drop of *dilute phosphoric acid*. To the colourless solution add 5 ml of freshly prepared *chromotropic acid solution* and heat on a water-bath at 60° for 10 minutes; no violet colour is produced.

**Foreign organic substances**. Clean a glass-stoppered cylinder thoroughly with *hydrochloric acid*, rinse with *water* and finally rinse with the substance under examination. Put 20 ml in the cylinder, cool to about 15° and then add from a carefully



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cleaned pipette 0.1 ml of *0.1 M potassium permanganate*. Mix at once by inverting the stoppered cylinder and allow to stand at 15° for 5 minutes; the pink colour does not entirely disappear.

**2-Propanol and 2-methyl-2-propanol**. To 1 ml add 3 ml of water and 10 ml of *mercuric sulphate solution* and heat in a boiling water-bath; no precipitate is formed within 3 minutes.

**Aldehydes**. Not more than 10 ppm, determined by the following method. To 5.0 ml add 5 ml of water and 1 ml of *decolorised magenta solution* and allow to stand for 30 minutes. Any colour produced is not more intense than that produced by treating in the same manner 5 ml of a 0.001 per cent w/v solution of redistilled *acetaldehyde* in *aldehyde-free ethanol (95 per cent)*.

**Benzene and related substances**. Determine by gas chromatography (2.4.13).

*Test solution*. The substance under examination.

*Reference solution (a)*. A 0.1 per cent v/v solution of *2-butanol reagent* in the test solution.

*Reference solution (b)*. A solution containing 0.1 per cent v/v each of *2-butanol reagent* and *1-propanol* in the test solution.

*Reference solution (c)*. A 0.0002 per cent v/v solution of *benzene* in the test solution.

Chromatographic system
- a glass column 1.8 m x 2 mm, packed with acid-washed diatomaceous support (80 to 100 mesh) coated with 15 per cent w/w of *polyethyelene glycol 400*,
- temperature:
  column. 50°,
  inlet port. 150°,
- flame ionisation detector at 250°,
- flow rate. 30 ml per minute of the carrier gas (nitrogen).

Inject alternately 2 µl of each of the test solution and reference solution (a). The chromatogram obtained with the test solution shows no peak with a retention time similar to the peak due to 2-butanol (retention time relative to isopropyl alcohol, about 1.5) obtained with reference solution (a). Inject 2 µl of reference solution (b) and adjust the sensitivity of the system so that the heights of the peaks due to 2-butanol and propanol in the chromatogram obtained with reference solution (b) are not less than 50 per cent of the full scale of the recorder.

The test is not valid unless the resolution between the peaks due to 2-butanol and propanol in the chromatogram obtained with reference solution (b) is at least 1.2.

Inject alternately 2 µl each of the test solution and reference solution (c). The area of any peak due to benzene in the chromatogram obtained with the test solution is not greater than the difference between the area of the peak due to benzene in the chromatogram obtained with reference solution (c) and that of the peak due to benzene in the chromatogram obtained with the test solution.

In the chromatogram obtained with reference solution (a) the sum of the areas of any peaks other than the principal peak and the peaks due to 2-butanol is not greater than 3 times the area of the peak due to 2-butanol (0.3 per cent).

**Fusel oil constituents**. Place 25 ml in a porcelain dish protected from dust and allow the liquid to evaporate on a water-bath until a little of the liquid remains. Remove the dish from the water-bath and allow the liquid to evaporate at room temperature till the dish is almost dry. No foreign odour is perceptible. Add 1 ml of *sulphuric acid*; no red or brown colour is produced.

**Non-volatile matter**. Evaporate 100.0 ml in a tared dish on a water-bath and dry the residue at 105°; the residue weighs not more than 5 mg.

**Assay**. Determine at 25° the relative density (2.4.29) and calculate from the Alcohol Table the content of $C_2H_6O$ at 15.56°.

**Storage**. Store in tightly-closed containers at a temperature not exceeding 30° and away from fire.

**Labelling**. The label states that it is flammable.

## Anaesthetic Ether

$CH_3CH_2OCH_2CH_3$

$C_4H_{10}O$ Mol. Wt. 74.1

Anaesthetic Ether is diethyl ether to which a suitable non-volatile stabiliser in a proportion not greater than 0.002 per cent w/v may have been added.

**Category**. General anaestheic.

**Description**. A clear, colourless, very mobile liquid; odour, characteristic; highly flammable.

*NOTE — It is absolutely essential that a preservative of the type of sodium pyrogallate, hydroquinone or propyl gallate in suitable concentrations shall be added in Anaesthetic Ether intended for use in tropical climates unless the Anaesthetic Ether is stored in a copper container or in a container copper-plated internally. The preservative used and its concentration shall be declared on the label.*

### Tests

**Relative density** (2.4.29). 0.714 to 0.716, determined at 20°.

**Boiling range** (2.4.8). 34° to 35°.



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*CAUTION — It is dangerous to determine the boiling range if the sample does not comply with the test for peroxides.*

**Acidity**. To 20 ml of *ethanol (95 per cent)* add 0.25 ml of *bromothymol blue solution* add dropwise *0.02 M sodium hydroxide* until the blue colour persists for 30 seconds. Add 25 ml of the substance under examination, shake and again add dropwise *0.2 M sodium hydroxide* until the blue colour reappears and persists for 30 seconds. Not more than 0.4 ml of *0.02 M sodium hydroxide* is required.

**Peroxides**. Place 8 ml of *potassium iodide and starch solution* in a 12-ml glass-stoppered cylinder of about 1.5 cm diameter. Fill completely with the substance under examination, insert the stopper, shake vigorously and allow to stand in the dark for 30 minutes; no colouration is produced.

**Acetone and aldehydes**. Place 2 ml of *alkaline potassium mercuri-iodide solution* in a 12-ml glass-stoppered cylinder of about 1.5 cm diameter and fill completely with the substance under examination, insert the stopper and shake vigorously for 15 seconds and set aside for 5 minutes, protected from light; no colour or turbidity, except for slight opalescence, is produced.

If the ether does not comply with the test, distil 40 ml *(after ensuring that it complies with the test for peroxides)* until only 5 ml remains and repeat the test using 10 ml of the distillate.

**Foreign odour**. Pour 10 ml in successive portions on to a clean filter paper and allow to evaporate spontaneously; no foreign odour is detectable at any stage of evaporation.

**Non-volatile matter**. Evaporate 50 ml in a tared dish on a water-bath and dry at 105° *(after ensuring that the sample complies with the test for peroxides)*; the residue weighs not more than 1.5 mg.

**Methanol**. To 10 ml, add 5 ml of *ethanol (20 per cent)* and 5 ml of *water*, in a separator, shake vigorously, set aside and allow the mixture to separate and draw off the lower layer. To 5 ml of the lower layer add 2.0 ml of *potassium permanganate and phosphoric acid solution*, set aside for 10 minutes and add 2.0 ml of *oxalic acid and sulphuric acid solution* and 5 ml of *decolorised magenta solution*. Set aside for 30 minutes; no colour is produced.

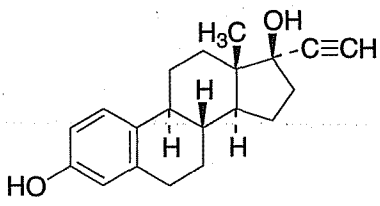
**Water** (2.3.43). Not more than 0.2 per cent, determined on 20.0 ml.

**Storage**. Store protected from light at a temperature not exceeding 30°. Ether remaining in a partly used container may deteriorate rapidly.

**Labelling**. The label states that (1) it is very flammable and should not be used near a naked flame; (2) the name and proportion of any stabiliser added.

# Ethinyloestradiol

Ethinylestradiol




$C_{20}H_{24}O_2$ Mol. Wt. 296.4

Ethinylestradiol is 19-nor-17α-pregna-1,3,5(10)-trien-20-yne-3,17β-diol.

Ethinyloestradiol contains not less than 97.0 per cent and not more than 102.0 per cent of $C_{20}H_{24}O_2$, calculated on the dried basis.

**Category**. Oestrogen.

**Dose**. For menopausal symptoms, 10 to 20 µg daily continuously or for 21 days, repeated after 7 days, with a progestogen from day 17 to day 26 of cycle if uterus is intact. For primary amenorrhoea, 10 µg on alternate days increasing to a maximum of 50 µg daily continuously with a progestogen for the last 5 days of month.

**Description**. A white or slightly yellowish-white, crystalline powder.

## Identification

*Test A may be omitted if tests B and C are carried out. Test C may be omitted if tests A and B are carried out.*

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

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

C. Dissolve about 1 mg in 1 ml of *sulphuric acid*; an orange-red colour develops which exhibits a greenish fluorescence when examined in ultraviolet light at 365 nm. Add the solution to 10 ml of *water*; the colour changes to violet and a violet precipitate is produced.

## Tests

**Appearance of solution**. A 5.0 per cent w/v solution in *ethanol* is clear (2.4.1), and not more intensely coloured than reference solution BYS6 (2.4.1).



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**Specific optical rotation** (2.4.22). –27.0° to –30.0°, determined at 20° in a 5.0 per cent w/v solution in *pyridine*.

**Light absorption** (2.4.7). Absorbance of a 0.01 per cent w/v solution in *ethanol (95 per cent)* at about 281 nm, 0.69 to 0.73.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 90 volumes of *toluene* and 10 volumes of *ethanol (95 per cent)*.

*Test solution (a)*. Dissolve 0.2 g of the substance under examination in 10 ml of a mixture of 9 volumes of *chloroform* and 1 volume of *methanol*.

*Test solution (b)*. Dilute 5 ml of test solution (a) to 100 ml with the same solvent mixture.

*Reference solution (a)*. Dilute 5 ml of test solution (b) to 25 ml with the same solvent mixture.

*Reference solution (b)*. A 0.1 per cent w/v solution of *ethinyloestradiol RS* in the same solvent mixture.

*Reference solution (c)*. A 0.02 per cent w/v solution of *estrone RS* in the same solvent mixture.

Apply to the plate 5 µl of each solution. After development, dry the plate in air until the odour of the solvent is no longer detectable, heat at 110° for 10 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v)*. Heat again at 110° for 10 minutes and examine in ultraviolet light at 365 nm. In the chromatogram obtained with test solution (a) any spot corresponding to estrone is not more intense than the spot in the chromatogram obtained with reference solution (c) and any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (a).

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 0.5 g by drying in an oven at 105° for 3 hours.

**Assay**. Dissolve 0.2 g in 40 ml of *tetrahydrofuran*, add 5 ml of a 10 per cent w/v solution of *silver nitrate* and titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.02964 g of $C_{20}H_{24}O_2$.

**Storage**. Store protected from light.

# Ethinyloestradiol Tablets

Ethinylestradiol Tablets

Ethinyloestradiol Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ethinyloestradiol, $C_{20}H_{24}O_2$.

**Usual strengths**. 10 µg; 20 µg; 50 µg.

## Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 90 volumes of *toluene* and 10 volumes of *ethanol (95 per cent)*.

*Test solution*. Shake a quantity of the powdered tablets containing 0.25 mg of Ethinyloestradiol with four quantities, each of 20 ml of *chloroform*, filter each extract in turn, evaporate the combined filtrates to dryness on a water-bath in a current of nitrogen and dissolve the residue in 0.25 ml of *chloroform*.

*Reference solution*. A 0.1 per cent w/v solution of *ethinyloestradiol RS* in *chloroform*.

Apply to the plate 20 µl of each solution. After development, dry the plate in air, spray with *ethanolic sulphuric acid (20 per cent v/v)*, heat at 110° for 10 minutes and examine in ultraviolet light at 365 nm and in daylight. By both methods of visualisation, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. Triturate a quantity of the powdered tablets containing 0.1 mg of Ethinyloestradiol with 0.5 ml of *0.1 M sodium hydroxide* and 5 ml of *water*, allow to stand for 5 minutes, filter, acidify the filtrate with 0.15 ml of *sulphuric acid*, add 3 ml of *ether*, shake and allow to separate. Evaporate the ether layer to dryness and heat the residue on a water-bath for 5 minutes with 0.2 ml of *glacial acetic acid* and 2 ml of *phosphoric acid*; a pink colour with an intense orange fluorescence is produced.

## Tests

**Uniformity of content**. Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14).

*Test solution*. Finely crush one tablet, add 20.0 ml of mobile phase, shake for 10 minutes, dilute to 100 ml with mobile phase and filter. Take 1 ml of this solution dilute 10 ml with the same solvent.

*Reference solution*. A 0.0025 per cent w/v solution of *ethinylestradiol RS* in mobile phase.

Chromatographic system

- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volume of *acetonitrile* and 40 volumes of *water*.
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume. 20 µl.

Inject the test solution and reference solution.



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Calculate the content of $C_{20}H_{24}O_2$ in the tablet.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

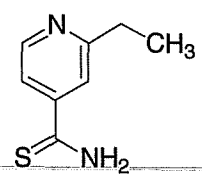
*Test solution*. Weigh and powder 20 tablets. Weight accurately a quantity of the powder containing 2.5 mg of ethinylestradiol, add 20.0 ml of mobile phase, shake for 10 minutes, dilute to 100 ml and filter. Take 1 ml of this solution and dilute to 10 ml with the same solvent.

*Reference solution*. A 0.0025 per cent w/v solution of *ethinylestradiol RS* in mobile phase.

Carry out the chromatographic procedure described under Uniformity of content. Calculate the content of $C_{20}H_{24}O_2$ in the tablets.

**Storage**. Store protected from light.

# Ethionamide




$C_8H_{10}N_2S$ Mol. Wt. 166.2

Ethionamide is 2-ethylpyridine-4-carbothioamide.

Ethionamide contains not less than 98.5 per cent and not more than 101.0 per cent of $C_8H_{10}N_2S$, calculated on the dried basis.

**Category**. Antitubercular; antileprotic.

**Dose**. 250 to 375 mg daily, usually in a three-drug regimen with Rifampicin and Dapsone.

**Description**. A yellow crystalline powder or small yellow crystals.

## Identification

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

B. Dissolve about 10 mg in 5 ml of *methanol* and add 5 ml of *0.1 M silver nitrate*; a dark brown precipitate is produced.

C. Melting point (2.4.21). 158° to 164°.

## Tests

**Appearance of solution**. Dissolve 0.5 g in 10 ml of *methanol*, heating to about 50° and allow to cool to room temperature. The solution is not more opalescent than opalescence standard OS2 (2.4.1).

**Acidity**. Dissolve 2.0 g in 20 ml of *methanol*, heating to about 50°, and add 20 ml of *water*. Cool slightly, shake until crystallisation occurs and allow to cool to room temperature. Add 60 ml of *water* and titrate with *0.1 M sodium hydroxide* using 0.2 ml of *cresol red solution* as indicator. Not more than 0.2 ml is required to change the colour of the solution to red.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 50 mg of the substance under examination in 100 ml of the mobile phase.

*Reference solution*. Dissolve 25 mg of the *ethionamide RS* in 100 ml of the mobile phase. Dilute 1 ml of this solution to100 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Intersil ODS-3),
- mobile phase: a mixture of 60 volumes of a buffer prepared by dissolving 2 ml of *triethylamine* in *water*, adjusting the pH to 6.0 with *orthophosphoric acid* and diluting to 1000 ml, and 40 volumes of *acetonitrile* and filtered,
- flow rate. 1 ml per minute,
- spectrophotometer set at 290 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution. Any individual impurity is not more than 0.5 per cent and the sum of all impurities found is not more than 1.0 per cent.

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

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

**Assay**. Determine by liquid chromatography (2.4.14) as given under the test for Related substances using the following solutions.

*Test solution*. Dissolve about 50 mg of the substance under examination in 100.0 ml of the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

*Reference solution*. Dissolve 50 mg of the *ethionamide RS* in 100.0 ml of the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more



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than 2.0 per cent, the tailing factor is not more than 2.0 and the column efficiency in not less than 5000 theoretical plates.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_8H_{10}N_2S$.

**Storage**. Store protected from light and moisture.

## Ethionamide Tablets

Ethionamide Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of ethionamide, $C_8H_{10}N_2S$.

**Usual strength**. 125 mg.

### Identification

A. Extract a quantity of the powdered tablets containing 25 mg of Ethionamide with 5 ml of *methanol,* filter and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ethionamide RS* or with the reference spectrum of ethionamide.

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

### Tests

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

*Test solution.* Weigh accurately a quantity of the powder containing 50 mg of Ethionamide and dissolve in 100 ml of the mobile phase.

*Reference solution*. Dissolve 25 mg of the *ethionamide RS* in 100 ml of the mobile phase. Dilute 1 ml of this solution to100 ml with the mobile phase.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution. Any individual impurity is not more than 0.5 per cent and the sum of all impurities found is not more than 1.0 per cent.

**Dissolution** (2.5.2).

Apparatus. No 2

Medium. 900 ml of *0.1 M hydrochloric acid*

Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium, filter and dilute a suitable volume of the filtrate with the same solvent. Measure the absorbance of the resulting solution at the maximum at about 274 nm (2.7.4). Calculate the content of $C_8H_{10}N_2S$ from the absorbance of a solution of known concentration of *ethionamide RS*.

D. Not less than 75 per cent of the stated amount of $C_8H_{10}N_2S$.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 50 mg of Ethionamide in 100.0 ml of the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

*Reference solution*. Dissolve 50 mg of the *ethionamide RS* in 100 ml of the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Intersil ODS-3),
- mobile phase: a mixture of 40 volumes of *acetonitrile* and 60 volumes of buffer pH 6.0 prepared by mixing 2 ml of *triethylamine* to 1000 ml with *water* and adjusting the pH to 6.0 with *phosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 290 nm,
- injection volume. 20 µl.

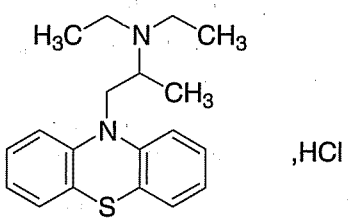
Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent, the tailing factor is not more than 2.0 and the column efficiency in not less than 5000 theoretical plates.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_8H_{10}N_2S$ in the tablets.

**Storage**. Store protected from light and moisture.

## Ethopropazine Hydrochloride




$C_{19}H_{24}N_2S,HCl$ Mol. Wt. 348.9

Ethopropazine Hydrochloride is 10-[2-(diethylamino)propyl]phenothiazine hydrochloride.

Ethopropazine Hydrochloride contains not less than 99.0 per cent and not more than 101.5 per cent of $C_{19}H_{24}N_2S,HCl$,



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calculated on the dried basis.

**Category**. Antiparkinsonian.

**Dose**. 50 mg daily, increasing gradually to 600 mg daily, in divided doses.

**Description**. A white or slightly creamy-white, crystalline powder.

### Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ethopropazine hydrochloride RS*.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.0005 per cent w/v solution in *ethanol (95 per cent)* shows an absorption maximum at about 252 nm and a less well-defined maximum at about 303 nm; absorbance at about 252 nm, about 0.42.

C. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (b).

D. Gives reaction A of chlorides (2.3.1).

### Tests

**Acidity or alkalinity**. Dissolve 0.15 g in 50 ml of *carbon dioxide-free water* and add 0.15 ml of *methyl red solution*; the solution is yellow and not more than 0.2 ml of *0.01 M hydrochloric acid* is required to change the colour of the solution to red.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A freshly prepared mixture of equal volumes of *ether* and *ethyl acetate* saturated with *strong ammonia solution*.

*Test solution*. Dissolve 0.5 g of the substance under examination in 100 ml of *methanol*.

*Reference solution (a)*. Dilute 1 volume of the test solution to 100 volumes with *methanol*.

*Reference solution (b)*. A 0.5 per cent w/v solution of *ethopropazine hydrochloride RS* in *methanol*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a).

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

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.7 g, dissolve in 200 ml of *acetone*, add 15 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, using 0.15 ml of a saturated solution of *methyl orange* in *acetone* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03489 g of $C_{19}H_{24}N_2S,HCl$.

**Storage**. Store protected from light.

## Ethopropazine Tablets

Ethopropazine Hydrochloride Tablets

Ethopropazine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of ethopropazine hydrochloride, $C_{19}H_{24}N_2S,HCl$.

**Usual strength**. 50 mg.

### Identification

A. Extract a quantity of the powdered tablets containing 50 mg of Ethopropazine Hydrochloride with 20 ml of *chloroform*, filter, evaporate the filtrate to dryness and dry the residue at 60° at a pressure not exceeding 0.7 kPa. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ethopropazine hydrochloride RS*.

B. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

C. To a quantity of the powdered tablets containing 5 mg of Ethopropazine Hydrochloride add 5 ml of *sulphuric acid* and allow to stand for 5 minutes; a red colour is produced.

### Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A freshly prepared mixture of equal volumes of *ether* and *ethyl acetate* saturated with *strong ammonia solution*.

*Test solution*. Shake a quantity of the powdered tablets containing 0.1 g of Ethopropazine Hydrochloride with 50 ml of *chloroform* for 15 minutes, centrifuge and use the supernatant liquid.



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*Reference solution (a)*. A 0.2 per cent w/v solution of *ethopropazine hydrochloride RS* in *chloroform*.

*Reference solution (b)*. A 0.002 per cent w/v solution of *ethopropazine hydrochloride RS* in *chloroform*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b).

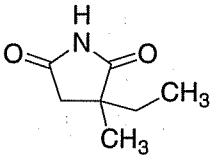
**Other tests**. Comply with the tests stated under Tablets.

**Assay**. *Protect the solution from light throughout the test.*

Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 50 mg of Ethopropazine Hydrochloride, extract with four quantities, each of 20 ml, of *ethanol (95 per cent)*. Filter and dilute the filtrate to 100.0 ml with *ethanol (95 per cent)*. Dilute 10.0 ml of this solution to 100.0 ml with *ethanol (95 per cent)*. Dilute 10.0 ml of this solution further to 100.0 ml and measure the absorbance of the resulting solution at the maximum at about 252 nm (2.4.7). Calculate the content of $C_{19}H_{24}N_2S,HCl$, taking 845 as the specific absorbance at 252 nm.

**Storage**. Store protected from light.

# Ethosuximide



$C_7H_{11}NO_2$ Mol. Wt. 141.2

Ethosuximide is (*RS*)-2-ethyl-2-methylsuccinimide.

Ethosuximide contains not less than 98.0 per cent and not more than 101.0 per cent of $C_7H_{11}NO_2$, calculated on the anhydrous basis.

**Category**. Anticonvulsant.

**Dose**. 500 mg daily, in divided doses, increasing to 2 g, as necessary; for a child, 50 to 125 mg twice daily, increasing to 250 mg, three to four times daily, as necessary.

**Description**. A white or almost white powder or waxy solid.

## Identification

*Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Melt a sufficient quantity at 50°, prepare a thin film between two previously warmed bromide plates and record the spectrum immediately. Compare the spectrum with that obtained with *ethosuximide RS*.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.1 per cent w/v solution in *ethanol (95 per cent)* shows an absorption maximum at about 248 nm; absorbance at 248 nm, about 0.85.

C. Dissolve 0.1 g in 3 ml of *methanol* and add 0.05 ml of a 10 per cent w/v solution of *cobalt chloride*, 0.05 ml of a 10 per cent w/v solution of *calcium chloride* and 0.1 ml of *2 M sodium hydroxide*; a purple colour develops and no precipitate is produced.

## Tests

**Appearance of solution**. Dissolve 2.5 g in sufficient *water* to produce 25 ml. The solution is clear (2.4.1) and colourless (2.4.1).

**Acidity**. Dissolve 5.0 g in 50 ml of *water* by warming on a water-bath for 5 minutes. Cool and titrate with *0.1 M sodium hydroxide* using *bromocresol green solution* as indicator. Not more than 0.7 ml of *0.1 M sodium hydroxide* is required.

**Cyanide**. Dissolve 1.0 g in 10 ml of *ethanol (90 per cent)* and add 0.5 ml of *ferrous sulphate solution*, 1 ml of *2 M sodium hydroxide* and 0.1 ml of *ferric chloride solution*. Heat to boiling, cool and acidify using 3 ml of *1 M sulphuric acid*. After 15 minutes, there is no blue colour and no blue precipitate is produced.

**Related substances**. Determine by gas chromatography (2.4.13).

*Test solution* (a) Dissolve 1 g of the substance under examination in sufficient *chloroform* to produce 10 ml.

*Test solution (b)*. Dilute 5 ml of test solution (a) to 10 ml with a 0.01 per cent w/v solution of *anthracene* (internal standard) in *chloroform*.

*Reference solution (a)*. Dissolve 10 mg of *2-ethyl-2-methylsuccinic acid* in sufficient *chloroform* to produce 10 ml.

*Reference solution (b)*. Dilute 1 ml of test solution (a) to 100 ml with *chloroform*. To 1 ml of this solution add 5 ml of the internal standard solution and sufficient *chloroform* to produce 10 ml.

*Reference solution (c)*. Dilute 1 ml of test solution (b) to 50 ml with *chloroform*. Add 1 ml of this solution to 1 ml of reference solution (a), add 5 ml of the internal standard solution and sufficient *chloroform* to produce 10 ml.



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Chromatographic system
- a glass column 2 m x 2 mm, packed with silanised diatomaceous support (125 to 180 mesh) impregnated with 3 per cent w/w polycyanopropylmethyl-phenylmethyl siloxane,
- temperature:
  column.165°,
  inlet port and detector. 240°,
- flow rate. 30 ml per minute of the carrier gas.

Inject 1 µl of reference solution (c) and adjust the sensitivity of the detector so that the heights of the three principal peaks are not less than 70 per cent of full-scale deflection. The peaks in order of emergence, are due to 2-ethyl-2-methylsuccinic acid, ethosuximide, and anthracene.

The test is not valid unless the resolution factor between the peaks corresponding to 2-ethyl-2-methylsuccinic acid and ethosuximide in the chromatogram obtained with test solution (a) is at least 4.

Inject 1 µl of test solution (a) and verify that there is no peak with the same retention time as the internal standard. Inject separately 1 µl of test solution (b) and reference solution (b) and record the chromatogram for twice the retention time of ethosuximide. Calculate the ratio (R) of the area of the peak due to ethosuximide to the area of the peak due to the internal standard in the chromatogram obtained with reference solution (b). In the chromatogram obtained with test solution (b) the ratio of the sum of the areas of any secondary peaks to the area of the peak due to the internal standard is not greater than R.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 0.5 per cent, determined on 1.0 g.

**Assay**. Weigh accurately about 0.12 g, dissolve 20 ml of *dimethylformamide* and add 0.2 ml of a 0.5 per cent w/v solution of *thymolphthalein* in *dimethylformamide*. Titrate with *0.1 M tetrabutylammonium hydroxide*, until a distinct blue colour is produced.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.01412 g of $C_7H_{11}NO_2$.

**Storage**. Store protected from light.

# Ethosuximide Capsules

Ethosuximide Capsules contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of ethosuximide, $C_7H_{11}NO_2$.

**Usual strength**. 250 mg.

## Identification

A. Heat a quantity of the contents of the capsules containing 0.1 g of Ethosuximide with 0.2 g of *resorcinol* and 0.1 ml of *sulphuric acid* at 140° for 5 minutes, add 5 ml of *water*, make alkaline with *5 M sodium hydroxide* and add 0.2 ml to a large volume of *water;* a bright green fluorescence is produced.

B. Shake a quantity of the contents of the capsules containing 0.25 g of Ethosuximide with 80 ml of *ethanol (95 per cent)* for a few minutes, add sufficient *ethanol (95 per cent)* to produce 100 ml, mix and filter. Dilute 20 ml of the filtrate to 100 ml with *ethanol (95 per cent)*. Absorbance of the resulting solution at the maximum at about 248 nm, about 0.43 (2.4.7).

## Tests

**Other tests**. Comply with the tests stated under Capsules.

**Assay**. Weigh accurately a quantity of the contents of the capsules containing about 0.2 g of Ethosuximide and dissolve in 30 ml of *dimethylformamide*. Titrate with *0.1 M tetrabutylammonium hydroxide,* using a 0.1 per cent w/v solution of *azo violet* in *dimethylformamide* as indicator. Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.01412 g of $C_7H_{11}NO_2$.

**Storage**. Store protected from moisture at a temperature not exceeding 30°.

# Ethosuximide Syrup

Ethosuximide Oral Solution

Ethosuximide Syrup is a solution of Ethosuximide in a suitable flavoured vehicle.

Ethosuximide Syrup contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of ethosuximide, $C_7H_{11}NO_2$.

**Usual strength**. 250 mg in 5 ml.

## Identification

A. Extract a quantity of the syrup containing 0.5 g of Ethosuximide with two quantities, each of 30 ml, of *chloroform,* filter the combined extracts through a plug of cotton and evaporate the filtrate to dryness. Heat 100 mg of the residue with 0.2 g *of resorcinol* and 0.1 ml of *sulphuric acid* at 140° for 5 minutes, cool, add 5 ml of *water,* make alkaline with *5 M sodium hydroxide* and add 0.2 ml to a large volume of *water*; a bright green fluorescence is produced.

B. In the Assay, the principal peak in the chromatogram obtained with test solution (b) has the same retention time as



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that of the peak due to ethosuximide in the chromatogram obtained with reference solution (a).

### Tests

**Other tests**. Complies with the tests stated under Oral liquids.

**Assay**. Determine by gas chromatography (2.4.13).

*Test solution (a)*. Add 10 ml of *water* and 2 g of *sodium bicarbonate* to a weighed quantity of the syrup containing about 0.25 g of Ethosuximide and extract with five quantities, each of 25 ml, of *chloroform*, washing each extract with the same 10 ml of *water*. To the combined extracts add 10 ml of a 3.0 per cent w/v solution of *dimethyl phthalate* (internal standard) in *chloroform*, shake with 10 g of *anhydrous sodium sulphate* and filter.

*Test solution (b)*. Prepare in the same manner as test solution (a) but omit the internal standard.

*Reference solution*. Add 2 ml of the internal standard solution to 25.0 ml of a 0.2 per cent w/v solution of *ethosuximide RS* in *chloroform*.

Chromatographic system
- a glass column 1.5 m x 4 mm, packed with acid-washed silanised diatomaceous support (80 to 100 mesh) impregnated with 3 per cent w/w of cyanopropylmethyl phenyl methyl silicone fluid (OV-225) of cyanopropylmethyl phenyl methyl silicone fluid (such as OV-225),
- temperature:
  column.165°,
  inlet port and detector. 240°,
- flow rate. 30 ml per minute of the carrier gas.

Determine the weight per ml of the syrup (2.4.29), and calculate the content of $C_7H_{11}NO_2$, weight in volume.

**Storage**. Store at a temperature not exceeding 30°.

## Ethylcellulose

Cellulose ethyl ether

Ethylcellulose is an ethyl ether of cellulose.

Ethylcellulose contains not less than 44.0 per cent and not more than 51.0 per cent of ethoxy ($-OC_2H_5$) groups, calculated on the dried basis.

**Category.** Pharmaceutical aid.

**Description**. A white to light tan powder; almost odourless.

### Identification

Dissolve 15 mg of the dried sample in 10 ml of dried *dichloromethane*. Grind 0.5 ml of this solution to dryness with 0.3 g of *potassium bromide*. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ethylcellulose RS*.

### Tests

**pH** (2.4.24). 5.5 to 8.0, determined in a solution prepared in the following manner. Stir 1.0 g with 50 ml of *carbon dioxide-free water* previously heated to 90°, then cool and dilute with sufficient *carbon dioxide-free water* to produce 100 ml.

**Apparent viscosity**. 90.0 to 110.0 per cent of that stated on the label for viscosity types of 10 mPa s or more; 80.0 to 120.0 per cent of that stated on the label for viscosity types of 6 to 10 millipascal seconds; 75.0 to 140.0 per cent of that stated on the label for viscosity types of 6 millipascal seconds or less, determined by the following method. Weigh accurately about 5.0 g, calculated on the dried basis and dissolve in 95.0 ± 0.05 g of a mixture of 80 parts of *toluene* and 20 parts of *ethanol* by weight. For ethylcellulose containing less than 46.5 per cent of ethoxy groups use a mixture of 60 parts of *toluene* and 40 parts of *ethanol*. Determine the viscosity at 25° by Method A (2.4.28).

**Arsenic** (2.3.10). Mix 1.0 g with 5 ml of *sulphuric acid AsT*, add a few glass beads and digest in a fumehood, preferably on a hot plate at a temperature not exceeding 120°, until charring begins. (Additional acid may be necessary to wet some samples completely but the total volume added should not exceed 10 ml). Cautiously add, dropwise, *hydrogen peroxide solution (30 per cent)* allowing the reaction to subside and again heating between additions of drops. Add the first few drops very slowly with sufficient mixing to prevent a rapid reaction. Discontinue heating if foaming becomes excessive. When the reaction has abated, heat cautiously, rotating the flask occasionally to prevent the sample from caking on glass exposed to the heating unit. *Maintain oxidising conditions at all times during the digestion by adding small quantities of the hydrogen peroxide solution whenever the mixture turns brown or darkens*. Continue the digestion until the organic matter is destroyed, gradually raising the temperature of the heating unit until fumes of sulphur trioxide are copiously evolved and the solution becomes colourless or retains only a light straw colour. Cool, add cautiously 10 ml of *water*, mix, and again evaporate till strong fuming, repeating this procedure to remove any trace of hydrogen peroxide. Cool, add cautiously 10 ml of *water*, wash the sides of the flask with a few ml of *water*, and dilute with *water* to 35 ml. The resulting solution complies with the limit test for arsenic (3 ppm).

**Heavy metals** (2.3.13). 0.5 g complies with the limit test for heavy metals, Method B (40 ppm).



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**Sulphated ash** (2.3.18). Not more than 0.5 per cent.

**Loss on drying** (2.4.19). Not more than 3.0 per cent, determined on 1.0 g by drying in an oven at 105° for 2 hours.

**Assay**. Weigh accurately about 50 mg in an empty, tared Hard Gelatin Capsule Shell and carry out the determination of methoxyl (2.3.29).

1 ml of *0.1 M sodium thiosulphate* is equivalent to 0.0007510 of ethoxy ($-OC_2H_5$) groups.

**Labelling**. The label states the apparent viscosity in mPa s of a 2.0 per cent w/v solution and its ethoxy content.

# Ethyl Chloride

$CH_3CH_2Cl$

$C_2H_5Cl$ Mol. Wt. 64.5

Ethyl Chloride is chloroethane.

**Category**. Anaesthetic.

**Description**. Gaseous at ambient temperatures and pressures but is generally compressed to a colourless, mobile, flammable and very volatile liquid; odour, pleasant and ethereal.

## Identification

A. Burns with a luminous flame with the production of hydrogen chloride.

B. Hydrolyse a few ml with *5 M sodium hydroxide*; the resulting solution gives the reactions of chlorides (2.3.1), and after the addition of *iodine solution* and warming, crystals of iodoform are produced.

## Tests

**Acidity or alkalinity**. Shake 10 ml with 10 ml of ice-cold *water* and allow the ethyl chloride to evaporate at room temperature; the residual liquid (liquid A) is neutral to *litmus solution*.

**Ionisable chlorides**. 5 ml of liquid A gives no turbidity with *silver nitrate solution*.

**Ethanol**. Warm 5 ml of liquid A with *iodine solution* and *sodium carbonate*; no iodoform is produced.

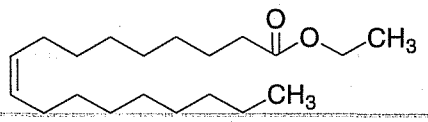
**Distillation range**. Into a dry 100-ml measuring cylinder insert a stopper carrying a short exit tube not less than 6 mm in internal diameter and an accurately standardised short-bulb thermometer covering the range –20° to +30° and graduated in tenths of a degree. Cover the bulb of the thermometer with a piece of very fine muslin, free from grease and sizing materials, so that one end hangs down about 10 mm below the bulb. Cool the cylinder in ice-water, transfer to it 100 ml of the sample, previously cooled in ice-water, insert the stopper and adjust the thermometer so that the end of the muslin dips into the liquid and the bulb is above the surface. Replace the ice-water with water at 24° to 26° and observe the temperature when 5 ml of sample has evaporated and again when 5 ml remains. Continually lower the thermometer to maintain its position relative to the liquid surface throughout the test. Correct the observed temperature by adding 0.26° for every kPa that the barometric pressure is below 101.3 kPa or by subtracting 0.26° for every kPa above. The corrected temperature is not lower than 12.0° and not higher than 12.5°.

**Other organic compounds**. On evaporation, no foreign odour is detectable at any stage.

**Non-volatile matter**. Not more than 0.01 per cent w/w, when evaporated and dried at 105°.

**Storage**. Store protected from light in a refrigerator (2° to 8°).

# Ethyl Oleate




$C_{20}H_{38}O_2$ Mol. Wt. 310.5

Ethyl Oleate consists of the ethyl esters of (*Z*)-oleic and related acids.

Ethyl Oleate contains not less than 100.0 per cent w/w and not more than 105.0 per cent w/w of the ethyl esters of (*Z*)-oleic and related acids, calculated as $C_{20}H_{38}O_2$.

**Category**. Pharmaceutical aid.

**Description**. A pale yellow oil; odour, slight but not rancid.

## Tests

**Weight per ml** (2.4.29). 0.869 g to 0.874 g, determined at 20°.

**Acid value** (2.3.23). Not more than 0.5.

**Peroxides**. Dissolve 5.0 g in 15 ml of *chloroform*, add 20 ml of *glacial acetic acid* and 0.5 ml of a saturated solution of *potassium iodide*, mix and allow to stand for exactly 1 minute in the dark. Add 30 ml of *water* and titrate with *0.01 M sodium thiosulphate* using *starch solution* as indicator; not more than 2.5 ml of *0.01 M sodium thiosulphate* is required.

**Iodine value** (2.3.28). 75 to 85.

**Assay**. Boil a suitable volume of *ethanol (95 per cent)* to expel carbon dioxide and neutralise it to *phenolphthalein*



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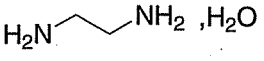


*solution*. Weigh accurately about 2.0 g of the substance under examination, dissolve in 5 ml of the neutralised ethanol contained in a hard-glass flask and neutralise the free acid in the solution with *0.1 M ethanolic potassium hydroxide* using 0.2 ml of *phenolphthalein solution* as indicator. Add 25.0 ml of *0.5 M ethanolic potassium hydroxide* and boil under a reflux condenser on a water-bath for 1 hour and continue boiling for 2 hours over a flame. Add 20 ml of *water* and titrate the excess of alkali with *0.5 M hydrochloric acid* using a further 0.2 ml of *phenolphthalein solution* as indicator. Repeat the operation without the substance under examination. The difference between the titres represents the alkali required to saponify the substance under examination.

1 ml of *0.5 M ethanolic potassium hydroxide* is equivalent to 0.1553 g of $C_{20}H_{38}O_2$.

**Storage**. Store protected from light in small, well-filled and well-closed containers under an atmosphere of nitrogen.

## Ethylenediamine Hydrate



$C_2H_8N_2,H_2O$ Mol. Wt. 78.1

Ethylenediamine Hydrate is ethane-1,2-diamine monohydrate.

Ethylenediamine Hydrate contains not less than 97.5 per cent w/w and not more than 101.5 per cent w/w of $C_2H_8N_2,H_2O$.

**Category**. Pharmaceutical aid (for Aminophylline Injection).

**Description**. A clear, colourless or slightly yellow liquid; odour, ammoniacal.

### Identification

A. Dilute 1 ml to 6 ml with *water*. To 3 drops of the solution add 2 ml of a 1 per cent w/v solution of *copper sulphate* and shake; a purple-blue colour is produced.

B. It is strongly alkaline.

### Tests

**Ammonia and other bases**. Weigh accurately about 1.5 ml and transfer with the aid of *ethanol (95 per cent)* to a small dish. Add, with stirring, 20 ml of *dilute hydrochloric acid*. Evaporate the solution to dryness on a water-bath, breaking up any cake formed with a glass rod, and dry at 105° for 1 hour.

1 g of residue is equivalent to 0.5872 g of $C_2H_8N_2,H_2O$.

Calculate the percentage of $C_2H_8N_2,H_2O$; the result is within 0.5 per cent of the percentage of ethylenediaminehydrate determined in the Assay.

**Heavy metals** (2.3.13). Evaporate 5.0 ml on a water-bath to dryness, add to the residue 1 ml of *hydrochloric acid* and 0.5 ml of *nitric acid* and evaporate to dryness. Dissolve the residue in 20 ml of warm *water*, cool, add sufficient *water* to produce 100 ml and mix. 20 ml of the resulting solution complies with the limit test for heavy metals, Method A (20 ppm).

**Iron** (2.3.14). To the residue obtained in the test for Non-volatile matter add 1 ml of *hydrochloric acid* and 0.5 ml of *nitric acid* and evaporate to dryness on a water-bath. Dissolve the residue in 20 ml of warm *water* and dilute with *water* to 100 ml. 40 ml of the solution complies with the limit test for iron (20 ppm).

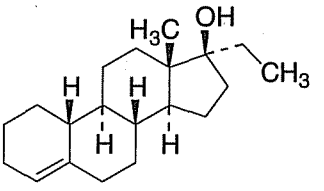
**Non-volatile matter**. Not more than 0.02 per cent w/v, determined on 5.0 ml by evaporating to dryness on a water-bath and drying at 105° for 1 hour.

**Assay**. Weigh accurately about 1.0 g, dissolve in 75 ml of *water* and titrate with *1 M hydrochloric acid* using *bromophenol blue solution* as indicator until a yellow colour is produced.

1 ml of *1 M hydrochloric acid* is equivalent to 0.03906 g of $C_2H_8N_2,H_2O$.

**Storage**. Store protected from light.

## Ethyloestrenol



$C_{20}H_{32}O$ Mol. Wt. 288.5

Ethyloestrenol is 17α-ethylestr-4-en-17β-ol containing a variable amount of methanol of crystallisation.

Ethyloestrenol contains not less than 95.0 per cent and not more than 103.0 per cent of $C_{20}H_{32}O$, calculated on the anhydrous and methanol-free basis.

**Category**. Anabolic steroid.

**Dose**. 2 to 4 mg daily.

**Description**. A white or almost white, crystalline powder; almost odourless.

### Identification

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



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B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 80 volumes of *heptane* and 20 volumes of *acetone*.

*Solvent mixture*. A mixture of 90 volumes of *chloroform* and 10 volumes of *methanol*.

*Test solution*. Dissolve 0.25 g of the substance under examination in 100 ml with solvent mixture.

*Reference solution (a)*. A 0.25 per cent w/v solution of *ethyloestrenol RS* in the same solvent mixture.

*Reference solution (b)*. A mixture of equal volumes of the test solution and reference solution (a).

Apply to the plate 2 µl of each solution. After development, dry the plate in air, heat it at 105° for 10 minutes, spray with *ethanolic sulphuric acid (20 per cent)* and heat at 105° for a further 10 minutes. Allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

## Tests

**Specific optical rotation** (2.4.22). +29.0° to +33.0°, determined in a 1.0 per cent w/v solution in *dioxan*.

**17α-Ethyloestran-17β-ol**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G* containing 20 per cent w/v of *silver nitrate*.

*Mobile phase*. A mixture of 75 volumes of *toluene* and 25 volumes of *nonan-5-one*.

*Solvent mixture*. A mixture of 9 volumes of *chloroform* and 1 volume of *methanol*.

*Test solution*. Dissolve 0.4 g of the substance under examination in 10 ml with solvent mixture.

*Reference solution*. A 0.08 per cent w/v solution of *17α–ethyloestran-17β-ol RS* in the same solvent mixture.

Apply to the plate 5 µl of each solution. After development, dry the plate, heat it at 105° for 10 minutes, spray with *ethanolic sulphuric acid (20 per cent)*, heat at 105° for a further 10 minutes and allow to cool. Any spot corresponding to 17α–ethyloestran-17β-ol in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Related substances**. Carry out Identification test B but using 10 µl of the following solutions.

*Test solution*. A 1 per cent w/v solution of the substance under examination.

*Reference solution (a)*. A 0.01 per cent w/v solution of the substance under examination.

*Reference solution (b)*. A 0.005 per cent w/v solution of the substance under examination.

Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

**Methanol**. Not more than 4.0 per cent w/w, determined by the following method.

Determine by gas chromatography (2.4.13).

*Test solution (a)*. A solution containing 10.0 per cent w/v of the substance under examination in *acetone*.

*Test solution (b)*. A solution containing 10.0 per cent w/v of the substance under examination and 0.4 per cent v/v of *ethanol* (internal standard) in *acetone*.

*Reference solution*. A solution containing 0.4 per cent v/v of *methanol* and 0.4 per cent v/v of the internal standard in *acetone*.

Chromatographic system
- a glass column 2.0 m x 0.4 mm, packed with porous polymer beads (100 to 120 mesh) (such as Porapak Q),
- temperature:
  column.170°,
  inlet port and detector. 240°,
- flame ionisation detector,
- flow rate. 30 ml per minute of the carrier gas (nitrogen).

Calculate the percentage w/w of methanol, assuming its weight per ml at 20° to be 0.792 g.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 0.5 per cent, determined on 5.0 g.

**Assay**. Determine by gas chromatography (2.4.13).

*Test solution (a)*. A solution containing 0.2 per cent w/v of the substance under examination in *chloroform*.

*Test solution (b)*. A solution containing 0.2 per cent w/v of the substance under examination and 0.1 per cent w/v of *arachidic alcohol* (internal standard) in chloroform.

*Reference solution*. A 0.2 per cent w/v solution of *ethyloestrenol RS* in *chloroform*.

Chromatographic system
- a glass column 1.0 m x 4 mm, packed with acid-washed, silanised diatomaceous support (80 to 100 mesh) coated with 3 per cent w/w of phenyl methyl silicone fluid (50 per cent phenyl) (such as OV-17),



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- temperature:
  column.200°,
  inlet port and detector. 280°,
- flame ionisation detector,
- flow rate. 30 ml per minute of the carrier gas (nitrogen).

Calculate the content of $C_{20}H_{32}O$.

**Storage**. Store protected from light in a refrigerator (2° to 8°).

# Ethyloestrenol Tablets

Ethyloestrenol Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ethyloestrenol, $C_{20}H_{32}O$.

**Usual strength**. 2 mg.

## Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 80 volumes of *heptane* and 20 volumes of *acetone*.

*Solvent mixture*. A mixture of 90 volumes of *chloroform* and 10 volumes of *methanol*.

*Test solution*. Extract a quantity of the powdered tablets containing 1 mg of Ethyloestrenol with *chloroform*, filter, evaporate the filtrate to dryness at room temperature at a pressure not exceeding 0.2 kPa and dissolve the residue in 0.4 ml with solvent mixture.

*Reference solution (a)*. A 0.25 per cent w/v solution of *ethyloestrenol RS* in the same solvent mixture.

*Reference solution (b)*. A mixture of equal volumes of the test solution and reference solution (a).

Apply to the plate 2 µl of each solution. After development, dry the plate in air, heat it at 105° for 10 minutes, spray with *ethanolic sulphuric acid (20 per cent)* and heat at 105° for a further 10 minutes. Allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

B. In the Assay, the principal peak the chromatogram obtained with the test solution has the same retention time as that of the peak due to *ethyloestrenol RS* in the chromatogram obtained with the reference solution (a).

## Tests

**17α-Ethyloestran-17β-ol**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G* containing 20 per cent w/v of *silver nitrate*.

*Mobile phase*. A mixture of 75 volumes of *toluene* and 25 volumes of *nonan-5-one*.

*Solvent mixture*. A mixture of 90 volumes of *chloroform* and 10 volumes of *methanol*.

*Test solution*. Dissolve 20 mg of the residue obtained in the test for Related substances in 0.5 ml with solvent mixture.

*Reference solution*. A 0.08 per cent w/v solution of *17α-ethyloestran-17β-ol RS* in the same solvent mixture.

Apply to the plate 5 µl of each solution. After development, dry the plate, heat it at 105° for 10 minutes, spray with *ethanolic sulphuric acid (20 per cent)*, heat at 105° for a further 10 minutes and allow to cool. Any spot corresponding to 17α-ethyloestran-17β-ol in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Related substances**. Carry out Identification test A but using 10 µl of the following solutions.

*Solvent mixture*. A mixture of 90 volumes of *chloroform* and 10 volumes of *methanol*.

*Test solution*. Extract a quantity of the powdered tablets containing 40 mg of Ethyloestrenol with *chloroform*, filter, evaporate the filtrate to dryness and dissolve 10 mg of the residue in 1 ml with solvent mixture.

*Reference solution (a)*. Dilute 1 volume of the test solution to 100 volumes with the same solvent mixture.

*Reference solution (b)*. Dilute 1 volume of reference solution (a) to 2 volumes with the same solvent mixture.

Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

**Uniformity of content**. Comply with the test stated under Tablets.

Proceed as directed in the Assay using the following solutions.

*Test solution*. Powder one tablet, extract with 5 ml of *chloroform* in a centrifuge tube, centrifuge, evaporate 2.0 ml of the supernatant liquid in a current of nitrogen, dissolve the residue in 2 ml of *acetone*, evaporate the solution to dryness on a water-bath and dissolve the residue in 0.4 ml of *chloroform*.

*Reference solution (a)*. A solution containing 0.2 per cent w/v of *ethyloestranol RS* and 0.1 per cent w/v of *arachidic alcohol* (internal standard) in *chloroform*.



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*Reference solution (b)*. Prepare in the same manner as the test solution on 2.0 ml of the supernatant liquid but by extracting with 2.0 ml of a 0.02 per cent w/v solution of *arachidic alcohol* in *acetone*.

Calculate the content of $C_{20}H_{32}O$ in the tablet.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by gas chromatography (2.4.13).

*Test solution*. Weigh and powder 20 tablets. Extract a quantity of the powdered tablets containing about 8 mg of Ethyloestrenol with 20 ml of *acetone*, filter, evaporate the filtrate to dryness on a water-bath and dissolve the residue in 4.0 ml of *chloroform*.

*Reference solution (a)*. A solution containing 0.2 per cent w/v of *ethyloestrenol RS* and 0.1 per cent w/v of *arachidic alcohol* (internal standard) in *chloroform*.

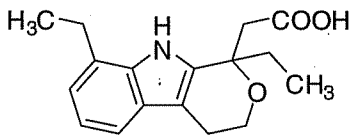
*Reference solution (b)*. Prepared in a similar manner as solution (1) but extracting with 20 ml of a 0.02 per cent w/v solution of *arachidic alcohol* in *acetone*.

Chromatographic system
- a glass column 1.0 m x 4 mm, packed with acid-washed, silanised diatomaceous support (80 to 100 mesh) coated with 3 per cent w/w of phenyl methyl silicone fluid (50 per cent phenyl) (such as OV-17),
- temperature:
  column.200°,
  inlet port and detector. 280°,
- flame ionisation detector,
- flow rate. 30 ml per minute of the carrier gas (nitrogen).

Calculate the content of $C_{20}H_{32}O$ in the tablets.

**Storage**. Store protected from light in a refrigerator (2° to 8°).

# Etodolac




$C_{17}H_{21}NO_3$ Mol. Wt. 287.4

Etodolac is 1,8-diethyl-1,3,4,9-tetrahydropyrano [3,4-*b*]indole-1-acetic acid.

Etodolac contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{17}H_{21}NO_3$, calculated on the anhydrous basis.

**Category**. Analgesic; antiinflammatory.

**Description**. A white or almost white, crystalline powder.

## Identification

*Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.*

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

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 0.5 volume of *glacial acetic acid*, 30 volumes of *anhydrous ethanol* and 70 volumes of *toluene*.

*Test solution*. Dissolve 10 mg of the substance under examination in 10 ml of *acetone*.

*Reference solution*. A 0.1 per cent w/v solution of *etodolac RS* in *acetone*.

Impregnate the TLC plate by heating at 105° for 1 hour. Place the plate in an unsaturated chamber containing a mixture of 20 volumes of a 2.5 per cent w/v solution of *ascorbic acid* and 80 volumes of *methanol*. Allow the solution to rise 1 cm above the line of application on the plate, remove the plate and allow it to dry for 30 minutes.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 15 cm of the plate. Dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

C. Melting point (2.4.21). 144° to 150°.

## Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 0.02 g of the substance under examination in *acetonitrile* and dilute to 50.0 ml with the same solvent.

*Reference solution (a)*. Dilute 1.0 ml of the test solution to 50.0 ml with *acetonitrile*. Dilute 1.0 ml of this solution to 20.0 ml with *acetonitrile*.

*Reference solution (b)*. Dissolve 4 mg of *2-(7-ethyl-1H-indol-3-yl)ethanol RS (etodolac impurity H)* in 10.0 ml of the test solution . Dilute 0.5 ml of this solution to 50.0 ml with *acetonitrile*.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with endcapped butylsilane bonded to porous silica (3.5 µm),
- mobile phase: A. 0.077 per cent w/v solution of *ammonium acetate*,



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B. a mixture of 10 volumes of mobile phase A and 90 volumes of *acetonitrile*,

- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume. 5 µl.

| Time (min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0-25 | 80→50 | 20→50 |
| 25-42 | 50 | 50 |
| 42-48 | 50→80 | 50→20 |

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to etodolac and etodolac impurity H is not less than 5.0. The relative retention time with reference to etodolac for 8-desethyl etodolac (etodolac impurity A) is about 0.68, for 8-methyl etodolac (etodolac impurity B) is about 0.83, for 1-methyl etodolac (etodolac impurity C) is about 0.85, for 2-(7-ethyl-1*H*-indol-3-yl)ethanol (etodolac impurity H) is about 1.09, for 8-isopropyl etodolac (etodolac impurity D) is about 1.17, for 1-propyl etodolac (etodolac impurity G) is about 1.19, for 8-propyl etodolac (etodolac impurity E) is about 1.2, for 1-isopropyl etodolac (etodolac impurity F) is about 1.22, for etodolac dimer (etodolac impurity I) is about 1.5 and for etodolac methyl ester (etodolac impurity K) is about 2.37.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of secondary peak due to etodolac impurity C is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent); the area of each secondary peak corresponding to etodolac impurity A, B, D, E, F, G, H, I, K is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent); the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of all other secondary peaks is not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

**Chlorides**. Dissolve 1.0 g of the substance under examination in 60 ml of *methanol*, add 10 ml of *water* and 20 ml of *dilute nitric acid*. Titrate with *0.01 M silver nitrate*, determining the end-point potentiometrically (2.4.25).

1 ml of *0.01 M silver nitrate* is equivalent to 0.0003545 g of Cl (300 ppm).

**Water** (2.3.43). Not more than 0.5 per cent, determined on 1.0 g.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Assay**. Weigh accurately about 0.25 g and dissolve in 60 ml of *methanol*. Titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.02874 g of $C_{17}H_{21}NO_3$.

# Etodolac Capsules

Etodolac Capsules contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of etodolac, $C_{17}H_{21}NO_3$.

**Usual strengths**. 200 mg; 300 mg.

## Identification

To a quantity of the contents of the capsules containing about 0.1 g of Etodolac, add 4 ml of *0.01 M hydrochloric acid* and mix with the aid of ultrasound for 5 minutes, shaking occasionally, centrifuge for 10 minutes, discard the supernatant liquid and wash the residue with 4 ml of *water*. Shake to disperse, centrifuge for 10 minutes and discard the supernatant liquid. Add 4 ml of *0.01 M sodium hydroxide* to the residue and mix with the aid of ultrasound for 5 minutes, shaking occasionally and centrifuge for 10 minutes. Transfer the supernatant liquid to a second centrifuge tube, add about 1 ml of *0.1 M hydrochloric acid,* the pH of the supernatant liquid should be 2 or less. Centrifuge for 10 minutes, discard the supernatant liquid and wash the residue with 4 ml of *water*, shake to disperse and centrifuge for 10 minutes. Discard the supernatant liquid and dry the residue at 105° for 1 hour. The residue complies with the following tests.

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

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

## Tests

**Dissolution** (2.5.2).

Apparatus No. 2,
Medium. 900 ml of *phosphate buffer pH 7.5,*



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Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter. Measure the absorbance of the filtrate, suitably diluted if necessary with dissolution medium at 278 nm (2.4.7). Calculate the content of etodolac, $C_{17}H_{21}NO_3$ in the medium from the absorbances obtained from a solution of known concentration of *etodolac RS*.

D. Not less than 70 per cent of the stated amount of $C_{17}H_{21}NO_3$.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 0.5 volumes of *glacial acetic acid*, 30 volumes of *absolute ethanol* and 70 volumes of *toluene*.

*Test solution*. Shake a quantity of the contents of capsules containing 0.2 g of Etodolac with 20.0 ml of *acetone*, mix with the aid of ultrasound for 5 minutes and filter.

*Reference solution (a)*. Dilute 1.0 ml of the test solution to 200.0 ml with *acetone*.

*Reference solution (b)*. Dilute 1.0 ml of reference solution (a) to 2.0 ml with *acetone*.

Place the plate in an unlined tank containing a solution prepared by dissolving 0.5 g of L-*ascorbic acid* in 20 ml of *water* and adding 80 ml of *methanol*. Allow the solution to ascend 1 cm above the line of application on the plate, remove the plate and allow it to dry for at least 30 minutes.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b) (0.25 per cent).

**Etodolac acid dimer**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 3 volumes of *glacial acetic acid*, 17 volumes of *1,4-dioxan* and 60 volumes of *toluene*.

*Test solution*. Shake a quantity of the contents of capsules containing 0.6 g of Etodolac with 20.0 ml of *acetone*, mix with the aid of ultrasound for 5 minutes and filter.

*Reference solution*. A 0.003 per cent w/v solution of *etodolac acid dimer RS* in *acetone*.

Place the plate in an unlined tank containing a solution prepared by dissolving 0.5 g of L-*ascorbic acid* in 20 ml of *water* and adding 80 ml of *methanol*. Allow the solution to ascend 1 cm above the line of application on the plate, remove the plate and allow it to dry for at least 30 minutes.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution corresponding to the acid dimer is not more intense than the spot in the chromatogram obtained with reference solution (0.1 per cent).

**Total methyl analogue impurities**. Determine by liquid chromatography (2.4.14).

*Test solution*. Shake a quantity of the contents of capsules containing 0.1 g of Etodolac with 40 ml of *methanol*, mix with the aid of ultrasound for 5 minutes, filter and dilute 10.0 ml of the filtrate to 25.0 ml with *water*.

*Reference solution*. Dilute 1.0 ml of a solution containing 0.025 per cent w/v each of *etodolac 1-methyl analogue RS* and *etodolac 8-methyl analogue RS* in *methanol* to 50.0 ml with *water*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 13 volumes of *acetonitrile*, 19 volumes of *methanol* and 68 volumes of a 1.74 per cent w/v solution of *dipotassium hydrogen phosphate*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to etodolac 8-methyl analogue and etodolac 1-methyl analogue is not less than 0.75.

Inject the reference solution and the test solution. Run the chromatogram twice the retention time of the principal peak.

Calculate the content of etodolac 1-methyl analogue and etodolac 8-methyl analogue in etodolac. Total content is not more than 1.0 per cent.

**Other tests**. Comply with the tests stated under Capsules.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve a quantity of the mixed contents of 20 capsules containing 50 mg of Etodolac with about 70 ml of *0.1 M sodium hydroxide* for 30 minutes, dilute to 100.0 ml with *0.1 M sodium hydroxide*, mix and filter through a glass-fibre filter. Dilute 2.0 ml of the filtrate to 100.0 ml with the mobile phase.

*Reference solution (a)*. Dilute 2.0 ml of a 0.05 per cent w/v solution of *etodolac RS* in *0.1 M sodium hydroxide* to 100.0 ml with the mobile phase.

*Reference solution (b)*. Add 2.0 ml of a 0.05 per cent w/v solution of *etodolac 1-methyl analogue RS* in *0.1 M sodium hydroxide* to 2.0 ml of a 0.05 per cent w/v solution of *etodolac*



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*RS* in *0.1 M sodium hydroxide* and dilute to 100 .0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 12.5 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Spherisorb ODS 1),
- mobile phase: a mixture of 45 volumes of *acetonitrile* and 55 volumes of *phosphate buffer pH 4.75*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to etodolac and etodolac 1-methyl analogue is not less than 1.5.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{17}H_{21}NO_3$ in the capsules.

# Etodolac Tablets

Etodolac Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of etodolac, $C_{17}H_{21}NO_3$.

**Usual strengths.** 200 mg; 300 mg; 400 mg.

## Identification

Shake a quantity of the powdered tablets containing 0.5 g of etodolac with 30 ml of *hexane* for 5 minutes, centrifuge, discard the clear hexane layer and add about 40 ml of *ether* to the residue, shake for 5 minutes, centrifuge for 5 minutes, decant the ether layer and filter if necessary. Evaporate the solution to dryness under nitrogen and add about 5 ml of *0.1 M hydrochloric acid* to the residue. Warm on a water-bath until the residue begins to crystallise and triturate with a glass rod to promote crystallisation. Cool the mixture in an ice bath, filter through a glass-fibre filter and dry the crystals at a pressure of 2 kPa at 60° for 1 hour, Complies with the following tests.

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

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

## Tests

**Dissolution** (2.5.2).

Apparatus No. 2,
Medium. 900 ml of *phosphate buffer pH 7.5,*
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter. Measure the absorbance of the filtrate, suitably diluted if necessary, at the maximum at about 278 nm (2.4.7). Calculate the content of etodolac, $C_{17}H_{21}NO_3$ in the medium from the absorbance obtained from a solution of known concentration of *etodolac RS*.

D. Not less than 70 per cent of the stated amount of $C_{17}H_{21}NO_3$.

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase.* A mixture of 0.5 volumes of *glacial acetic acid*, 30 volumes of *absolute ethanol* and 70 volumes of *toluene*.

*Test solution.* Shake a quantity of the powdered tablets containing 0.2 g of Etodolac with 20.0 ml of *acetone*, mix with the aid of ultrasound for 5 minutes and filter.

*Reference solution (a).* Dilute 1.0 ml of the test solution to 200.0 ml with *acetone*.

*Reference solution (b).* Dilute 1.0 ml of reference solution (a) to 2.0 ml with *acetone*.

Place the plate in an unlined tank containing a solution prepared by dissolving 0.5 g of L-*ascorbic acid* in 20 ml of *water* and adding 80 ml of *methanol*. Allow the solution to ascend 1 cm above the line of application on the plate, remove the plate and allow it to dry for at least 30 minutes.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b) (0.25 per cent).

**Etodolac acid dimer.** Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase.* A mixture of 3 volumes of *glacial acetic acid*, 17 volumes of *1,4-dioxan* and 60 volumes of *toluene*.

*Test solution.* Dissolve a quantity of the powdered tablets containing about 0.6 g of Etodolac with 20.0 ml of *acetone*, mix with the aid of ultrasound for 5 minutes and filter.

*Reference solution.* A 0.003 per cent w/v solution of *etodolac acid dimer RS* in *acetone*.

Place the plate in an unlined tank containing a solution prepared by dissolving 0.5 g of L-*ascorbic acid* in 20 ml of *water* and adding 80 ml of *methanol*. Allow the solution to ascend 1 cm above the line of application on the plate, remove the plate and allow it to dry for at least 30 minutes.



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Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution corresponding to the acid dimer is not more intense than the spot in the chromatogram obtained with the reference solution (0.1 per cent).

**Total methyl analogue impurities**. Total content is not more than 1.0 per cent.

Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh a quantity of the powdered tablets containing 0.1 g of Etodolac with 40 ml of *methanol*, mix with the aid of ultrasound for 5 minutes and filter. Dilute 10.0 ml of the filtrate to 25.0 ml with *water*.

*Reference solution*. Dilute 1.0 ml of a solution containing 0.025 per cent w/v each of *etodolac 1-methyl analogue RS* and *etodolac 8-methyl analogue RS* in *methanol* to 50.0 ml with *water*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 13 volumes of *acetonitrile*, 19 volumes of *methanol* and 68 volumes of a 1.74 per cent w/v solution of *dipotassium hydrogen phosphate*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to etodolac 8-methyl analogue and etodolac 1-methyl analogue is not less than 0.75.

Inject the reference solution and the test solution. Run the chromatogram twice the retention time of the principal peak.

Calculate the content of etodolac 1-methyl analogue and etodolac 8-methyl analogue in etodolac.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh and powder 20 tablets, shake a quantity of the powder containing 50 mg of Etodolac with 70 ml of *0.1 M sodium hydroxide* for 30 minutes, dilute to 100.0 ml with *0.1 M sodium hydroxide*, mix and filter through a glass-fibre filter. Dilute 2.0 ml of the solution to 100.0 ml with the mobile phase.

*Reference solution (a)*. Dilute 2.0 ml of a 0.05 per cent w/v solution of *etodolac RS* in *0.1 M sodium hydroxide* to 100.0 ml with the mobile phase.

*Reference solution (b)*. Add 2.0 ml of a 0.05 per cent w/v solution of *etodolac 1-methyl analogue RS* in *0.1 M sodium hydroxide* to 2.0 ml of a 0.05 per cent w/v solution of *etodolac RS* in *0.1 M sodium hydroxide* and dilute to 100 .0 ml with the mobile phase.

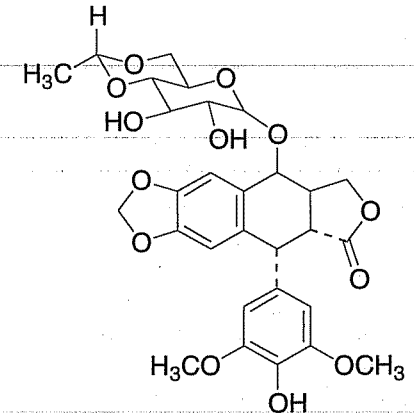
Chromatographic system
- a stainless steel column 12.5 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 45 volumes of *acetonitrile* and 55 volumes of *phosphate buffer pH 4.75*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between peaks due to etodolac and etodolac 1-methyl analogue is not less than 1.5.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{17}H_{21}NO_3$ in the tablets.

# Etoposide



$C_{29}H_{32}O_{13}$ Mol. Wt. 588.6

Etoposide is 4′-demethylepipodophyllotoxin 9-[4,6-*O*-ethylidene-β-D-glucopyranoside].

Etoposide contains not less than 95.0 per cent and not more than 105.0 per cent of $C_{29}H_{32}O_{13}$, calculated on the anhydrous basis.

*CAUTION — Etoposide is potentially cytotoxic. Great care should be taken in handling the powder and preparing solutions.*

**Category**. Anticancer.

**Description**. A white or almost white crystalline powder.



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## Identification

*Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *etoposide RS*.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H*.

*Solvent mixture*. 1 volume of *methanol* and 9 volumes of *dichloromethane*.

*Mobile phase*. A mixture of 1.5 volumes of *water*, 8 volumes of *glacial acetic acid*, 20 volumes of *acetone* and 100 volumes of *dichloromethane*.

*Test solution*. Dissolve 50 mg of the substance under examination in 10 ml of the solvent mixture.

*Reference solution*. A 0.5 per cent w/v solution of *etoposide RS* in the solvent mixture.

Apply to the plate 5µl of each solution as bands 10 mm by 3 mm. Allow the mobile phase to rise 17 cm. Dry the plate in warm air, spray it with a mixture of 1 volume of *sulphuric acid* and 9 volumes of *ethanol* and heat at 140° for 15 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

C. Dissolve 5 mg in 5 ml of *glacial acetic acid* and add 0.05 ml of *ferric chloride solution*. Mix and cautiously add 2 ml of *sulphuric acid*. Avoid mixing the 2 layers. Allow to stand for about 30 minutes; a pink to reddish-brown ring develops at the interface and the upper layer is yellow.

## Tests

**Appearance of solution**. A 3.0 per cent w/v solution in a mixture of 1 volume of *methanol* and 9 volumes of *dichloromethane* is clear (2.4.1) and not more intensely coloured than reference solution Y6 or BY6 (2.4.1).

**Specific optical rotation** (2.4.22). – 106.0° to - 114.0°, determined in a 0.5 per cent w/v solution in a mixture of 1 volume of *methanol* and 9 volumes of *dichloromethane*.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. Equal volumes of mobile phase A and mobile phase B.

*Test solution (a)*. Dissolve 40 mg of the substance under examination in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

*Test solution (b)*. Dissolve 50.0 mg of the substance under examination in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

*Reference solution (a)*. Dilute 1.0 ml of test solution (a) to 10.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 20.0 ml with the solvent mixture.

*Reference solution (b)*. Dilute 4.0 ml of reference solution (a) to 10.0 ml with the solvent mixture.

*Reference solution (c)* A 0.1 per cent w/v solution of *etoposide RS* in the solvent mixture.

*Reference solution (d)* To 10 ml of test solution (b), add 0.1 ml of a 4 per cent v/v solution of *glacial acetic acid* and 0.1 ml of *phenolphthalein solution*. Add *1 M sodium hydroxide* until the solution becomes faintly pink (about 0.15 ml). After 15 minutes, add 0.1 ml of a 4 per cent v/v solution of *glacial acetic acid*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature 40°,
- mobile phase: A. a mixture of 1 volume of *triethylamine*, 1 volume of *anhydrous formic acid* and 998 volumes of *water*,
  B. a mixture of 1 volume of *triethylamine*, 1 volume of *anhydrous formic acid* and 998 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 285 nm,
- injection volume. 10 µl.

| Time (min) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) | Comment |
|---|---|---|---|
| 0 | 75 | 25 | isocratic |
| 7 | 75 | 25 | begin linear gradient |
| 23 | 27 | 73 | end chromatogram, return to 75A |
| 25 | 75 | 25 | end equilibration, begin next chromatogram |

Inject reference solution (d). Continue the chromatography until the peak due to phenolphthalein is eluted. The chromatogram shows two principal peaks corresponding to etoposide and to *cis*-etoposide. Ignore any peak due to phenolphthalein.

Inject test solution (a) and reference solutions (a), (b) and (d). The resolution between the peaks due to etoposide and to *cis*-etoposide is not less than 3.0. The area of any impurity peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent) and not more than two such peaks have an area more



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than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent), the sum of the impurity peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1 per cent). Ignore any peak that is less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a).

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

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 6.0 per cent, determined on 0.25 g.

**Assay**. Determine by liquid chromatography (2.4.14).

Follow the chromatographic procedure described under Related substances.

Inject reference solution (c). The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately test solution (b) and reference solution (c).

Calculate the content of $C_{29}H_{32}O_{13}$.

**Storage**. Store protected from moisture.

# Etoposide Capsules

Etoposide Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of etoposide, $C_{29}H_{32}O_{13}$.

*CAUTION —Etoposide is potentially cytotoxic. Great care should be taken in handling the powder and preparing solutions.*

**Usual strengths**. 50 mg; 100 mg.

## Identification

Add a quantity of the contents of the capsules containing 0.1 g of Etoposide to a separating funnel containing 100 ml of *water*, extract with two quantities, each of 20 ml, of *dichloromethane*, dry the combined organic extracts over *anhydrous sodium sulphate* and filter. Extract the filtrate with 30 ml of *water*, filter the dichloromethane layer through *anhydrous sodium sulphate* and evaporate to dryness at 25° to 35° under reduced pressure. Dissolve the oily residue in 5 ml of *water*, shake gently and allow to stand for 30 minutes. Filter through a sintered-glass funnel, wash the precipitate in the funnel with three quantities, each of 20 ml, of *water* and dry the precipitate in the funnel at 40° at a pressure of 2 kPa for 90 minutes. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *etoposide RS*.

## Tests

***cis*-Etoposide**. Determine by liquid chromatography (2.4.14).

*Test solution (a)*. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 0.5 g of Etoposide, dissolve in the mobile phase and dilute to 100 ml with the mobile phase; use immediately.

*Test solution (b)*. Dilute 1 ml of test solution (a) to 50 ml with the mobile phase.

*Reference solution*. A 0.5 per cent w/v solution of *etoposide RS* in a mixture of 50 volumes of *acetonitrile*, 50 volumes of *water* and 0.1 volume of *triethylamine* and allow to stand for 40 minutes.

Use the chromatographic system described under Dissolution.

Inject the reference solution. The test is not valid unless the resolution between the principal peak and the peak immediately following the principal peak (*cis*-etoposide) is at least 1.0.

Inject test solution (a). The area of any peak corresponding to cis-etoposide is not more than the area of the peak in the chromatogram obtained with test solution (b) (2 per cent).

**Dissolution** (2.5.2).

Apparatus No. 2,
Medium. 900 ml of a pH 4.5 buffer prepared by dissolving 2.99 g of *sodium acetate* and 14 ml of *2 M acetic acid* in 1000 ml of *water*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Determine by liquid chromatography (2.4.14).

*Test solution*. Use the filtrate.

*Reference solution (a)*. A 0.005 per cent w/v solution of *etoposide RS* in the dissolution medium.

*Reference solution (b)*. A solution containing 0.005 per cent w/v of *etoposide RS* and 0.00025 per cent w/v of *ethyl parahydroxybenzoate* in the dissolution medium.

Chromatographic system
- a stainless steel column 30 cm × 3.9 mm, packed with phenyl silica gel (10 µm) (such as Bondapak phenyl),
- mobile phase: a mixture of 26 volumes of *acetonitrile* and 74 volumes of a 0.272 per cent w/v solution of *sodium acetate* adjusted to pH 4.0 with *glacial acetic acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.



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D. Not less than 80 per cent of the stated amount of $C_{29}H_{32}O_{13}$.

**Other tests.** Comply with the tests stated under Capsules.

**Assay.** Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh accurately a quantity of the mixed contents of 20 capsules containing about 40 mg of Etoposide dissolve in the mobile phase and dilute to 100.0 ml with the mobile phase; use immediately.

*Reference solution (a).* A 0.04 per cent w/v solution of *etoposide RS* in the mobile phase.

*Reference solution (b).* A solution containing 0.005 per cent w/v of *etoposide RS* and 0.00025 per cent w/v of *ethyl parahydroxybenzoate* in the mobile phase.

Use the chromatographic system described under Dissolution.

Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is at least 2.0.

Inject alternately the test solution and reference solution (a).

Calculate the content of $C_{29}H_{32}O_{13}$ in the capsules.

**Storage.** Store protected from moisture at a temperature not exceeding 30°. The capsules should not be stored in a refrigerator.

# Etoposide Injection

Etoposide Injection is a sterile material consisting of Etoposide Concentrate. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections in accordance with the manufacturer's instructions, immediately before use.

*The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Infusions).*

**Usual strength.** 20 mg per ml.

**Storage.** The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

## Etoposide Concentrate

Etoposide Concentrate is a sterile solution of Etoposide in a suitable ethanolic vehicle.

*The concentrate complies with the requirements stated under Parenteral Preparations (Concentrated Solutions for Injection) and with the following requirements.*

Etoposide Concentrate contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of etoposide, $C_{29}H_{32}O_{13}$.

### Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

*Mobile phase.* A mixture of 80 volumes of *dichloromethane*, 25 volumes of *acetone*, 2.5 volumes of *ethanol (95 per cent)* and 0.5 volume of *water.*

*Test solution.* Dilute a volume containing 20 mg of Etoposide to 25 ml with a mixture of 9 volumes of *dichloromethane* and 1 volume of *methanol.*

*Reference solution.* A 0.08 per cent w/v solution of *etoposide RS* in a mixture of 9 volumes of *dichloromethane* and 1 volume of *methanol.*

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 17 cm. Dry the plate in air, and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

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

### Tests

**pH** (2.4.24). 3.0 to 4.0, determined in a solution prepared by diluting a volume of the concentrate containing 0.1 g of Etoposide to 50 ml with *carbon dioxide-free water.*

***cis*-Etoposide.** Determine by liquid chromatography (2.4.14).

*Test solution.* Dilute a volume of the concentrate containing 0.5 g of Etoposide to 100 ml with the mobile phase.

*Reference solution (a).* Dilute 1.0 ml of the test solution to 50 ml with the mobile phase.

*Reference solution (b).* A 0.5 per cent w/v solution of *etoposide RS* in a mixture of 50 volumes of *acetonitrile*, 50 volumes of *water* and 0.1 volume of *triethylamine* and allow to stand for 40 minutes.

Use the chromatographic system described under Assay.

Inject reference solution (b). The test is not valid unless the resolution between the principal peak and the peak immediately after the principal peak (*cis*-etoposide) is not less than 1.0.

Inject the test solution and reference solution (a). The area of any peak corresponding to *cis*-etoposide is not more than the area of the principal peak in the chromatogram obtained with reference solution (a).



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**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Dilute a volume of the concentrate containing about 40 mg of Etoposide to 100.0 ml with the mobile phase.

*Reference solution.* A 0.04 per cent w/v solution of *etoposide RS* in the mobile phase.

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm, packed with phenyl silica gel (10 µm)(such as ìBondapak phenyl),
- mobile phase: a mixture of 26 volumes of *acetonitrile* and 74 volumes of a 0.272 per cent w/v solution of *sodium acetate* adjusted to pH 4.0 with *glacial acetic acid,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_{29}H_{32}O_{13}$ in the concentrate.

**Storage**. Store protected from light.

**Labelling**. The label states: (1) the directions for dilution of the contents; (2) that the diluted injection is to be given by intravenous injection; (3) that the concentrate should be protected from light.



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Famotidine Tablets .... 1332
Felodipine .... 1333
Felodipine Sustained-release Tablets .... 1335
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Fenofibrate .... 1337
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Furazolidone Oral Suspension .... 1395
Furazolidone Tablets .... 1395
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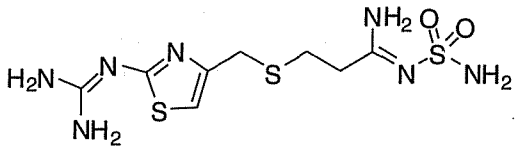
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# Famotidine



$C_8H_{15}N_7O_2S_3$ Mol. Wt. 337.5

Famotidine is [1-amino-3-[[[2-(diaminomethylene)amino]-4-thiazolyl]methyl]thio]propylidene]sulphamide.

Famotidine contains not less than 98.5 per cent and not more than 101.5 per cent of $C_8H_{15}N_7O_2S_3$, calculated on the dried basis.

**Category**. Antiulcer.

**Description**. A white or yellowish-white, crystalline powder or crystals.

## Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *famotidine RS* or with the reference spectrum of famotidine.

## Tests

**Appearance of solution**. Dissolve 0.2 g in a 5.0 per cent w/v solution of *hydrochloric acid*, heating to 40°, if necessary, and dilute to 20.0 ml with the same acid. The solution is clear (2.4.1) and not more intensely coloured than reference solution BYS7 (2.4.1).

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 12.5 mg of the substance under examination in 25.0 ml of mobile phase A.

*Reference solution (a)*. Dilute 1.0 ml of the test solution to 10.0 ml with mobile phase A. Dilute 1.0 ml of this solution to 100.0 ml with mobile phase A.

*Reference solution (b)*. Dissolve 2.5 mg of *3-[[[2-[(diaminomethylene)amino]thiazol-4-yl]methyl]sulphanyl] propanamide RS* ( *famotidine impurity D RS)* in *methanol* and dilute to 10.0 ml with the same solvent. To 1.0 ml of the solution, add 0.5 ml of the test solution and dilute to 100.0 ml with mobile phase A.

*Reference solution (c)*. Dissolve 5 mg of *famotidine for system suitability RS* (famotidine impurities A, B, C, D, E, F, G) in mobile phase A and dilute to 10.0 ml with mobile phase A.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature 50°,
- mobile phase: A. a mixture of 6 volumes of *methanol*, 94 volumes of *acetonitrile* and 900 volumes of a 0.1882 per cent w/v solution of *sodium hexanesulphonate* previously adjusted to pH 3.5 with *acetic acid*,
  B. *acetonitrile*,
- a linear gradient programme using the conditions given below,
- flow rate. 2 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume. 20 µl.

| Time (min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0 - 23 | 100 → 96 | 0 → 4 |
| 23 - 27 | 96 | 4 |
| 27 - 47 | 96 → 78 | 4 → 22 |
| 47 - 50 | 78 → 100 | 22 → 0 |
| 50 - 54 | 100 | 0 |

Inject reference solution (c). The relative retention time with reference to famotidine for 3-[[[2-[(diaminomethylene)amino] thiazol-4-yl]methyl]sulphanyl]propanamide ( famotidine impurity D) is about 1.1, for 3-[[[2-[(diaminomethylene) amino]amino]thiazol-4-yl]methyl]sulphanyl]-N-sulphamoylpropanamide ( famotidine impurity C ) is about 1.2, for N-cyano-3-[[[2-[(diaminomethylene)amino]thiazol-4-yl]methyl]sulphanyl]propanimidamide (famotidine impurity G ) is about 1.4, for 3-[[[2-[(diaminomethylene)amino]thiazol-4-yl]methyl]sulphanyl]propanoic acid ( famotidine Impurity F ) is about 1.5 , for 3-[[[2-[(diaminomethylene)amino]thiazol-4-yl]methyl]sulphanyl]propanimidamide (famotidine impurity A ) is about 1. 6, for 3,5-bis[2-[[[2-[(diaminomethylene) amino]thiazol-4-yl]methyl]sulphanyl]ethyl]-4H-1,2,4,6-thiatriazine1,1-dioxide ( famotidine impurity B) is about 2.0 , for 2,2'-[disulphanediylbis (methylenethiazole-4,2-diyl)] diguanidine ( famotidine Impurity E ) is about 2.1.

For calculation of contents, multiply the areas of each known impurity by its response factor, for impurity A=1.9, impurity B=2.5, impurity C=1.9, impurity F=1.7, impurity G=1.4 .

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to famotidine and famotidine impurity D is not less than 3.5.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of each secondary peak due to famotidine impurity A and G is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent ) and for each famotidine impurity B,C,D and E is not more than three times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent ), and the area of the peak due to famotidine impurity F is



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not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent ). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent); the sum of the areas of all other secondary peaks is not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent); Ignore any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 80° at a pressure not exceeding 0.67 kPa for 5 hours.

**Assay**. Weigh accurately about 0.12 g dissolve in 60 ml of *anhydrous acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01687 g of $C_8H_{15}N_7O_2S_3$.

**Storage**. Store protected from light.

# Famotidine Tablets

Famotidine Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of famotidine, $C_8H_{15}N_7O_2S_3$.

**Usual strengths**. 20 mg; 40 mg.

## Identification

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

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 2 volumes of *13.5 M ammonia*, 20 volumes of *toluene*, 25 volumes of *methanol* and 40 volumes of *ethyl acetate*.

*Test solution*. Shake a quantity of the powdered tablets containing about 40 mg of Famotidine with 4 ml of *glacial acetic acid*, dilute to 10 ml with the same solvent, centrifuge and use the clear supernatant liquid.

*Reference solution*. A 0.4 per cent w/v solution of *famotidine RS* in *glacial acetic acid*.

Apply to the plate 10 µl of each solution. Dry the plate in air and examine at 254 nm, The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

## Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of phosphate buffer prepared by dissolving 13.61 g of *potassium dihydrogen orthophosphate* in *water*, adjust the pH to 4.5 with *orthophosphoric acid* or *1 M potassium hydroxide*, add sufficient *water* to produce 1000 ml,
Speed and time. 50 rpm and 45 minutes.

Determine by liquid chromatography (2.4.14).

*Test solution*. Withdraw a suitable volume of the medium, filter and centrifuge at 2000 rpm for 20 minutes, diluted if necessary to prepare a solution containing 0.001 per cent w/v of Famotidine with the dissolution medium.

*Reference solution*. A 0.001 per cent w/v solution of *famotidine RS* in the dissolution medium.

Use chromatographic system as described under Related substances.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0.

Inject the test solution and the reference solution.

Calculate the content of $C_8H_{15}N_7O_2S_3$ in the tablet.

D. Not less than 70 per cent of the stated amount of $C_8H_{15}N_7O_2S_3$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. A solution prepared by dissolving *0.05 M potassium dihydrogen phosphate*, adjusted to pH 6.0 with *1 M potassium hydroxide*.

*Test solution*. Shake a quantity of whole tablets containing about 0.2 g of Famotidine, with 200 ml of the solvent mixture, add 200 ml of *methanol*. Shake for 60 minutes and dilute to 500 ml with the solvent mixture.

*Reference solution (a)*. Dilute 1 ml of the test solution to 100 ml with the solvent mixture.

*Reference solution (b)*. Dilute 1 ml of the test solution to 10 ml with the solvent mixture. Dilute 1 ml of this solution to 50 ml with the same solvent.



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*Reference solution (c).* Dissolve 2 mg each of *3-[[[2-[(diaminomethylene)amino]-1,3-thiazol-4-yl]methyl]sulphinyl]-N-sulphamoylpropanamidine RS (famotidine impurity C RS)*, *3-[2-(Diaminomethylene-amino)-1,3-thiazol-4-ylmethylthio]propanoic acid RS (famotidine degradation impurity 1)* and *3-[2-(Diaminomethyleneamino)-1,3-thiazol-4-ylmethylthio] propanamide RS (famotidine degradation impurity 2)* in 40 ml of *acetonitrile*. Add 40 ml of *methanol* and dilute to 200 ml with the solvent mixture. Dilute 1 ml of this solution to 5 ml with the solvent mixture.

*Reference solution (d).* Dissolve 8 mg of *famotidine RS* in 20 ml of solvent mixture (solution A). To 1 ml of this solution, add 0.05 ml of *hydrogen peroxide solution* (generates famotidine degradation impurity 3).

*Reference solution (e).* Dilute 1.0 ml of solution A with 100.0 ml of the solvent mixture. Further dilute a suitable volume of this solution with an equal volume of reference solution (c).

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm packed with endcapped octadecylsilane bonded to porous silica (5 µm) (such as Inertsil ODS-2),
- temperature. 40°,
- mobile phase: a mixture of 7 volumes of *acetonitrile* and a mixture of 93 volumes of *0.1 M sodium acetate* containing 0.1 per cent v/v of *triethylamine*, adjusted to pH 6.0 with *glacial acetic acid*,
- flow rate. 1.4 ml per minute,
- spectrophotometer set at 275 nm,
- injection volume. 50 µl.

Inject reference solution (e). The test is not valid unless the resolution between the peaks due to famotidine and famotidine impurity C is not less than 1.4 and between the peaks due to famotidine and famotidine degradation impurity 2 is not less than 1.4.

Inject reference solution (a), (b), (c) and (d) and the test solution. In the chromatogram obtained with the test solution the areas of peaks corresponding to famotidine impurity C or famotidine degradation impurities 1 and 2 is not more than the area of the corresponding peaks in the chromatogram obtained with reference solution (c) (0.5 per cent each), the area of peak corresponding to famotidine degradation impurity 3 is not more than the area of the peak in the chromatogram obtained with reference solution (a) (1 per cent) and the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent). The sum of the areas of all the secondary peaks is not more than 2.5 per cent. Ignore any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Shake a quantity of whole tablets containing about 0.2 g of Famotidine with 200 ml of the solvent mixture, add 200 ml of *methanol.* Shake for 60 minutes and dilute to 500 ml with the solvent mixture. Dilute 1.0 ml of this solution to 5.0 ml with the solvent mixture.

*Reference solution.* Dissolve 8 mg of *famotidine RS* in 4 ml of *methanol*, dilute to 20 ml with the solvent mixture. Dilute 1.0 ml of this solution to 5.0 ml with the solvent mixture.

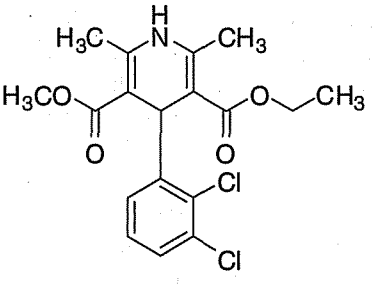
Use chromatographic system as described under Related substances.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0.

Inject the reference solution and the test solution.

Calculate the content of famotidine, $C_8H_{15}N_7O_2S_3$.

# Felodipine



$C_{18}H_{19}Cl_2NO_4$ Mol. Wt. 384.3

Felodipine is ethyl methyl (4*RS*)-4-(2,3-dichlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate.

Felodipine contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{18}H_{19}Cl_2NO_4$, calculated on the dried basis.

**Category**. Antihypertensive.

**Description**. A white or light yellow crystalline powder.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

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



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B. When examined in the range 220 nm to 400 nm (2.4.7), a 0.0015 per cent w/v solution in *methanol*, shows absorption maxima at about 238 nm and 361 nm; the absorbane ratio of 361 nm and 238 nm is between 0.34 to 0.36.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 40 volumes of *ethyl acetate* and 60 volumes of *cyclohexane*.

*Test solution*. Dissolve 10 mg of the substance under examination in 10.0 ml of *methanol*.

*Reference solution (a)*. Dissolve 10 mg of *felodipine RS* in 10.0 ml of *methanol*.

*Reference solution (b)*. Dissolve 5 mg of *nifedipine RS* in 5.0 ml of reference solution (a).

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows 2 clearly separated spots.

D. Dissolve 0.15 g in a mixture of 25 ml of *2-methyl-2-propanol* and 25 ml of *perchloric acid solution*. Add 10 ml of *0.1 M cerium sulphate*, allow to stand for 15 minutes, add 3.5 ml of *strong sodium hydroxide solution* and neutralise with *dilute sodium hydroxide solution*. Shake with 25 ml of *dichloromethane*, evaporate the lower layer to dryness on a water-bath under nitrogen (the residue is also used in the test for related substances). Dissolve 20 mg of the residue in 50.0 ml of *methanol*, Dilute 2 .0 ml of this solution to 50.0 ml with *methanol*. When examined in the range 220 nm to 400 nm (2.4.7), the resulting solution shows an absorption maximum only at about 273 nm.

## Tests

**Appearance of solution**. A 5 per cent w/v solution in *methanol* (Solution A) is clear (2.4.1).

**Light absorption**. Not more than 0.1, determined at 440 nm (2.4.7) on solution A.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 25 mg of the substance under examination in 50.0 ml of the mobile phase.

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

*Reference solution (b)*. Dilute 1.0 ml of reference solution (a) to 10.0 ml with the mobile phase.

*Reference solution (c)*. Dissolve 10 mg of the residue obtained in identification test D [ethyl methyl 4-(2,3-dichlorophenyl)-2,6-dimethylpyridine-3,5-dicarboxylate] (felodipine impurity A) and 5 mg of *felodipine RS* in the mobile phase, then dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 12.5 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 20 volumes of *methanol*, 40 volumes of *acetonitrile* and 40 volumes of a *phosphate buffer solution pH 3.0* containing 0.08 per cent w/v of *orthophosphoric acid* and 0.8 per cent w/v of *sodium dihydrogen phosphate*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to felodipine and felodipine impurity A is not less than 2.5. The order of elution is dimethyl 4-(2,3-dichlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate, (felodipine impurity B), ethyl methyl 4-(2,3-dichlorophenyl)-2,6-dimethylpyridine-3,5-dicarboxylate (felodipine impurity A), felodipine and diethyl 4-(2,3-dichlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (felodipine impurity C).

Inject reference solution (a), (b) and the test solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of secondary peak corresponding to felodipine impurity B and C is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). Sum of all other secondary peaks is not more than three times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent). Ignore any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.02 per cent).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.16 g, dissolve in a mixture of 25 ml of *2-methyl-2-propanol* and 25 ml of *perchloric acid solution*, add 0.05 ml of *ferroin*. Titrate with *0.1 M cerium sulphate* until the pink colour disappears and titrate slowly towards the end of the titration.

1 ml of *0.1 M cerium sulphate* is equivalent to 0.01921 g of $C_{18}H_{19}C_{l2}NO_4$.



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**Storage**. Store protected from light.

## Felodipine Sustained-release Tablets

Felodipine Sustained-release Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of felodipine, $C_{18}H_{19}Cl_2NO_4$.

**Usual strengths**. 2.5 mg; 5 mg; 10 mg.

### Identification

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

### Tests

**Dissolution** (2.5.2).

Apparatus No.1,
Medium. 500 ml of phosphate buffer pH 6.5 with 1 per cent *sodium lauryl sulphate* prepared by diluting 206 ml of *1 M monobasic sodium phosphate monohydrate*, 196 ml of *0.5 M dibasic sodium phosphate anhydrous*, and 50.0 g of *sodium lauryl sulphate* to a 5000-ml volumetric flask. Add approximately 4000 ml of *water*, adjust with *1 M sodium hydroxide* to a pH of 6.5. Dilute to volume with *water*,
Speed and time. 50 rpm and 2, 6 and 10 hours.

Withdraw a suitable volume of the medium at each time interval and filter through a membrane filter.

*Test solution.* Use the filtrate.

*Stock solution.* Dissolve a quantity of *felodipine RS* in *ethanol (95 per cent)* to obtain a solution having a concentration of 0.25 mg per ml.

*Reference solution.* Dilute an accurately measured volume of the stock solution quantitatively, and stepwise if necessary, with medium to obtain a solution having a known concentration of *felodipine RS* equivalent to the concentration that would result from about 60 per cent dissolution of a single Tablet in 500 ml of medium.

Use chromatographic system as directed in the Assay.

Inject 100 µl of the reference solution and the test solution.

Calculate the content of felodipine, $C_{18}H_{19}Cl_2NO_4$.

D. Not less than 10 to 30 per cent in 2 hours, not less than 42 and not more than 68 per cent in 6 hours and not less than 75 per cent of the stated amount of $C_{11}H_{11}F_3N_2O_3$ in 10 hours.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution.* Proceed as directed in the Assay, except that after centrifuging a portion of the solution at high speed for 15 minutes, filter.

*Reference solution (a).* A 0.02 per cent w/v solution of *felodipine impurity A RS* in *methanol.* Dilute 10.0 ml of this solution to 100 ml with the mobile phase.

*Reference solution (b).* A 0.2 per cent w/v solution of *felodipine RS* in *methanol.*

*Reference solution (c).* Dilute 15.0 ml of reference solution (a) and 5.0 ml of reference solution (b) to 100 ml with the mobile phase.

*Reference solution (d).* Dilute 10.0 ml of reference solution (a) to 100 ml with the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture 40 volumes of buffer solution, 40 volumes of *acetonitrile* and 20 volumes of *methanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 40 µl.

Inject reference solution (c). The test is not valid unless the relative retention time with reference to felodipie for felodipine impurity A is about 0.75 and the resolution between the peaks due to felodipine and felodipine impurity A is not less than 1.5 and the theoretical plates is not less than 1500.

Inject reference solution (d) and the test solution. In the chromatogram obtained with the test solution, the area of the peak due to felodipine impurity A is not more than 2.0 per cent the area of the principal peak in the chromatogram obtained with reference solution (d).

**Uniformity of content.** Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

*Test solution.* Sonicate 1 tablet with 40 ml of *acetonitrile* for 20 minutes and add 20 ml of *methanol*, and then shake by mechanical means for 30 minutes. Allow to cool to room temperature, dilute to 100.0 ml with the buffer solution. Centrifuge for 15 minutes. Dilute a portion of the supernatant with mobile phase to obtain a solution containing about 20 µg of felodipine per ml, filter.

Inject 40 µl of the reference solution and the test solution.

Calculate the content of felodipine, $C_{18}H_{19}Cl_2NO_4$ in the Tablet.

**Assay**. Determine by liquid chromatography (2.4.14).

*Buffer solution.* Dissolve 6.9 g of *monobasic sodium phosphate* in about 800 ml of *water*, adjust to pH 3.0 with *orthophosphoric acid* and dilute to 1000 ml with *water.*



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*Test solution.* Weigh and powder 10 tablets. Disperse a quantity of powder containing about 10 mg of Felodipine with 40 ml of *acetonitrile* and 20 ml of *methanol,* and sonicate for 5 minutes. Add about 30 ml of buffer solution, and shake by mechanical means for 30 minutes. Allow the solution to cool to room temperature, dilute to 100.0 ml with buffer solution. Centrifuge for 15 minutes. Dilute 10 ml of the supernatant to 50 ml with the mobile phase, filter.

*Reference solution (a).* A 0.2 per cent w/v solution of *felodipine RS* in *methanol.*

*Reference solution (b).* Dilute a volume of reference solution (a) with mobile phase to obtain a solution having a concentration of 0.02 mg per ml.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 40 volumes of buffer solution, 40 volumes of *acetonitrile* and 20 volumes of *methanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 362 nm,
- injection volume. 40 µl.

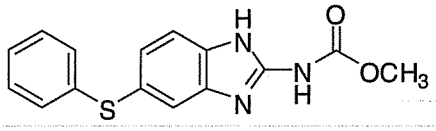
Inject reference solution (b). The test is not valid unless the theoretical plates is not less than 1500 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (b) and the test solution.

Calculate the content of felodipine, $C_{18}H_{19}Cl_2NO_4$ in the tablets.

**Storage**. Store protected from moisture.

# Fenbendazole



$C_{15}H_{13}N_3O_2S$ Mol. Wt. 299.4

Fenbendazole is methyl 5-(phenylthio)-2-benzimidazolecarbamate.

Fenbendazole contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{15}H_{13}N_3O_2S$, calculated on the dried basis.

**Category**. Anthelminthic.

**Description**. A white or almost white powder.

## Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fenbendazole RS* or with the reference spectrum of fenbendazole.

## Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve 50 mg of the substance under examination in 10.0 ml of *methanolic hydrochloric acid.*

*Reference solution (a).* Dissolve 50 mg of *fenbendazole RS* in 10.0 ml of *methanolic hydrochloric acid.* Dilute 1.0 ml of this solution to 200.0 ml with *methanol.* Dilute 5.0 ml of the solution to 10.0 ml with *methanolic hydrochloric acid.*

*Reference solution (b).* Dissolve 10 mg of *methyl (1H-benzimidazol-2-yl)carbamate RS ( fenbendazole impurity A RS)* in 100.0 ml of *methanol.* Dilute 1.0 ml of this solution to 10.0 ml with *methanolic hydrochloric acid.*

*Reference solution (c).* Dissolve 10 mg of *methyl (5-chloro-1H-benzimidazol-2-yl)carbamate RS (fenbendazole impurity B RS)* in 100.0 ml of *methanol.* Dilute 1.0 ml of the solution to 10.0 ml with *methanolic hydrochloric acid.*

*Reference solution (d).* Dissolve 10 mg of *fenbendazole RS* and 10 mg of *mebendazole RS* in 100.0 ml of *methanol.* Dilute 1.0 ml of this solution to 10.0 ml with *methanolic hydrochloric acid.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 1 volume of *anhydrous acetic acid,* 30 volumes of *methanol* and 70 volumes of *water*,

  B. a mixture of 1 volume of *anhydrous acetic acid*, 30 volumes of *water* and 70 volumes *methanol*,
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume. 10 µl.

| Time (min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0-10 | 100→0 | 100 |
| 10-40 | 0 | 100 |
| 40-50 | 0→100 | 100→0 |
| 50-55 | 100 | 0 |

Inject reference solution (d). The test is not valid unless the resolution between the peaks due to fenbendazole and mebendazole is not less than 1.5.

Inject reference solution (a), (b), (c) and the test solution. In the chromatogram obtained with the test solution, the area of secondary peak due to fenbendazole impurity A is not more than 2.5 times the area of the corresponding peak in the



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chromatogram obtained with reference solution (b) (0.5 per cent). The area of secondary peak corresponding to fenbendazole impurity B is not more than 2.5 times the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.5 per cent ), the area of any other secondary peak is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent); The sum of the areas of all other secondary peaks is not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

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

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

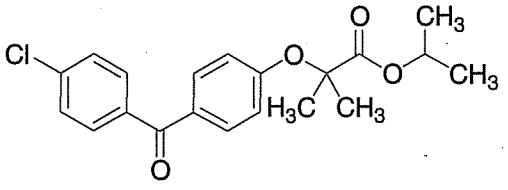
**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

**Assay**. Dissolve 0.2 g in 30 ml of *anhydrous acetic acid*, warming gently, if necessary. Cool and titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02994 g of $C_{15}H_{13}N_3O_2S$.

**Storage**. Store protected from light.

# Fenofibrate




$C_{20}H_{21}ClO_4$ Mol. Wt. 360.8

Fenofibrate is isopropyl [4-(4-chlorophenyl)-2-phenoxy-2-methyl]propionate

Fenofibrate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{20}H_{21}ClO_4$, calculated on the dried basis.

**Category.** Antihyperlipidemic.

**Description**. A white or almost white, crystalline powder.

## Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fenofibrate RS* or with the reference spectrum of fenofibrate.

## Tests

**Appearance of solution**. A 5.0 per cent w/v solution in *acetone* is clear (2.4.1) and not more intensely coloured than reference solution BYS6 (2.4.1).

**Acidity**. Dissolve 1.0 g in 50 ml of *ethanol (95 per cent)*, previously neutralized using 0.2 ml of *phenolphthalein* and titrate with *0.1 M sodium hydroxide*. Not more than 0.2 ml of *0.1 M sodium hydroxide* is required to change the color of the indicator to pink.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve 0.1 g of the substance under examination in 100.0 ml of the mobile phase.

*Reference solution (a).* A 0.1 per cent w/v solution of *fenofibrate RS* in the mobile phase.

*Reference solution (b).* A solution containing 0.005 per cent w/v each of *fenofibrate RS*, *fenofibrate impurity A RS*, *fenofibrate impurity B RS* and 0.01 per cent w/v of *fenofibrate impurity G RS* in the mobile phase. Dilute 1.0 ml of this solution to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 70 volumes of *acetonitrile* and 30 volumes of *water*, adjusted to pH 2.5 with *orthophosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 286 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to fenofibrate impurity A and fenofibrate impurity B is not less than 1.5. The relative retention time with reference to fenofibrate for (4-chlorophenyl)(4-hydroxyphenyl)methanone (fenofibrate impurity A) is about 0.34, for fenofibric acid (fenofibrate impurity B) is about 0.36, for (3*RS*)-3-[4-(4-chlorobenzoyl)phenoxy]butan-2-one (fenofibrate impurity C) is about 0.50, for methyl 2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropanoate (fenofibrate impurity D) is about 0.65, for ethyl 2-[4-(4-chlorobenzoyl) phenoxy]-2-methylpropanoate (fenofibrate impurity E) is about 0.80, for (4-chlorophenyl)[4-(1-methylethoxy)phenyl] methanone (fenofibrate impurity F) is about 0.85 and for 1-methylethyl 2-[[2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropanoyl]oxy]-2-methylpropanoate (fenofibrate impurity G) is about 1.35.

Inject reference solution (b) and the test solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of each peak due to fenofibrate impurity A and B is not more



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than the area of the corresponding peaks in the chromatogram obtained with reference solution (b) (0.1 per cent), the area of the peak due to fenofibrate impurity G is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.2 per cent), the area of any any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The sum of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.01 per cent).

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

**Chlorides** (2.3.12). Dilute 5 ml of solution A to 10 ml of *distilled water*, complies with the limit test for chlorides (100 ppm).

**Sulphates** (2.3.17). Dissolve 5 g in 25 ml of *distilled water* and heat at 50° for 10 minutes. Cool and dilute to 50.0 ml with the same solvent, filter (Solution A), complies with the limit test for sulphates (100 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent determined on 1 g by drying in vacuum over phosphorus pentoxide at 60°.

**Assay**. Determine by liquid chromatography (2.4.14), as described under Related substances using injection volume. 5 µl.

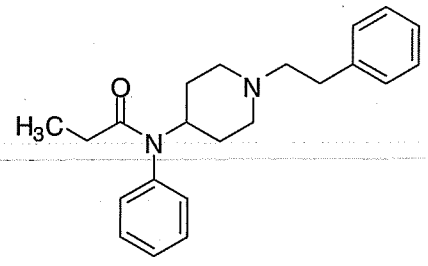
Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{20}H_{21}ClO_4$.

**Storage**. Store protected from light.

# Fentanyl




$C_{22}H_{28}N_2O$ Mol. Wt. 336.5

Fentanyl is *N*-phenyl-*N*-[1-(2-phenylethyl)piperidin-4-yl]propanamide.

Fentanyl contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{22}H_{28}N_2O$, calculated on the dried basis.

**Category**. Analgesic.

**Description**. A white or almost white powder.

## Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fentanyl RS* or with the reference spectrum of fentanyl.

## Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 0.1 g of the substance under examination in 10.0 ml of *methanol*.

*Reference solution (a)*. Dilute 1.0 ml of the test solution to 100.0 ml with *methanol*. Dilute 5.0 ml of this solution to 20.0 ml with *methanol*.

*Reference solution (b)*. Dissolve 10 mg of the substance under examination in 10.0 ml of *dilute hydrochloric acid*. Heat on a water-bath under a reflux condenser for 4 hours. Neutralise with 10.0 ml of *dilute sodium hydroxide solution* and evaporate to dryness on a water-bath, cool and dissolve the residue in 10.0 ml of *methanol*, filter.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- mobile phase: A. a 0.5 per cent w/v solution of *ammonium carbonate* in a mixture of 10 volumes of *tetrahydrofuran* and 90 volumes of *water*,
  B. *acetonitrile*,
- a linear gradient programme using the conditions given below,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 10 µl.

| Time (min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0 - 15 | 90 → 40 | 10 → 60 |
| 15 - 20 | 40 | 60 |
| 20 - 25 | 40 → 90 | 60 → 10 |
| 25 - 30 | 90 | 10 |

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to fentanyl and fentanyl impurity D is not less than 8.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution the area of secondary peak corresponding to *N*-phenyl-*N*-[*cis,trans*-1-



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oxido-1-(2-phenylethyl)piperidin-4-yl]propanamide (fentanyl impurity A), *N*-phenyl-*N*-(piperidin-4-yl)propanamide (fentanyl impurity B), *N*-phenyl-*N*-[1-(2-phenylethyl)piperidin-4-yl]acetamide (fentanyl impurity C), *N*-phenyl-1-(2-phenylethyl)piperidin-4-amine (fentanyl impurity D), for each impurity is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.25 per cent). The sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

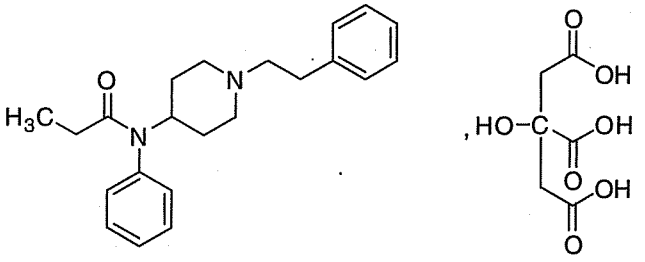
**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 50° under vacuum.

**Assay**. Dissolve 0.2 g in 50 ml of a mixture of 1 volume of *anhydrous acetic acid* and 7 volumes of *methyl ethyl ketone*. Titrate with *0.1 M perchloric acid*, using 0.2 ml of *naphtholbenzein solution* as indicator, until the colour changes from orange-yellow to green. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03365 g of $C_{22}H_{28}N_2O$.

**Storage**. Store protected from light.

# Fentanyl Citrate



$C_{22}H_{28}N_2O,C_6H_8O_7$ Mol. Wt. 528.6

Fentanyl Citrate is *N*-(phenylethyl-4-piperidinyl)-*N*-phenylpropionamide citrate.

Fentanyl Citrate contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{22}H_{28}N_2O,C_6H_8O_7$, calculated on the dried basis.

**Category**. Analgesic.

**Description**. A white or almost white powder.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fentanyl citrate RS* or with the reference spectrum of fentanyl citrate.

B. Melting point (2.4.21). 152°.

## Tests

**Appearance of solution**. A 1.0 per cent w/v solution in *water* is clear (2.4.1) and colourless (2.4.1).

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 0.1 g of the substance under examination in 10.0 ml of *methanol*.

*Reference solution (a)*. Dilute 1.0 ml of the test solution to 100.0 ml with *methanol*. Dilute 5.0 ml of this solution to 20.0 ml with *methanol*.

*Reference solution (b)*. Dissolve 10 mg of the substance under examination in 10.0 ml of *dilute hydrochloric acid*. Heat on a water-bath under a reflux condenser for 4 hours. Neutralise with 10.0 ml of *dilute sodium hydroxide solution* and evaporate to dryness on a water-bath, cool and dissolve the residue in 10.0 ml of *methanol* and filter.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- mobile phase: A. 0.5 per cent w/v solution of *ammonium carbonate* in a mixture of 10 volumes of *tetrahydrofuran* and 90 volumes of *water*,
  B. *acetonitrile*,
- a linear gradient programme using the conditions given below,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 10 µl.

| Time (min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0 - 15 | 90 → 40 | 10 → 60 |
| 15 - 20 | 40 | 60 |
| 20 - 25 | 40 → 90 | 60 → 10 |
| 25 - 30 | 90 | 10 |

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to fentanyl and fentanyl impurity D is not less than 8.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution the area of secondary peak corresponding to *N*-phenyl-*N*-[*cis,trans*-1-oxido-1-(2-phenylethyl)piperidin-4-yl]propanamide (fentanyl impurity A), *N*-phenyl-*N*-(piperidin-4-yl)propanamide (fentanyl



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impurity B), *N*-phenyl-*N*-[1-(2-phenylethyl)piperidin-4-yl]acetamide (fentanyl impurity C), *N*-phenyl-1-(2-phenylethyl)piperidin-4-amine (fentanyl impurity D), for each impurity is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.25 per cent). The sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 50° under vacuum.

**Assay**. Weigh accurately 0.3 g, dissolve in 50 ml of a mixture of 1 volume of *anhydrous acetic acid* and 7 volumes of *methyl ethyl ketone*. Titrate with *0.1 M perchloric acid*, using 0.2 ml of *naphtholbenzein solution* as indicator, until the colour changes from orange-yellow to green. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.05286 g of $C_{28}H_{36}N_2O_8$.

**Storage**. Store protected from light.

# Fentanyl Injection

Fentanyl Citrate Injection

Fentanyl Injection is a sterile solution of Fentanyl Citrate in Water for Injections.

Fentanyl Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of fentanyl, $C_{22}H_{28}N_2O$.

**Usual strength**. 50 µg per ml.

## Identification

A. When examined in the range 230 to 350 nm (2.4.7), a solution containing 0.005 per cent w/v of fentanyl exhibits two maxima at 251 and 257 nm.

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

C. Gives reaction B for citrates (2.3.1).

## Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution.* Dilute a volume of injection containing about 5 mg of Fentanyl to 100.0 ml with the mobile phase.

*Reference solution (a).* Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 20.0 ml with the mobile phase.

*Reference solution (b).* A 0.00005 per cent w/v solution of *N-phenyl-N-[cis,trans-1-oxido-1-(2-phenylethyl)piperidin-4-yl]propanamide RS (fentanyl impurity A RS)* in the mobile phase.

*Reference solution (c).* Dissolve 10 mg of *fentanyl citrate RS* in 10 ml of *2 M hydrochloric acid*, heat on a water-bath under a reflux condenser for 4 hours and neutralise with 10 ml of *2 M sodium hydroxide*. Evaporate to dryness on a water-bath, cool, dissolve the residue in 10 ml of *methanol* and filter. Dilute 1 ml of the filtrate to 10 ml with the mobile phase {generation of *N*-phenyl-1-(2-phenylethyl)piperidin-4-amine (fentanyl impurity D)}.

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm, packed with endcapped octadecylsilane bonded to porous silica (10 µm) (Such as Bondclone C18),
- mobile phase: a solution containing 0.3 per cent w/v of *potassium dihydrogen phosphate* in a mixture of 4 volumes of *acetonitrile*, 40 volumes of *methanol* and 56 volumes of *water*, adjusted to pH to 3.2 with *orthophosphoric acid*,
- flow rate. 1.25 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume. 100 µl.

Inject reference solution (c).The relative retention time with reference to fentanyl for fentanyl impurity D is about 0.8.

Inject reference solution (a), (b), (c) and the test solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with test solution the area of peak corresponding to fentanyl impurity A is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent), the area of peak corresponding to fentanyl impurity D is not more than twice the area of the peak in the chromatogram obtained with reference solution (a) (0.5 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.25 per cent) and the sum of the areas of all secondary peaks other than fentanyl impurity A and D is not more than three times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.75 per cent). Ignore any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. Determine by liquid chromatography (2.4.14).



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*Test solution.* Dilute a volume of injection containing about 5 mg of Fentanyl to 100 ml with the mobile phase.

*Reference solution.* A 0.008 per cent w/v solution of *fentanyl citrate RS* in the mobile phase.

Use chromatographic system as described under Related substances.

Inject the test solution and the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not less than 2.0.

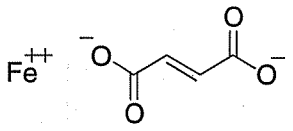
Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{28}N_2O$ in the injection.

**Storage**. Store protected from light.

**Labelling**. The quantity of active ingredient is stated in terms of the equivalent amount of fentanyl.

# Ferrous Fumarate



$C_4H_2FeO_4$ Mol. Wt. 169.9

Ferrous Fumarate contains not less than 93.0 per cent and not more than 101.0 per cent of $C_4H_2FeO_4$, calculated on the dried basis.

**Category**. Haematinic.

**Dose**. Prophylactic, 200 mg daily; therapeutic, 400 to 600 mg daily, in divided doses. (200 mg of ferrous fumarate is approximately equivalent to 65 mg of ferrous iron).

**Description**. A reddish orange to reddish brown, fine powder; may contain soft lumps that produce a yellow streak when crushed; odour, slight.

## Identification

A. Heat 1 g with 25 ml of a mixture of equal volumes of *hydrochloric acid* and *water* on a water-bath for 15 minutes, cool and filter. Reserve the precipitate for test B; the filtrate gives reaction A of ferrous salts (2.3.1).

B. Wash the precipitate reserved in test A with a mixture of 1 volume of *dilute hydrochloric acid* and 9 volumes of *water* and dry at 105°. Suspend 0.1 g of the residue in 2 ml of *sodium carbonate solution* and add *dilute potassium permanganate solution* dropwise; the permanganate is decolorised and a brownish solution is obtained.

C. Mix 0.5 g with 1 g of *resorcinol*. To 0.5 g of the mixture in a crucible, add a few drops of *sulphuric acid* and heat gently; a deep red semi-solid mass is formed. Add the mass to a large volume of *water*; an orange-yellow solution without any fluorescence is obtained.

## Tests

**Arsenic** (2.3.10). Mix 2.0 g with 1.5 g of *anhydrous sodium carbonate*, add 10 ml *bromine water* and mix thoroughly. Evaporate to dryness on a water-bath, ignite gently and dissolve the cooled residue in 20 ml of *brominated hydrochloric acid* and 10 ml of *water*. Transfer to a small flask, add sufficient *stannous chloride solution AsT* to remove the yellow colour, connect to a condenser and distil 22 ml. The distillate complies with the limit test for arsenic (5 ppm).

**Heavy metals**. Not more than 20 ppm, determined by the following method. Ignite 1.0 g gently until free from carbon, dissolve in 5 ml of *hydrochloric acid* by heating on a water-bath and evaporate to dryness. Dissolve the residue in a mixture of 15 ml of *hydrochloric acid*, 4 ml of *nitric acid* and 6 ml of *water*. Boil gently for 1 minute, cool and extract with three quantities, each of 20 ml, of *ether*. If the aqueous layer is more than slightly yellow, extract with a fourth quantity of 20 ml of *ether* and reject the ether extracts, heat the aqueous solution gently to remove the dissolved ether, add 1 g of *citric acid*, make alkaline with *5 M ammonia* and add 1 ml of *potassium cyanide solution*. Dilute to 50 ml with *water* and add 0.1 ml of *sodium sulphide solution*. Any brown colour produced is not more intense than that produced by treating 1.0 ml of *lead standard solution (20 ppm Pb)* in a similar manner.

**Sulphates** (2.3.17). Boil 0.15 g with 10 ml of *2 M hydrochloric acid* and 20 ml of freshly boiled and cooled *water*, cool in ice and filter; the filtrate complies with the limit test for sulphates (0.1 per cent).

**Ferric iron**. Not more than 2.0 per cent, determined by the following method. Weigh accurately about 3.0 g and dissolve in a mixture of 200 ml of *water* and 20 ml of *hydrochloric acid* by heating rapidly to boiling point. Boil for 15 seconds, cool rapidly, add 3 g of *potassium iodide*, close the flask, allow to stand in the dark for 15 minutes and titrate the liberated iodine with *0.1 M sodium thiosulphate* using *starch solution*, added towards the end of the titration, as indicator. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of iodine liberated by the ferric iron.

1 ml of *0.1 M sodium thiosulphate* is equivalent to 0.005585 g of ferric iron.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.3 g and dissolve in 15 ml of *dilute sulphuric acid* with the aid of gentle heat. Cool, add



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50 ml of *water* and immediately titrate with *0.1 M ceric ammonium sulphate* using *ferroin sulphate solution* as indicator.

1 ml of *0.1 M ceric ammonium sulphate* is equivalent to 0.01699 g of $C_4H_2FeO_4$.

## Ferrous Fumarate Tablets

Ferrous Fumarate Tablets contain not less than 90.0 per cent and not more than 105.0 per cent of the stated amount of ferrous fumarate, $C_4H_2FeO_4$.

**Usual strength**. 200 mg. (200 mg of ferrous fumarate is approximately equivalent to 65 mg of ferrous iron).

### Identification

The powdered tablets comply with the following tests.

A. Heat 1 g with 25 ml of a mixture of equal volumes of *hydrochloric acid* and *water* on a water-bath for 15 minutes, cool and filter. Reserve the precipitate for test B; the filtrate gives reaction A of ferrous salts (2.3.1).

B. Wash the precipitate reserved in test A with a mixture of 1 volume of *dilute hydrochloric acid* and 9 volumes of *water* and dry at 105°. Suspend 0.1 g of the residue in 2 ml of *sodium carbonate solution* and add *dilute potassium permanganate solution* dropwise; the permanganate is decolorised and a brownish solution is obtained.

C. Mix 0.5 g with 1 g of *resorcinol*. To 0.5 g of the mixture in a crucible, add a few drops of *sulphuric acid* and heat gently; a deep red semi-solid mass is formed. Add the mass to a large volume of *water*; an orange-yellow solution without any fluorescence is obtained.

### Tests

**Ferric iron**. Weigh accurately a quantity of the powder prepared for the Assay, containing about 1.5 g of Ferrous Fumarate, in a stoppered flask, dissolve as completely as possible with the aid of heat in a mixture of 100 ml of freshly boiled and cooled *water* and 10 ml of *hydrochloric acid*, boil for 15 seconds, cool rapidly, add 3 g of *potassium iodide*, close the flask and allow to stand in the dark for 15 minutes. Titrate the liberated iodine with *0.1 M sodium thiosulphate* using *starch solution*, added towards the end of the titration, as indicator. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of iodine liberated by the ferric iron. Not more than 13.5 ml of *0.1 M sodium thiosulphate* is required.

**Disintegration** (2.5.1). 60 minutes.

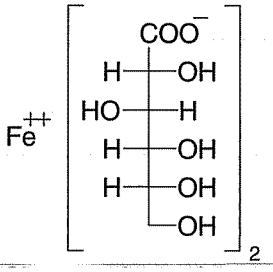
**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.3 g of Ferrous Fumarate and dissolve in 15 ml of *dilute sulphuric acid* with the aid of gentle heat. Cool, add 50 ml of *water* and immediately titrate with *0.1 M ceric ammonium sulphate* using *ferroin sulphate solution* as indicator.

1 ml of *0.1 M ceric ammonium sulphate* is equivalent to 0.01699 g of $C_4H_2FeO_4$.

**Labelling**. The label states the quantity of the active ingredient both as the amount of Ferrous Fumarate and in terms of the equivalent amount of ferrous iron in each tablet.

## Ferrous Gluconate




$C_{12}H_{22}FeO_{14},xH_2O$ Mol. Wt. 446.1 (anhydrous)

Ferrous Gluconate is ferrous di(D-gluconate).

Ferrous Gluconate contains not less than 95.0 per cent and not more than 102.0 per cent of $C_{12}H_{22}FeO_{14}$, calculated on the dried basis.

**Category**. Haematinic.

**Dose**. Prophylactic, 600 mg daily; therapeutic, 1.2 to 1.8 g daily, in divided doses.

(300 mg of ferrous gluconate is approximately equivalent to 35 mg of ferrous iron).

**Description**. A yellowish grey or pale greenish-yellow, fine powder or granules; odour, slight, resembling that of burnt sugar.

### Identification

A. Dissolve 5 g in *carbon dioxide-free water* at 60°, cool and dilute to 50 ml with *water*. 1 ml of the resulting solution gives reaction A of ferrous salts (2.3.1).

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *Silica gel G*



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*Mobile phase*. A mixture of 10 volumes of *concentrated ammonia*, 10 volumes of *ethyl acetate*, 30 volumes of *water* and 50 volumes of *ethanol (95 per cent)*.

*Test solution*. Dissolve 20 mg of the substance under examination in 2 ml of *water*, heating if necessary in a water-bath at 60°.

*Reference solution*. Dissolve 20 mg of *ferrous gluconate RS* in 2 ml of *water*, heating if necessary in a water-bath at 60°.

Apply to the plate 5µl of each solution. After development, dry the plate at 105° for 20 minutes and spray with 5 per cent w/v solution of *potassium dichromate* in a 40 per cent w/v solution of *sulphuric acid*. The principle spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

## Tests

**Appearance of solution**. Dissolve 5.0 g in *carbon dioxide-free water* at 60°, cool and dilute to 50 ml with the same solvent (solution A). Dilute 2 ml of solution A to 10 ml with *water*. When examined against the light, the resulting solution is clear (2.4.1).

**pH** (2.4.24). 4.0 to 5.5, determined in solution A, 3 to 4 hours after preparation.

**Arsenic** (2.3.10). To 5.0 g add 15 ml of *water* and 15 ml of *stannated hydrochloric acid*, distil 22 ml and add to the distillate 40 ml of *water* and 0.2 ml of *stannous chloride solution AsT*. The resulting solution complies with the limit test for arsenic (2 ppm).

**Heavy metals** (2.3.13). Warm 2.0 g gently with 10 ml of *nitric acid* until reaction begins and allow to stand until the evolution of nitrous fumes subsides. Boil gently to complete oxidation, adding a further 5 ml of *nitric acid*, if necessary, and continue boiling until the volume is reduced to about 5 ml. Add 20 ml of *hydrochloric acid*, boil gently for 1 minute, cool and extract with three quantities, each of 20 ml, of *ether*. If the acid solution is still more than faintly yellow, extract with a fourth quantity of 20 ml of *ether* and discard the ether extracts. Transfer the acid solution to a narrow-necked flask, rinse the separator with 5 ml of *water*, and add the rinsings to the flask. Heat to remove the dissolved ether and part of the hydrochloric acid. Cool and dilute to 50 ml with *water*. 25 ml of the resulting solution complies with the limit test for heavy metals, Method A (20 ppm).

**Chlorides** (2.3.12). 0.4 g complies with the limit test for chlorides (625 ppm).

**Sulphates** (2.3.17). 0.3 g complies with the limit test for sulphates (500 ppm).

**Barium**. Dissolve 0.1 g in 50 ml of *distilled water*, and 5 ml of *dilute sulphuric acid*, and allow to stand for 5 minutes. The solution is not more opalescent than a mixture of 10 ml of solution A and 45 ml of *distilled water*, when examined against the light.

**Ferric iron**. Not more than 1.0 per cent, determined by the following method. Weigh accurately about 5.0 g, transfer to a glass-stoppered flask and dissolve in a mixture of 100 ml of freshly boiled and cooled *water* and 10 ml of *hydrochloric acid*. Add 3 g of *potassium iodide*, shake well and allow to stand in the dark for 5 minutes. Titrate the liberated iodine with *0.1 M sodium thiosulphate* using *starch solution*, added towards the end of the titration, as indicator. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of iodine liberated by the ferric iron.

1 ml of *0.1 M sodium thiosulphate* is equivalent to 0.005585 g of ferric iron.

**Oxalic acid**. Dissolve 1 g in 5 ml of *water*, add 2 ml of *hydrochloric acid* and transfer to a separator. Extract with two quantities, each of 20 ml, of *ether*. Evaporate the combined ether extracts to dryness on a water-bath and dissolve the residue in 5 ml of *water*. Add 0.05 ml of *acetic acid* and 3 ml of *calcium chloride solution*; no turbidity is produced.

**Reducing sugars**. Dissolve 0.5 g in 10 ml of *water* and make alkaline with *dilute ammonia solution*. Pass *hydrogen sulphide* into the solution and allow to stand for 30 minutes. Filter and wash the precipitate with two quantities, each of 5 ml, of *water*. Combine the filtrate and the washings and acidify with *dilute hydrochloric acid*. Add 2 ml of *dilute hydrochloric acid* in excess. Boil the solution until the vapours no longer darken *lead acetate paper* and, if necessary, boil further to concentrate the solution to about 10 ml. Cool and add 10 ml of *sodium carbonate solution*, set aside for 5 minutes, filter and dilute the filtrate to 100 ml with *water*. To 5 ml of the filtrate add 2 ml of *potassium cupri-tartrate solution* and boil for 1 minute; no red precipitate is formed.

**Loss on drying** (2.4.19). 5.0 per cent to 10.0 per cent, determined on 1.0 g by drying in an oven at 105°.

**Microbial Contamination** (2.2.9). Total viable aerobic count, not more than 1000 micro-organisms per g, determined by plate count.

**Assay**. Dissolve 0.5 g of *sodium bicarbonate* in a mixture of 70 ml of *water* and 30 ml of *1 M sulphuric acid*. When effervescence ceases, add about 1.0 g, accurately weighed, of the substance under examination, shake gently to dissolve and titrate with *0.1 M ceric ammonium nitrate*, using 0.1 ml of *ferroin solution* as indicator, until the red colour disappears.

1 ml of *0.1 M ceric ammonium nitrate* is equivalent to 0.04461 g of $C_{12}H_{22}FeO_{14}$.

**Storage**. Store protected from light.



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# Ferrous Gluconate Tablets

Ferrous Gluconate Tablets contain not less than 90.0 per cent and not more than 105.0 per cent of the stated amount of ferrous gluconate, $C_{12}H_{22}FeO_{14},2H_2O$.

**Usual strength**. 300 mg. (300 mg of ferrous gluconate is approximately equivalent to 35 mg of ferrous iron).

## Identification

Dissolve a quantity of the powdered tablets containing about 1 g of Ferrous Gluconate in 10 ml of *water* and filter; the filtrate complies with the following tests.

A. 1 ml of the filtrate gives reaction A of ferrous salts (2.3.1).

B. To 7.5 ml of the filtrate add 1 ml of *glacial acetic acid* and 1 ml of freshly distilled *phenylhydrazine*. Heat the mixture on a water-bath for 30 minutes. Cool and scratch the inner surface of the test tube with a glass rod until crystals of gluconic acid phenylhydrazide begin to form. Set aside for 10 minutes, filter, dissolve the precipitate in hot *water*, mix a small amount of *decolorising charcoal* and filter into a test tube. Allow the filtrate to cool, and scratch the inner surface of the test tube; white crystals are obtained which melt at about 202°, with decomposition (2.4.21).

C. Shake a quantity of the powdered tablets containing 0.5 g of Ferrous Gluconate with 10 ml of *dilute hydrochloric acid*, filter and add to the filtrate 1 ml of *barium chloride solution*; an opalescence may be produced but no precipitate is formed.

## Tests

**Ferric iron**. Weigh accurately a quantity of the powder prepared for the Assay, containing about 5.0 g of Ferrous Gluconate, in a stoppered flask, dissolve as completely as possible without the aid of heat in a mixture of 100 ml of freshly boiled and cooled *water* and 10 ml of *hydrochloric acid*, add 3 g of *potassium iodide*, close the flask and allow to stand in the dark for 5 minutes. Titrate the liberated iodine with *0.1 M sodium thiosulphate* using *starch solution*, added towards the end of the titration, as indicator. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of iodine liberated by the ferric iron. Not more than 11.2 ml of *0.1 M sodium thiosulphate* is required.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 1.0 g of Ferrous Gluconate, dissolve in a mixture of 140 ml of *water* and 60 ml of *1 M sulphuric acid* and titrate with *0.1 M ceric ammonium sulphate*, using *ferroin solution* as indicator, until the red colour disappears.

1 ml of *0.1 M ceric ammonium sulphate*, is equivalent to 0.04822 g of $C_{12}H_{22}FeO_{14},2H_2O$.

**Storage**. Store protected from light.

**Labelling**. The label states the quantity of the active ingredient both as the amount of Ferrous Gluconate and in terms of the equivalent amount of ferrous iron in each tablet.

# Ferrous Sulphate

$FeSO_4,7H_2O$ Mol. Wt. 278.0

Ferrous Sulphate contains not less than 98.0 per cent and not more than 105.0 per cent of $FeSO_4,7H_2O$.

**Category**. Haematinic.

**Dose**. Prophylatic, 300 mg; therapeutic 600 to 900 mg daily, in divided doses. (300 mg of ferrous sulphate is approximately equivalent to 60 mg of ferrous iron).

**Description**. Bluish green crystals or a light green, crystalline powder; odourless. Efflorescent in air. On exposure to moist air, the crystals rapidly oxidise and become brown.

## Identification

Gives reaction A of ferrous salts and the reactions of sulphates (2.3.1).

## Tests

**Appearance of solution**. Dissolve 2.5 g in *carbon dioxide-free water*, add 0.5 ml of *1 M sulphuric acid* and dilute to 50.0 ml with *water* (solution A). The solution is not more opalescent than opalescence standard OS2 (2.4.1).

**pH** (2.4.24). 3.0 to 4.0, determined in a 5.0 per cent solution.

**Arsenic** (2.3.10). Dissolve 5.0 g in 10 ml of *water*, add 15 ml of *stannated hydrochloric acid* and distil 20 ml. To the distillate add a few drops of *bromine solution*, remove the excess of bromine with a few drops of *stannous chloride solution AsT* and add 40 ml of *water*. The resulting solution complies with the limit test for arsenic (2 ppm).

**Copper**. Dissolve 8.0 g in 40 ml of *hydrochloric acid*, add 10 ml of *nitric acid* and 15 ml of *water*, boil gently for 5 minutes and cool. Shake with four quantities, each of 30 ml, of *ether* and discard the ether extracts. Heat the acid solution on a water-bath to remove the dissolved *ether*, cool and add sufficient *water* to produce 100.0 ml (solution B). To 10.0 ml of solution B add 1 g of *citric acid*, make alkaline with *dilute ammonia solution*, add 25 ml of *water* and 5 ml of *sodium diethyldithiocarbamate solution*. Extract successively with 5, 3 and 2-ml quantities of *carbon tetrachloride*, mix the carbon



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tetrachloride extracts and add sufficient *carbon tetrachloride* to produce 100.0 ml. The resulting solution is not more intensely coloured than a solution prepared by treating 4.0 ml of *copper standard solution (10 ppm Cu)* and 7.5 ml of *water* in the same manner (50 ppm).

**Lead**. Make 25.0 ml of solution B alkaline with *dilute ammonia solution*, add 1 ml of *potassium cyanide solution* and sufficient *water* to produce 50.0 ml. Add 0.1 ml of *sodium sulphide solution*; the solution is not more intensely coloured than a mixture of 10 ml of *hydrochloric acid*, 0.5 ml of *nitric acid*, 5.0 ml of *lead standard solution (20 ppm Pb)*, 0.1 ml of *sodium sulphide solution* and sufficient *water* to produce 50.0 ml (50 ppm).

**Zinc**. To 2.5 ml of solution B add 1 g of *citric acid* and 1 g of *resorcinol*, neutralise the solution with *dilute ammonia solution* using *thymol blue solution* as indicator and shake for 1 minute with two quantities, each of 20 ml, of *dithizone solution*. To the combined extracts add 10 ml of *0.1 M hydrochloric acid* and shake for 1 minute. Separate the acid layer, add 3 ml of *1 M hydrochloric acid* and 20 ml of *ammonium chloride solution* and adjust the volume to 50.0 ml with *water*. Add 1.0 ml of *potassium ferrocyanide solution* and allow to stand for 15 minutes. Any turbidity produced is not greater than that developed in 15 minutes by the addition of 1.0 ml of *potassium ferrocyanide solution* to a freshly prepared mixture of 10.0 ml of *zinc standard solution (10 ppm Zn)*, 4 ml of *1 M hydrochloric acid*, 20 ml of *ammonium chloride solution* and sufficient *water* to produce 50.0 ml (500 ppm).

**Manganese**. Dissolve 1.0 g in 40 ml of *water*, add 10 ml of *nitric acid* and boil until red fumes are evolved. Add 0.5 g of *ammonium persulphate* and boil for 10 minutes. Discharge any pink colour by the dropwise addition of a 5 per cent w/v solution of *sodium sulphite* and boil until any odour of sulphur dioxide is eliminated. Add 10 ml of *water*, 5 ml of *phosphoric acid* and 0.5 g of *sodium periodate*, boil for 1 minute and allow to cool. The resulting solution is not more intensely coloured than that of a solution prepared at the same time and in the same manner using 1.0 ml of *0.02 M potassium permanganate* in place of the substance under examination (0.1 per cent).

**Ferric ions**. Not more than 0.5 per cent

Dissolve 5 g ferrous sulphate in a mixture of 10 ml of *hydrochloric acid* and 100 ml of *water* in a stoppered flask, add 3 g of *potassium iodide*, close the flask and allow to stand in the dark for 5 minutes. Titrate the liberated iodine with *0.1 M sodium thiosulphate*, using 0.5 ml of *starch solution* as indicator. Carry out the blank titration. Not more than 4.5 ml of *0.1 M sodium thiosulphate* is required.

**Chlorides** (2.3.12). 20 ml of solution A complies with the limit test for chlorides (250 ppm).

**Assay**. Dissolve 2.5 g of *sodium bicarbonate* in a mixture of 150 ml of *water* and 10 ml of *sulphuric acid*. When effervescence ceases, add about 0.5 g of the substance under examination, accurately weighed, shake gently to dissolve and titrate with *0.1 M ceric ammonium nitrate*, using 0.1 ml of *ferroin solution* as indicator, until the red colour disappears.

1 ml of *0.1 M ceric ammonium nitrate* is equivalent to 0.02780 g of $FeSO_4,7H_2O$.

## Dried Ferrous Sulphate

Dried Ferrous Sulphate is Ferrous Sulphate from which a part of the water of crystallisation has been removed by drying at a temperature of 40°.

Dried Ferrous Sulphate contains not less than 86.0 per cent and not more than 90.0 per cent of $FeSO_4$.

**Category**. Haematinic.

**Dose**. Prophylatic, 200 mg; therapeutic, 400 to 600 mg daily, in divided dosage.

**Description**. A greyish white to buff coloured powder.

### Identification

Gives reaction A of ferrous salts and the reactions of sulphates (2.3.1).

### Tests

**Copper**. Dissolve 8.0 g in 40 ml of *hydrochloric acid*, add 10 ml of *nitric acid* and 15 ml of *water*, boil gently for 5 minutes and cool. Shake with four quantities, each of 30 ml, of *ether* and discard the ether extracts. Heat the acid solution on a water-bath to remove the dissolved *ether*, cool and add sufficient *water* to produce 100.0 ml (solution A). To 10.0 ml of solution A add 1 g of *citric acid*, make alkaline with *dilute ammonia solution*, add 25 ml of *water* and 5 ml of *sodium diethyldithiocarbamate solution*. Extract successively with 5, 3 and 2 ml quantities of *carbon tetrachloride*, mix the carbon tetrachloride extracts and add sufficient *carbon tetrachloride* to produce 100.0 ml. The resulting solution is not more intensely coloured than a solution prepared by treating 4.0 ml of *copper standard solution (10 ppm Cu)* and 7.5 ml of *water* in the same manner (50 ppm).

**Lead**. Make 25.0 ml of solution A alkaline with *dilute ammonia solution*, add 1 ml of *potassium cyanide solution* and sufficient *water* to produce 50.0 ml. Add 0.1 ml of *sodium sulphide solution*; the solution is not more intensely coloured than a mixture of 10 ml of *hydrochloric acid*, 0.5 ml of *nitric acid*, 5.0 ml of *lead standard solution (20 ppm Pb)*, 0.1 ml of *sodium sulphide solution* and sufficient *water* to produce 50.0 ml (50 ppm).



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**Zinc**. To 2.5 ml of solution A add 1 g of *citric acid* and 1 g of *resorcinol*, neutralise the solution with *dilute ammonia solution* using *thymol blue solution* as indicator and shake for 1 minute with two quantities, each of 20 ml, of *dithizone solution*. To the combined extracts add 10 ml of *0.1 M hydrochloric acid* and shake for 1 minute. Separate the acid layer, add 3 ml of *1 M hydrochloric acid* and 20 ml of *ammonium chloride solution* and adjust the volume to 50.0 ml with *water*. Add 1.0 ml of *potassium ferrocyanide solution* and allow to stand for 15 minutes. Any turbidity produced is not greater than that developed in 15 minutes by the addition of 1.0 ml of *potassium ferrocyanide solution* to a freshly prepared mixture of 10.0 ml of *zinc standard solution (10 ppm Zn)*, 4 ml of *1 M hydrochloric acid*, 20 ml of *ammonium chloride solution* and sufficient *water* to produce 50.0 ml (500 ppm).

**Manganese**. Dissolve 1.0 g in 40 ml of *water*, add 10 ml of *nitric acid* and boil until red fumes are evolved. Add 0.5 g of *ammonium persulphate* and boil for 10 minutes. Discharge any pink colour by the dropwise addition of a 5 per cent w/v solution of *sodium sulphite* and boil until any odour of sulphur dioxide is eliminated. Add 10 ml of *water*, 5 ml of *phosphoric acid* and 0.5 g of *sodium periodate*, boil for 1 minute and allow to cool. The resulting solution is not more intensely coloured than that of a solution prepared at the same time and in the same manner using 1.0 ml of *0.02 M potassium permanganate* in place of the substance under examination (0.1 per cent).

**Arsenic** (2.3.10). Dissolve 3.3 g in 10 ml of *water*, add 15 ml of *stannated hydrochloric acid* and distil 20 ml. To the distillate add a few drops of *bromine solution*, remove the excess of bromine with a few drops of *stannous chloride solution AsT* and add 40 ml of *water*. The resulting solution complies with the limit test for arsenic (3 ppm).

**Basic sulphate**. 2.0 g dissolves slowly in a mixture of 7.5 ml of freshly boiled and cooled *water* and 0.5 ml of *0.5 M sulphuric acid*, producing a solution that is not more than faintly turbid.

**Ferric ions.** Not more than 0.5 per cent

Dissolve 5 g ferrous sulphate in a mixture of 10 ml of *hydrochloric acid* and 100 ml of *water* in a stoppered flask, add 3 g of *potassium iodide*, close the flask and allow to stand in the dark for 5 minutes. Titrate the liberated iodine with *0.1 M sodium thiosulphate*, using 0.5 ml of *starch solution* as indicator. Carry out the blank titration. Not more than 4.5 ml of *0.1 M sodium thiosulphate* is required.

**Assay.** Weigh accurately about 0.5 g, dissolve in a mixture of 30 ml of *water* and 20 ml of *1 M sulphuric acid* and titrate with *0.1 M ceric ammonium sulphate* using *ferroin solution* as indicator.

1 ml of *0.1 M ceric ammonium sulphate* is equivalent to 0.01519 g of $FeSO_4$.

## Ferrous Sulphate Tablets

Ferrous Sulphate Tablets contain Dried Ferrous Sulphate.

Ferrous Sulphate Tablets contain not less than 80.0 per cent and not more than 90.0 per cent of the stated amount of dried ferrous sulphate, $FeSO_4$. The tablets are coated.

**Usual strength**. 200 mg. (200 mg of Dried Ferrous Sulphate is approximately equivalent to 60 mg of ferrous iron).

### Identification

A. The powdered tablets give reaction A of ferrous salts (2.3.1).

B. Extract the powdered tablets with *2 M hydrochloric acid* and filter. The filtrate gives reaction A of sulphates (2.3.1).

### Tests

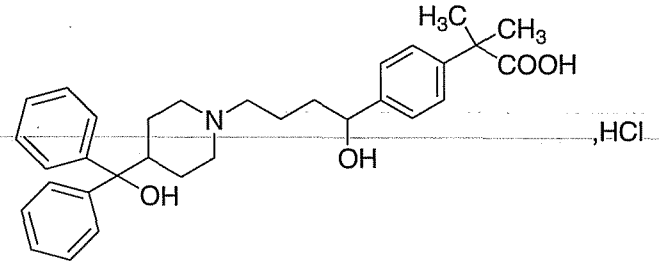
**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.5 g of Dried Ferrous Sulphate, dissolve in a mixture of 30 ml of *water* and 20 ml of *1 M sulphuric acid* and titrate with *0.1 M ceric ammonium sulphate* using *ferroin solution* as indicator.

1 ml of *0.1 M ceric ammonium sulphate* is equivalent to 0.01519 g of $FeSO_4$.

**Labelling**. The label states the quantity of the active ingredient both as the amount of Dried Ferrous Sulphate and in terms of the equivalent amount of ferrous iron in each tablet.

## Fexofenadine Hydrochloride




$C_{32}H_{39}NO_4,HCl$ Mol. Wt. 538.1

Fexofenadine Hydrochloride is (*RS*) α,α-dimethyl-4-[1-hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]butyl] benzene acetic acid hydrochloride.

Fexofenadine Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{32}H_{39}NO_4,HCl$, calculated on the anhydrous basis.

**Categrory**. Antihistaminic.

**Description**. A white to off-white crystalline powder.



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## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fexofenadine hydrochloride RS* or with the reference spectrum of fexofenadine hydrochloride.

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

## Tests

**Fexofenadine impurity B**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 25 mg of the substance under examination in 100.0 ml of the mobile phase.

*Reference solution (a)*. A solution containing 0.025 per cent w/v of *fexofenadine hydrochloride RS* and 0.0005 per cent w/v of *{3-[1-hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]butyl]-α,α-dimethylbenzeneacetic acid hydrochloride} RS* (*fexofenadine impurity B RS*) in the mobile phase.

*Reference solution (b)*. Dilute 1.0 ml of reference solution (a) to 10.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with beta cyclodextrin bonded to porous silica (5 µm),
- mobile phase: a mixture of 80 volumes of *ammonium acetate buffer* prepared by mixing 1.15 ml of *glacial acetic acid* in 1000 ml of *water* and adjusting the pH to 4.0 with *6 M ammonium hydroxide* and 20 volumes of *acetonirile*,
- flow rate. 0.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 20 µl.

Inject reference solution (a). The relative retention time with respect to fexofenadine, for fexofenadine impurity B is about 0.7 and the resolution between fexofenadine and fexofenadine impurity B is not less than 3.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any peak due to fexofenadine impurity B is not more than the area of the peak due to fexofenadine impurity B in the chromatogram obtained with reference solution (b) ( 0.2 per cent ).

**Related substances**. Determine by liquid chromatography (2.4.14).

Use the phosphate-perchlorate buffer, solvent mixture and chromatographic system as described in the Assay.

*Test solution*. Dissolve 50 mg of the substance under examination in 50 ml of the solvent mixture.

*Reference solution*. A solution containing 0.0005 per cent w/v of each of *fexofenadine hydrochloride RS* and *[benzeneacetic acid, 4-(1-oxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl] butyl]-α,α-dimethyl] RS* (*fexofenadine impurity A RS*) in the mobile phase.

Inject the test solution and the reference solution. In the chromatogram obtained with the test solution, the area of any peak due to the peak of fexofenadine impurity A is not more than 0.4 times the area of the peak due to fexofenadine impurity A in the chromatogram of the reference solution ( 0.2 per cent), area of any peak due to decarboxylated degradant [(+)-4-[1-hydroxy-4-(hydroxydi-phenylmethyl)-1-piperidinyl]-butyl]-isopropylbenzene], with a relative retention time of 3.2 with respect to fexofenadine hydrochloride, is not more than 0.3 times the area of the peak due to fexofenadine hydrochloride in the chromatogram of the reference solution ( 0.15 per cent ) and the area of any peak due to other unknown impurity is not more than 0.2 times the area of the peak due to fexofenadine hydrochloride in the chromatogram of the reference solution (0.1 per cent). The sum of all the impurities is not more than 0.5 per cent.

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

**Chlorides**. 6.45 per cent to 6.75 per cent.

Dissolve about 0.3 g of the substance under examination in 50 ml of *methanol*. Titrate with *0.1 M silver nitrate*. Determine the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M silver nitrate* is equivalent to 0.00354 g of chloride.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 0.5 per cent for the anhydrous form and 6.0 per cent to 10.0 per cent for the hydrate form (dihydrate and trihydrate forms of fexofenadine hydrochloride), determined on 1 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. 50 volumes of *acetonitrile* and 50 volumes of *phosphate-perchlorate buffer*.

*Test solution*. Dissolve 50 mg of the substance under examination in 50 ml of the solvent mixture. Dilute 3.0 ml of the solution to 50.0 ml with the mobile phase.

*Reference solution*. A solution containing 0.006 per cent w/v of *fexofenadine hydrochloride RS* and 0.0005 per cent w/v of *fexofenadine impurity A RS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with phenyl groups chemically bonded to porous silica (5 µm),
- mobile phase: a mixture of 65 volumes of *phosphate-perchlorate buffer* prepared by dissolving 6.64 g of



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*monobasic sodium phosphate* and 0.84 g of *sodium perchlorate* in 1000 ml of *water* and adjusting the pH to 2.0 with *orthophosphoric acid*, and 35 volumes of *acetonitrile*. Add 3 ml of *triethylamine* to 1000 ml of the mixture,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the resolution between fexofenadine and fexofenadine impurity A is not less than 10, tailing factor is not more than 2.0 and the relative standard deviation for replicate injections determined from fexofenadine and fexofenadine impurity A are not more than 2.0 per cent and 3.0 per cent respectively.

Inject the test solution and the reference solution.

Calculate the content of $C_{32}H_{39}NO_4$, HCl.

**Storage**. Store protected from light and moisture, at a temperature not exceeding 30°.

# Fexofenadine Capsules

Fexofenadine Hydrochloride Capsules

Fexofenadine Capsules contain not less than 93.0 per cent and not more than 105.0 per cent of the stated amount of fexofenadine hydrochloride, $C_{32}H_{39}NO_4HCl$.

**Usual strengths**. 120 mg; 180 mg.

## Identification

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

## Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *water*,
Speed and time. 50 rpm and 15 and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

*Test solution*. Dilute the filtrate, if necessary, with the dissolution medium.

*Reference solution (a)*. A 0.007 per cent w/v solution of *fexofenadine hydrochloride RS* in the dissolution medium.

*Note — A small amount of methanol, not exceeding 0.5 per cent of the total volume, can be used to dissolve fexofenadine hydrochloride.*

*Reference solution (b)*. A solution containing 0.001 per cent w/v of *benzene acetic acid-4-[1-oxy-4(4-(hydroxydiphenyl-methyl)-1-piperidinyl]butyl)-α,α-dimethyl RS (fexofenadine impurity A RS)* and 0.006 per cent w/v of *fexofenadine hydrochloride RS* in *water*.

*Note — A small amount of acetic acid, not exceeding 5 per cent of the total volume, can be used to dissolve fexofenadine impurity A*.

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 30 volumes of a buffer solution prepared by dissolving 1.0 g of *monobasic sodium phosphate*, 0.5 g of *sodium perchlorate*, and 0.3 ml of *orthophosphoric acid* in 300 ml of *water* and 70 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 50 µl.

Inject reference solution (b). The resolution between fexofenadine and fexofenadine impurity A is not less than 2.0.

Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

D. Not less than 50 per cent in 15 minutes and not less than 75 per cent in 45 minutes, of the stated amount of $C_{32}H_{39}NO_4,HCl$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Phosphate-perchlorate buffer*. Dissolve 6.64 g of *monobasic sodium phosphate* and 0.84 g of *sodium perchlorate* in 1000 ml of *water*. Adjust with *orthophosphoric acid* to a pH of 2.0.

*Solvent mixture*. 50 volumes of *acetonitrile* and 50 volumes of *phosphate-perchlorate buffer*.

*Test solution (a)*. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 50 mg of Fexofenadine Hydrochloride, add 40 ml of solvent mixture and shake by mechanical means for 60 minutes. Mix with the aid of ultrasound for about 2 minutes. Cool and dilute to 50.0 ml with the solvent mixture.

*Test solution (b)*. Dilute 3.0 ml of test solution (a) to 50.0 ml with the mobile phase.

*Reference solution*. A solution containing 0.006 per cent w/v of *fexofenadine hydrochloride RS* and 0.0005 per cent w/v of *fexofenadine impurity A RS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with phenyl groups chemically bonded to porous silica (5 µm),



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- mobile phase: a mixture of 65 volumes of *phosphate-perchlorate buffer* and 35 volumes of *acetonitrile*. Add 0.3 ml of *triethylamine* and mix,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 20 µl.

Inject reference solution.The resolution between fexofenadine and fexofenadine impurity A is not less than 10, tailing factor is not more than 2.0 and the relative standard deviation for replicate injections determined from fexofenadine and fexofenadine impurity A are not more than 2.0 per cent and 3.0 per cent respectively.

Inject test solution (a) and the reference solution. In the chromatogram obtained with test solution (a) the area due to fexofenadine impurity A is not more than 0.8 times the area of the peak due to fexofenadine impurity A in the chromatogram obtained with the reference solution (0.4 per cent), the area of the peak due to decarboxilated degradant [(+)-4-[1-hydroxy-4-[4-(hydroxydi-phenylmethyl)-1-piperidinyl]-butyl]-isopropylbenzene having a relative retention time of 3.2 is not more than 0.03 times the area of the principal peak in the chromatogram obtained with the reference solution (0.2 per cent), the area of any other individual impurity is not more than 0.03 times the area of the principal peak in the chromatogram obtained with the reference solution (0.2 per cent), and the sum of all the impurities is not more than 0.5 per cent.

**Other tests**. Comply with the tests stated under Capsules.

**Assay**. Determine by liquid chromatography (2.4.14) as described under Related substances.

Inject test solution (b) and the reference solution.

Calculate the content of $C_{32}H_{39}NO_4$, HCl in the capsules.

**Storage**. Store protected from moisture, at a temperature not exceeding 30°.

# Fexofenadine Tablets

## Fexofenadine Hydrochloride Tablets

Fexofenadine Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of fexofenadine hydrochloride, $C_{32}H_{39}NO_4$,HCl.

**Usual strengths**. 120 mg; 180 mg.

### Identification

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

### Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.001 M hydrochloric acid,*
Speed and time. 50 rpm and 10 and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

*Test solution*. Dilute the filtrate, if necessary, with the dissolution medium.

*Reference solution (a)*. Dissolve an accurately weighed quantity of *fexofenadine hydrochloride RS* in the dissolution medium to obtain a solution having a known concentration similar to that expected for the solution under test.

*Note — A small amount of methanol, not exceeding 0.5 per cent of the total volume, can be used to dissolve fexofenadine hydrochloride.*

*Reference solution (b)*. A 0.044 per cent w/v solution of *benzene acetic acid,-4-[1-oxy-4(4-(hydroxydiphenyl-methyl)-1-piperidinyl]butyl)-α,α-dimethyl RS (fexofenadine impurity A RS)* in *water*. To 1.0 ml of this solution add 40 ml of reference solution (a).

*Note — A small amount of acetic acid, not exceeding 5 per cent of the total volume, can be used to dissolve fexofenadine impurity A.*

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 30 volumes of a buffer solution prepared by dissolving 1.0 g of *monobasic sodium phosphate*, 0.5 g of *sodium perchlorate*, and 0.3 ml of *orthophosphoric acid* in 300 ml of *water* and 70 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 20 µl.

Inject reference solution (b). The resolution between fexofenadine and fexofenadine impurity A is not less than 2.0.

Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution (a).

D. Not less than 60 per cent in 10 minutes and not less than 80 per cent in 30 minutes, of the stated amount of $C_{32}H_{39}NO_4$,HCl.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. 75 volumes of *acetonitrile* and 25 volumes of a 0.17 per cent v/v solution of *glacial acetic acid.*



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*Test solution.* Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 120 mg of Fexofenadine Hydrochloride, disperse in 20 ml of 0.17 per cent v/v solution of *glacial acetic acid*, with vigorous shaking for 30 minutes and dilute to 100.0 ml with *acetonitrile*, shake vigorously for 60 minutes, filter.

*Reference solution (a).* A solution containing 0.025 per cent w/v of *fexofenadine hydrochloride RS* and 0.005 per cent w/v of *fexofenadine impurity A RS* in the solvent mixture. Dilute 3 ml and 4.5 ml, respectively, of the solutions to 50.0 ml with the mobile phase.

*Reference solution (b).* A 0.025 per cent w/v solution of *fexofenadine hydrochloride RS* in the solvent mixture. Dilute 4 ml of the solution to 100.0 ml with the mobile phase. Dilute 6 ml of this solution to 100.0 ml with the mobile phase.

Use the chromatographic system described under Assay.

Inject reference solution (b). The test is not valid unless the relative standard deviation for replicate injections is not more than 6.0 per cent.

Inject reference solution (a). The relative retention time with respect to fexofenadine for fexofenadine impurity A is about 1.6. The resolution between fexofenadine and fexofenadine impurity A is not less than 7, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent and not more than 3.0 per cent for fexofenadine and fexofenadine impurity A, respectively.

Inject the test solution and reference solution (a). In the chromatogram obtained with the test solution the area of the peak due to fexofenadine impurity A is not more than the area of the peak due to fexofenadine impurity A in the chromatogram obtained with reference solution (a) (0.4 per cent), the area of any individual impurity is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.25 per cent), the area of the peak due to decarboxilated degradant [(+)-4-[1-hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]-butyl]-isopropylbenzene having a relative retention time of 6.7 is not more than 0.12 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent) and the sum of all the impurities is not more than 0.5 per cent. Ignore any peak with an area less than 0.04 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Determine by liquid chromatography (2.4.14).

*Solvent mixture.* 75 volumes of *acetonitrile* and 25 volumes of 0.17 per cent v/v *glacial acetic acid* in *water.*

*Test solution.* Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 120 mg of Fexofenadine Hydrochloride, disperse in 20 ml of a 0.17 per cent v/v solution of *glacial acetic acid*, with vigorous shaking for 30 minutes and dilute to 100.0 ml with *acetonitrile*, shake vigorously for 60 minutes and filter. Dilute 1.5 ml of this solution to 100.0 ml with the mobile phase.

*Reference solution.* A 0.025 per cent w/v solution of *fexofenadine hydrochloride RS* in the solvent mixture. Dilute 3 ml of the solution to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with phenyl groups chemically bonded to porous silica (5 µm),
- column temperature 35°,
- mobile phase: a mixture of 64 volumes of a buffer solution prepared by diluting 7.5 ml of *acetonitrile* and 7.5 ml of *triethylamine* to 1000 ml with 0.17 per cent v/v *glacial acetic acid* in *water*, adjust the pH to 5.2 with *orthophosphoric acid* and 36 volumes of *acetonitrile*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 20 µl.

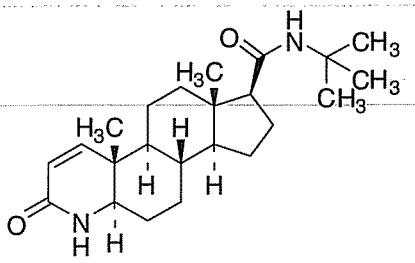
Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{32}H_{39}NO_4$, HCl in the tablets.

**Storage.** Store protected from moisture, at a temperature not exceeding 30°.

## Finasteride



$C_{23}H_{36}N_2O_2$ Mol. Wt. 372.6

Finasteride is 17β-(*N-tert*-butylcarbamoyl)-4-aza-5α-androst-1-en-3-one.

Finasteride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{23}H_{36}N_2O_2$, calculated on the dried basis.

**Category.** Antiandrogen.

**Description.** A white or almost white, crystalline powder.



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### Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *finasteride RS* or with the reference spectrum of finasteride.

### Tests

**Specific optical rotation** (2.4.22). +12.0° to +14.0°, determined in a 1.0 per cent w/v solution in *methanol*.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. 50 volumes of *acetonitrile* and 50 volumes of *water*.

*Test solution (a)*. Dissolve 25 mg of the substance under examination in 50.0 ml of the solvent mixture.

*Test solution (b)*. Dissolve 0.1 g of the substance under examination in 10.0 ml of the solvent mixture.

*Reference solution (a)*. A 0.05 per cent w/v solution of *finasteride RS* in the solvent mixture.

*Reference solution (b)*. A 1.0 per cent w/v solution of *finasteride for system suitability RS* in the solvent mixture.

*Reference solution (c)*. Dilute 2.0 ml of test solution (b) to 100.0 ml in solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature 60°,
- mobile phase: a mixture of 10 volumes of *acetonitrile*, 10 volumes of *tetrahydrofuran* and 80 volumes of *water*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

Inject reference solution (b). Run the chromatogram twice the retention time of the principal peak. The test is not valid unless the height above the baseline of the peak due to finasteride impurity A and the height above the baseline of the lowest point of the curve separating this peak from the peak due to finasteride, is not less than 2.5. The relative retention time with reference to finasteride for *N*-(1,1-dimethylethyl)-3-oxo-4-aza-5α-androstane-17β-cabroxamide (dihydrofinasteride) (finasteride impurity A ) is about 0.94, for methyl 3-oxo-4-aza-5α-androst-1-ene-17β-carboxylate (Δ-1-aza ester) (finasteride impurity B) is about 1.22, and for *N*-(1,1-dimethylethyl)-3-oxo-4-azaandrosta-1,5-diene-17β-carboxamide (Δ-1,5-aza amide) (finasteride impurity C ) is about 1. 36.

Inject reference solution (b), (c) and test solution (b). In the chromatogram obtained with the test solution (b) the area of secondary peak corresponding to finasteride impurity A is not more than 0.3 per cent , calculated from the area of the corresponding peak in the chromatogram obtained with reference solution (b) and taking into account the assigned value of impurity A in *finasteride for system suitability RS*. The area of each the secondary peak corresponding to finasteride impurity B and finasteride impurity C is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent). The area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent). The sum of all other secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.6 per cent). Ignore any peak with an area less than 0. 25 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Determine by liquid chromatography (2.4.14) as described under Related substances.

Inject reference solution (a) and test solution (a).

Calculate the percentage content of $C_{23}H_{36}N_2O_2$.

**Storage**. Store protected from light.

## Finasteride Tablets

Finasteride Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of finasteride, $C_{23}H_{36}N_2O_2$.

**Usual strength**. 5 mg.

### Identification

In the test for Dissolution, the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

### Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *water*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

*Test solution*. The filtrate obtained as given above.

*Reference solution*. Dissolve an accurately weighed quantity of *finasteride RS* in a mixture of 3 volumes of *water* and



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7 volumes of *acetonitrile* and dilute with the same mixture to obtain a solution having a known concentration similar to the expected concentration of the test solution.

Chromatographic system
- a stainless steel column 5.0 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- column temperature. 45°,
- mobile phase: a mixture of 42 volumes of *water* and 58 volumes of *acetonitrile*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 200 µl.

Inject the reference solution. The test is not valid unless the tailing factor of the principal peak is less than 2.0.

Inject the reference solution and the test solution.

Calculate the content of $C_{23}H_{36}N_2O_2$ in the medium.

D. Not less than 75 per cent of the stated amount of $C_{23}H_{36}N_2O_2$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. 50 volumes of *acetonitrile* and 50 volumes of *water*.

*Test solution*. Dissolve a quantity of powdered tablets containing about 100 mg of Finasteride in 7 ml of solvent mixture and dilute to 10.0 ml with the solvent mixture, centrifuge and filter the supernatant liquid.

*Reference solution (a)*. Dilute 2.0 ml of test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 with the solvent mixture.

*Reference solution (b)*. A 1.0 per cent w/v solution of *finasteride for system suitability RS* in the solvent mixture.

*Reference solution (c)*. Dilute 25.0 ml of reference solution (a) to 100.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 60°,
- mobile phase: a mixture of 10 volumes of *acetonitrile*, 10 volumes of *tetrahydrofuran* and 80 volumes of *water*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

Inject reference solution (b) and (c). The relative retention time with reference to finasteride for finasteride impurity A is about 0.9, for finasteride impurity B is about 1.2 and for finasteride impurity C is about 1.4. Run the chromatogram twice the retention time of the principal peak. The test is not valid unless: (a) in the chromatogram obtained with reference solution (b), the peak to valley ratio between finasteride impurity A and finasteride is at not less than 2.5 and (b) in the chromatogram obtained with reference solution (c), the peak due to finasteride has a signal to noise ratio of not less than 10.

Inject reference solution (a), (b), (c) and the test solution. In the chromatogram obtained with the test solution the area of each of any peak corresponding to finasteride impurity A, finasteride impurity B and finasteride impurity C is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent); the area of any other peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of the areas of all other secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.6 per cent). Ignore any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

**Uniformity of content** (*For tablets containing less than 10 mg*). Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), using the chromatographic conditions and reference solution as described under Assay.

*Test solution*. Powder one tablet, add 25 ml of *water*, shake with the aid of ultrasound until the tablet is completely dispersed. Add 35 ml of a mixture of 3 volumes of *water* and 7 volumes of *acetonitrile*, mix with the aid of ultrasound for a further 30 minutes. Cool to room temperature, dilute to 50 ml with *acetonitrile*, centrifuge and filter the supernatant liquid.

Calculate the content of $C_{23}H_{36}N_2O_2$ in the tablet.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh and powder 20 tablets. Disperse a quantity of powder containing about 50 mg of Finasteride with 25 ml of *water*. Add 350 ml of a mixture of 3 volumes of *water* and 7 volumes of *acetonitrile*, mix with the aid of ultrasound for 30 minutes, dilute to 500 ml with *acetonitrile*, centrifuge and filter the supernatant liquid.

*Reference solution*. A 0.01 per cent w/v solution of *finasteride RS* in *acetonitrile*.

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Hypersil ODS),
- column temperature. 45°,
- mobile phase: a mixture of equal volumes of *acetonitrile* and *0.0025 M* of *orthophosphoric acid*,



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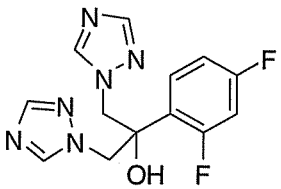
– flow rate. 1.5 ml per minute,
– spectrophotometer set at 240 nm,
– injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor of the principal peak is less than 2.0 and the relative standard deviation for replicate injections is not more than 2.0.

Inject the reference solution and the test solution.

Calculate the content of $C_{23}H_{36}N_2O_2$ in the tablets.

# Fluconazole



$C_{13}H_{12}F_2N_6O$ Mol. Wt. 306.3

Fluconazole is 2-(2,4-difluorophenyl)-1,3-bis(1*H*-1,2,4-triazo-1-yl)propan-2-ol.

Fluconazole contains not less than 98.5 per cent and not more than 101.5 per cent of $C_{13}H_{12}F_2N_6O$, calculated on the dried basis.

**Category**. Antifungal.

**Description**. A white or almost white, crystalline powder.

## Identification

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

B. When examined in the range 200 nm to 350 nm (2.4.7), a 0.025 per cent w/v solution in *methanol* shows absorption maxima at about 266 nm and 261 nm.

## Tests

**Appearance of solution**. A 5 per cent w/v solution in *methanol* is clear (2.4.1) and colourless (2.4.1).

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 300 mg of the substance under examination in 100 ml of the mobile phase.

*Reference solution*. A 0.003 per cent w/v solution of *fluconazole* RS in the mobile phase.

Chromatographic system
– a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3.5 µm),
– column temperature. 40°,
– mobile phase: 80 volumes of *water* and 20 volumes of *acetonitrile*,
– flow rate. 0.5 ml per minute.
– spectrophotometer set at 260 nm,
– injection volume. 20 µl.

The relative retention time with reference to fluconazole for fluconazole impurity A is about 0.49, for fluconazole impurity B is about 0.81 and for fluconazole impurity C is about 0.86.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution the area of impurity peak at relative retention time about 0.6 is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent), the area of secondary peak due to fluconazole impurity A or fluconazole impurity C is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (0.2 per cent); the area of any peak corresponding to fluconazole impurity B is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (0.1 per cent), the area of any other secondary peak is not more than 0.1 times the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent) and the sum of the areas of other secondary peaks is not more than 1.5 times the area of the principal peak in the chromatogram obtained with the reference solution (1.5 per cent).

**Iron** (2.3.14). Dissolve 0.5 g in 5 ml of *ethanol* and 5 ml of *water* and mix; the resulting solution complies with the limit test for iron (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

**Assay**. Dissolve 0.2 g in 100 ml of *glacial acetic acid*. Titrate with *0.1 M perchloric acid*. Determine the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01531 g of $C_{13}H_{12}F_2N_6O$.

**Storage**. Store protected from moisture.

# Fluconazole Capsules

Fluconazole Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of fluconazole, $C_{13}H_{12}F_2N_6O$.

**Usual strengths**. 50 mg; 150 mg; 200 mg.



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### Identification

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

### Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter disc with an average pore diameter not more than 1.0 µm, rejecting the first few ml of the filtrate.

Determine by liquid chromatography (2.4.14), using the chromatographic system as described under Assay.

*Test solution*. Dilute the filtrate, if necessary, with the dissolution medium.

*Reference solution*. Dissolve an accurately weighed quantity of *fluconazole RS* in the dissolution medium and dilute with the dissolution medium to obtain a solution having a known concentration similar to the expected concentration of the test solution.

D. Not less than 80 per cent of the stated amount of $C_{13}H_{12}F_2N_6O$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh accurately a quantity of the powder containing 100 mg of Fluconazole, disperse in 100.0 ml of the mobile phase and filter.

*Reference solution (a)*. A 0.1 per cent w/v solution of *fluconazole RS* in the mobile phase.

*Reference solution (b)*. Dilute 1 ml of reference solution (a) to 100 ml with the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 80 volumes of *water* and 20 volumes of *acetonitrile*,
- flow rate. 0.5 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume. 50 µl.

Inject reference solution (b). The test is not valid unless the theoretical plates is not less than 1000 and the tailing factor is not more than 2.0.

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

**Other tests**. Comply with the tests stated under Capsules.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh accurately a quantity of the powder containing 50 mg of Fluconazole, add 70 ml of the mobile phase, sonicate for 15 minutes, and dilute to 100.0 ml with the mobile phase and filter.

*Reference solution*. A 0.05 per cent w/v solution of *fluconazole RS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of buffer solution prepared by dissolving 1.36 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water* and 40 volumes of *methanol*, adjusted to pH 3.6 with *orthophosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 261 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the theoretical plates is not less than 1000, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{13}H_{12}F_2N_6O$ in the capsules.

**Storage**. Store protected from moisture.

## Fluconazole Tablets

Fluconazole Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of fluconazole, $C_{13}H_{12}F_2N_6O$.

**Usual strengths**. 50 mg; 100 mg; 150 mg; 200 mg.

### Identification

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



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## Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter disc with an average pore diameter not more than 1.0 µm, rejecting the first few ml of the filtrate.

Determine by liquid chromatography (2.4.14), using the chromatographic system as described under Assay.

*Test solution.* Dilute the filtrate, if necessary, with the dissolution medium.

*Reference solution.* Dissolve an accurately weighed quantity of *fluconazole RS* in the dissolution medium and dilute with the dissolution medium to obtain a solution having a known concentration similar to the expected concentration of the test solution.

D. Not less than 80 per cent of the stated amount of $C_{13}H_{12}F_2N_6O$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh accurately a quantity of the powdered tablets containing 100 mg of Fluconazole, disperse in 100.0 ml of the mobile phase and filter.

*Reference solution (a).* A 0.1 per cent w/v solution of *fluconazole RS* in the mobile phase.

*Reference solution (b).* Dilute 1 ml of reference solution (a) to 100 ml with the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 80 volumes of *water* and 20 volumes of *acetonitrile*,
- flow rate. 0.5 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume. 50 µl.

Inject reference solution (b). The test is not valid unless the theoretical plates is not less than 1000 and the tailing factor is not more than 2.0.

Inject reference solution (b) and the test solution. Run the chromatogram 4 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with reference solution (b) (2.0 per cent).

**Other tests.** Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Disperse a quantity of the powdered tablets containing about 50 mg of Fluconazole with 70 ml of the mobile phase, sonicate for 15 minutes and dilute to 100.0 ml with the mobile phase, filter.

*Reference solution.* A 0.05 per cent w/v solution of *fluconazole RS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of buffer solution prepared by dissolving 1.36 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water* and 40 volumes of *methanol*, adjusted to pH 3.6 with *orthophosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 261 nm,
- injection volume. 20 µl.

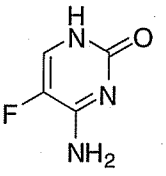
Inject the reference solution. The test is not valid unless the theoretical plates is not less than 1000, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{13}H_{12}F_2N_6O$.

**Storage**. Store protected from moisture.

# Flucytosine




$C_4H_4FN_3O$ Mol. Wt. 129.1

Flucytosine is 4-amino-5-fluoro-2-(1*H*)-pyrimidinone.

Flucytosine contains not less than 98.5 per cent and not more than 101.0 per cent of $C_4H_4FN_3O$, calculated on the dried basis.

**Category**. Antifungal.

**Description**. A white or almost white, crystalline powder.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*



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

B. In the test for Related Substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

C. Mix about 5 mg with 45 mg of *heavy magnesium oxide* and ignite in a crucible until an almost white residue is obtained (usually less than 5 minutes). Allow to cool, add 1 ml of *water*, 0.05 ml of *phenolphthalein solution* and about 1 ml of *dilute hydrochloric acid* to render the solution colourless. Filter and add to the filtrate a freshly prepared mixture of 0.1 ml of *alizarin solution* and 0.1 ml of *zirconyl nitrate solution*. Mix, allow to stand for 5 minutes and compare the colour of the solution with that of a blank prepared in the same manner. The colour of the solution changes from red to yellow.

D. To 5 ml of solution A, add 0.15 ml of *bromine water* and shake. The colour of the solution is discharged.

## Tests

**Appearance of solution**. A 1.0 per cent w/v solution in *carbon dioxide-free water* (solution A) is clear (2.4.1) and not more intensely coloured than reference solution BYS7 (2.4.1).

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Solvent mixture*. 40 volumes of *water* and 60 volumes of *methanol*.

*Mobile phase*. A mixture of 1 volume of *anhydrous formic acid*, 15 volumes of *water*, 25 volumes of *methanol* and 60 volumes of *ethyl acetate*.

*Test solution (a)*. Dissolve 50 mg of the substance under examination in 5.0 ml of the solvent mixture.

*Test solution (b)*. Dilute 1.0 ml of test solution (a) to 10.0 ml with the solvent mixture.

*Reference solution (a)*. A 0.1 per cent w/v solution of *flucytosine RS* in the solvent mixture.

*Reference solution (b)*. Dilute 1.0 ml of test solution (b) to 100.0 ml with the solvent mixture.

*Reference solution (c)*. Dissolve 5 mg of *fluorouracil RS* in 5.0 ml of reference solution (a).

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of cold air, heat at 110° for 10 minutes, place the plate, while hot, in a tank of chlorine vapours for 5 minutes, prepared by adding a mixture of 2 volumes of a 1.5 per cent w/v solution of *potassium permanganate*, 1 volume of *hydrochloric acid* and 1 volume of *water* contained in a beaker placed at the bottom of tank and allow the plate to stand for 15 minutes. Remove the plate from the tank and dry it in a current of cold air until the excess of chlorine is removed and an area of the plate below the line of application gives not more than a faint blue colour with a drop of *potassium iodide and starch solution*; avoid prolonged exposure to cold air. Spray the plate with *potassium iodide and starch solution* and examine the plate in daylight. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.1 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated principal spots.

**Fluoride**. Not more than 200 ppm.

*NOTE—Prepare and store all solutions in plastic containers.*

*Buffer solution*. Dissolve 58 g of *sodium chloride* in 500 ml of *water*. Add 57 ml of *glacial acetic acid* and 200 ml of a 10.0 per cent w/v solution of *cyclohexylenedinitrilotetra-acetic acid* in *1 M sodium hydroxide*. Adjust the pH to 5.0 to 5.5 with a 20.0 per cent w/v solution of *sodium hydroxide* and dilute to 1000.0 ml with *water*.

*Test solution*. Dissolve 1.0 g of the substance under examination in *water* and dilute to 100.0 ml with the same solvent.

*Reference solutions*. Dissolve 4.42 g of *sodium fluoride*, previously dried at 120° for 2 hours, in 300 ml of *water* and dilute to 1000.0 ml with the same solvent (0.19 per cent w/v of fluoride). Prepare three reference solutions by diluting 1ml in 100 ml, 1ml in 1000 ml and 1 ml in 10000 ml.

To 20.0 ml of each reference solution, add 10.0 ml of the buffer solution and stir with a magnetic stirrer. Introduce the electrodes into the solution and allow to stand for 5 minutes with constant stirring, determining the end point potentiometrically (2.4.25) for fluoride ion, using a fluoride-selective indicator electrode and a silver-silver chloride reference electrode. Determine the potential difference between the electrodes. Plot on semi-logarithmic graph paper the potential difference obtained for each solution as a function of concentration of fluoride. Using exactly the same conditions, determine the potential difference obtained with the test solution and calculate the content of fluoride.

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

**Sulphated ash** (2.3.18). Not more than 0.1 per cent, using platinum crucible.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.1 g, dissolve in 40 ml of *anhydrous acetic acid* and add 100 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.



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1 ml of *0.1 M perchloric acid* is equivalent to 0.01291 g of $C_4H_4FN_3O$.

**Storage**. Store protected from light.

## Flucytosine Capsules

Flucytosine Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of flucytosine, $C_4H_4FN_3O$.

**Usual strengths**. 250 mg; 500 mg.

### Identification

A. When examined in the range 200 nm to 400 nm (2.4.7), a 0.002 per cent w/v solution shows absorption maxima at about 260 nm and 350 nm.

B. Shake a portion of the contents of capsules containing about 500 mg of Flucytosine with 10 ml of *water* and filter. To 2 ml of the filtrate, add 1 ml of *sodium pentacyanoaminoferrate reagent* prepared by dissolving 100 mg of *sodium (tri)pentacyanoaminoferrate* in 20 ml of 1 per cent w/v solution of *sodium carbonate solution* and 1 ml of 3 per cent v/v *hydrogen peroxide*; a darker green colour is produced.

### Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *water*,
Speed and time. 75 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter. Dilute the filtrate, if necessary, with the same solvent. Measure the absorbance at the maximum at about 276 nm (2.4.7). Calculate the content of $C_4H_4FN_3O$ in the medium from the absorbance obtained from a solution of known concentration of *flucytosine RS*.

D. Not less than 80 per cent of the stated amount of $C_4H_4FN_3O$.

**Other tests**. Complies with the tests stated under Capsules.

**Assay**. Weigh accurately a quantity of the contents of 20 capsules containing about 250 mg of Flucytosine, add about 50 ml of *0.1 M hydrochloric acid*, shake for 30 minutes, then dilute with *0.1 M hydrochloric acid* to produce 250.0 ml. Mix, and filter, discard the first 20 ml of the filtrate and dilute 10.0 ml of the filtrate to 250.0 ml with *0.1 M hydrochloric acid*. Further dilute 10.0 ml of this solution to 50.0 ml. with *0.1 M hydrochloric acid*. Measure the absorbance of the resulting solution at the maximum at about 285 nm (2.4.7). Calculate the content of $C_4H_4FN_3O$ from the absorbance obtained with 0.0008 per cent w/v solution of *flucytosine RS* in the same medium.

**Storage**. Store protected from light.

## Flucytosine Oral Suspension

Flucytosine Oral Suspension is a suspension of Flucytosine in a suitable flavoured vehicle.

The suspension is constituted by dispersing the contents of the sealed container in the specified volume of water just before use.

Flucytosine Oral Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of flucytosine, $C_4H_4FN_3O$.

**Usual strength**. 10 mg per ml.

### Identification

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

### Tests

**pH** (2.4.24). 4.0 to 5.0.

**Other tests**. Complies with the tests stated under Oral Liquids.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Shake a quantity of the oral suspension containing about 5 mg of Flucytosine with 100 ml of the mobile phase.

*Reference solution*. A 0.005 per cent w/v solution of *flucytosine RS* in the mobile phase.

Chromatographic system
- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of *methanol* and 50 volumes of buffer solution prepared by dissolving 1 g of *ammonium acetate* and 1.0 ml of *di-isopropylamine* in 1000 ml of *water*, adjust the pH to 7.5 with *glacial acetic acid* ,
- flow rate. 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_4H_4FN_3O$ in the suspension.

Determine the weight per ml (2.4.29) of the suspension and calculate the content of $C_4H_4FN_3O$, weight in volume.

Repeat the procedure using a portion of the suspension that has been stored at the temperature and for the period stated



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on the label during which it may be expected to be satisfactory for use.

**Storage**. Store protected from light and moisture.

**Labelling**. The constitued suspension should be used within 60 days.

## Flucytosine Tablets

Flucytosine Tablets contains not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of flucytosine, $C_4H_4FN_3O$.

**Usual strengths**. 250 mg; 500 mg.

### Identification

A. Extract a quantity of the powdered tablets containing 0.5 g of flucytosine with 100 ml of *methanol* for 30 minutes, filter and evaporate the filtrate to dryness. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *flucytosine RS* or with the reference spectrum of flucytosine.

B. When examined in the range 230 to 350 nm (2.4.7), the solution obtained in the Assay shows absorption maxima only at about 286 nm.

### Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *water*,
Speed and time. 75 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter. Dilute the filtrate, if necessary, with the same solvent. Measure the absorbance at the maximum at about 276 nm (2.4.7). Calculate the content of $C_4H_4FN_3O$ in the medium from the absorbance obtained from a solution of known concentration of *flucytosine RS*.

D. Not less than 80 per cent of the stated amount of $C_4H_4FN_3O$.

**Related substances**. Determine by thin layer chromatography (2.4.17), coating the plate with *silica gel*.

*Mobile phase*. A mixture of 1 volume of *anhydrous formic acid* , 15 volumes of *water*, 25 volumes of *methanol* and 60 volumes of *ethyl acetate*.

*Test solution*. Shake a quantity of the powdered tablets containing about 0.1 g of Flucytosine with 10.0 ml of a mixture of equal volumes of *13.5 M ammonia* and *methanol*, filter.

*Reference solution (a)*. Dilute 1.0 ml of test solution to 10.0 ml with *methanol (60 per cent)*. Dilute 1.0 ml of the solution to 100.0 ml with *methanol (60 per cent)*.

*Reference solution (b)*. Dilute 1.0 ml of test solution to 10.0 ml and dissolve 5 mg of *fluorouracil RS* in 5.0 ml of the resulting solution.

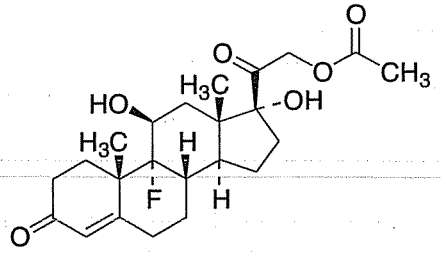
Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of cold air, heat at 110° for 10 minutes, place the plate, while hot, in a tank of chlorine gas prepared by adding *hydrochloric acid* to a 5 per cent w/v solution of *potassium permanganate* contained in a beaker placed in the tank and allow to stand for 15 minutes. Dry it in a current of cold air until an area of the plate below the line of application gives at most a very faint blue colour with a 0.5 per cent w/v solution of *potassium iodide* in *starch solution*; avoid prolonged exposure to cold air. Spray the plate with *potassium iodide* and *starch solution* and examine the plate in daylight. Any secondary spot in the chromatogram obtained with test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.1 per cent). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated principal spots.

**Other tests**. Comply with the tests stated under tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 0.1 g of Flucytosine add 80 ml of *0.1 M hydrochloric acid*, shake for 15 minutes and Dilute to 100 .0 ml with *0.1 M hydrochloric acid* and filter. Dilute 10.0 ml of the filtrate to 100.0 ml with *0.1 M hydrochloric acid*. Further dilute 10.0 ml of the solution to 100.0 ml with *0.1 M hydrochloric acid*. Measure the absorbance of the resulting solution at the maximum at about 286 nm (2.4.7). Calculate the content of $C_4H_4FN_3O$ taking 709 as the specific absorbance at 286 nm.

**Storage**. Store protected from light.

## Fludrocortisone Acetate




$C_{23}H_{31}FO_6$ Mol. Wt. 422.5

Fludrocortisone Acetate is 9α-fluoro-11β,17α,21-trihydroxy-pregn-4-ene-3,20-dione 21-acetate.



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Fludrocortisone Acetate contains not less than 96.0 per cent and not more than 104.0 per cent of $C_{23}H_{31}FO_6$, calculated on the dried basis.

**Category**. Mineralocorticoid.

**Dose**. In adrenocortical insufficiency, 50 to 300 µg daily.

**Description**. A white or almost white, crystalline powder; odourless or almost odourless; hygroscopic.

## Identification

*Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fludrocortisone acetate RS* or with the reference spectrum of fludrocortisone acetate.

B. To a warm 1 per cent w/v solution in *methanol* add an equal volume of *potassium cupri-tartrate solution*; a red precipitate is produced.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Solvent mixture*. A mixture of 90 volumes of *acetone* and 10 volumes of *formamide*.

*Mobile phase*. A mixture of 30 volumes of *toluene* and 10 volumes of *chloroform*.

*Test solution*. Dissolve 25 mg of the substance under examination in 10 ml of the solvent mixture.

*Reference solution (a)*. Dissolve 25 mg of *fludrocortisone RS* in 10 ml of the solvent mixture.

*Reference solution (b)*. Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v)*. Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

## Tests

**Specific optical rotation** (2.4.22). +148° to +156°, determined in a 1.0 per cent w/v solution in *dioxan*.

**Light absorption** (2.4.7). Absorbance of a 0.001 per cent w/v solution in *ethanol* at the maximum at about 240 nm, 0.39 to 0.42.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 20 mg of the substance under examination in the mobile phase and dilute to 10 ml with the mobile phase.

*Reference solution (a)*. Dissolve 2 mg of *fludrocortisone acetate RS* and 2 mg of *hydrocortisone acetate RS* in the mobile phase and dilute to 50 ml with the mobile phase.

*Reference solution (b)*. Dilute 1 ml of the test solution to 50 ml with the mobile phase.

Chromatographic system

- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 35 volumes of *tetrahydrofuran* and 65 volumes of *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Equilibrate the column with the mobile phase for about 30 minutes.

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (b) is 70 per cent to 90 per cent of the full scale of the recorder.

Inject reference solution (a). The retention times are: hydrocortisone acetate, about 8.5 minutes and fludrocortisone acetate about 10 minutes. The test is not valid unless the resolution between the peaks corresponding to hydrocortisone acetate and fludrocortisone acetate is at least 1.0. If this is not achieved, adjust the concentration of tetrahydrofuran in the mobile phase. Increasing the concentration of tetrahydrofuran reduces the retention times.

Inject the test solution and reference solution (b). Continue the chromatography for twice the retention time of the principal peak. In the chromatogram obtained with the test solution: the area of any peak other than the principal peak is not greater than half the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of the areas of all such peaks is not greater than 0.75 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent). Ignore any peak due to the solvent and any peak with an area less than 0.025 times



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that of the principal peak in the chromatogram obtained with reference solution (b).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 10 mg, dissolve in 50 ml of *ethanol* and add sufficient *ethanol* to produce 100.0 ml. Dilute 5.0 ml of this solution to 50.0 ml with *ethanol* and mix. Measure the absorbance of the resulting solution (2.4.7) at the maximum at about 238 nm. Calculate the content of $C_{23}H_{31}FO_6$ taking 405 as the specific absorbance at 238 nm.

**Storage**. Store protected from light at a temperature not exceeding 30°.

# Fludrocortisone Tablets

Fludrocortisone Acetate Tablets

Fludrocortisone Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of fludrocortisone acetate, $C_{23}H_{31}FO_6$.

**Usual strength**. 100 µg.

## Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Solvent mixture*. A mixture of 90 volumes of *acetone* and 10 volumes of *formamide*.

*Mobile phase*. A mixture of 30 volumes of *toluene* and 10 volumes of *chloroform*.

*Test solution*. Shake a quantity of the powdered tablets containing 1 mg of Fludrocortisone Acetate with 20 ml of *chloroform* for 5 minutes, filter and evaporate the filtrate to dryness. Dissolve the residue in 4 ml of a mixture of 90 volumes of *chloroform* and 10 volumes of *methanol*.

*Reference solution (a)*. Dissolve 25 mg of *fludrocortisone RS* in 10 ml of the solvent mixture.

*Reference solution (b)*. Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 20 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v)*. Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

## Tests

**Uniformity of content**. Comply with the test stated under Tablets.

Powder one tablet, disperse in 10 ml of *water* and extract with three quantities, each of 5 ml, of *chloroform*. Filter the extracts through a plug of cotton wool moistened with *chloroform*. Evaporate the chloroform on a water-bath just to dryness. Cool and dissolve the residue in 10.0 ml of *ethanol*. Measure the absorbance of the resulting solution at the maximum at about 240 nm (2.4.7). Calculate the content of $C_{23}H_{31}FO_6$ taking 405 as the specific absorbance at 240 nm.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution (a)*. Weigh and powder 20 tablets. Shake a weighed quantity of the powdered tablets containing about 0.5 mg of Fludrocortisone Acetate with 2 ml of *water* for one minute, add 8 ml of *acetonitrile* and shake on a mechanical shaker for 40 minutes. Dilute the mixture to 20.0 ml with *acetonitrile*, centrifuge and use the supernatant liquid.

*Test solution (b)*. Prepare in the same manner as test solution (a) but using 4.0 ml of a solution containing 0.01 per cent w/v of *norethisterone RS* (internal standard) in *acetonitrile* and 4.0 ml of *acetonitrile* in place of 8 ml of *acetonitrile*.

*Reference solution*. Mix 20.0 ml of internal standard, 25.0 ml of a 0.01 per cent w/v solution of *fludrocortisone acetate RS* in *acetonitrile* and 10 ml of *water* and dilute to 100.0 ml with *acetonitrile*.

Chromatographic system

- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (Such as Spherisorb ODS 1),
- mobile phase: a mixture of 40 volumes of *acetonitrile* and 60 volumes of *water*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume. 20 µl.

Calculate the content of $C_{23}H_{31}FO_6$ in the tablets.

**Storage**. Store protected from light.

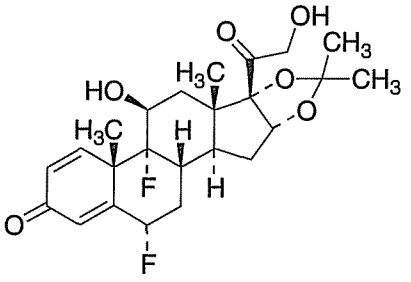


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# Fluocinolone Acetonide



$C_{24}H_{30}F_2O_6$ Mol. Wt. 452.5

Fluocinolone Acetonide is 6α,9α-difluoro-11β,21-dihydroxy-16α,17α-isopropylidenedioxypregna-1,4-diene-3,20-dione.

Fluocinolone Acetonide contains not less than 96.0 per cent and not more than 104.0 per cent of $C_{24}H_{30}F_2O_6$, calculated on the dried basis.

**Category**. Adrenocortical steroid.

**Description**. A white or almost white, crystalline powder.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests C and D may be omitted if tests A and B are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fluocinolone acetonide RS*.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Solvent mixture*. A mixture of 90 volumes of *acetone* and 10 volumes of *formamide*.

*Mobile phase*. A mixture of 30 volumes of *toluene* and 10 volumes of *chloroform*.

*Test solution*. Dissolve 25 mg of the substance under examination in 10 ml of the solvent mixture.

*Reference solution (a)*. Dissolve 25 mg of *fludrocortisone acetonide RS* in 10 ml of the solvent mixture.

*Reference solution (b)*. Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v)*. Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

C. Carry out the procedure given in Test B but using solutions prepared in the following manner.

*Test solution*. Dissolve 10 mg in 1.5 ml of *glacial acetic acid* in a separating funnel, add 0.5 ml of a 2 per cent w/v solution of *chromium trioxide* and allow to stand for 30 minutes. Add 5 ml of *water* and 2 ml of *dichloromethane* and shake vigorously for 2 minutes. Allow to separate and use the lower layer.

*Reference solution (a)*. Prepare in the same manner as the test solution but using 10 mg of *fluocinolone acetonide RS*.

The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

D. Heat 0.5 ml of *chromic-sulphuric acid* in a test-tube (5 cm x about 6 mm) in a naked flame until white fumes are evolved; the solution wets the sides of the tube readily and there is no greasiness. Add 2 or 3 mg of the substance under examination and again heat in a naked flame until white fumes appear; the solution does not wet the sides of the tube and does not pour easily from the tube.

## Tests

**Specific optical rotation** (2.4.22). +92.0° to +96.0°, determined in a 1.0 per cent w/v solution in *dioxan*.

**Light absorption**. Dissolve 15 mg in about 50 ml of *ethanol* and dilute to 100.0 ml with *ethanol*. Dilute 10.0 ml of the solution to 100.0 ml with *ethanol*. Absorbance of the resulting solution at the maximum at about 239 nm, 0.52 to 0.56 (2.4.7).

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 25 mg of the substance under examination in *acetonitrile* and dilute to 10 ml with the same solvent.

*Reference solution (a)*. Dissolve 2.5 mg of *fluocinolone acetonide RS* and 2.5 mg of *triamcinolone acetonide RS* in 45 ml of *acetonitrile* and dilute to 100 ml with *water*.

*Reference solution (b)*. Dilute 1 ml of the test solution to100 ml with *acetonitrile*.

Chromatographic system

– a stainless steel column 25 cm x 4.6 mm, packed with base-deactivated end-capped octadecylsilane bonded to porous silica (5 µm),



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- mobile phase: a mixture of 450 ml of *acetonitrile* and 500 ml of *water*, allowed to equilibrate, the volume adjusted to 1000 ml with *water* and mixed again,
- flow rate. 1 ml per minute,
- spectrophotometer set at 238 nm,
- injection volume. 20 µl.

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (b) is not less than 50 per cent of the full scale of the recorder.

Inject reference solution (a). The retention times are: triamcinolone acetonide about 8.5 minutes and fluocinolone acetonide about 10 minutes. The test is not valid unless the resolution between the peaks corresponding to triamcinolone acetonide and fluocinolone acetonide is not less than 3.0.

Inject the test solution and reference solution (b). Continue the chromatography for 4 times the retention time of fluocinolone acetonide. In the chromatogram obtained with the test solution the area of any peak other than the principal peak is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of the areas of all such peaks is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.5 per cent). Ignore any peak due to the solvent and any peak with an area less than 0.05 times that of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

**Assay**. Weigh accurately about 50 mg, dissolve in *ethanol*, add sufficient *ethanol* to produce 50.0 ml and mix. Dilute 2.0 ml of this solution to 100.0 ml with *ethanol*. Measure the absorbance of the resulting solution at the maximum at about 238 nm. Calculate the content of $C_{24}H_{30}F_2O_6$ taking 355 as the specific absorbance at 238 nm.

**Storage**. Store protected from light.

# Fluocinolone Cream

Fluocinolone Acetonide Cream

Fluocinolone Cream contains Fluocinolone Acetonide in a suitable base.

Fluocinolone Cream contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of fluocinolone acetonide, $C_{24}H_{30}F_2O_6$.

**Usual strengths**. 0.0025 per cent; 0.00625 per cent; 0.01 per cent; 0.025 per cent; 0.2 per cent w/w.

## Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 60 volumes of *n-hexane*, 40 volumes of *chloroform*, 10 volumes of *methanol* and 1 volume of *triethylamine*.

*Test solution*. Disperse, by shaking a quantity of the cream containing 0.25 mg of Fluocinolone Acetonide in 2 ml of *chloroform*, add 10 ml of *methanol*, shake vigorously, cool in ice for 15 minutes, centrifuge at 3000 rpm for 15 minutes, decant the clear supernatant liquid, evaporate to dryness on a water-bath in a current of nitrogen and dissolve the residue in 1 ml of *chloroform*.

*Reference solution*. A 0.025 per cent w/v solution of *fluocinolone acetonide RS* in *chloroform*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air until the odour of the solvent is no longer detectable, heat at 105° for 5 minutes and spray whilst hot with *blue tetrazolium solution*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution (a) has the same retention time as that of the peak due to Fluocinolone Acetonide in the chromatogram obtained with the reference solution.

## Tests

**Other tests**. Complies with the tests stated under Creams.

**Assay**. Determine by liquid chromatography (2.4.14).

*For creams containing 0.025 per cent to 0.2 per cent w/w of fluocinolone acetonide:*

*Test solution (a)*. Weigh accurately a quantity of the cream containing about 2.5 mg of Fluocinolone Acetonide, add 60 ml of a solution prepared by adding 80 ml of *methanol* to 20 ml of a 25 per cent w/v solution of *lithium chloride* and disperse by shaking vigorously. Add 100 ml of *cyclohexane*, shake gently for 2 minutes and separate the lower, aqueous methanolic layer, taking care to exclude any solid matter that separates at the interface. Repeat the extraction using a further 25 ml of the *lithium chloride solution*. To the combined extracts add a solution containing 11 g of *alum* in 214 ml of *water* followed by 50 ml of *chloroform*, shake vigorously for about 3 minutes, allow the layers to separate and filter the chloroform extract through filter paper (such as Whatman No 1), previously moistened with *chloroform*, again excluding any solid matter at the interface. Repeat the extraction with 50- and 10-ml quantities of *chloroform*, filtering the extracts as before. Evaporate the combined extracts to dryness on a water-bath in a current of nitrogen, dissolve the residue in 5 ml of



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*chloroform*, transfer to a 10-ml volumetric flask with the aid of *chloroform* and add sufficient *chloroform* to produce 10.0 ml.

*Test solution (b)*. Prepare in the same manner as test solution (a) but adding 1.0 ml of a 0.05 per cent w/v solution of *phenacetin* (internal standard) to the chloroform solution before dilution to 10.0 ml.

*Reference solution*. A solution containing 0.025 per cent w/v of *fluocinolone acetonide RS* and 0.005 per cent w/v of *phenacetin* in *chloroform*.

*For creams containing 0.01 per cent w/w of fluocinolone acetonide:*

*Test solution (a)*. Prepare as described above but using a quantity of the cream containing about 1 mg of Fluocinolone Acetonide.

*Test solution (b)*. Prepare in the same manner as test solution (a) but adding 1.0 ml of a 0.02 per cent w/v solution of *phenacetin* (internal standard) to the chloroform solution before diluting to 10.0 ml.

*Reference solution*. A solution containing 0.01 per cent w/v of *fluocinolone acetonide RS* and 0.002 per cent w/v of *phenacetin* in *chloroform*.

*For creams containing 0.00625 per cent w/w of fluocinolone acetonide:*

*Test solution (a)*. Prepare as described above but using a quantity of the cream containing about 0.625 mg of Fluocinolone Acetonide.

*Test solution (b)*. Prepare in the same manner as test solution (a) but adding 1.0 ml of a 0.0125 per cent w/v solution of *phenacetin* (internal standard) to the chloroform solution before diluting to 10.0 ml.

*Reference solution*. A solution containing 0.00625 per cent w/v of *fluocinolone acetonide RS* and 0.00125 per cent w/v of *phenacetin* in *chloroform*.

*For creams containing 0.0025 per cent w/w of fluocinolone acetonide:*

*Test solution (a)*. Prepare as described above but using a quantity of the cream containing about 0.25 mg of Fluocinolone Acetonide.

*Test solution (b)*. Prepare in the same manner as test solution (a) but adding 1.0 ml of a 0.005 per cent w/v solution of *phenacetin* (internal standard) to the chloroform solution before diluting to 10.0 ml.

*Reference solution*. A solution containing 0.0025 per cent w/v of *fluocinolone acetonide RS* and 0.0005 per cent w/v of *phenacetin* in *chloroform*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with porous silica particles (5 µm),
- mobile phase: a mixture of 58 volumes of *n-hexane*, 40 volumes of *chloroform*, 2 volumes of *methanol* and 0.1 volume of *glacial acetic acid*,
- flow rate. 1.8 ml per minute,
- spectrophotometer set at 243 nm,
- injection volume. 20 µl.

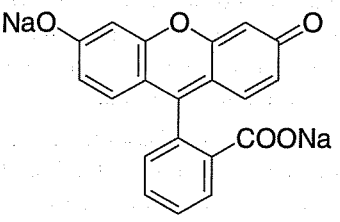
The assay is not valid unless the resolution between the peaks due to fluocinolone acetonide and phenacetin is more than 2, and the capacity factors of fluocinolone acetonide and phenacetin are about 3 and 2, respectively. If these conditions are not achieved, adjust the concentration of methanol and chloroform in the mobile phase. Repeat the adjustment of chloroform and methanol concentration until correct values for both resolution and capacity factors have been obtained.

Calculate the content of $C_{24}H_{30}F_2O_6$ in the cream.

**Storage**. Store at a temperature not exceeding 30°.

# Fluorescein Sodium

Soluble Fluorescein




$C_{20}H_{10}Na_2O_5$ Mol. Wt. 376.3

Fluorescin Sodium is disodium 2-(3-oxo-6-oxido-3*H*-xanthen-9-yl)benzoate.

Fluorescein Sodium contains not less than 98.5 per cent and not more than 100.5 per cent of $C_{20}H_{10}Na_2O_5$, calculated on the dried basis.

**Category**. Diagnostic aid (dye for detection of corneal lesions and foreign bodies).

**Description**. An orange-red powder; odourless or almost odourless; hygroscopic.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fluorescein sodium RS* or with the reference spectrum of fluorescein sodium.

B. A solution is strongly fluorescent, even in extreme dilutions. The fluorescence disappears when the solution is made acidic and reappears when it is made alkaline.



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C. A drop of a 0.05 per cent w/v solution, absorbed on a piece of filter paper, colours the paper yellow. On exposing the moist paper to the vapours of bromine for 1 minute and then to the vapours of ammonia, the yellow colour becomes deep pink.

D. The residue after incineration gives the reactions of sodium salts (2.3.1).

## Tests

**pH** (2.4.24). 7.0 to 9.0, determined in a 2.0 per cent w/v solution.

**Related substances.** Determine by thin-layer chromatography (2.4.17), using a silica gel GF254 precoated plate (such as Merck silica gel 60 GF254 plate).

*Mobile phase*. A mixture of 80 volumes of *chloroform* and 20 volumes of *methanol*.

*Test solution*. Dissolve 0.1 g of the substance under examination in 10 ml of *0.1 M methanolic hydrochloric acid*.

*Reference solution*. A 0.002 per cent w/v solution of the substance under examination in 100 ml of *0.1 M methanolic hydrochloric acid*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 365 nm. Expose the plate to iodine vapour for 30 minutes. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Chloroform-soluble matter**. Dissolve 0.2 g in 10 ml of *0.1 M sodium hydroxide* and extract with 10 ml of *chloroform*. Dry the chloroform layer with *anhydrous sodium sulphate* and filter. Absorbance of the resulting solution at about 480 nm, using *chloroform* as the blank, not more than 0.10 (2.4.7).

**Zinc**. Dissolve 0.1 g in 10 ml of *water*, add 2 ml of *hydrochloric acid*, filter and add 0.1 ml of *potassium ferrocyanide solution*; no turbidity or precipitate is produced immediately.

**Chlorides** (2.3.12). Dissolve 75 mg in 20 ml of *water*, add 2 ml of *nitric acid* and filter; the filtrate complies with the limit test for chlorides (0.33 per cent).

**Sulphates** (2.3.17). Dissolve 62.5 mg in 100 ml of *water*. To 20 ml add 2.5 ml of *dilute hydrochloric acid* and filter; the filtrate complies with the limit test for sulphates (1.2 per cent).

**Dimethylformamide**. Determine by gas chromatography (2.4.13).

*Test solution (a)*. Dissolve 1.0 g of the substance under examination in 10 ml of *water*, add, with stirring, 10 ml of *0.6 M hydrochloric acid*, allow to stand for 15 minutes and centrifuge. To 5 ml of the supernatant liquid add 0.1 g of *trisodium phosphate* and shake to dissolve.

*Test solution (b)*. Prepare in the same manner as test solution (a) but using 10 ml of a 0.02 per cent w/v solution of *dimethylacetamide* (internal standard) in place of *water*.

*Reference solution*. Mix 10 ml of a 0.02 per cent w/v solution of *dimethylformamide* with 10 ml of the internal standard.

Chromatographic system
- a glass column 1.5m x 4 mm, packed with acid-washed, silanised diatomaceous support (80 to 100 mesh) coated with 10 per cent w/w of *polyethylene glycol 1000*,
- temperature:
  column.120°,
  inlet port and detector. 180°,
- flame ionisation detector,
- flow rate. 30 ml per minute of the carrier gas (nitrogen).

In the chromatogram obtained with test solution (b) the ratio of the area of any peak corresponding to dimethylformamide to the area of the peak due to the internal standard is not greater than the corresponding ratio in the chromatogram obtained with the reference solution.

**Resorcinol**. Determine by thin-layer chromatography (2.4.17), using a silica gel GF254 precoated plate (such as Merck silica gel 60 GF254 plate).

*Mobile phase*. A mixture of 60 volumes of *hexane* and 40 volumes of *ethyl acetate*.

*Test solution*. Dissolve 1 g of the substance under examination in 10 ml of *water*, add slowly with constant stirring, 10 ml of *0.6 M hydrochloric acid*, allow to stand for 15 minutes, centrifuge and use the supernatant liquid.

*Reference solution*. A 0.025 per cent w/v solution of *resorcinol* in *water*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and expose to iodine vapour for 30 minutes. Any spot corresponding to resorcinol in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Acriflavin**. Dissolve 10 mg in 5 ml of *water*, and add a few drops of *sodium salicylate solution*; no precipitate is formed.

**Loss on drying** (2.4.19). Not more than 10.0 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.5 g, dissolve in 20 ml of *water*, add 5 ml of *dilute hydrochloric acid*, and extract with four quantities, each of 20 ml, of a mixture of equal volumes of *2-methyl-1-propanol* and *chloroform*. Wash the combined extracts with 10 ml of *water*, extract the washings with 5 ml of the mixture of *2-methyl-1-propanol* and *chloroform* and add to the combined extracts. Evaporate the combined extracts to dryness on a water-bath in a current of air, dissolve the residue in 10 ml of *ethanol (95 per cent)*, evaporate to dryness on a water-bath and dry to constant weight at 105°.

1 g of the residue is equivalent to 1.132 g of $C_{20}H_{10}Na_2O_5$.

**Storage**. Store protected from light.



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# Fluorescein Eye Drops

Fluorescein Sodium Eye Drops

Fluorescein Eye Drops are a sterile solution of Fluorescein Sodium in Purified Water.

Fluorescein Eye Drops contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of fluorescein sodium, $C_{20}H_{10}Na_2O_5$.

**Usual strengths**. 1 per cent w/v; 2 per cent w/v.

## Identification

A. Evaporate a volume of the eye drops containing 20 mg of Fluorescein Sodium and dry at 105° for 30 minutes. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fluorescein sodium RS* or with the reference spectrum of fluorescein sodium.

B. Dilute the eye drops with *water* to produce a solution containing 0.05 per cent w/v of Fluorescein Sodium. One drop of the solution, absorbed by a piece of filter paper, colours the paper yellow. On exposing the moist paper to the vapours of bromine for 1 minute and then to vapours of ammonia, the yellow colour becomes deep pink.

C. The eye drops are strongly fluorescent, even in extreme dilution. The fluorescence disappears when the solution is made acidic and reappears when it is made alkaline.

## Tests

**pH** (2.4.24). 7.0 to 9.0.

**Related substances**. Determine by thin-layer chromatography (2.4.17), using a silica gel *GF254* precoated plate (such as Merck silica gel 60 GF254 plate).

*Mobile phase*. A mixture of 80 volumes of *chloroform* and 20 volumes of *methanol*.

*Test solution*. Dilute a suitable volume of the eye drops, if necessary, with an equal volume of *0.1 M methanolic hydrochloric acid* so as it give a concentration of 1.0 per cent w/v of fluorescein sodium.

*Reference solution*. Dilute 1 volume of the test solution to 500 volumes with *0.1 M methanolic hydrochloric acid*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 365 nm. Expose the plate to iodine vapour for 30 minutes. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Chloroform-soluble matter**. To a volume of the eye drops containing 0.1 g of Fluorescein Sodium add 1 ml of *2 M sodium hydroxide*, extract with 10 ml of *chloroform*, dry the chloroform layer with *anhydrous sodium sulphate* and filter; absorbance of the resulting solution at about 480 nm, using *chloroform* as the blank, not more than 0.05 (2.4.7).

**Dimethylformamide**. Determine by gas chromatography (2.4.13).

*Test solution (a)*. Dilute the eye drops with *water*, if necessary, to produce a solution containing 1.0 per cent w/v of Fluorescein Sodium. To 5 ml of this solution add, with stirring, 0.3 ml of *1 M hydrochloric acid*, allow to stand for 15 minutes and centrifuge; dissolve 10 mg of *trisodium phosphate* in 2 ml of the supernatant liquid.

*Test solution (b)*. Prepare in the same manner as test solution (a) but adding 1.0 ml of a 0.01 per cent v/v solution of *dimethylacetamide* (internal standard) before the hydrochloric acid.

*Reference solution*. A solution containing 0.002 per cent v/v of *dimethylformamide* and 0.002 per cent v/v of the internal standard.

Chromatographic system
- a glass column 1.5 m x 4 mm, packed with acid-washed, silanised diatomaceous support (80 to 100 mesh) coated with 10 per cent w/w of *polyethylene glycol 1000*,
- temperature:
  column.120°,
  inlet port and detector. 180°,
- flame ionisation detector,
- flow rate. 30 ml per minute of the carrier gas (nitrogen).

In the chromatogram obtained with test solution (b) the ratio of the area of any peak corresponding to dimethylformamide to the area of the peak due to the internal standard is not greater than the corresponding ratio in the chromatogram obtained with the reference solution.

**Resorcinol**. Determine by thin-layer chromatography (2.4.17), using a silica gel *GF254* precoated plate (such as Merck silica gel 60 GF254 plate).

*Mobile phase*. A mixture of 60 volumes of *hexane* and 40 volumes of *ethyl acetate*.

*Test solution*. Dilute the eye drops with *water*, if necessary, to produce a solution containing 1.0 per cent w/v of Fluorescein Sodium and to 10 ml add, with stirring, 2.5 ml of *0.25 M hydrochloric acid*. Allow to stand for 15 minutes, centrifuge and dissolve 0.1g of *trisodium phosphate* in 5ml of the supernatant liquid.

*Reference solution*. A 0.004 per cent w/v solution of *resorcinol* in *water*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and expose to iodine vapour for 30 minutes.



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Any spot corresponding to resorcinol in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Other tests**. Comply with the tests stated under Eye Drops.

**Assay**. Dilute an accurately measured volume of the eye drops containing about 0.1 g of Fluorescein Sodium to 20 ml with *water*. Add 5 ml of *dilute hydrochloric acid* and extract with four quantities, each of 20 ml, of a mixture of equal volumes of *2-methyl-1-propanol* and *chloroform*. Wash the combined extracts with 10 ml of *water*, extract the washings with 5 ml of the mixture of 2-methyl-1-propanol and chloroform and add to the combined extracts. Evaporate the combined extracts to dryness on a water-bath in a current of air, dissolve the residue in 10 ml of *ethanol (95 per cent)*, evaporate to dryness on a water-bath and dry to constant weight at 105°.

1 g of the residue is equivalent to 1.132 g of $C_{20}H_{10}Na_2O_5$.

**Storage**. Store protected from light.

**Labelling**. The label states that the eye drops should be discarded after use on a single occasion.

# Fluorescein Injection

Fluorescein Sodium Injection

Fluorescein Injection is a sterile solution of Fluorescein Sodium in Water for Injections.

Fluorescein Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of fluorescein sodium, $C_{20}H_{10}Na_2O_5$.

**Usual strengths**. 100 mg per ml; 200 mg per ml.

## Identification

A. Evaporate 1 ml of the injection to dryness on a water bath and dry the residue at 105° for 30 minutes. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fluorescein sodium RS* or with the reference spectrum of fluorescein sodium.

B. The injection is strongly fluorescent, even in extreme dilution. The fluorescence disappears when the solution is made acidic and reappears when it is made alkaline.

C. One drop of the solution containing 0.05 per cent w/v of Fluorescein Sodium, absorbed by a piece of filter paper, colours the paper yellow. On exposing the moist paper to bromine vapour for 1 minute and then to ammonia vapour the yellow colour becomes deep pink.

## Tests

**pH** (2.4.24). 8.0 to 9.8.

**Chloroform-soluble matter**. Dilute a volume of injection containing about 0.5 g of Fluorescein Sodium in 10 ml of *water*. To 4 ml of this solution add 1 ml of *1 M sodium hydroxide*, dilute to 10 ml with *water*, extract with 10 ml of *chloroform* and dry the chloroform layer over *anhydrous sodium sulphate*. The absorbance of this solution at 480 nm (2.4.7) is not more than 0.1.

**Dimethylformamide**. Determine by gas chromatography (2.4.13).

*Test solution.* Dilute a volume of injection containing about 0.5 g of fluorescein sodium to 10 ml with *water*. To 10 ml of this solution add, with stirring, 1 ml of *3 M hydrochloric acid,* allow to stand for 15 minutes and centrifuge; dissolve 0.1 g of *trisodium orthophosphate* in 5 ml of the supernatant liquid.

*Reference solution (a).* A solution containing 0.002 per cent v/v each of *dimethylformamide* and *dimethylacetamide* (internal standard).

*Reference solution (b).* Dilute a volume of injection containing about 0.5 g of fluorescein sodium to 10 ml with *water*. To 10 ml of this solution add, with stirring, add 1.0 ml of a 0.10 per cent v/v solution of *dimethylacetamide* (internal standard) and 1 ml of *3 M hydrochloric acid,* allow to stand for 15 minutes and centrifuge; dissolve 0.1 g of *trisodium orthophosphate* in 5 ml of the supernatant liquid.

Chromatographic system

- a glass column 1.5 m x 4 mm, packed with *acid-washed, silanised diatomaceous support* (80 to 100 mesh) coated with 10 per cent w/w of *polyethylene glycol 1000,*
- temperature :
  column 120°,
  inlet port and detector at 120°,
- flow rate. 30 ml per minute of the carrier gas.

Inject the test solution, reference solution (a) and (b). In the chromatogram obtained with test solution the ratio of the area of any peak corresponding to dimethylformamide to the area of the peak due to the internal standard is not more than the corresponding ratio in the chromatogram obtained with reference solution (a) (0.2 per cent).

**Related substances and resorcinol**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 10 volumes of *methanol* and 90 volumes of *dichloromethane*.

*Test solution.* Dilute a volume of injection containing about 1 g of Fluorescein Sodium with *0.1 M methanolic hydrochloric acid.*



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*Reference solution (a)*. A 5.0 per cent w/v solution of *fluorescein sodium RS* in *water*. To 5 ml of this solution add 2 ml of *phosphate buffer pH 8.0*, 3 ml of *water* and 2.5 g of *sodium chloride*, shake to dissolve the *sodium chloride* and extract with two 25-ml quantities of *peroxide-free ether*. Dry the combined extracts over *anhydrous sodium sulphate*, evaporate to dryness under reduced pressure and dissolve the residue in 10 ml of *0.1 M methanolic hydrochloric acid*.

*Reference solution (b)*. Dilute 1 ml of the test solution to 200 ml *with 0.1 M methanolic hydrochloric acid.*

*Reference solution (c)*. Dilute 2 ml of reference solution (b) to 5 ml with *0.1 M methanolic hydrochloric acid.*

*Reference solution (d)*. A 0.0125 per cent w/v solution of *resorcinol* in *0.1 M methanolic hydrochloric acid.*

*Reference solution (e)*. A mixture of 9 ml of reference solution (d) and 1 ml of the test solution.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and expose the plate to iodine vapour for 30 minutes and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (d) (0.2 per cent). On examination in daylight, any spot corresponding to resorcinol in the chromatogram obtained with reference solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (d) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (e) shows two clearly separated spots in daylight.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Dilute a volume of injection containing about 1.0 per cent w/v of Fluorescein Sodium. Dilute 1.0 ml of this solution to 200.0 ml with the mobile phase.

*Reference solution.* Dissolve 55 mg of *diacetylfluorescein RS* in a mixture of 5 ml of *ethanol (95 per cent)* and 1 ml of *2.5 M sodium hydroxide*, heat on a water bath for 20 minutes, mixing frequently, cool and add sufficient *water* to produce 50 ml. Dilute 5.0 ml of this solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with endcapped octadecylsilane bonded to porous silica (5 µm) (such as Spherisorb ODS 2),
- mobile phase: a mixture of 5 volumes of *triethylamine*, 400 volumes of *acetonitrile* and 595 volumes of *water*, adjusted to pH 3.0 with *orthophosphoric acid*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution. The test is not valid unless the relative standard deviation for replicate injections is not less than 2.0 per cent.

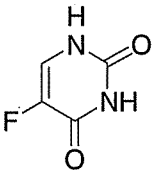
Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{10}Na_2O_5$ in the injection.

1 mg of anhydrous diacetylfluorescein is equivalent to 0.9037 mg of $C_{20}H_{10}Na_2O_5$.

**Storage**. Store protected from light.

# Fluorouracil




$C_4H_3FN_2O_2$ Mol. Wt. 130.1

Fluorouracil is 5-fluoro-1*H*,3*H*-pyrimidine-2,4-dione.

Fluorouracil contains not less than 98.0 per cent and not more than 101.0 per cent of $C_4H_3FN_2O_2$, calculated on the dried basis.

**Category**. Anticancer.

**Dose**. By intravenous injection, 3 to 12 mg per kg daily for 4 days followed by 6 mg per kg on alternate days, to a maximum of 800 mg daily.

**Description**. A white or almost white, crystalline powder.

*CAUTION - Great care should be taken to avoid inhaling particles of Fluorouracil and exposing the skin to it.*

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

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

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *acetate buffer pH 4.7* shows an absorption maximum only at about 266 nm.

C. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).



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D. To 5 ml of a 1 per cent w/v solution add 1 ml of *bromine water*; the colour of bromine is discharged.

### Tests

**Appearance of solution**. A 1.0 per cent w/v solution in carbon *dioxide-free water* is clear (2.4.1), and not more intensely coloured than reference solution YS7 or BYS7 (2.4.1).

**pH** (2.4.24). 4.5 to 5.0, determined in a 1.0 per cent w/v solution.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 70 volumes of *ethyl acetate*, 15 volumes of *methanol* and 15 volumes of *water*.

*Test solution (a)*. Dissolve 0.1 g of the substance under examination in 10 ml of *methanol (50 per cent)*.

*Test solution (b)*. Dilute 5 ml of the test solution to 25 ml with *methanol (50 per cent)*.

*Reference solution (a)*. A 0.2 per cent w/v solution of *fluorouracil RS* in *methanol (50 per cent)*.

*Reference solution (b)*. A 0.0025 per cent w/v solution of *fluorouracil RS* in *methanol (50 per cent)*.

*Reference solution (c)*. A 0.0025 per cent w/v solution of *5-hydroxyuracil* in *methanol (50 per cent)*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b). Spray the plate with a freshly prepared 0.5 per cent w/v solution of *fast blue B salt* and then with *0.1 M sodium hydroxide*. Any spot corresponding to 5-hydroxyuracil in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (c). Ignore any secondary spot on or near the line of application.

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

**Sulphated ash** (2.3.18). Not more than 0.1 per cent, determined on 1.0 g in a platinum crucible.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying over *phosphorus pentoxide* in an oven at 80° at a pressure of 1.5 to 2.5 kPa for 4 hours.

**Assay**. Weigh accurately about 0.2 g, dissolve in 80 ml of *dimethylformamide* with the aid of gentle heat and cool. Titrate with *0.1 M tetrabutylammonium hydroxide* in *methanol*, using 0.25 ml of a 1 per cent w/v solution of *thymol blue* in *dimethylformamide* as indicator. Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.01301 g of $C_4H_3FN_2O_2$.

**Storage**. Store protected from light.

## Fluorouracil Injection

Fluorouracil Injection is a sterile solution in Water for Injections of fluorouracil sodium, prepared by the interaction of Fluorouracil and Sodium Hydroxide.

Fluorouracil Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of fluorouracil, $C_4H_3FN_2O_2$.

**Usual strengths**. 25 mg per ml; 50 mg per ml.

**Description**. A colourless or almost colourless solution.

### Identification

A. Acidify carefully a volume of the injection containing 0.1 g of Fluorouracil with *glacial acetic acid*, stir, cool and filter. Wash the precipitate with 1 ml of *water* and dry over *phosphorus pentoxide* at 80° at a pressure of 2 kPa for 4 hours. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fluorouracil RS* or with the reference spectrum of fluorouracil.

B. When examined in the range 230 nm to 360 nm (2.4.7), the final solution obtained in the Assay shows an absorption maximum only at about 266 nm.

C. To a volume of the injection containing 50 mg of Fluorouracil add 1 ml of *bromine water*; the colour of bromine is discharged.

### Tests

**pH** (2.4.24). 8.5 to 9.5.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 70 volumes of *ethyl acetate*,15 volumes of *methanol* and 15 volumes of *water*.

*Test solution*. Dilute a suitable quantity of the injection with *water* to produce a solution containing the equivalent of 2 per cent w/v of Fluorouracil.

*Reference solution (a)*. Dilute 1 volume of test solution to 400 volumes with *methanol (50 per cent)*.

*Reference solution (b)*. A 0.005 per cent w/v solution of *5-hydroxyuracil* in *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a). Spray with a freshly prepared solution containing 0.5 per cent w/v of *fast blue B salt* and then with *0.1 M sodium hydroxide*. Any spot corresponding to 5-hydroxyuracil in the chromatogram obtained with the test solution is not more intense than the



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spot in the chromatogram obtained with reference solution (b). Ignore any secondary spot on or near the line of application.

**Urea**. Carry out the method described under Related substances applying separately to the plate 20 µl of the following solutions. For the test solution dilute a suitable quantity of the injection with *water* to produce a solution containing the equivalent of 0.5 per cent w/v of Fluorouracil. The reference solution contains 0.02 per cent w/v of *urea* in *water*. After development, dry the plate in air, spray with a mixture of 10 volumes of a 1 per cent w/v solution of *4-dimethylaminobenzaldehyde* in *ethanol (95 per cent)* and 1 volume of *hydrochloric acid* and heat at 105° until maximum intensity of the spots is obtained. Any spot corresponding to urea in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

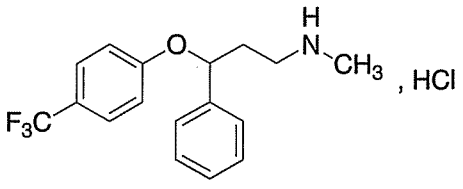
**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. To an accurately measured volume containing about 50 mg of Fluorouracil add 20 ml of *1 M hydrochloric acid* and sufficient *water* to produce 250.0 ml. Dilute 5.0 ml to 100.0 ml with *0.1 M hydrochloric acid* and measure the absorbance of the resulting solution at the maximum at about 266 nm (2.4.7). Calculate the content of $C_4H_3FN_2O_2$ taking 552 as the specific absorbance at 266 nm.

**Storage**. Store protected from light in single dose containers at a temperature not exceeding 30°. The injection should not be allowed to freeze.

**Labelling**. The label states (1) the strength in terms of the equivalent amount of Fluorouracil in a suitable dose-volume; (2) that, if separation has occurred, the injection should be heated to 60°, shaken vigorously and allowed to cool to body temperature prior to use.

# Fluoxetine Hydrochloride




$C_{17}H_{18}F_3NO,HCl$ Mol. Wt. 345.8

Fluoxetine Hydrochloride is (*RS*)-*N*-methyl-3-phenyl-3-[4-trifluoromethyl)phenoxy]propylamine hydrochloride.

Fluoxetine Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{17}H_{18}F_3NO,HCl$, calculated on the anhydrous basis.

**Category**. Antidepressant.

**Description**. A white or almost white, crystalline powder.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fluoxetine hydrochloride RS* or with the reference spectrum fluoxetine hydrochloride.

B. Gives the reaction A of chlorides (2.3.1).

## Tests

**Appearance of solution**. Dissolve 2.0 g in a mixture of 15 volumes of *water* and 85 volumes of *methanol* and dilute to 100 ml with the same solvent mixture (solution A). Solution A is clear (2.4.1) and colourless (2.4.1).

**pH** (2.4.24). 4.5 to 6.5. determined in a solution of 0.2 g in sufficient *carbon dioxide-free water* to produce 20 ml.

**Specific optical rotation** (2.4.22). -0.05° to +0.05°, determined in solution A.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution (a)*. Dissolve 0.055 g of the substance under examination in the mobile phase and dilute to 10.0 ml with the mobile phase.

*Test solution (b)*. Dilute 2.0 ml of test solution (a) to 10.0 ml with the mobile phase.

*Reference solution*. Dissolve 0.022 g of *fluoxetine hydrochloride RS* in 10.0 ml of *0.5 M sulphuric acid*. Heat at about 85° for 3 hours. Allow to cool. The resulting solution contains mainly of (1*RS*)-3-(methylamino)-1-phenylpropan-1-ol (fluoxetine impurity A) and some 4-trifluoromethylphenol. To 0.4 ml of the solution add 28 mg of *fluoxetine hydrochloride RS*, about 1 mg of *N-methyl-3-phenylpropan-1-amine RS* (*fluoxetine impurity B RS*) and about 1 mg of *(3RS)-N-methyl-3-phenyl-3-[3-(trifluoromethyl) phenoxy]propan-1-amine RS* (*fluoxetine impurity C RS*) and dilute to 25.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 8 volumes of *methanol*, 30 volumes of *tetrahydrofuran* and 62 volumes of a solution of *triethylamine* prepared by adding 980 ml of *water* to 10 ml of *triethylamine*, mixing and adjusting the pH to 6.0 with *orthophosphoric acid* and diluting to 1000 ml with *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume. 10 µl.



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Inject the reference solution. The relative retention time with respect to fluoxetine for fluoxetine impurity A is about 0.24, for fluoxetine impurity B is about 0.27 and for fluoxetine impurity C is about 0.94.

The test is not valid unless the retention time of the peak due to fluoxetine is 10-18 minutes; the retention time of the peak due to 4-trifluoromethylphenol is not greater than 35 minutes (if no peak due to 4-trifluoromethylphenol is seen in the chromatogram, inject a 0.02 per cent solution of *4-trifluoromethylphenol* in the mobile phase); the peak to valley ratio for fluoxetine impurity C is not greater than 1.1. If the ratio is greater than 1.1, reduce the volume of methanol and increase the volume of the solution of triethylamine in the mobile phase.

Inject test solutions (a) and (b). Continue the chromatography for 3 times the retention time of fluoxetine. In the chromatogram obtained with test solution (b), the area of any peak due to fluoxetine impurity C is not greater than 0.0015 times the area of the principal peak in the chromatogram obtained with test solution (a) (0.15 per cent).

In the chromatogram obtained with test solution (a) the areas of any peaks due to fluoxetine impurity A and fluoxetine impurity B are not greater than 0.0125 times the area of the principal peak in the chromatogram obtained with test solution (b) (0.25 per cent); none of the peaks other than the principal peak and the peaks due to fluoxetine impurity A and fluoxetine impurity B, has an area greater than 0.005 times the area of the principal peak in the chromatogram obtained with test solution (b) (0.1 per cent);the sum of the areas of all the peaks, other than the principal peak, is not greater than 0.025 times the area of the principal peak obtained with test solution (b) (0.5 per cent). Ignore any peak with an area less than 0.0025 times that of the principal peak in the chromatogram obtained with test solution (b) (0.05 per cent).

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

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 0.5 per cent, determined on 1.0 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh accurately about 0.055 g of the substance under examination, dissolve in the mobile phase and add sufficient mobile phase to produce 50.0 ml. Dilute 10.0 ml of the solution to 100.0 ml with the mobile phase.

*Reference solution*. Weigh accurately about 0.055 g of *fluoxetine hydrochloride RS* in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 10.0 ml of this solution to 100.0 ml with the mobile phase.

Use the chromatographic system described under the test for Related substances and set the spectrophotometer at 227 nm. Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with the reference solution is at least 50 per cent of the full scale of the recorder.

Adjust the volumes of methanol and the solution of triethylamine in the mobile phase so that the retention time of fluoxetine is between 10 minutes and 18 minutes.

The assay is not valid unless the symmetry factor calculated at 10 per cent of the height of the peak due to fluoxetine is at most 2.0.

Inject the test solution and reference solution.

Calculate the content of $C_{17}H_{18}F_3NO,HCl$.

# Fluoxetine Capsules

## Fluoxetine Hydrochloride Capsules

Fluoxetine Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of fluoxetine, $C_{17}H_{18}F_3NO$.

**Usual strengths**. 10 mg; 20 mg.

### Identification

A. Shake a quantity of the contents of the capsules containing 10 mg of fluoxetine with 10 ml of *methanol*, centrifuge for 10 minutes and filter. Evaporate the filtrate to dryness with the aid of a current of air and mild heat. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fluoxetine hydrochloride RS* or with thre reference spectrum of fluoxetine hydrochloride.

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

### Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

*Diethylamine phosphate suspension.* To 250 ml of *acetonitrile*, add 1.0 ml of *diethylamine*, mix, and adjust the pH to 3.5 with *orthophosphoric acid*.



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*NOTE — Keep the suspension well-mixed.*

*Test solution.* To 5.0 ml of the filtrate obtained as given above, add 2.0 ml of the diethylamine phosphate suspension and mix well.

*Reference solution.* Dissolve 0.022 g of *fluoxetine hydrochloride RS* in sufficient *0.1 M hydrochloric acid* to produce 100.0 ml and mix. Dilute 10.0 ml of the solution to 100.0 ml with *0.1 M hydrochloric acid.* To 5.0 ml of the resulting solution, add 2.0 ml of the diethylamine phosphate suspension and mix well.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with particles of silica the surface of which has been modified with chemically-bonded cyano groups (5 µm),
- mobile phase: a mixture of 0.4 volume of *diethylamine*, 40 volumes of *acetonitrile* and 60 volumes of *water,* adjusted to pH 3.5 with *orthophosphoric acid,*
- flow rate. 2 ml per minute,
- spectrophotometer set at 226 nm,
- injection volume. 50 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

D. Not less than 70 per cent of the stated amount of $C_{17}H_{18}F_3NO$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh accurately a quantity of the contents of the capsules containing 20 mg of fluoxetine, disperse in 10.0 ml of the mobile phase, mix and centrifuge.

*Reference solution.* A solution of *fluoxetine hydrochloride RS* equivalent to 0.001 w/v of fluoxetine in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with particles of silica the surface of which has been modified with chemically-bonded cyano groups (5 µm),
- mobile phase: a mixture of 35 volumes of *acetonitrile* and 65 volumes of a solution of *triethylamine,* prepared by adding to 10 ml of *triethylamine* 980 ml of *water*, mixing, adjusting the pH to 6.0 with *orthophosphoric acid* (about 4.5 ml) and diluting to 1000 ml with *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 1100 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and the reference solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with reference solution (0.5 per cent) and the sum of areas of all secondary peaks is not more than twice the area of the peak in the chromatogram obtained with the reference solution (1.0 per cent). Ignore any peaks with an area 0.05 times the area of the principal peak in the chromatogram obtained with reference solution ( 0.05 per cent).

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh a quantity of the contents of the capsules containing about 20 mg of fluoxetine, disperse in 200.0 ml of the mobile phase, mix and filter.

*Reference solution.* A 0.011 per cent w/v solution of *fluoxetine hydrochloride RS* in the mobile phase.

Chromatographic system
- a stainless steel column 7.5 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 33 volumes of a solution containing 0.3 per cent w/v of *glacial acetic acid* and 0.64 per cent w/v of *sodium pentanesulphonate*, adjusted to pH 5.0 with *5 M sodium hydroxide*, and 67 volumes of *methanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 227 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{17}H_{18}F_3NO$ in the capsules.

**Storage**. Store protected from moisture.

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

## Fluoxetine Oral Solution

Fluoxetine Oral Liquid; Fluoxetine Hydrochloride Oral Solution

Fluoxetine Oral Solution is a solution of Fluoxetine Hydrochloride in a suitable aqueous vehicle. It may contain one or more preservatives.

Fluoxetine Oral Solution contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of fluoxetine, $C_{17}H_{18}F_3NO$.



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**Usual strength**. 20 mg per 5 ml.

## Identification

Transfer a volume of the oral solution containing about 20 mg of fluoxetine, to a separating funnel, add 5 ml of *water* and 0.5 ml of *1 M sodium hydroxide;* extract with 5 ml of *chloroform* and discard the aqueous layer. Evaporate the chloroform layer to dryness. The residue dissolved in 0.4 ml of *chloroform* complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fluoxetine hydrochloride RS* treated in the same manner.

## Tests

**pH** (2.4.24). 2.5 to 4.5.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Ion pair solution.* Dissolve about 4.3 g of *sodium 1-octanesulphonate* and 13.8 g of *monobasic sodium phosphate* in 1000 ml of *water*, and adjust to pH 3.0 with *orthophosphoric acid.*

*Solvent mixture*. 60 volumes of *ion pair solution*, 30 volumes of *methanol* and 10 volumes of *acetonitrile*.

*Test solution (a)* Weigh accurately a quantity of the oral solution containing about 19 mg of fluoxetine and dilute to 10.0 ml with the solvent mixture.

*Test solution (b)*. Dilute 1.0 ml of test solution (a) to 25.0 ml with the solvent mixture.

*Reference solution*. A 0.2 per cent w/v solution of *fluoxetine hydrochloride RS* in *1 M sulphuric acid.* Heat this solution at 85° for 1 hour. To 1.0 ml of this solution add about 10 mg *fluoxetine hydrochloride RS*, dissolve in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase : A. a mixture of 53 volumes of *ion-pair solution*, 26 volumes of *methanol* and 21 volumes of *acetonitrile,*

  B. a mixture of 43 volumes of *ion-pair solution*, 35 volumes of *acetonitrile* and 22 volumes of *methanol,*
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 215 nm,
- injection volume. 20 µl.

| Time (in min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) | Comment |
|---|---|---|---|
| 0 | 100 | 0 | equilibration |
| 0-13 | 100 | 0 | isocratic |
| 13-15 | 100→0 | 0→100 | linear gradient |
| 15-29 | 0 | 100 | isocratic |
| 29-30 | 0→100 | 100→0 | linear gradient |
| 30-end | 100 | 0 | isocratic |

Inject the reference solution. The retention time of any secondary peak is not more than 13 minutes.

Inject test solutions (a) and (b). In the chromatogram obtained with test solution (a), the area of any secondary peak is not more than 0.1 times the area of the principal peak in the chromatogram obtained with test solution (b) (0.4 per cent). The sum of the areas of the peaks, other than the principal peak, is not more than 0.2 times the area of the principal peak in the chromatogram obtained with test solution (b) (0.8 per cent).

**Other tests**. Complies with the tests stated under Oral Liquids.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh accurately a quantity of the oral solution containing about 4.0 mg of fluoxetine and dilute to 100.0 ml with the mobile phase.

*Reference solution.* A 0.0045 per cent w/v solution of *fluoxetine hydrochloride RS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with nitrile groups chemically bonded to porous silica (10 µm),
- mobile phase: a mixture of equal volumes of a buffer solution prepared by adding 10 ml of *triethylamine* to 980 ml with *water* and adjusting the pH to 6.0, and *acetonitrile,*
- flow rate 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume. 20 µl.

Inject the reference solution. The tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Determine the weight per ml (2.4.29) of the oral solution and calculate the content of $C_{17}H_{18}F_3NO$, weight in volume.

**Storage**. Store protected from light.

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



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# Fluoxetine Tablets

Fluoxetine Hydrochloride Tablets

Fluoxetine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of fluoxetine, $C_{17}H_{18}F_3NO$.

**Usual strengths**. 10 mg; 20 mg.

## Identification

A. Shake a quantity of the contents of the tablets containing 10 mg of fluoxetine with 10 ml of *methanol*, centrifuge for 10 minutes and filter. Evaporate the filtrate to dryness with the aid of a current of air and mild heat. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fluoxetine hydrochloride RS*.

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

## Tests

**Dissolution** (2.5.2).

Apparatus No.1,

Medium. 900 ml of *0.1 M hydrochloric acid,*

Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

*Diethylamine phosphate suspension.* To 250 ml of *acetonitrile* add 1.0 ml of *diethylamine*, mix, and adjust the pH to 3.5 with *orthophosphoric acid*.

*Note—Keep the suspension well-mixed.*

*Test solution.* To 5.0 ml of the filtrate obtained as given above, add 2.0 ml of the diethylamine phosphate suspension and mix well.

*Reference solution.* Dissolve 0.022 g of *fluoxetine hydrochloride RS* in sufficient *0.1 M hydrochloric acid* to produce 100.0 ml and mix. Dilute 10.0 ml of the solution to 100.0 ml with *0.1 M hydrochloric acid.* To 5.0 ml of the resulting solution, add 2.0 ml of the diethylamine phosphate suspension and mix well.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with particles of silica the surface of which has been modified with chemically-bonded cyano groups (5 µm),
- mobile phase: a mixture of 0.4 volume of *diethylamine*, 40 volumes of *acetonitrile* and 60 volumes of *water*, adjusted to pH 3.5 with *orthophosphoric acid*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 226 nm,
- injection volume. 50 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

D. Not less than 70 per cent of the stated amount of $C_{17}H_{18}F_3NO$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 20 mg of fluoxetine, disperse in 10.0 ml of the mobile phase, mix and centrifuge.

*Reference solution.* A solution of *fluoxetine hydrochloride RS* containing the equivalent of 0.001 per cent w/v of fluoxetine in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with particles of silica the surface of which has been modified with chemically-bonded cyano groups (5 µm),
- mobile phase: a mixture of 35 volumes of *acetonitrile* and 65 volumes of a solution of *triethylamine,* prepared by adding to 10 ml of *triethylamine* 980 ml of *water*, mixing, adjusting the pH to 6.0 with *orthophosphoric acid* (about 4.5 ml) and diluting to 1000 ml with *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 1100 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and the reference solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of areas of all secondary peaks is not more than twice the area of the peak in the chromatogram obtained with the reference solution (1.0 per cent). Ignore any peaks with an area 0.05 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 20 mg of fluoxetine, disperse in 200.0 ml of the mobile phase, mix and filter.

*Reference solution.* A 0.011 per cent w/v solution of *fluoxetine hydrochloride RS* in the mobile phase



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Chromatographic system
- a stainless steel column 25 cm x 4.0 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 33 volumes of a solution containing 0.3 per cent w/v of *glacial acetic acid* and 0.64 per cent w/v of *sodium pentanesulphonate*, adjusted to pH 5.0 with *5 M sodium hydroxide*, and 67 volumes of *methanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 227 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

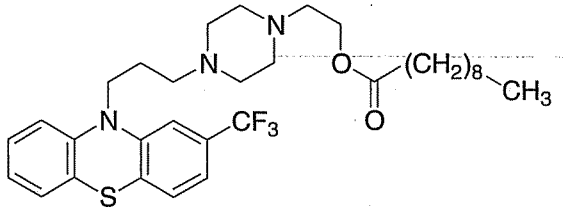
Calculate the content of $C_{17}H_{18}F_3NO$ in the tablets.

**Storage**. Store protected from moisture.

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

# Fluphenazine Decanoate

Fluphenazine Decanoate Ester



$C_{32}H_{44}F_3N_3O_2S$ Mol. Wt. 591.8

Fluphenazine Decanoate is 2-{4-[3-(2-trifluoromethylphenothiazin-10-yl)propyl]piperazin-1-yl}ethyl decanoate.

Fluphenazine Decanoate contains not less than 98.5 per cent and not more than 101.5 per cent of $C_{32}H_{44}F_3N_3O_2S$, calculated on the dried basis.

**Category**. Antipsychotic.

**Dose**. By deep intramuscular injection, test dose 6.25 to 12.5 mg, thereafter 4 to 7 days 12.5 to 100 mg repeated as required.

**Description**. A pale yellow, viscous liquid or yellow, crystalline, oily solid; odour, faint and ester-like.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fluphenazine decanoate RS* or with the reference spectrum of fluphenazine decanoate.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *ethanol* shows an absorption maximum at about 261 nm and a less well-defined maximum at about 310 nm; absorbance at about 261 nm, about 0.60.

C. Determine in subdued light by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*. Impregnate the dry plate by placing it in a tank containing a 5 per cent v/v solution of *n-tetradecane* in *n-hexane*, allowing the impregnating solvent to ascend to the top and allowing to dry.

*Mobile phase. Methanol (90 per cent).*

*Test solution*. Dissolve 0.2 g of the substance under examination in 10 ml of *ethanol (95 per cent)*.

*Reference solution*. A 2.0 per cent w/v solution of *fluphenazine decanoate RS* in *ethanol (95 per cent)*.

Apply to the plate 1 µl of each solution. After development, dry the plate dry in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

D. Dissolve 5 mg in 2 ml of *sulphuric acid* and allow to stand for 5 minutes; a reddish-brown colour is produced.

## Tests

**Related substances**. Determine in subdued light by thin-layer chromatography (2.4.17), coating the plate with *silica gel F254*.

*Mobile phase*. A mixture of 80 volumes of *acetone*, 30 volumes of *cyclohexane* and 5 volumes of *strong ammonia solution*.

*Test solution*. Dissolve 0.25 g of the substance under examination in 10 ml of *methanol*.

*Reference solution*. A 0.025 per cent w/v solution of the substance under examination in *methanol*.

Apply to the plate 20 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Spray the plate with *sulphuric acid (50 per cent v/v)* and examine in daylight. By both methods of visualisation, any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa for 3 hours.

**Assay**. Weigh accurately about 0.6 g and dissolve in 50 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric*



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*acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02959 g of $C_{32}H_{44}F_3N_3O_2S$.

**Storage**. Store protected from light.

## Fluphenazine Decanoate Injection

Fluphenazine Decanoate Injection is a sterile solution of Fluphenazine Decanoate in Sesame Oil.

Fluphenazine Decanoate Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of fluphenazine decanoate, $C_{32}H_{44}F_3N_3O_2S$.

**Usual strengths**. 25 mg per ml; 100 mg per ml.

### Identification

A.Determine in subdued light by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. *Chloroform* for the first development and *methanol (90 per cent)* for the second development.

*Test solution*. Dilute a suitable volume of the injection with *ethanol (95 per cent)* to produce a solution containing 2.5 mg of Flufenazine Decanoate per ml.

*Reference solution*. A 0.25 per cent w/v solution of *fluphenazine decanoate RS* in *ethanol (95 per cent)*.

Apply to the bottom right-hand corner of the plate 10 µl of the test solution. After development, dry the plate in air, turn the plate through 90° in a clockwise direction, impregnate the coating with a 5 per cent v/v solution of *n-tetradecane* in *n-hexane* and allow it to dry in air. Apply to the bottom right-hand corner of the plate, to the right of the solvent front of the first development, 10 µl of the reference solution. After the second development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. Shake a volume of the injection containing 5 mg of Fluphenazine Decanoate with 1 ml of a 1 per cent w/v solution of *sucrose* in *hydrochloric acid* and allow to stand for 5 minutes; a red colour is produced in the acid layer.

### Tests

**Related substances**. Determine in subdued light by thin-layer chromatography (2.4.17),coating the plate with *silica gel GF254*.

*Mobile phase*. *Chloroform* for the first development and a mixture of 80 volumes of *acetone*, 30 volumes of *cyclohexane* and 5 volumes of *strong ammonia solution* for the second development.

*Test solution*. Dilute a suitable volume of the injection with *ethanol (95 per cent)* to produce a solution containing 2.5 mg of Flufenazine Decanoate per ml.

*Reference solution*. A 0.1 per cent w/v solution of *fluphenazine hydrochloride RS* in *methanol*.

Apply to the bottom right-hand corner of the plate 10 µl of the test solution. After development, dry the plate in air, turn it through 90° in a clockwise direction. Apply to the bottom right-hand corner of the plate, to the right of the solvent front of the first development, 1 µl of the reference solution. After the second development, dry the plate in air and examine in ultraviolet light at 254 nm. Spray the plate with *sulphuric acid (50 per cent v/v)*. By both methods of visualisation, any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. *Carry out the following procedure protected from light.*

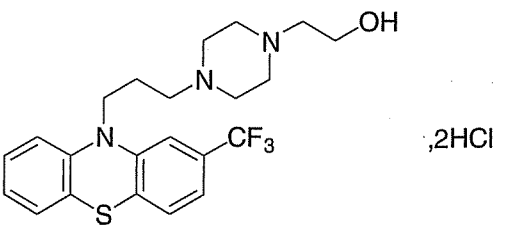
Measure accurately a volume of the injection containing about 0.25 g of Fluphenazine Decanoate and dilute with 75 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02959 g of $C_{32}H_{44}F_3N_3O_2S$.

**Storage**. Store protected from light.

**Labelling**. The label states that the injection is for intramuscular injection only.

## Fluphenazine Hydrochloride




,2HCl

$C_{22}H_{26}F_3N_3OS,2HCl$ Mol. Wt. 510.5

Fluphenazine Hydrochloride is 2-{4-[3-(2-trifluoromethylphenothiazin-10-yl)propyl]piperazin-1-yl}ethanol dihydrochloride.



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Fluphenazine Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent w/v of $C_{22}H_{26}F_3N_3OS,2HCl$, calculated on the dried basis.

**Category**. Antipsychotic.

**Dose**. Orally, 1 to 2 mg twice daily, in anxiety states; 2.5 to 10 mg daily in 2 to 3 divided doses in schizophrenia and other psychoses. By intramuscular injection, 1.25 to 10 mg daily.

**Description**. A white or almost white, crystalline powder.

## Identification

*Test A may be omitted if tests B, C, D and E are carried out. Tests B, C and D may be omitted if tests A and E are carried out.*

A. Dissolve 0.1 g in 10 ml of *water*, make alkaline with *1 M sodium hydroxide*, extract with 5 ml of *chloroform*, filter through *anhydrous sodium sulphate* and evaporate the solvent in a current of nitrogen. The oily residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fluphenazine hydrochloride RS* treated in the same manner or with the reference spectrum of fluphenazine.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *methanol* shows an absorption maximum at about 258 nm and a less well-defined maximum at about 310 nm; absorbance at about 258 nm, between 0.63 and 0.70.

C. Determine in subdued light by thin-layer chromatography (2.4.17), coating the plate with *kieselguhr G*. Impregnate the dry plate by placing in a tank containing a shallow layer of a mixture of 36 volumes of *acetone*, 3 volumes of *formamide* and 1 volume of *2-phenoxyethanol*. Allow the impregnating solvent to ascend to the top, remove the plate from the tank and use it immediately.

*Mobile phase*. A mixture of 100 volumes of *light petroleum (40° to 60°)* saturated with *2-phenoxyethanol* and 2 volumes of *diethylamine*.

*Test solution*. Dissolve 0.2 g of the substance under examination in 100 ml of *methanol*.

*Reference solution*. A 0.2 per cent w/v solution of *fluphenazine hydrochloride RS* in *methanol*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air, examine in ultraviolet light at 365 nm and observe the fluorescence produced after about 2 minutes. Heat the plate at 120° for 20 minutes, cool, spray with *ethanolic sulphuric acid (20 per cent)* and observe the colour produced. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

D. Mix about 5 mg with 45 mg of *heavy magnesium oxide* and ignite in a crucible until an almost white residue is obtained (usually less than 5 minutes). Allow to cool, add 1 ml of *water*, 0.05 ml of *dilute phenolphthalein solution* and about 1 ml of *2 M hydrochloric acid* to render the solution colourless and filter. To a freshly prepared mixture of 0.1 ml of *alizarin red S solution* and 0.1 ml of *zirconyl nitrate solution*, add 1 ml of the filtrate. Mix, allow to stand for 5 minutes and examine the colour of the solution as well as of a blank prepared in the same manner. The colour of the test solution is yellow and that of the blank is red.

E. Gives the reaction of chlorides (2.3.1).

## Tests

**pH** (2.4.24). 1.9 to 2.3, determined in a 5.0 per cent w/v solution in *carbon dioxide-free water*.

**Related substances**. Determine in subdued light by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 80 volumes of *acetone*, 30 volumes of *cyclohexane* and 5 volumes of *strong ammonia solution*.

*Test solution*. Dissolve 0.1 g of the substance under examination in 10 ml of *0.1 M methanolic sodium hydroxide*.

*Reference solution (a)*. Dilute 1 ml of the test solution to 100 ml with *0.1 M methanolic sodium hydroxide*.

*Reference solution (b)*. Dilute 5 ml of reference solution (a) to 10 ml with *0.1 M methanol sodium hydroxide*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b). Ignore any spot remaining on the line of application.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

**Assay**. Weigh accurately about 0.25 g and dissolve in a mixture of 10 ml of *anhydrous formic acid* and 40 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02552 g of $C_{22}H_{26}F_3N_3OS,2HCl$.



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**Storage**. Store protected from light.

## Fluphenazine Hydrochloride Injection

Fluphenazine Hydrochloride Injection is a sterile solution of Fluphenazine Hydrochloride in Water for Injection.

Fluphenazine Hydrochloride Injection contains not less than 95.0 per cent and not more than 110.0 per cent of the stated amount of fluphenazine hydrochloride, $C_{22}H_{26}F_3N_3OS,2HCl$.

**Usual strengths**. 2.5 mg per ml; 10 mg per ml.

**Description**. A clear, colourless solution.

### Identification

A. Determine in subdued light by thin-layer chromatography (2.4.17), coating the plate with *kieselguhr G*. Impregnate the dry plate by placing in a tank containing a shallow layer of a mixture of 36 volumes of *acetone*, 3 volumes of *formamide* and 1 volume of *2-phenoxyethanol*. Allow the impregnating solvent to ascend to the top, remove the plate from the tank and use it immediately.

*Mobile phase*. A mixture of 100 volumes of *light petroleum (40° to 60°)* saturated with *2-phenoxyethanol* and 2 volumes of *diethylamine*.

*Test solution*. Use a quantity of the injection containing 2 mg of Fluphenazine Hydrochloride and dilute it to 1 ml with *methanol*.

*Reference solution*. A 0.2 per cent w/v solution of *fluphenazine hydrochloride RS* in *methanol*.

After development, dry the plate in air, examine in ultraviolet light at 365 nm and observe the fluorescence produced after about 2 minutes. Heat the plate at 120° for 20 minutes, cool, spray with *ethanolic sulphuric acid (20 per cent)* and observe the colour produced. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. To a quantity of the injection containing 5 mg of Fluphenazine Hydrochloride add 2 ml of *sulphuric acid* and allow to stand for 5 minutes; an orange colour is produced.

### Tests

**pH** (2.4.24). 4.8 to 5.2.

**Related substances**. Determine in subdued light by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 80 volumes of *acetone*, 30 volumes of *cyclohexane* and 5 volumes of *strong ammonia solution*.

*Test solution*. Dilute a quantity of the injection containing about 20 mg of Fluphenazine Hydrochloride with sufficient *0.1 M methanolic sodium hydroxide* to make 10 ml.

*Reference solution (a)*. Dilute 1 volume of the test solution to 50 volumes with *0.1 M methanolic sodium hydroxide*.

*Reference solution (b)*. Dilute 1 volume of the test solution to 100 volumes with *0.1 M methanolic sodium hydroxide*.

Apply to the plate 50 µl of the test solution and 25 µl of reference solutions (a) and (b). After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than two such spots are more intense than the spot in the chromatogram obtained with reference solution (b). Ignore any spot remaining on the line of application.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. *Carry out the following procedure protected from light.*

To an accurately measured quantity of the injection containing about 5 mg of Fluphenazine Hydrochloride add a mixture of 1 volume of *1 M hydrochloric acid* and 99 volumes of *ethanol (90 per cent)* to produce 50.0 ml. Dilute 10.0 ml of this solution to 100.0 ml with the acid-ethanol mixture and measure the absorbance of the resulting solution at the maximum at about 258 nm (2.4.7). Calculate the content of $C_{22}H_{26}F_3N_3OS,2HCl$ taking 620 as the specific absorbance at 258 nm.

**Storage**. Store protected from light.

## Fluphenazine Tablets

Fluphenazine Hydrochloride Tablets

Fluphenazine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of fluphenazine hydrochloride, $C_{22}H_{26}F_3N_3OS,2HCl$. The tablets are coated.

**Usual strengths**. 1 mg; 2.5 mg; 5 mg.

### Identification

A. Determine in subdued light by thin-layer chromatography (2.4.17), coating the plate with *kieselguhr G*. Impregnate the dry plate by placing in a tank containing a shallow layer of a mixture of 36 volumes of *acetone*, 3 volumes of *formamide* and 1 volume of *2-phenoxyethanol*. Allow the impregnating solvent to ascend to the top, remove the plate from the tank and use it immediately.



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*Mobile phase.* A mixture of 100 volumes of *light petroleum (40° to 60°)* saturated with *2-phenoxyethanol* and 2 volumes of *diethylamine.*

*Test solution.* Shake a quantity of the powdered tablets with sufficient *methanol* to produce a solution containing 0.2 per cent w/v of Fluphenazine Hydrochloride, centrifuge and use the supernatant liquid.

*Reference solution.* A 0.2 per cent w/v solution of *fluphenazine hydrochloride RS* in *methanol.*

After development, dry the plate in air, examine in ultraviolet light at 365 nm and observe the fluorescence produced after about 2 minutes. Heat the plate at 120° for 20 minutes, cool, spray with *ethanolic sulphuric acid (20 per cent)* and observe the colour produced. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. Extract a quantity of the powdered tablets containing 5 mg of Fluphenazine Hydrochloride with 5 ml of *acetone,* filter and evaporate the filtrate to dryness. Add 2 ml of *sulphuric acid* to the residue and allow to stand for 5 minutes; an orange colour is produced.

C. Extract a quantity of the powdered tablets containing 10 mg of Fluphenazine Hydrochloride with 10 ml of *ethanol* containing 0.2 per cent v/v of *strong ammonia solution* and evaporate the extract to dryness. Heat 0.5 ml of *chromic-sulphuric acid mixture* in a small test-tube in a water-bath for 5 minutes; the solution wets the sides of the tube readily and there is no greasiness. Add 2 or 3 mg of the residue and again heat in a water-bath for 5 minutes; the solution does not wet the sides of the tube and does not pour easily from the tube.

## Tests

**Related substances.** Determine in subdued light by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

*Mobile phase.* A mixture of 80 volumes of *acetone,* 30 volumes of *cyclohexane* and 5 volumes of *strong ammonia solution.*

*Test solution.* Remove the coating from a suitable quantity of tablets; shake a quantity of the powdered tablet cores containing 20 mg of Fluphenazine Hydrochloride with 10 ml of *0.1 M methanolic sodium hydroxide* for 5 minutes, centrifuge and use the supernatant liquid.

*Reference solution (a).* Dilute 1 volume of the test solution to 50 volumes with *0.1 M methanolic sodium hydroxide.*

*Reference solution (b).* Dilute 1 volume of the test solution to 100 volumes with *0.1 M methanolic sodium hydroxide.*

Apply to the plate 50 µl of the test solution and 25 µl of reference solutions (a) and (b). After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than two such spots is more intense than the spot in the chromatogram obtained with reference solution (b). Ignore any spot remaining on the line of application.

**Uniformity of content.** Comply with the test stated under Tablets.

*Carry out the procedure protected from light.*

Powder 1 tablet and dissolve the powder as completely as possible in a mixture of 99 volumes of *ethanol (80 per cent)* and 1 volume of *1 M hydrochloric acid.* Add sufficient of the acid-ethanol mixture to produce 100.0 ml and filter. Dilute suitably, if necessary with the acid-ethanol mixture and measure the absorbance of the resulting solution at the maximum at about 258 nm (2.4.7). Calculate the content of $C_{22}H_{26}F_3N_3OS,2HCl$ taking 620 as the specific absorbance at 258 nm.

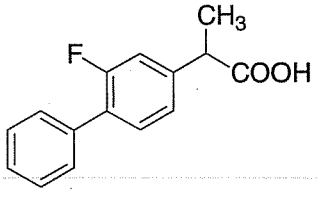
**Other tests.** Comply with the tests stated under Tablets.

**Assay.** *Carry out the following procedure protected from light.*

Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 5 mg of Fluphenazine Hydrochloride, dissolve as completely as possible in a mixture of 99 volumes of *ethanol (80 per cent)* and 1 volume of *1 M hydrochloric acid,* add sufficient of the acid-ethanol mixture to produce 100.0 ml and filter. Dilute 10.0 ml of the filtrate to 50.0 ml with the acid-ethanol mixture and measure the absorbance of the resulting solution at the maximum at about 258 nm (2.4.7). Calculate the content of $C_{22}H_{26}F_3N_3OS,2HCl$ taking 620 as the specific absorbance at 258 nm.

**Storage.** Store protected from light.

# Flurbiprofen




$C_{15}H_{13}FO_2$ Mol. Wt. 244.3

Flurbiprofen is (*RS*)-2-(2-fluorobiphenyl-4-yl)propionic acid.

Flurbiprofen contains not less than 99.0 per cent and not more than 100.5 per cent of $C_{15}H_{13}FO_2$, calculated on the dried basis.

**Category.** Antiinflammatory; analgesic.



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**Dose**. 150 to 200 mg, daily in divided doses, increased in acute conditions to 300 mg daily.

**Description**. A white or almost white, crystalline powder.

### Identification

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

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *0.1 M sodium hydroxide* shows an absorption maximum only at about 247 nm; absorbance at about 247 nm, about 0.8.

C. Heat 0.5 ml of *chromic-sulphuric acid mixture* in a small test-tube in a water-bath for 5 minutes; the solution wets the sides of the tube readily and there is no greasiness. Add 2 or 3 mg of the substance under examination and heat in a water-bath for 5 minutes; the solution does not wet the sides of the tube and does not pour easily from the tube.

### Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. 45 volumes of *acetonitrile* and 55 volumes of *water*.

*Test solution (a)*. Dissolve 0.2 g of the substance under examination in 100 ml with solvent mixture.

*Test solution (b)*. Dissolve 0.2 g of the substance under examination and 1 mg of *2-(biphenyl- 4-yl) propionic acid RS* in 100 ml of the solvent mixture.

*Reference solution*. A 0.001 per cent w/v solution of *2-(biphenyl-4-yl) propionic acid RS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of *water*, 35 volumes of *acetonitrile* and 5 volumes of *glacial acetic acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Adjust the sensitivity of the instrument so that with the reference solution the height of the peak due to 2-(biphenyl-4-yl)propionic acid is about 40 per cent of the full-scale deflection on the recorder.

In the chromatogram obtained with test solution (a) the area of any secondary peak is not greater than the area of the peak in the chromatogram obtained with the reference solution and the sum of the areas of any such peaks is not greater than twice the area of the peak in the chromatogram obtained with the reference solution.

The test is not valid unless a peak due to 2-(biphenyl-4-yl) propionic acid appears immediately before the principal peak in the chromatogram obtained with test solution (b) and the height of the trough separating the two peaks is less than 4 per cent of the full-scale deflection on the chart paper.

**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying**. Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa.

**Assay**. Weigh accurately about 0.5 g, dissolve in 100 ml of *ethanol (95 per cent)* previously neutralised to *phenolphthalein solution* and titrate with *0.1 M sodium hydroxide* using *phenolphthalein solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.02443 g of $C_{15}H_{13}FO_2$.

## Flurbiprofen Tablets

Flurbiprofen Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of flurbiprofen, $C_{15}H_{13}FO_2$. The tablets are coated.

**Usual strengths**. 50 mg; 100 mg.

### Identification

Extract a quantity of the powdered tablets containing 0.5 g of Flurbiprofen with 25 ml of *acetone*, filter, evaporate the filtrate to dryness with the aid of a current of air without heating and dry at 60° at a pressure of 2 kPa. The residue complies with the following tests.

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

B. Heat 0.5 ml of *chromic-sulphuric acid mixture* in a small test-tube in a water-bath for 5 minutes; the solution wets the sides of the tube readily and there is no greasiness. Add 2 or 3 mg of the substance under examination and heat in a water-bath for 5 minutes; the solution does not wet the sides of the tube and does not pour easily from the tube.

### Tests

**Related substances**. Determine by liquid chromatography (2.4.14).



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*Test solution*. Disperse a quantity of the powdered tablets containing 0.5 g of Flurbiprofen in 50 ml of *water*, add 200 ml of *acetonitrile*, mix and centrifuge. Use the supernatant liquid.

*Reference solution*. A 0.001 per cent w/v solution of *2-(biphenyl-4-yl)propionic acid RS* in the test solution.

Chromatographic system
- a stainless steel column 15 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of *water*, 35 volumes of *acetonitrile* and 5 volumes of *glacial acetic acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

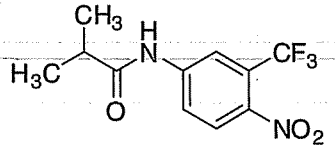
Adjust the sensitivity of the instrument so that with the reference solution the height of the peak due to 2-(biphenyl-4-yl) propionic acid is about 40 per cent of the full-scale deflection on the recorder.

In the chromatogram obtained with the test solution the area of any secondary peak is not greater than the area of the peak in the chromatogram obtained with the reference solution and the sum of the areas of any such peaks is not greater than twice the area of the peak in the chromatogram obtained with the reference solution.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.1 g of Flurbiprofen, shake with 60 ml of *0.1 M sodium hydroxide* for 5 minutes, dilute to 100.0 ml with *0.1 M sodium hydroxide*, filter if necessary and dilute 10.0 ml of the filtrate to 100.0 ml with the same solvent. Further dilute 10.0 ml to 100.0 ml with the same solvent and measure the absorbance of the resulting solution at the maximum at about 247 nm (2.4.7). Calculate the content of $C_{15}H_{13}FO_2$ taking 802 as the specific absorbance at 247 nm.

# Flutamide




$C_{11}H_{11}F_3N_2O_3$ Mol. Wt. 276.2

Flutamide is 2-methyl-*N*-[4-nitro-3-(trifluoromethyl)phenyl] propanamide.

Flutamide contains not less than 97.0 per cent and not more than 103.0 per cent of $C_{11}H_{11}F_3N_2O_3$, calculated on the dried basis.

**Category**. Antiandrogen.

**Description**. A pale yellow crystalline powder.

## Identification

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

B. Melting point (2.4.21). About112°.

## Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 20 mg of the substance under examination in 20.0 ml of the mobile phase.

*Reference solution (a)*. Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 2.0 ml of this solution to 20.0 ml with the mobile phase.

*Reference solution (b)*. Dissolve 2 mg of *flutamide RS* and 2 mg of *N-[4-nitro-3-(trifluoromethyl)phenyl]propanamide RS (flutamide impurity C RS)* in the mobile phase and dilute to 50.0 ml with the mobile phase. Further dilute 1.0 ml of this solution to 20.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of equal volumes of *acetonitrile* and *water*,
- flow rate. 0.5 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to flutamide impurity C and flutamide is not less than 10.5. The relative retention time with reference to flutamide for flutamide impurity C is about 0.72.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of secondary peak corresponding to flutamide impurity C is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent) and the sum of the areas of all secondary peaks is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.25 times the area of the principal peak in the



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chromatogram obtained with reference solution (a) (0.05 per cent).

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

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 60° for 3 hours.

**Assay**. Weigh accurately about 25 mg, dissolve in *methanol* and dilute to 25.0 ml with the *methanol*. Dilute 2.0 ml of this solution to 100.0 ml with *methanol* and measure the absorbance of the resulting solution at the maximum at about 295 nm (2.4.7). Calculate the content of $C_{11}H_{11}F_3N_2O_3$ taking the specific absorbance at about 295 nm.

**Storage**. Store protected from light.

## Flutamide Capsules

Flutamide Capsules contain not less than 93.0 per cent and not more than 107.0 per cent of the stated amount of flutamide, $C_{11}H_{11}F_3N_2O_3$.

**Usual strength**. 125 mg.

### Identification

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

B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel *GF254*.

*Solvent mixture.* 50 volumes of *chloroform* and 10 volumes of *methanol*.

*Mobile phase.* A mixture of 30 volumes of *chloroform* and 10 volumes of *ethyl acetate*.

*Test solution.* Dissolve the content of the capsules containing about 30 mg of Flutamide in 10.0 ml of the solvent mixture.

*Reference solution.* A 0.3 per cent w/v solution of *flutamide RS* in the solvent mixture.

Apply to the plate 20 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

### Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 1000 ml of 2.0 per cent w/v solution of *sodium lauryl sulphate*,

Speed and time. 75 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter. Measure the absorbance of the filtrate, suitably diluted if necessary, at the maximum at about 306 nm (2.4.7). Calculate the content of $C_{11}H_{11}F_3N_2O_3$ in the medium from a known concentration of *flutamide RS* in the same medium.

D. Not less than 75 per cent of the stated amount of $C_{11}H_{11}F_3N_2O_3$.

**Uniformity of content**. Comply with the test stated under Capsules.

Determine by liquid chromatography (2.4.14), as described under Assay using the following solution as the test solution.

*Test solution.* Disperse 1 capsule in 250 ml of the solvent mixture. Dilute 10 ml of this solution to 25 ml with *water*.

Calculate the content of $C_{11}H_{11}F_3N_2O_3$ in the capsule.

**Chromatographic purity**. Determine by liquid chromatography (2.4.14), as described under Assay using the following modifications.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 10 per cent.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of the secondary peak having relative retention time of 0.45 is not more than 0.2 per cent the area of the principal peak in the chromatogram obtained with the reference solution, the area of any other secondary peak is not more than 0.1 per cent the area of the principal peak in the chromatogram obtained with the reference solution and the sum of all the secondary peaks is not more than 0.3 per cent the area of the principal peak in the chromatogram obtained with the reference solution.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture.* 50 volumes of *acetronitrile* and 50 volumes of *water*.

*Test solution.* Mix the content of 20 Capsules. Disperse the content of the capsules containing about 125 mg of Flutamide in 250 ml of the solvent mixture, filter. Dilute 10.0 ml of the filtrate to 25 ml with *water*.

*Reference solution.* A 0.05 per cent w/v solution of *flutamide RS* in the solvent mixture. Dilute 10.0 ml of this solution to 25 ml with *water*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Inertsil),
- mobile phase: a mixture 55 volumes of *water* and 45 volumes of *acetonitrile*,



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- flow rate. 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume. 20 µl.

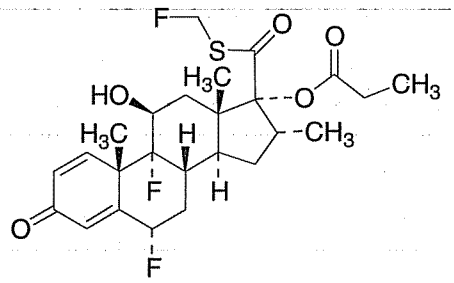
Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 1.5 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{11}H_{11}F_3N_2O_3$.

**Storage**. Store protected from light.

## Fluticasone Propionate



$C_{25}H_{31}F_3O_5S$ Mol. Wt. 500.6

Fluticasone Propionate is *S*-fluoromethyl 6α,9α-difluoro-11β-hydroxy-16α-methyl-17α-propionyloxy-3-oxoandrosta-1,4-diene-17β-carbothioate.

Fluticasone Propionate contains not less than 96.0 per cent and not more than 102.0 per cent of fluticasone, $C_{25}H_{31}F_3O_5S$, calculated on the anhydrous basis.

**Category**. Corticosteroid.

**Description**. A white or almost white powder.

### Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fluticasone propionate RS* or with the reference spectrum of fluticasone propionate.

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

### Tests

**Specific optical rotation** (2.4.22). +32.0° to +36.0°, determined in a 0.5 per cent w/v solution in *dichloromethane*.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. 50 volumes of mobile phase A and 50 volumes of mobile phase B.

*Test solution*. Dissolve 20 mg of the substance under examination in 100 ml of the solvent mixture.

*Reference solution*. A 0.02 per cent w/v solution of *fluticasone propionate RS* in the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature 40°,
- mobile phase: A. 0.05 per cent v/v *orthophosphoric acid* and 3 per cent v/v *methanol* in *acetonitrile*.
  B. 0.05 per cent v/v *orthophosphoric acid* and 3 per cent v/v *methanol* in *water*.
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 239 nm,
- injection volume. 50 µl.

| Time (min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0 | 43 | 57 |
| 40 | 55 | 45 |
| 60 | 90 | 10 |
| 70 | 90 | 10 |
| 75 | 43 | 57 |
| 85 | 43 | 57 |

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the column efficiency in not less than 20,000 theoretical plates.

Inject the test solution. Any individual impurity is not more than 0.5 per cent and sum of all the impurities is not more than 2.0 per cent.

**Water** (2.3.43). Not more than 0.5 per cent, determined on 0.25 g, using as solvent a mixture of equal volumes of *chloroform* and *methanol*.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 40 mg of the substance under examination in 100.0 ml of the mobile phase. Dilute 10.0 ml of this solution to 100.0 ml with the mobile phase.

*Reference solution*. A 0.004 per cent w/v solution of *fluticasone propionate RS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature 40°,



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- mobile phase: a mixture of 15 volumes of *acetonitrile*, 35 volumes of a buffer solution prepared by dissolving 1.15 g of *ammonium dihydrogen phosphate* in 1000 ml of *water* and adjusted to pH 3.5 with *orthophosphoric acid* and 50 volumes of methanol,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 239 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{25}H_{31}F_3O_5S$.

**Storage**. Store protected from light.

## Fluticasone Propionate Inhalation

Fluticasone Propionate Inhalation is a suspension of microfine Fluticasone Propionate in a suitable liquid filled in a suitable pressurized container. It may contain suitable pharmaceutical aids such as surfactants, stabilizing agents.

Fluticasone Propionate Inhalation delivers not less than 80.0 per cent and not more than 120.0 per cent of the stated amount of fluticasone propionate, $C_{25}H_{31}F_3O_5S$, per inhalation by actuation of the valve.

**Usual strengths**. 44 µg per metered dose; 110 µg per metered dose; 220 µg per metered dose.

### Identification

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

### Tests

**Other tests**. Complies with the tests stated under Inhalation Preparations (Pressurised Metered-dose Preparations).

Follow the procedure described under Assay with suitable dilution of the reference solution wherever the amount of active substance is to be determined in any test.

**Assay**. Carry out the test for Content of active ingredient delivered per actuation stated under Inhalation Preparations (Pressurised Metered-dose Preparations).

Determine by liquid chromatography (2.4.14).

*Test solution*. Prepare using the mobile phase as described under the test for Content of active ingredient delivered per actuation stated under Inhalation Preparations (Pressurised Metered-dose Preparations).

*Reference solution (a)*. A 0.05 per cent w/v solution of *fluticasone propionate RS* in *acetonitrile*.

*Reference solution (b)*. Dilute reference solution (a) with the mobile phase to obtain a solution containing 25 µg of fluticasone propionate per ml.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 40°,
- mobile phase: a mixture of 40 volumes of a buffer solution prepared by dissolving 1.3 g of *diammonium hydrogen orthophosphate* in 1000 ml of *water* and adjusted to pH 7.0 with *orthophosphoric acid*, and 60 volumes of *acetonitrile*.
- flow rate. 2 ml per minute,
- spectrophotometer set at 238 nm,
- inject volume. 200 µl.

Inject the reference solution (b). The test is not valid unless the column efficiency is not less than 1500 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and reference solution (b).

Calculate the content of $C_{25}H_{31}F_3O_5S$ in the solution and the content of $C_{25}H_{31}F_3O_5S$ delivered per actuation of the valve.

Determine the content of active ingredient a second and third time by repeating the procedure on the middle ten and on the last ten successive combined actuations of the valve. For each of the three determinations the average content of $C_{25}H_{31}F_3O_5S$ delivered per actuation of the valve meets the requirements.

**Storage**. Store protected from moisture at a temperature not exceeding 30°.

**Labelling**. The label states the amount of active ingredient delivered per inhalation.

## Fluticasone Propionate Powder for Inhalation

Fluticasone Propionate Powder for Inhalation consists of Fluticasone propionate in microfine powder either alone or admixed with Lactose in a pre-metered unit for use in a suitable powder inhaler.

Fluticasone Propionate Powder for Inhalation contains not less than 90.0 per cent and not more than 125.0 per cent of the stated amount of fluticasone propionate, $C_{25}H_{31}F_3O_5S$ per unit dose.

**Usual strengths**. 50 mg; 100 mg; 250 mg.



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## Identification

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

## Tests

**Other tests**. Complies with the tests stated under Inhalation Preparations (Powders for Inhalation).

Follow the procedure described under Assay with suitable dilution of the reference solution wherever the amount of active substance is to be determined in any test.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve a quantity of the mixed contents of 20 capsules in sufficient of the mobile phase to get a solution containing 25 µg of Fluticasone Propionate per ml.

*Reference solution*. A solution containing 0.5 mg of fluticasone propionate per ml prepared by dissolving 10 mg of *fluticasone propionate RS* in 10 ml *acetonitrile* and adding sufficient of the mobile phase to produce 20 ml and further dilute with mobile phase to obtain a solution containing 25 µg of Fluticasone Propionate per ml.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 40°,
- mobile phase: a mixture of 40 volumes of a buffer solution prepared by dissolving 1.3 g of *diammonium hydrogen orthophosphate* in 1000 ml of *water* and adjusted to pH 7.0 with *orthophosphoric acid* and 60 volumes of *acetonitrile*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 238 nm,
- injection volume. 200 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 1500 theoretical plates and the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

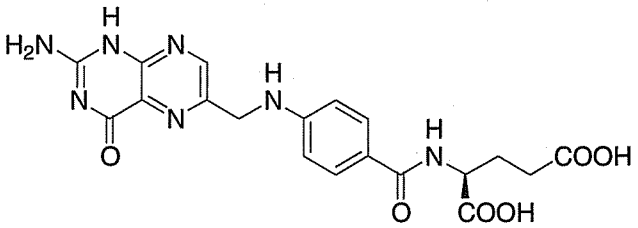
Calculate the content of $C_{25}H_{31}F_3O_5S$ per unit.

**Storage**. Store protected from moisture, at temperature not exceeding 30°.

**Labelling**. The label states the quantity of active ingredient per pre-metered unit.

# Folic Acid

Pteroylglutamic Acid



$C_{19}H_{19}N_7O_6$ Mol. Wt. 441.4

Folic Acid is (2*S*)-[4-[(2-amino-4-hydroroxypteridin-6-yl) methylamino]benzamido]glutamic acid.

Folic acid contains not less than 95.0 per cent and not more than 102.0 per cent of $C_{19}H_{19}N_7O_6$, calculated on the anhydrous basis.

**Category**. B-group vitamin (hematopoietic).

**Dose**. In the treatment of megaloblastic anaemia associated with folic acid deficiency, 5 to 20 mg daily. In the prophylaxis of megaloblastic anaemia of pregnancy, 200 to 500 mg daily.

**Description**. A yellow to yellowish-orange, crystalline powder; odourless or almost odourless.

## Identification

A. When examined in the range 230 nm to 380 nm (2.4.7), a 0.001 per cent w/v solution in *0.1 M sodium hydroxide* shows three absorption maxima, at about 256 nm, 283 nm and 365 nm; absorbance at about 256 nm, about 0.59, at about 283 nm, about 0.575 and at about 365 nm, about 0.206.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 60 volumes of *ethanol (95 per cent)*, 20 volumes of *strong ammonia solution* and 20 volumes of *1-propanol*.

*Solvent mixture*. 9 volumes of *methanol* and 2 volumes of *strong ammonia solution*.

*Test solution*. Dissolve 50 mg of the substance under examination in 100 ml of the solvent mixture.

*Reference solution*. A 0.05 per cent w/v solution of *folic acid RS* in the solvent mixture.

Apply to the plate 2 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.



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### Tests

**Specific optical rotation** (2.4.22). About +20°, determined in a 1.0 per cent w/v solution in *0.1 M sodium hydroxide*.

**Related substances.** Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve 100 mg of the substance under examination in 5 ml of a 2.86 per cent w/v solution of *sodium carbonate* and dilute to 100.0 ml with the mobile phase. Dilute 2.0 ml of this solution to 10.0 ml with the mobile phase.

*Reference solution (a).* Dissolve 100 mg of *folic acid RS* in 5 ml of a 2.86 per cent w/v solution of *sodium carbonate* and dilute 100 ml with the mobile phase. Dilute 2.0 ml of this solution to 10.0 ml with the mobile phase.

*Reference solution (b).* To 20 mg of *pteroic acid*, add 5 ml of a 2.86 per cent w/v solution of *sodium carbonate*, dilute to 100 ml with the mobile phase. Mix 1.0 ml of this solution with 1.0 ml of reference solution (a) and dilute to 100 ml with the mobile phase.

*Reference solution (c).* Dilute 2.0 ml of the test solution to 20 ml with the mobile phase. Dilute 1.0 ml of this solution to 20.0 ml with the mobile phase.

*Reference solution (d).* Dissolve 10 mg of *N-(4-aminobenzoyl)-l-gulatamic acid (folic acid impurity A)* in 1 ml of a 2.86 per cent w/v solution of *sodium carbonate* and dilute to 100 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

*Reference solution (e).* To 12 mg of *pteroic acid (folic acid impurity D),* add 1 ml of a 2.86 per cent w/v solution of *sodium carbonate*, dilute to 100 ml with the mobile phase. Dilute 1.0 ml of this solution to 100 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 12 volumes of *methanol* and 88 volumes of a solution containing 1.1 per cent w/v solution of *potassium dihydrogen phosphate* and 0.6 per cent w/v solution of *dipotassium hydrogen phosphate,*
- flow rate. 0.6 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume. 5 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to folic acid and folic acid impurity D is not less than 4.0. The relative retention time with reference to folic acid for N-(4-aminobenzoyl)-L-glutamic acid ( folic acid impurity A) is about 0.5; for 2,5,6-triaminopyrimidin-4(1H)-one (folic acid impurity B) is about 0.6; for isofolic acid (folic acid impurity C) is about 0.9, for pteroic acid (folic acid impurity D) is about 1.33; for 6-pterinylfolic acid (folic acid impurity E) is about 1.27.

Inject the test solution, reference solution (c), (d) and (e). In the chromatogram obtained with the test solution the area of peak corresponding to folic acid impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (0.5 per cent), the area of the peak corresponding to folic acid impurity D is not more than the area of the principal peak in the chromatogram obtained with reference solution (e) (0.6 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent), The sum of areas of all other secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

**Free amines**. The absorbance (2.4.7) of the unreduced solution as determined in the Assay is not more than one-sixth of the absorbance of the reduced solution.

**Sulphated ash** (2.3.18). Not more than 0.5 per cent.

**Water** (2.3.43). 5.0 to 8.5 per cent, determined on 0.15 g.

**Assay**. Determine by liquid chromatography (2.4.14), as described under Related substances.

Inject the test solution and reference solution (a).

Calculate the content of $C_{19}H_{19}N_7O_6$.

**Storage**. Store protected from light.

## Folic Acid Tablets

Folic Acid Tablets contain not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of folic acid, $C_{19}H_{19}N_7O_6$.

**Usual strengths**. 100 µg; 5 mg.

### Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase.* A mixture of 60 volumes of *ethanol (95 per cent)*, 20 volumes of *strong ammonia solution* and 20 volumes of *1-propanol.*

*Solvent mixture.* 9 volumes of *methanol* and 2 volumes of *strong ammonia solution.*

*Test solution.* Extract a quantity of the powdered tablets containing 0.5 mg of Folic Acid with 1 ml of the solvent mixture.



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*Reference solution.* A 0.05 per cent w/v solution of *folic acid RS* in the solvent mixture.

Apply to the plate 2 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. Dissolve a quantity of the powdered tablets containing 5 mg of Folic Acid as completely as possible in 5 ml of *0.1 M sodium hydroxide* and filter. To the filtrate add 45 ml of *0.5 M hydrochloric acid* and 5 g of *zinc powder* and shake for 30 minutes. To 5 ml of the reduced solution, add 2 ml of a 0.1 per cent w/v solution of *sodium nitrite*, allow to stand for 2 minutes, add 2 ml of a 0.5 per cent w/v solution of *ammonium sulphamate*, mix, allow to stand for 3 minutes and add 2 ml of a 0.1 per cent w/v solution of *N-(1-naphthyl)ethylenediamine dihydrochloride*; a deep magenta colour is produced.

## Tests

**Hydrolysis products**. Determine by liquid chromatography (2.4.14).

*Protect the solutions from light.*

*Test solution.* Shake a quantity of the powdered tablets containing 5.0 mg of Folic Acid with 50 ml of the mobile phase, centrifuge and use the supernatant liquid.

*Reference solution.* A solution containing 0.00005 per cent w/v of *4-aminobenzoic acid* and 0.0002 per cent w/v of *N-(4-aminobenzoyl)-L-glutamic acid* in the mobile phase.

Chromatographic system

- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Spherisorb ODS 1),
- mobile phase: *0.05 M potassium dihydrogen phosphate*, adjusted to pH 5.5 with *5 M sodium hydroxide*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 269 nm,
- injection volume. 20 µl.

In the chromatogram obtained with the reference solution two peaks due to N-(4-aminobenzoyl)-L-glutamic acid and 4-aminobenzoic acid are obtained in the order of their emergence.The areas of the peaks due to 4-aminobenzoic-acid and N-(4-amino- benzoyl)-L-glutamic acid in the chromatogram obtained with the reference solution are greater than the areas of any corresponding peaks in the chromatogram obtained with the test solution. The test is not valid unless the resolution between the two peaks in the chromatogram obtained with the reference solution is greater than 3.

**Uniformity of content**. Comply with the test stated under Tablets.

*Test solution.* Shake 1 tablet with 5.0 ml of *0.1 M sodium hydroxide*, add sufficient mobile phase to produce a solution containing 0.001 per cent w/v of Folic Acid, centrifuge and use the supernatant liquid.

*Reference solution.* Add 1.0 ml of *0.5 M hydrochloric acid* to 5.0 ml of a 0.002 per cent w/v solution of *folic acid RS* in *0.1 M sodium hydroxide* and dilute to 10.0 ml with the mobile phase.

The chromatographic procedure described under Assay may be carried out. Calculate the content of $C_{19}H_{19}N_7O_6$ in the tablet.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh and powder 20 tablets. Shake a quantity of the powdered tablets containing about 20 mg of Folic Acid with 50 ml of *0.1 M sodium hydroxide*, dilute to 100.0 ml with the same solvent, centrifuge and dilute 5.0 ml of the supernatant liquid to 100.0 ml with the mobile phase.

*Reference solution.* Dilute 5.0 ml of a 0.02 per cent w/v solution of *folic acid RS* in *0.1 M sodium hydroxide* to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 93 volumes of *0.05 M potassium dihydrogen phosphate* and 7 volumes of *acetonitrile* adjusted to pH 6.0 with *5 M sodium hydroxide*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 283 nm,
- injection volume. 20 µl.

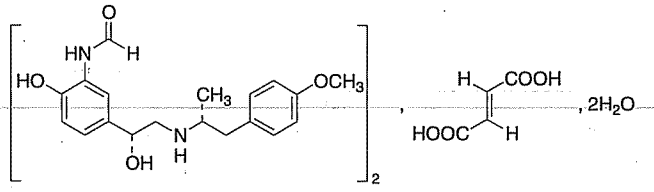
Inject the test solution and the reference solution.

Calculate the content of $C_{19}H_{19}N_7O_6$ in the tablets.

*NOTE — For tablets containing 2 mg or less of Folic Acid the average of the 10 individual results obtained in the test for Uniformity of content may be taken in lieu of the Assay.*

**Storage**. Store protected from light.

# Formoterol Fumarate Dihydrate



$(C_{19}H_{24}N_2O_4)_2, C_4H_4O_4, 2H_2O$ Mol. Wt. 840.9

Formoterol Fumarate Dihydrate is dihydrate salt of fumaric acid with (*RS*)-2′-hydroxy-5′-[(*RS*)-1-hydroxy-2-[[(*RS*)-*p*-methoxy-α-methylphenethyl]amino]ethyl]formanilide.



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Formoterol Fumarate Dihydrate contains not less than 98.5 per cent and not more than 101.5 per cent of formoterol fumarate, $C_{42}H_{52}N_4O_{12}$, calculated on the anhydrous basis.

**Category**. Bronchodilator; adrenergic.

**Description**. A white or almost white or slightly yellow powder.

### Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *formoterol fumarate dihydrate RS* or with the reference spectrum of formoterol fumarate dihydrate.

### Tests

**pH** (2.4.24). 5.5 to 6.5, determined in a 0.1 per cent w/v solution in *carbon dioxide-free water*.

**Optical rotation** (2.4.22). – 0.10° to + 0.10°, determined in a 1.0 per cent w/v solution in *methanol*.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. 84 volumes of a buffer solution prepared by dissolving 6.10 g of *sodium dihydrogen phosphate monohydrate* and 1.03 g of *disodium hydrogen phosphate dihydrate* in 1000 ml *water*, and 16 volumes of *acetonitrile*.

*Test solution*. Dissolve 20 mg of the substance under examination in 100 ml of the solvent mixture.

*Reference solution*. A 0.00004 per cent w/v solution of *formoterol fumarate RS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with spherical octylsilane bonded to porous silica (5 µm),
- mobile phase: A. *acetonitrile*,
  B. a buffer solution prepared by dissolving 3.73 g of *sodium dihydrogen phosphate monohydrate* and 0.35 g of *orthophosphoric acid* in 1000 ml of *water*,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 214 nm,
- injection volume. 20 µl.

| Time (min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0 | 16 | 84 |
| 10 | 16 | 84 |
| 37 | 70 | 30 |
| 40 | 16 | 84 |
| 55 | 16 | 84 |

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the column efficiency in not less than 4000 theoretical plates.

Inject the test solution. Any individual impurity is not more than 0.5 per cent and the sum of all the impurities is not more than 1.0 per cent. Ignore the peak corresponding to fumaric acid.

**Water** (2.3.43). Not more than 5.0 per cent, determined on 0.1 g.

**Assay**. Weigh accurately about 0.35 g and dissolve in 50 ml of *anhydrous acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04024 g of $C_{42}H_{52}N_4O_{12}$.

**Storage**. Store protected from light and moisture.

## Formoterol Fumarate and Budesonide Powder for Inhalation

Formoterol Fumarate and Budesonide Powder for Inhalation consists of Formoterol Fumarate and Budesonide in microfine powder either alone or admixed with Lactose in a pre-metered unit for use in a suitable powder inhaler.

Formoterol Fumarate and Budesonide Powder for Inhalation contains not less than 90.0 per cent and not more than 125.0 per cent of the stated amounts of formoterol fumarate $C_{42}H_{52}N_4O_{12}$ and budesonide $C_{25}H_{34}O_6$ per pre-metered unit.

**Usual Strengths.** Formoterol Fumarate 6 µg and Budesonide 100 µg; Formoterol Fumarate 6 µg and Budesonide 200 µg; Formoterol Fumarate 6 µg and Budesonide 400 µg.

### Identification

In the Assay, the principal peaks in the chromatogram obtained with the test solution correspond to the peaks in the chromatogram obtained with reference solution (c).

### Tests

**Other tests**. Complies with the tests stated under the Inhalation Preparations (Powders for Inhalation).

Follow the procedure described under Assay with suitable dilution of the reference solution wherever the amount of active substance is to be determined in any test.



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**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. To a suitable number of intact capsules add 10 ml of *water* and disperse with the aid of ultrasound till the shells get disintegrated. Add 60 ml of the mobile phase and mix further with the aid of ultrasound for 10 minutes with intermittent shaking. Add sufficient of the mobile phase to produce 100.0 ml. Dilute suitably with the mobile phase, if required, to get a final concentration of 0.6 µg per ml of Formoterol Fumarate in the mobile phase.

*Reference solution (a)*. A 0.06 mg per ml solution of *formoterol fumarate RS* prepared by initially dissolving in 5 ml *acetonitrile* and then making up to volume with the mobile phase.

*Reference solution (b)*. A 0.2 mg per ml solution of *budesonide RS* prepared by initially dissolving in 5 ml *acetonitrile* and then making up to volume with the mobile phase.

*Reference solution (c)*. Dilute suitable volumes of reference solution (a) and reference solution (b) with the mobile phase to obtain a solution containing 0.6 µg of Formoterol Fumarate and 40 µg per ml of Budesonide per ml.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 65 volumes of a buffer solution prepared by dissolving 0.138 g *sodium dihydrogen orthophosphate monohydrate* and 0.122 g of *decane sulphonic acid sodium salt* in 100 ml of *water* and adjusting the pH to 3.0 with *orthophosphoric acid*, and 35 volumes of *acetonitrile*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 220 nm,
- inject 200 µl.

Inject reference solution (c). The order of elution is formoterol fumarate, budesonide epimer B and epimer A. The test is not valid unless the column efficiency determined from the formoterol fumarate and both the epimer peaks of Budesonide is not less than 1800 and 4000 theoretical plates respectively, the resolution between budesonide epimer peaks is not less than 1.5 and the relative standard deviation for formoterol fumarate and sum of peaks of budesonide epimer A and epimer B in replicate injections is not more than 2.0 per cent.

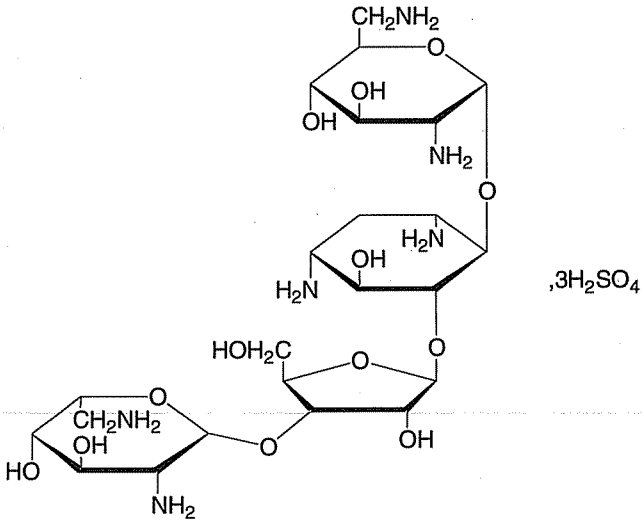
Inject the test solution and reference solution (c).

Calculate the contents of $C_{42}H_{52}N_4O_{12}$ and $C_{25}H_{34}O_6$ per unit.

**Storage**. Store protected from moisture, at temperature not exceeding 30°.

**Labelling**. The label states the quantities of active ingredients per pre-metered unit.

# Framycetin Sulphate




$C_{23}H_{46}N_6O_{13},3H_2SO_4$ Mol. Wt. 908.9

Framycetin Sulphate is 2-deoxy-4-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-5-*O*-[3-*O*-(2,6-diamino-2,6-dideoxy-β-L-idopyranosyl)-β-D-ribofuranosyl]-D-streptamine (neomycin B) sulphate. The base is produced by the growth of selected strains of *Streptomyces fradiae* or *Streptomyces decaris* or by any other means.

Framycetin Sulphate contains an amount of framycetin sulphate equivalent to not less than 630 µg of neomycin B per mg, calculated on the dried basis.

**Category**. Antibacterial (topical).

**Description**. A white or yellowish-white powder; odourless or almost odourless; hygroscopic.

## Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate in the following manner. Mix 0.3 g of *carbomer* with 240 ml of *water*, allow to stand for 1 hour with moderate shaking, adjust the pH to 7 by the gradual addition, with shaking, of *2 M sodium hydroxide* and add 30 g of *silica gel H*. Spread a uniform layer of the suspension 0.75 mm thick, heat at 110° for 1 hour and allow to cool. Use the plate immediately.

*Mobile phase*. A 10 per cent w/v solution of *potassium dihydrogen phosphate*.

*Test solution*. A 0.1 per cent w/v solution of the substance under examination.

*Reference solution (a)*. A 0.1 per cent w/v solution of *framycetin sulphate RS*.



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*Reference solution (b)*. A solution containing 0.1 per cent w/v each of *framycetin sulphate RS*, *kanamycin sulphate RS* and *streptomycin sulphate RS*.

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of warm air, spray with a mixture of equal volumes of a 46 per cent w/v solution of *sulphuric acid* and of a 0.2 per cent w/v solution of *1,3-naphthalenediol* in *ethanol (95 per cent)* and heat at 150° for about 10 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows three clearly separated spots.

B. Dissolve 10 mg in 5 ml of *water*, add 0.1 ml of *pyridine* and 2 ml of a 0.1 per cent w/v solution of *ninhydrin* and heat in a water-bath at 65° to 70° for 10 minutes; an intense violet colour is produced.

C. A 5 per cent w/v solution gives the reactions of sulphates (2.3.1).

## Tests

**pH** (2.4.24). 6.0 to 7.0, determined in a 1.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). +52.0° to +55.5°, determined at 20° in a 10.0 per cent w/v solution.

**Neamine**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H*.

*Mobile phase*. A mixture of 30 volumes of *methanol*, 20 volumes of *strong ammonia solution* and 10 volumes of *dichloromethane*.

*Test solution*. Dissolve 0.25 g of the substance under examination in *water* and dilute to 10 ml with *water*.

*Reference solution*. Dissolve 0.5 mg of *neamine RS* in 2 ml of *water*.

Apply to the plate as 5-mm bands 5 µl of each solution. Dry the bands and develop over a path of at least 8 cm. Dry the plate at 105° for 10 minutes. Spray it with *ninhydrin and stannous chloride reagent* and heat at 110° for 15 minutes. Spray the plate again with the same reagent and heat at 110° for 15 minutes. Any band corresponding to neamine in the chromatogram obtained with the test solution is not more intense than the band in the chromatogram obtained with the reference solution (1 per cent).

**Neomycin C**. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel of a suitable grade.

*Mobile phase*. A mixture of 80 volumes of a 20 per cent w/v solution of *sodium chloride* and 20 volumes of *methanol*.

*Test solution*. Dissolve 40 mg of the substance under examination in *water* and dilute to 5 ml with *water*.

*Reference solution (a)*. Dissolve 40 mg of *framycetin sulphate RS* in *water* and dilute to 5 ml with *water*.

*Reference solution (b)*. Dissolve 30 mg of *framycetin sulphate RS* in *water* and dilute to 25 ml with *water*. Dilute 5 ml of this solution to 25 ml with *water*.

*Reference solution (c)*. Dissolve 40 mg of *neomycin sulphate RS* in *water* and dilute to 5 ml with *water*.

Apply to the plate as 5-mm bands 10 µl of each solution. Dry the bands and develop over a path of at least 12 cm. Dry the plate at 100° to 105° for 10 minutes. In the chromatogram obtained with the test solution, the principal band corresponds to the principal band in the chromatogram obtained with reference solution (a) and the band for neomycin C with Rf value slightly less than that of the principal band is not more intense than the band in the chromatogram obtained with reference solution (b) (3 per cent).

The test is not valid unless in the chromatogram obtained with reference solution (c), a band appears with $R_f$ value slightly less than that of the principal band.

**Sulphate**. 27.0 to 31.0 per cent of $SO_4$, calculated on the dried basis, determined by the following method. Weigh accurately about 0.25 g, dissolve in 100 ml of *water*, adjust the pH to 11 with *strong ammonia solution* and add 10.0 ml of *0.1 M barium chloride*. Titrate with *0.1 M disodium edetate* using 0.5 mg of *metalphthalein* as indicator; add 50 ml of *ethanol (95 per cent)* when the colour of the solution begins to change and continue the titration until the violet-blue colour disappears.

1 ml of *0.1 M barium chloride* is equivalent to 0.009606 g of $SO_4$.

**Alcohols**. Not more than 2 per cent w/w, calculated as *methanol*, $CH_4O$, determined by the following method. Dissolve 0.2 g in 5 ml of *water* and add 0.05 ml of *0.05 M sulphuric acid*. Distil the mixture and collect about 2.5 ml of the distillate in a flask. Add 25.0 ml of a *0.0167 M potassium dichromate* in a mixture of 30 volumes of *water* and 20 volumes of *sulphuric acid*. Heat on a water-bath for 30 minutes, cool and add sufficient *water* to produce 500.0 ml. Add 10 ml of *potassium iodide solution*, allow to stand for 5 minutes and titrate with *0.1 M sodium thiosulphate* using *starch solution*, added towards the end of the titration, as indicator, until the solution becomes pale green. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of *potassium dichromate* equivalent to the alcohols present.

1 ml of *0.0167 M potassium dichromate* is equivalent to 0.000534 g of $CH_4O$.

**Sulphated ash** (2.3.18). Not more than 1.0 per cent.

**Loss on drying** (2.4.19). Not more than 8.0 per cent, determined on 1.0 g by drying over *phosphorus pentoxide* at 60° at a pressure not exceeding 0.7 kPa for 3 hours.



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**Assay**. Determine by the microbiological assay of antibiotics, Method A (2.2.10), and express the results in µg of neomycin B per mg.

*Framycetin Sulphate intended for administration into internal body cavities without a further appropriate procedure for removal of bacterial endotoxins complies with the following additional requirement.*

**Bacterial endotoxins** (2.2.3). Not more than 1.3 Endotoxin Units per mg.

*Framycetin Sulphate intended for administration into internal body cavities without a further appropriate sterilisation procedure complies with the following additional requirement.*

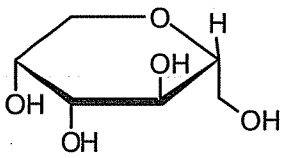
**Sterility** (2.2.11). Complies with the test for sterility.

**Storage**. Store protected from light and moisture at a temperature not exceeding 30°. If the material is sterile, the container should be tamper-evident and sealed so as to exclude micro-organisms.

**Labelling**. The label states (1) the strength in terms of µg of neomycin B per mg; (b) where applicable, that the material is free from bacterial endotoxins; (c) where applicable, that the material is sterile.

# Fructose

D-Fructose




$C_6H_{12}O_6$ Mol. Wt. 180.2

Fructose is D-(-)-fructopyranose.

**Category**. Nutrient; fluid replenisher.

**Description**. A white, crystalline powder with a very sweet taste.

## Identification

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

B. Dissolve 0.1 g in 10 ml of *water*, add 3 ml of *potassium cupri-tartrate solution*; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.

C. Dissolve 5 g in *water* and dilute to 10 ml with the same solvent. To 0.5 ml of this solution add 0.2 g of *resorcinol* and 9 ml of *dilute hydrochloric acid* and heat on a water-bath for 2 minutes; a red colour is produced.

## Tests

**Appearance of solution**. Dissolve 5.0 g in *water* and dilute to 10 ml with the same solvent. The solution is clear (2.4.1). Add 10 ml of *water*. The solution is colourless (2.4.1).

**Acidity or alkalinity**. Dissolve 6.0 g in 25 ml of *carbon dioxide-free water* and add 0.3 ml of *phenolphthalein solution*. The solution is colourless. Not more than 0.15 ml of *0.1 M sodium hydroxide* is required to change the colour of the solution to pink.

**Specific optical rotation** (2.4.22). -91.0° to –93.5°, calculated on the anhydrous basis and determined on a solution prepared by dissolving 10.0 g in 80 ml of *water*, adding 0.2 ml of *5 M ammonia*, mixing well, allowing to stand for 30 minutes and diluting to 100.0 ml with *water*.

**5-Hydroxymethylfurfural and related compounds**. To 5 ml of solution A add 5 ml of *water* and measure the absorbance (2.4.7) of the resulting solution at the maximum at about 284 nm; absorbance at about 284 nm, not more than 0.32).

**Arsenic** (2.3.10). Dissolve 10.0 g in 50 ml of *water* and add 10 ml of *stannated hydrochloric acid*. The resulting solution complies with the limit test for arsenic (1 ppm).

**Heavy metals** (2.3.13). A solution of 4.0 g in 10 ml of *water*, 2 ml of *dilute acetic acid* and sufficient *water* to produce 25.0 ml complies with the limit test for heavy metals, Method A (5 ppm).

**Chlorides** (2.3.12). 20 ml of a 10 per cent w/v solution (solution A) complies with the limit test for chlorides (125 ppm).

**Sulphates** (2.3.17). 7.5 ml of solution A diluted to 15 ml with *water* complies with the limit test for sulphates (200 ppm).

**Foreign sugars.** Dissolve 5.0 g in *water* and dilute to 10 ml with the same solvent. To 1 ml of the solution add 9 ml of *ethanol (95 per cent)*. Any opalescence in the solution is not more intense than that in a mixture of 1 ml of the initial solution and 9 ml of *water*.

**Barium**. To 10 ml of solution A add 1 ml of *1 M sulphuric acid*. Examine exactly after 1 hour; any opalescence in the solution is not more intense than that in a mixture of 10 ml of solution A and 1 ml of *water*.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 0.5 per cent, determined on 1.0 g.

*Fructose intended for use in the manufacture of parenteral preparations without a further appropriate procedure for*



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*the removal of bacterial endotoxins complies with the following additional requirement.*

**Bacterial endotoxins** (2.2.3). Not more than 0.5 Endotoxin Unit per ml of a 5 per cent w/v solution in Water for Injections.

**Storage**. Store protected from moisture.

**Labelling**. The label states whether or not the contents are intended for use in the manufacture of parenteral preparations.

## Fructose Injection

Fructose Intravenous Infusion

Fructose Injection is a sterile solution of Fructose in Water for Injections.

Fructose Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of fructose, $C_6H_{12}O_6$. It contains no antimicrobial agent.

**Usual strength**. 10 per cent w/v.

**Description**. A clear, colourless solution.

### Identification

A. The solution prepared as directed in the Assay is laevorotatory.

B. To 1 ml add 0.05 ml of *potassium cupri-tartrate solution*; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.

### Tests

**pH** (2.4.24). 3.0 to 6.0, determined in a solution diluted, if necessary, with Water for Injections to contain not more than 5.0 per cent w/v of Fructose and to which 0.30 ml of a saturated solution of *potassium chloride* has been added for each 100 ml of solution.

**5-Hydroxymethylfurfural and Related substances**. Dilute a volume containing 1.0 g of Fructose to 500.0 ml with *water* and measure the absorbance (2.4.7) of the resulting solution at the maximum at about 284 nm; absorbance at about 284 nm, not more than 0.50.

**Heavy metals** (2.3.13). A volume of the injection containing 4.0 g of fructose that has been evaporated to a volume of about 10 ml, cooled and diluted to 25 ml with *water* complies with the limit test fpr heavy metals, Method A (5 ppm).

**Bacterial endotoxins** (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

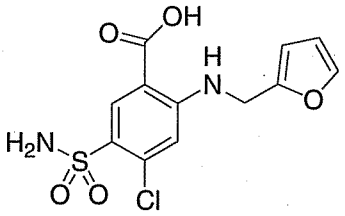
**Assay**. To an accurately measured volume containing about 5.0 g of Fructose, add 0.2 ml of *5 M ammonia* and sufficient *water* to produce 100.0 ml. Mix well, allow to stand for 30 minutes and determine the optical rotation in a 2-dm tube (2.4.22). The observed rotation in degrees multiplied by 0.5427 represents the weight, in g, of fructose, $C_6H_{12}O_6$, in the volume taken for Assay.

**Storage**. Store in single dose containers at a temperature not exceeding 30°.

**Labelling**. The label states (1) the strength as the percentage w/v of fructose, $C_6H_{12}O_6$; (2) that the injection should not be used if it contains visible particles.

## Frusemide

Furosemide




$C_{12}H_{11}ClN_2O_5S$ Mol. Wt. 330.7

Frusemide is 4-chloro-*N*-furfuryl-5-sulphamoylanthranilic acid.

Frusemide contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{12}H_{11}ClN_2O_5S$, calculated on the dried basis.

**Category**. Diuretic.

**Dose**. Orally, in oedema, 20 to 40 mg daily; in oliguvria, 250 mg 4 to 6 times daily; by intramuscular or slow intravenous injection, 20 to 50 mg (rate not exceeding 4 mg per minute).

**Description**. A white or almost white, crystalline powder.

### Identification

*Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.*

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

B. When examined in the range 220 nm to 360 nm (2.4.7), a 0.0005 per cent w/v solution in *0.1 M sodium hydroxide* shows three absorption maxima at about 228 nm, 271 nm and 333 nm. The ratio of the absorbance at the maximum at about 271 nm to that at the maximum at about 228 nm is 0.52 to 0.57.



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C. Dissolve about 5 mg in 10 ml of *methanol*. Transfer 1 ml of this solution to a flask, add 10 ml of *dilute hydrochloric acid* and boil under a reflux condenser on a water-bath for 15 minutes. Cool, add 15 ml of *1 M sodium hydroxide* and 5 ml of a 0.1 per cent w/v solution of *sodium nitrite*. Allow to stand for 3 minutes, add 5 ml of a 0.5 per cent w/v solution of *ammonium sulphamate*, mix and add 5 ml of a 0.1 per cent w/v solution of *N-(1-naphthyl)ethylenediamine dihydrochloride*; a red-violet colour is produced.

### Tests

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

**Chlorides** (2.3.12). Shake 1 g with 40 ml of *water* for 5 minutes and filter. The filtrate complies with the limit test for chlorides (250 ppm).

**Sulphates** (2.3.17). Shake 1.0 g with a mixture of 30 ml of *distilled water* and 0.2 ml of *5 M acetic acid* for 5 minutes and filter. 15 ml of the filtrate complies with the limit test for sulphates (300 ppm).

**Related substances.** Determine by liquid chromatography (2.4.14).

*Note—Prepare the solutions immediately before use.*

*Test solution.* Dissolve 50 mg of the substance under examination in 50.0 ml of the mobile phase.

*Reference solution.* Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 70 volumes of solution prepared by dissolving 0.2 g of *potassium dihydrogen phosphate* and 0.25 g of *cetrimide* in 70 ml of *water* adjusted to pH 7.0 with *ammonia* and 30 volumes of *propanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 238 nm,
- injection volume. 20 µl.

Inject the reference solution. Adjust the sensitivity so that the height of the peak is not less than 20 per cent of the full scale of the recorder.

Inject the test solution and the reference solution. Run the chromatogram three times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area any secondary peak is not more than 0.25 times the area of the peak obtained with the reference solution (0.25 per cent) and sum of areas of all the secondary peaks is not more than 0.5 times the area of the peak in the chromatogram obtained with the reference solution (0.5 per cent).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.5 g, dissolve in 40 ml of *dimethylformamide* and titrate with *0.1 M sodium hydroxide* using *bromothymol blue solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.03307 g of $C_{12}H_{11}ClN_2O_5S$.

**Storage**. Store protected from light.

## Frusemide Injection

Furosemide Injection

Frusemide Injection is a sterile solution of Frusemide in Water for Injections prepared with the aid of Sodium Hydroxide.

Frusemide Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of frusemide, $C_{12}H_{11}ClN_2O_5S$.

**Usual strength**. 10 mg per ml.

**Description**. A clear, colourless or almost colourless solution.

### Identification

A. When examined in the range 220 nm to 360 nm (2.4.7), the final solution obtained in the Assay shows three absorption maxima at about 228 nm, 271 nm and 333 nm.

B. To a volume of the injection containing 5 mg of Frusemide add 10 ml of *dilute hydrochloric acid* and boil under a reflux condenser on a water-bath for 15 minutes. Cool, add 15 ml of *1 M sodium hydroxide* and 5 ml of a 0.1 per cent w/v solution of *sodium nitrite*. Allow to stand for 3 minutes, add 5 ml of a 0.5 per cent w/v solution of *ammonium sulphamate*, mix and add 5 ml of a 0.1 per cent w/v solution of *N- (1-naphthyl) ethylenediamine dihydrochloride*; a red-violet colour is produced.

### Tests

**pH** (2.4.24). 8.0 to 9.3.

**Related substances.** Determine by liquid chromatography (2.4.14).

*Note—Prepare the solutions immediately before use.*

*Test solution.* Dilute a volume of injection containing about 0.1 g of Frusemide to 100 ml with the mobile phase.

*Reference solution.* Dilute 1.0 ml of the test solution to 100 ml with the mobile phase.



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Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm) (such as ChromSpher C8),
- mobile phase: mixture of 70 volumes of solution prepared by dissolving 0.2 g of *potassium dihydrogen phosphate* and 0.25 g of *cetrimide* in 70 ml of *water* adjusted to pH 7.0 with *ammonia* and 30 volumes of *propanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 238 nm,
- injection volume. 100 µl.

Inject the test solution and the reference solution. Run the chromatogram three times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the principal peak obtained with the reference solution (1 per cent), and sum of areas of all the secondary peaks is not more than 1.5 times the area of the peak in the chromatogram obtained with the reference solution (1.5 per cent).

**Bacterial endotoxins** (2.2.3). Not more than 3.5 Endotoxin Units per mg of frusemide.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. Dilute an accurately measured volume of the injection containing about 20 mg of Frusemide with *water* to produce 100.0 ml. Dilute 5.0 ml to 100.0 ml with *0.1 M sodium hydroxide*. Measure the absorbance of the resulting solution at the maximum at about 271 nm (2.4.7). Calculate the content of $C_{12}H_{11}ClN_2O_5S$ taking 580 as the specific absorbance at 271 nm.

**Storage**. Store protected from light.

# Frusemide Tablets

Furosemide Tablets

Frusemide Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of frusemide, $C_{12}H_{11}ClN_2O_5S$.

**Usual strengths**. 20 mg; 40 mg.

## Identification

A. When examined in the range 220 nm to 360 nm (2.4.7), the final solution obtained in the Assay shows three absorption maxima at about 228 nm, 271 nm and 333 nm.

B. Shake a quantity of the powdered tablets containing 80 mg of Frusemide with 10 ml of *ethanol (95 per cent)*, filter and evaporate the filtrate to dryness. Dissolve 25 mg of the residue obtained in 2.5 ml of *ethanol (95 per cent)* and add 2 ml of *4-dimethylaminobenzaldehyde solution*; a green colour is produced which changes to deep red.

## Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *phosphate buffer pH 5.8*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 277 nm (2.4.7). Calculate the content of $C_{12}H_{11}ClN_2O_5S$, in the medium from the absorbance obtained by using a solution of known concentration of *furosemide RS*.

D. Not less than 70 per cent of the stated amount of $C_{12}H_{11}ClN_2O_5S$.

**Related substances.** Determine by liquid chromatography (2.4.14).

*Note—Prepare the solutions immediately before use.*

*Test solution*. Disperse a quantity of powdered tablets containing about 20 mg of Frusemide in 50.0 ml of the mobile phase.

*Reference solution.* Dilute 1.0 ml of the test solution to 100 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm) (such as ChromSpher C8),
- mobile phase: a mixture of 70 volumes of solution prepared by dissolving 0.2 g of *potassium dihydrogen phosphate* and 0.25 g of *cetrimide* in 70 ml of *water* adjusted to pH 7.0 with *ammonia* and 30 volumes of *propanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 238 nm,
- injection volume. 100 µl.

Inject the test solution and the reference solution. Run the chromatogram three times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the principal peak obtained with the reference solution (1 per cent), and sum of areas of all the secondary peaks is not more than 1.5 times the area of the peak in the chromatogram obtained with the reference solution (1.5 per cent).

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.1 g of Frusemide and shake with 150 ml of *0.1 M sodium hydroxide* for



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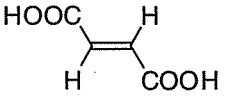
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10 minutes. Add sufficient *0.1 M sodium hydroxide* to produce 250.0 ml and filter. Dilute 5.0 ml to 200.0 ml with *0.1 M sodium hydroxide* and measure the absorbance of the resulting solution at the maximum at about 271 nm (2.4.7). Calculate the content of $C_{12}H_{11}ClN_2O_5S$ taking 580 as the specific absorbance at 271 nm.

**Storage**. Store protected from light.

# Fumaric Acid




$C_4H_4O_4$ Mol. Wt. 116.1

Fumaric acid is (2*E*)-butanedioic acid.

Fumaric Acid contains not less than 99.5 per cent and not more than 100.5 per cent of $C_4H_4O_4$, calculated on the anhydrous basis.

**Category**. Pharmaceutical aid.

## Identification

Dissolve about 10 mg of substance under examination in 25 ml of *water*, and add 1.0 ml of a solution prepared by mixing 20 ml of 20 per cent w/v solution of *copper sulphate* and 8.0 ml of *pyridine*, a blue precipitate is formed in the solution within 1 minute.

## Tests

**Heavy metals** (2.3.13). Weigh about 2.0 g of the substance under examination in a crucible and sufficient *sulphuric acid* to wet the substance, carefully ignite at a low temperature untill throughly charred. Add to the carbonized mass 2 ml of *nitric acid* and 5 drops of *sulphuric acid*, heat cautiously until white fumes no longer are evolved. Ignite, preferably in a muffle furnace, at 500° to 600°, until the carbon is completely burned off. Cool, add 4 ml of *dilute hydrochloric acid*, cover, digest on a stream bath for 15 minutes, and slowely evaporate on a stream bath to dryness. Moisten the residue with 1 drop of *hydrochloric acid*, add 10 ml of hot *water*, and digest for 2 minutes. Add *dilute ammonium hydroxide* dropwise until the solution is just alkaline to litmus paper, dilute with *water* to 25 ml, and adjust at pH 3.0 with *dilute acitic acid*, filter. Rinse the crucible and the filter with 10 ml of *water*, combine the filtrate and rinsing in a 50 ml Nessler cylinder, dilute with water to 40 ml, and mix.

To each of the cylinder containing the standard solution and the test solution, add 2 ml of *acetate buffer pH 3.5*, then add 1.2 ml of *thioacetamide reagent*, dilute with water to 50 ml, mix allow to stand for 2 minutes, and view downward over a white surface: the colour of the solution obtained from the test solution is not more intense than that of the solution obtained from the standard solution, produced by treating 2 ml of *lead standard solution* (1 ppm) in a similar manner.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 0.5 per cent, determined on 1 g.

**Maleic acid**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 100 mg of the substance under examination in 100.0 ml of the mobile phase.

*Reference solution (a)*. A 0.1 per cent w/v solution of *malic acid RS* in the mobile phase.

*Reference solution (b)*. A 0.001 per cent w/v solution of *fumaric acid RS* and 0.0005 per cent w/v solution of *maleic acid RS* in the mobile phase.

Chromatographic system
- a stainless steel column 20 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase. *0.005 M sulphuric acid,*
- flow rate. 0.3 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 5 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks corresponding to maleic acid and fumaric acid is not less than 2.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (a) and the test solution. The relative retention time with reference to fumaric acid for maleic acid is about 0.5.

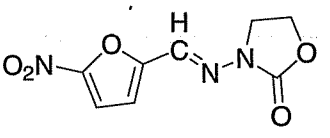
Calculate the content of maleic acid, $C_4H_4O_4$.

**Assay**. Weigh accurately about 1 g of Fumaric Acid and dissolve in 50 ml of *methanol*, warm gently on a steam bath to effect solution, cool. Titrate with *0.5 M sodium hydroxide*, using *phenolphthalein solution* as indicator . Carry out a blank titration.

1 ml of *0.5 M sodium hydroxide* is equivalent to 0.02902 g of $C_4H_4O_4$.

**Storage**. Store protected from moisture.

# Furazolidone




$C_8H_7N_3O_5$ Mol. Wt. 225.2

Furazolidone is 3-(5-nitrofurfurylideneamino)oxazolidin-2-one.



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Furazolidone contains not less than 97.0 per cent and not more than 103.0 per cent of $C_8H_7N_3O_5$, calculated on the dried basis.

**Category**. Antibacterial; antiprotozoal; antifungal.

**Dose**. 400 mg daily, in divided doses.

**Description**. A yellow, crystalline powder; odourless or almost odourless.

### Identification

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

B. Dissolve 1 mg in 1 ml of *dimethylformamide* and add 0.05 ml of *1 M ethanolic potassium hydroxide*; a deep blue colour is produced.

### Tests

**pH** (2.4.24). 4.5 to 7.0, determined in a solution prepared by shaking 1.0 g for 15 minutes with 100 ml of *carbon dioxide-free water* and filtering.

**Nitrofurfural diacetate**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 95 volumes of *toluene* and 5 volumes of *dioxan*.

*Test solution*. Dissolve 50 mg of the substance under examination in 5 ml of *dimethylformamide* by heating on a water-bath for a few minutes, allow to cool and dilute to 10 ml with *acetone*.

*Reference solution*. A solution containing 0.01 per cent w/v of *nitrofurfural diacetate RS* in a mixture of equal volumes of *dimethylformamide* and *acetone*.

Apply to the plate 20 µl of the test solution and 10 µl of the reference solution. After development, dry the plate in air and heat it at 105° for 5 minutes. Spray with a solution prepared by dissolving 0.75 g of *phenylhydrazine hydrochloride* in 10 ml of *ethanol (95 per cent)*, diluting to 50 ml with *water*, adding *activated charcoal*, filtering and then adding 25 ml of *hydrochloric acid* and sufficient *water* to produce 200 ml. Any spot corresponding to nitrofurfural diacetate in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. *Carry out the following procedure protected from light.*

Weigh accurately about 80 mg, add 150 ml of *dimethylformamide*, swirl to dissolve and add sufficient *water* to produce 500.0 ml. Dilute 5.0 ml to 100.0 ml with *water* and mix. Measure the absorbance of the resulting solution at the maximum at about 367 nm (2.4.7). Calculate the content of $C_8H_7N_3O_5$ taking 750 as the specific absorbance at 367 nm.

**Storage**. Store protected from light.

## Furazolidone Oral Suspension

Furazolidone Oral Suspension is a suspension of Furazolidone in a suitable aqueous flavoured vehicle.

Furazolidone Oral Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of furazolidone, $C_8H_7N_3O_5$.

**Usual strength**. 25 mg per 5 ml.

### Identification

Add a quantity of the suspension containing 50 mg of Furazolidone to 10 ml of a freshly prepared mixture of 9 volumes of *dimethylformamide* and 1 volume of *1 M ethanolic potassium hydroxide*. The solution turns purple, immediately changes to deep blue and on standing for about 10 minutes, again turns purple.

### Tests

**pH** (2.4.24). 6.0 to 8.5.

**Other tests**. Complies with the tests stated under Oral Liquids.

**Assay**. *Carry out the following procedure protected from light.*

To an accurately measured volume of the suspension containing about 50 mg of Furazolidone add 5 ml of *water* and mix. Transfer this mixture to a 250-ml volumetric flask with the aid of *dimethylformamide*. Add about 150 ml of *dimethylformamide*, shake by mechanical means for 10 minutes, dilute to volume with *dimethylformamide* and mix. Dilute 5.0 ml of this solution to 100.0 ml with *water* and mix well. Measure the absorbance of the resulting solution at the maximum at about 367 nm (2.4.7), using *dimethylformamide solution (5 per cent v/v)* as the blank. Calculate the content of $C_8H_7N_3O_5$ taking 750 as the specific absorbance at 367 nm.

**Storage**. Store protected from light at a temperature not exceeding 30°.

## Furazolidone Tablets

Furazolidone Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of furazolidone, $C_8H_7N_3O_5$.



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**Usual strengths.** 100 mg; 200 mg.

### Identification

To a quantity of the powdered tablets containing 50 mg of Furazolidone add 10 ml of a freshly prepared mixture of 9 volumes of *dimethylformamide* and 1 volume of *1 M ethanolic potassium hydroxide*. The solution turns purple, immediately changes to deep blue and on standing for 10 minutes, again turns purple.

### Tests

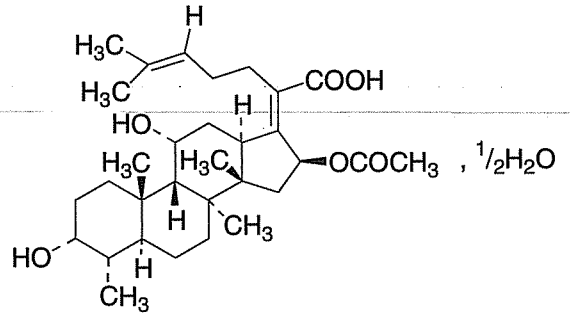
**Other tests.** Comply with the tests stated under Tablets.

**Assay.** *Carry out the following procedure protected from light.*

Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 80 mg of Furazolidone into a 200-ml volumetric flask, add 100 ml of *dimethylformamide*, warm to about 50° and shake well. Cool, dilute to volume with *dimethylformamide*, mix and centrifuge a small quantity of the mixture. Dilute 5.0 ml of the clear, supernatant liquid to 250.0 ml with *water* and mix. Measure the absorbance of the resulting solution at the maximum at about 367 nm (2.4.7), using *dimethylformamide* diluted 50 times with *water* as the blank. Calculate the content of $C_8H_7N_3O_5$ taking 750 as the specific absorbance at 367 nm.

**Storage.** Store protected from light at a temperature not exceeding 30°.

## Fusidic Acid



$C_{31}H_{48}O_6,½H_2O$ Mol. Wt. 525.7

Fusidic Acid is *ent*-16α-acetoxy-3β,11β-dihydroxy-4β,8β,14β-trimethyl-18-nor-5β,10α-cholesta(17Z)-17(20),24-dien-21-oic acid hemihydrate, an antimicrobial substance produced by the growth of certain strains of *Fusidium coccineum* or by any other means.

Fusidic Acid contains not less than 97.5 per cent and not more than 101.0 per cent of $C_{31}H_{48}O_6$, calculated on the anhydrous basis.

**Category.** Antibacterial.

**Dose.** 500 mg to 1 g every 8 hours.

**Description.** A white, crystalline powder.

### Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fusidic acid RS* or with the reference spectrum of fusidic acid.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase.* A mixture of 80 volumes of *chloroform*, 10 volumes of *glacial acetic acid*, 10 volumes of *cyclohexane* and 2.5 volumes of *methanol*.

*Test solution.* Dissolve 0.2 g of the substance under examination in 100 ml of *ethanol (95 per cent)*.

*Reference solution.* A 0.24 per cent w/v solution of *diethanolamine fusidate RS* in *ethanol (95 per cent)*.

Apply to the plate 5 µl of each solution. After development, dry the plate at 105° for 10 minutes and examine in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

### Tests

**Related substances.** Determine by liquid chromatography (2.4.17).

*Test solution.* Dissolve 0.5 g of the substance under examination in 100 ml of the mobile phase.

*Reference solution (a).* Dissolve 5 mg of *3-ketofusidic acid RS* in 5 ml of the mobile phase. To 1 ml of this solution add 0.2 ml of the test solution and dilute to 20 ml with the mobile phase.

*Reference solution (b).* Dilute 20 µl of the test solution to 100 ml with the mobile phase.

Chromatographic system

- a stainless steel column 12.5 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of *acetonitrile*, 20 volumes of *water*, 20 volumes of a 1 per cent w/v solution of *phosphoric acid* and 10 volumes of *methanol*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume. 20 µl.



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Continue the chromatography for at least 3.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution the sum of the areas of any secondary peaks is not greater than 4 times the area of the peak corresponding to fusidic acid in the chromatogram obtained with reference solution (a). Ignore any peak with an area less than that of the principal peak in the chromatogram obtained with reference solution (b). The test is not valid unless the resolution factor between the peaks corresponding to 3-ketofusidic acid and fusidic acid in the chromatogram obtained with reference solution (a) is not less than 2.5 and unless the principal peak in the chromatogram obtained with reference solution (b) has a signal-to-noise ratio of not less than 3.

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Water** (2.3.43). 1.4 to 2.0 per cent, determined on 0.5 g.

**Assay**. Weigh accurately about 0.5 g and dissolve in 10 ml of *ethanol (95 per cent)*. Titrate with *0.1 M sodium hydroxide* using *phenolphthalein solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.05167 g of $C_{31}H_{48}O_6$.

**Storage**. Store protected from light.



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# G


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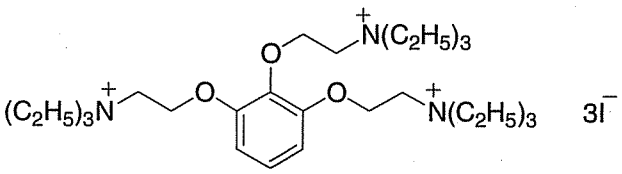
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# Gallamine Triethiodide



$C_{30}H_{60}I_3N_3O_3$ Mol. Wt. 891.5

Gallamine Triethiodide is 2,2',2"-(benzene-1,2,3-triyltrioxy)tris(tetraethylammonium) triiodide.

Gallamine Triethiodide contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{30}H_{60}I_3N_3O_3$, calculated on the dried basis.

**Category**. Skeletal muscle relaxant.

**Dose**. By intravenous injection, 80 to 120 mg followed by 20 to 40 mg as required.

**Description**. A white or almost white powder; odourless or almost odourless; hygroscopic.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *gallamine triethiodide RS*.

B. When examined in the range 220 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *0.01 M hydrochloric acid* shows an absorption maximum at about 225 nm; absorbance at about 225 nm, 0.50 to 0.55.

C. To 5 ml of a 1 per cent w/v solution add 1 ml of *potassium mercuri-iodide solution*; a yellow precipitate is produced.

D. Acidify 2 ml of a 0.5 per cent w/v solution with 0.2 ml of *2 M nitric acid*; the resulting solution gives reaction A of iodides (2.3.1).

## Tests

**Appearance of solution**. A 2.0 per cent w/v solution is clear (2.4.1), and, when examined immediately after preparation, not more intensely coloured than reference solution YS7 (2.4.1).

**Acidity or alkalinity**. To 50 ml of *water* add 0.2 ml of *methyl red solution* and adjust the pH to 6 by adding either *0.01 M sulphuric acid* or *0.02 M sodium hydroxide* until the colour is orange-yellow. Add 1 g of the substance under examination and shake to dissolve. Not more than 0.2 ml of *0.01 M sulphuric acid* or *0.02 M sodium hydroxide* is required to restore the orange-yellow colour.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve 30 mg of the substance under examination in 50 ml of the mobile phase.

*Reference solution.* Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: dissolve 1.4 g of *sodium perchlorate* in 85 ml of *phosphate buffer pH 3.0* and 15 ml of *methanol,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 205 nm,
- injection volume. 20 µl.

The relative retention time with reference to gallamine for 2,2',2"-[benzene-1,2,3-triyltris(oxy)]tris(*N,N*-diethylamine) (gallamine impurity A) is about 0.45, for 2,2'-[2-[2-(triethylammonio)ethyl]-1,3-phenylenebis(oxy)]bis(*N,N,N*-triethylethanaminium)triiodide (gallamine impurity B) is about 0.5, for 2,2'-[2-[2-(diethylmethylammonio)ethoxy]-1,3-phenylenebis(oxy)]bis(*N,N,N*-triethylethanaminium)triiodide (gallamine impurity C) is about 0.65, for 2,2'-[3-[2-(diethylmethylammonio)ethoxy]-1,2-phenylenebis(oxy)bis(*N,N,N*-triethylethanaminium)triiodide (gallamine impurity D) is about 0.75, for 2,2'-[3-[2-(diethylamino)ethoxy-1,2-phenylenebis(oxy)bis(*N,N,N*-triethylethanaminium)diiodide (gallamine impurity E) is about 0.85, for 2,2',2"-[4-[2-(triethylammonio)ethyl]benzene-1,2,3triyltris(oxy)tris(*N,N,N*-triethylethanaminium)tetraidide (gallamine impurity F) is about 0.9.

Inject the test solution and the reference solution. Run the chromatogram 1.5 times the peak due to triethylgallamine as perchlorate. In the chromatogram obtained with the test solution the area of secondary peak corresponding to gallamine impurity A, B, C, D, E and F is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent). The sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (2.0 per cent). Ignore the peak due to iodide (non-retained peak).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 1.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.27 g and dissolve in a mixture of 40 ml of *acetone* and 15 ml of *mercuric acetate solution.* Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02972 g of $C_{30}H_{60}I_3N_3O_3$.

**Storage**. Store protected from light.



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# Gallamine Injection

Gallamine Triethiodide Injection

Gallamine Injection is a sterile solution of Gallamine Triethiodide in Water for Injections.

Gallamine Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of gallamine triethiodide, $C_{30}H_{60}I_3N_3O_3$.

**Usual strength**. 40 mg per ml.

**Description**. A clear, colourless or almost colourless solution.

## Identification

A. When examined in the range 220 nm to 360 nm (2.4.7), the final solution obtained in the Assay shows an absorption maximum only at about 225 nm.

B. To 1 ml add 1 ml of iodinated *potassium iodide solution*; a brown precipitate is produced.

C. To 1 ml add 1 ml of *potassium mercuri-iodide solution*; a yellow precipitate is produced.

## Tests

**pH** (2.4.24). 5.5 to 7.5.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution.* Dilute a volume of injection containing 60 mg of Gallamine Triethiodide in 100 ml of the mobile phase.

*Reference solution.* Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with silica whose surface is chemically modified with a mixture of chemically- bonded octylsilane and octadecylsilane groups (5µm) (Hichrom RPB is suitable),
- column temperature. 40°,
- mobile phase: dissolve 14 g of *sodium perchlorate* in 850 ml of a solution prepared by diluting 0.7 ml of *orthophosphoric acid* to 900 ml with *water*, adjust the pH to 3.0 with *10 M sodium hydroxide* and dilute to 1000 ml with *water*, and add 130 ml of *methanol*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 205 nm,
- injection volume. 20 µl.

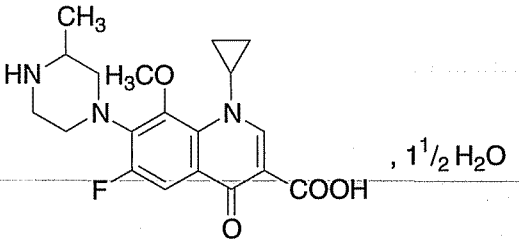
Inject the test solution and the reference solution. Run the chromatogram 1.5 times the retention time of the principal peak obtained with the test solution. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the principle peak in the chromatogram obtained with the reference solution (1.0 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (2.0 per cent). Ignore the peak due to iodide (non-retained peak).

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. Dilute an accurately measured volume containing about 40 mg of Gallamine Triethiodide with sufficient *0.01 M hydrochloric acid* to produce 200.0 ml. Dilute 5.0 ml of this solution to 100.0 ml with *0.01 M hydrochloric acid* and measure the absorbance of the resulting solution at the maximum at about 225 nm (2.4.7). Calculate the content of $C_{30}H_{60}I_3N_3O_3$ taking 525 as the specific absorbance at 225 nm.

**Storage**. Store protected from light.

# Gatifloxacin




$C_{19}H_{22}FN_3O_4,1\frac{1}{2}H_2O$ Mol.Wt. 402.4

Gatifloxacin is (*RS*)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-3-quinolinecarboxylic acid sesquihydrate

Gatifloxacin contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{19}H_{22}FN_3O_4$, calculated on the anhydrous basis.

**Category**. Antibacterial.

**Description**. A white to light yellow crystalline powder.

## Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *gatifloxacin RS*.

## Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 30 mg of the substance under examination in 100 ml of the mobile phase.



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*Reference solution (a)*. A 0.03 per cent w/v solution of *gatifloxacin RS* in the mobile phase.

*Reference solution (b)*. Dilute 1 ml of reference solution (a) to 100 ml with mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm),
- column temperature. 50°,
- mobile phase: a mixture of 85 volumes of buffer solution prepared by dissolving 4.1 g of *sodium acetate anhydrous* in 1000 ml with *water*, add 4 ml *triehylamine*. Adjust the pH to 4.0 with *acetic acid* and 15 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 295 nm,
- injection volume. 20 µl.

Inject reference solution (a). The test is not valid unless the column effciency is not less than 4000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent).

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

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Water** (2.3.43). 4.0 to 8.0 per cent, determined on 0.1 g.

**Assay**. Weigh accurately about 0.3 g, dissolve in 20 ml of *N,N'-dimethylformamide*, add 50 ml of *methanol*. Titrate with *0.1 M hydrochloric acid*. Determine the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml *0.1 M hydrochloric acid* is equivalent of 0.03754 g of $C_{19}H_{22}FN_3O_4$.

**Storage**. Store protected from light.

# Gatifloxacin Infusion

Gatifloxacin Infusion contains Gatifloxacin.

Gatifloxacin Infusion contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of gatifloxacin $C_{19}H_{22}FN_3O_4$.

**Usual strength**. 0.2 per cent w/v.

## Identification

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

## Tests

**pH** (2.4.24). 3.5 to 5.5.

**Related substances**. Determine by liquid chromatography (2.4.14).

*NOTE — Protect the solutions from light.*

*Test solution*. Dilute the Infusion to obtain 0.1 per cent w/v solution in mobile phase.

*Reference solution (a)*. A 0.1 per cent w/v solution of *gatifloxacin RS* in the mobile phase.

*Reference solution (b)*. Dilute 1 ml of reference solution (a) to 100 ml with mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase : a mixture of 80 volumes of 1.6 per cent v/v solution of *orthophosphoric acid*, adjusted to pH 3.0 with *triethylamine* and 20 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume. 20 µl.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 2500 theoretical plates and the tailing factor is not more than 3.0.

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

**Other tests**. Comply with the tests stated under Parenteral Preparations (Infusion).

**Assay**. Determine by liquid chromatography (2.4.14).

*NOTE — Protect the solutions from light.*

*Test solution*. Dilute the Infusion to obtain 0.1 per cent w/v solution in mobile phase.

*Reference solution*. A 0.1 per cent w/v solution of *gatifloxacin RS* in the mobile phase.

Use the chromatographic system described in the test for Related substances.



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Inject the reference solution. The test is not valid unless the column efficiency determined from the principal peak is not less than 2500 theoretical plates, the tailing factor is not more than 3.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{19}H_{22}FN_3O_4$ in the infusion.

**Storage**. Store protected from light and moisture.

## Gatifloxacin Tablets

Gatifloxacin Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of gatifloxacin, $C_{19}H_{22}FN_3O_4$.

**Usual strengths**. 200 mg; 400 mg.

### Identification

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

### Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *acetate buffer pH 4.0*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

*Test solution*. Dilute the filtrate, if necessary, with the dissolution medium.

*Reference solution*. A 0.01 per cent w/v solution of *gatifloxacin RS* in dissolution medium.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 80 volumes of 0.16 v/v solution of *phosphoric acid* adjusted to pH 3.0 with *triethylamine* and 20 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume. 50 µl.

D. Not less than 70 per cent of the stated amount of $C_{19}H_{22}FN_3O_4$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*NOTE — Protect the solutions from light.*

*Test solution*. Disperse a quantity of powdered tablets containing 100 mg of Gatifloxacin in 100.0 ml of the mobile phase.

*Reference solution (a)*. A 0.1 per cent w/v solution of *gatifloxacin RS* in the mobile phase.

*Reference solution (b)*. Dilute 1 ml of reference solution (a) to 100 ml with mobile phase.

Chromatographic system as described under Assay.

Inject reference solution (b). The test is not valid unless the column effciency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than 3 times the area of the peak in the chromatogram obtained with the reference solution (b) (3.0 per cent).

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 100 mg of Gatifloxacin, dissolve in 50 ml of *0.1 per cent phosphoric acid solution* and dilute to 100 ml with same solvent and filter. Dilute 5 ml of the solution to 100.0 ml with mobile phase.

*Reference solution*. A 0.005 per cent of *gatifloxacin RS* in mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 80 volumes of 0.16 per cent v/v solution of *phosphoric acid* adjusted to pH 3.0 with *triethylamine* and 20 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume. 50 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 3.0. The column efficiency is not less than 2500 theoretical plates. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{19}H_{22}FN_3O_4$ in the tablets.

**Storage**. Store protected from light.

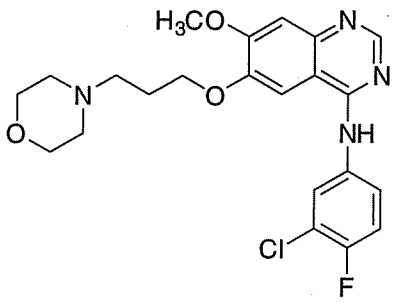


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## Gefitinib



$C_{22}H_{24}O_3N_4FCl$ Mol. Wt. 446.9

Gefitinib is *N*-(3-chloro-4-fluorophenyl)-7-methoxy-6-[3-(4-morpholinyl)propoxy]-4-quinazolinoamine.

Gefitinib contains not less than 98.0 per cent and not more than 102.0 per cent $C_{22}H_{24}O_3N_4FCl$, calculated on the dried basis.

**Category**. Anticancer.

**Description**. A white or off white crystalline powder.

*CAUTION — Gefitinib is cytotoxic; extra care required to prevent inhaling particles and exposing the skin to it.*

### Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *gefitinib RS* or with the reference spectrum of gefitinib.

### Tests

**Melting range** (2.4.21). 193° to 197°.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 100 mg of the substance under examination in 100 ml of *methanol*.

*Reference solution (a)*. Dilute 1.0 ml of the test solution to 100.0 ml with *methanol*.

*Reference solution (b)*. A 0.1 per cent w/v solution of *gefitinib RS* in the *methanol*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm),
- mobile phase: a mixture of 40 volumes of 1 per cent w/v solution of *ammonium acetate* and 60 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (a). The test is not valid unless the theoretical plates are not less than 2000 and the tailing factor is not more than 2.0.

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

**Heavy metals** (2.3.13). 1 g complies with limit test for heavy metals, Method B (10 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Determine by liquid chromatography (2.4.14) as described under Related substances.

Inject reference solution (b). The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (b) and the test solution.

Calculate the content of $C_{22}H_{24}O_3N_4FCl$.

**Storage**. Store protected from light and moisture, at a temperature not exceeding 25°.

## Gefitinib Tablets

Gefitinib Tablets contains not less than 90.0 per cent and not more than 110.0 per cent of stated amount of gefitinib, $C_{22}H_{24}O_3N_4FCl$.

**Usual strength**. 250 mg.

### Identification

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

### Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *acetate buffer pH 4.0,*
Speed and time. 75 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter. Dilute a suitable volume of the filtrate with the same solvent, if necessary and measure the absorbance (2.4.7) of the resulting



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solution at the maximum at about 254 nm. Calculate the content of $C_{22}H_{24}O_3N_4FCl$ in the medium from the absorbance obtained from a solution of known concentration of *gefitinib RS* in the same medium.

D. Not less than 75 per cent of the stated amount of $C_{22}H_{24}O_3N_4FCl$.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 100 mg of Gefitinib, disperse in 100 ml of the *methanol*, filter. Dilute 5.0 ml of the filtrate to 50.0 ml with the mobile phase.

*Reference solution.* A 0.1 per cent w/v solution of *gefitinib RS* in *methanol.* Dilute 5 ml of this solution to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 40 volumes of 1.0 per cent w/v solution of *ammonium acetate* and 60 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{24}O_3N_4FCl$ in the tablets.

**Storage**. Store protected from light and moisture.

# Gelatin

Gelatin is a purified protein obtained by partial hydrolysis of animal collagen. Gelatin used in the manufacture of capsule shells or as a pharmaceutical aid in the manufacture of tablets may contain suitable antimicrobial agents.

**Category**. Pharmaceutical aid (encapsulating agent; suspending agent; tablet binding and coating agent).

**Description**. Light amber to faintly yellow, translucent flakes, sheets, shreds, powder or granules; odour, slight. Stable in air but is subject to microbial decomposition when moist or in solution.

## Identification

A. Dissolve 1 g in sufficient *carbon dioxide-free water* at about 55° to produce 100 ml and maintain the solution at this temperature until required for use (solution A). To 2 ml of solution A add 0.05 ml of *copper sulphate solution*, mix and add 0.5 ml of *2 M sodium hydroxide*; a violet colour is produced.

B. Add 10 ml of *water* to 0.5 g in a test-tube, allow to stand for 10 minutes, heat at 60° for 15 minutes, allow to stand upright at 0° for 6 hours and invert the test-tube; the contents do not immediately flow out.

## Tests

**Appearance of solution**. Solution A is not more opalescent than opalescence standard OS4 (2.4.1), and not more intensely coloured than reference solution YS4 (2.4.1).

**pH** (2.4.24). 3.8 to 7.6, determined in solution A.

**Arsenic** (2.3.10). To 5.0 g add 10 ml of *water* and allow to stand for 1 hour. Warm to dissolve and add 10 ml of *hydrochloric acid* and a slight excess of *bromine solution*. Add 2 ml of *stannated hydrochloric acid*, heat under a reflux condenser for 1 hour, cool and add 10 ml of *water* and 10 ml of *hydrochloric acid*. The resulting solution complies with the limit test for arsenic (2 ppm).

**Heavy metals** (2.3.13). To the residue obtained in the test for Ash add 2 ml of *hydrochloric acid* and 0.5 ml of *nitric acid*, and evaporate to dryness. To the residue add 1 ml of *1 M hydrochloric acid* and 15 ml of *water* and warm for a few minutes. Filter, wash with *water* and dilute the filtrate to 100 ml with *water*. Dilute 8 ml of this solution to 25 ml with *water*. The resulting solution complies with the limit test for heavy metals, Method A (50 ppm).

**Sulphur dioxide**. Not more than 200 ppm, determined by the following method. Add 150 ml of *water* to a 500-ml three-necked, round-bottomed flask fitted with a water-cooled reflux condenser 200 mm long the upper end of which is connected to an absorption tube. The flask is fitted with a 100-ml dropping funnel and a gas inlet tube that reaches nearly to the bottom of the flask. Pass a stream of carbon dioxide through the flask at a rate of 100 ml per minute for 15 minutes. Connect an absorption tube containing 10 ml of *hydrogen peroxide solution (10 vol)* previously neutralised to a 0.1 per cent w/v solution of *bromophenol blue* in *ethanol (20 per cent)* and without interrupting the flow of carbon dioxide, introduce through the funnel 25 g of the substance under examination and 80 ml of *2 M hydrochloric acid*. Boil for 1 hour, disconnect the absorption tube and stop the flow of carbon dioxide. Wash the contents of the absorption tube into a 250-ml conical flask, heat on a water-bath for 15 minutes and allow to cool. Titrate with *0.1 M sodium hydroxide* using a 0.1 per cent w/v solution of *bromophenol blue* in *ethanol (20 per cent)* as indicator, until the colour changes from yellow to violet-blue.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.003203 g of sulphur dioxide.



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**Microbial contamination** (2.2.9). Total microbial count, not more than 1000 per g; 1 g is free from *Escherichia coli;* 10 g is free from *Salmonellae.*

**Ash**. Not more than 3.25 per cent, determined by the following method. Weigh accurately 5.0 g, add about 2 g of liquid paraffin (to avoid loss due to swelling) and incinerate at a temperature not exceeding 500° until free from carbon. Cool and weigh.

**Loss on drying** (2.4.19). Not more than 16.0 per cent, determined by the following method. Weigh accurately about 1.0 g in a stainless steel dish weighing about 25 g and with a diameter of 70 mm and a height of 15 mm, fitted with a cover. Add 10 ml of *water* and allow to soak. Heat on a water-bath to form a homogeneous solution and continue heating until most of the water has evaporated. Dry for 2 hours at 105° and for further periods of 30 minutes until two successive weighings do not differ by more than 1 mg (Do not powder sheet gelatin while preparing for this test).

*Gelatin intended for use in the preparation of pessaries and suppositories complies with the following additional requirement.*

**Jelly strength** (2.4.18). Between 150 and 250 g. (The exact jelly strength may be negotiated between the manufacturer and user on the basis of end use of the gelatin).

**Storage**. Store protected from moisture.

**Labelling**. The label states, where applicable, that the material is suitable for the preparation of pessaries and suppositories and, if so, the jelly strength.

# Hard Gelatin Capsule Shells

Hard Gelatin Capsule Shells are soluble containers for incorporation of drugs, usually in the form of powders, pellets or granules, and are commonly intended for oral administration. The shells are acted upon by digestive fluids and the filled contents are released. They are composed of gelatin, water and additives such as plasticizers, humectants, surfactants, dispersing agents, flavouring agents, antimicrobial agents, sweetening agents, opacifying agents and one or more colouring agents permitted under the Drugs and Cosmetics Rules, 1945. Ingredients other than colouring agents and opacifying agents comply with the standards of this Pharmacopoeia.

**Category**. Pharmaceutical aid.

**Description**. Hard Gelatin Capsule Shells (shells or cases) consist of two cylindrical, telescoping pieces (cap and body), one end of which is rounded and closed and the other, open. Shapes other than cylindrical can also be formed as per requirements. The two pieces are uncoloured or coloured; if coloured, of identical or different colours; transparent or opaque, partially or completely and printed or unprinted or bear other surface markings. The cap overlaps the body and maintains a tight friction closure. The closure may be strengthened by suitable means.

The shells are of various sizes, usually designated by different numbers, 5 being the smallest and 000 the biggest. Shells of sizes 0 to 4 are commonly used. Shells of special shapes, sizes, lengths and designations are also available. The shells are smooth and uniform in size, shape and colour. Guidelines on dimensions with respect to different sizes of commonly used capsules are given in chapter 5.8.

## Identification

Boil one capsule shell with 20 ml of *water*, allow to cool and centrifuge. To 5 ml of the supernatant liquid add 1 ml of *picric acid solution* and to another 5 ml add 1 ml of *tannic acid solution*; a precipitate is produced in each case.

## Tests

**Odour**. Keep 100 capsule shells in a well-closed bottle for 24 hours at a temperature between 30° and 40°; the shells do not develop any foreign odour.

*NOTE - In order to ensure that the quality of the shells is not affected by temperature and humidity, the capsule shells should be conditioned at a temperature of 25° ± 2° and a relative humidity of 50 ± 5 per cent for not less than 12 hours before conducting the test for* Average weight.

**Average weight**. Weigh 100 capsule shells and determine the average weight of a capsule. The average weight is within ± 10 per cent of the target weight shown in Table 1. (As sizes 0 to 4 are commonly used, detailed requirements are included for these sizes only. Requirements for other sizes may be decided upon mutually between the manufacturer of the Hard Gelatin Capsule Shells and the user).

Table 1 - Target weight of capsules

| Size | Target weight (mg) |
|---|---|
| 0 | 96 |
| 1 | 76 |
| 2 | 63 |
| 3 | 50 |
| 4 | 40 |

**Disintegration** (2.5.1). 15 minutes, using discs.

**Microbial contamination** (2.2.9). Total microbial count, not more than 1000 per g. 1 g is free from *Escherichia coli* and *Salmonellae*.

**Loss on drying** (2.4.19). 12.5 to 16.0 per cent, determined on 1.0 g by drying in an oven at 105° for 4 hours or to constant weight.



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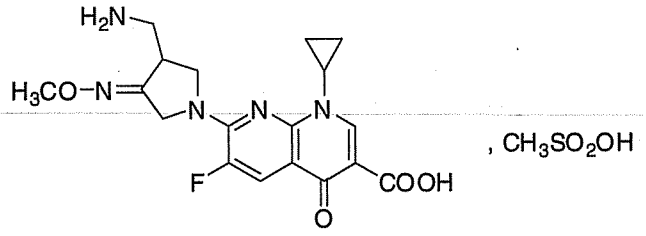
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**Storage**. Store protected from moisture at a temperature not exceeding 30°.

**Labelling**. The label states (1) the size of the capsule shells; (2) that only permitted colours, if any, have been used; (3) the storage conditions.

## Gemifloxacin Mesylate




$C_{19}H_{24}FN_5SO_7$ Mol. Wt. 485.5

Gemifloxacin Mesylate is 7-[3-(aminomethyl)-4-(methoxyimino)-1-pyrrolidinyl]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid methanesulphonate.

Gemifloxacin Mesylate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{19}H_{24}FN_5SO_7$, calculated on the anhydrous basis.

**Category**. Antibacterial.

**Description**. An off white to light brown coloured powder.

### Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *gemifloxacin mesylate RS* or with the reference spectrum of gemifloxacin mesylate.

### Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture.* 80 volumes of *water* and 20 volumes of *acetonitrile.*

*Test solution.* Dissolve 50 mg of the substance under examination in 100.0 ml of solvent mixture.

*Reference solution.* Dilute 5.0 ml of the test solution to 100.0 ml with the solvent mixture. Further dilute 5.0 ml of this solution to 25.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Cosmosil C18),
- column temperature. 45°,
- mobile phase: A. a solution containing 2.0 g of *ammonium acetate* and 3.5 g of *sodium perchlorate* in 650 ml of *water*, adjusted to pH 2.2 with *orthophosphoric acid*,
  B. *acetonitrile*,
- a linear gradient programme using the conditions given below,
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 270 nm,
- injection volume. 20 µl.

| Time (in min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0 | 81 | 19 |
| 25 | 81 | 19 |
| 35 | 47 | 53 |
| 40 | 47 | 53 |
| 45 | 81 | 19 |
| 50 | 81 | 19 |

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent) and the sum of areas of all the secondary peaks is not more than 1.5 times the area of the principal peak in the chromatogram obtained with the reference solution (1.5 per cent).

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Water** (2.3.43). 4.0 per cent to 7.0 per cent, determined on 0.4 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture.* 80 volumes of *water* and 20 volumes of *acetonitrile.*

*Test solution (a).* Dissolve 50 mg of the substance under examination in 100.0 ml of the solvent mixture.

*Test solution (b).* Dilute 5.0 ml of test solution (a) to 100.0 ml with the solvent mixture.

*Reference solution (a).* A 0.05 per cent w/v solution of *gemifloxacin mesylate RS* in the solvent mixture.

*Reference solution (b).* Dilute 5.0 ml of reference solution (a) to 100.0 ml with the solvent mixture.



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Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Cosmosil C18),
- column temperature. 45°,
- mobile phase: a mixture of 70 volumes of buffer solution prepared by dissolving 2.0 g of *ammonium acetate* and 3.5 g of *sodium perchlorate* in 650 ml *water*, adjusted to pH 2.2 with *orthophosphoric acid,* and 30 volumes of *acetonitrile*,
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 270 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the theoretical plates is not less than 2000, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (b) and test solution (b).

Calculate the content of $C_{19}H_{24}FN_5SO_7$.

**Storage**. Store protected from light and moisture.

# Gemifloxacin Tablets

Gemifloxacin Mesylate Tablets

Gemifloxacin Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of gemifloxacin, $C_{18}H_{20}FN_5O_4$.

**Usual strength**. 320 mg.

## Identification

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

## Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.01 M hydrochloric acid,*
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

*Test solution*. Use the filtrate. Dilute the filtrate, if necessary with *water*.

*Reference solution*. A 0.047 per cent w/v solution of *gemifloxacin mesylate RS* in the dissolution medium. Dilute 5 ml of this solution to 50 ml with *water*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 mm) ( Such as Hypersil BDS C18 ),
- column temperature. 45°,
- mobile phase: a mixture of 75 volumes of a buffer solution prepared by dissolving 6.0 g of *ammonium acetate* and 10.5 g of *sodium perchlorate monohydrate* in 1950 ml of *water*, adjusted to pH 2.2 with *orthophosphoric acid* and 25 volumes of *acetonitrile,*
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 272 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the theoretical plates is not less than 2000, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injection is not more than 2.0 per cent.

Inject the reference solution the test solution.

Calculate the content of $C_{18}H_{20}FN_5O_4$ in the tablet.

D. Not less than 70 per cent of the stated amount of $C_{18}H_{20}FN_5O_4$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture.* A mixture of 80 volumes of *water* and 20 volumes of *acetonitrile.*

*Test solution.* Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 20 mg of the gemifloxacin with 100.0 ml of the solvent mixture, filter.

*Reference solution (a).* A 0.025 per cent w/v solution of *gemifloxacin mesylate RS* in the solvent mixture.

*Reference solution (b).* Dilute 1.0 ml of reference solution (a) to 100.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 mm) ( Such as phenomenex Luna C18),
- mobile phase: A. buffer solution prepared by dissolving 6.0 g of *ammonium acetate* and 10.5 g of *sodium perchlorate monohydrate* in 1950 ml of *water*, adjusted to pH to 2.2 with *orthophosphoric acid*,
  B. *acetonitrile,*
  C. *tetrahydrofuran,*
- a linear gradient programme using the conditions given below,
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 272 nm,
- injection volume. 10 µl.



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| Time (in min.) | Mobile Phase A (per cent v/v) | Mobile Phase B (per cent v/v) | Mobile Phase C (per cent v/v) |
|---|---|---|---|
| 0 | 92 | 5 | 3 |
| 30 | 42 | 55 | 3 |
| 32 | 92 | 5 | 3 |
| 40 | 92 | 5 | 3 |

Inject reference solution (b). The test is not valid unless the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent) and the sum of areas of all the secondary peaks is not more than three times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent).

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh and powder 20 tablets. Disperse a quantity of powder containing about 10 mg of gemifloxacin, with 100.0 ml of *water*, filter.

*Reference solution*. A 0.01 per cent w/v solution of *gemifloxacin mesylate RS* in *water*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 mm) ( Such as Hypersil BDS C18 ),
- column temperature. 45°,
- mobile phase: a mixture of 75 volumes of a buffer solution prepared by dissolving 6.0 g of *ammonium acetate* and 10.5 g of *sodium perchlorate monohydrate* in 1950 ml of *water*, adjusted to pH 2.2 with *orthophosphoric acid* and 25 volumes of a*cetonitrile,*
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 272 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the theoretical plates is not less than 2000, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

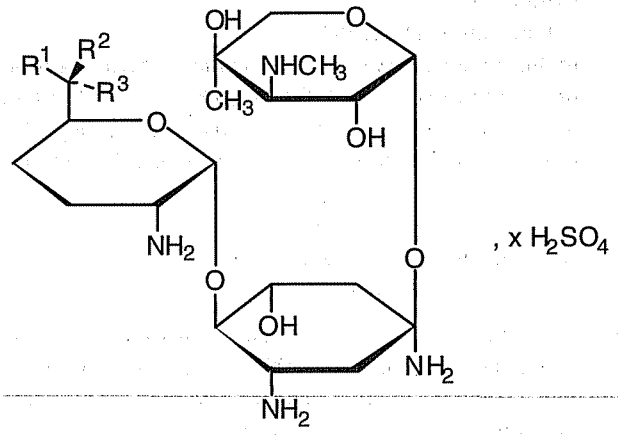
Inject the reference solution and the test solution.

Calculate the content of $C_{18}H_{20}FN_5O_4$ in the tablets.

**Storage**. Store protected from light and moisture.

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

# Gentamicin Sulphate




| Gentamycin | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|
| $C_1$ | $CH_3$ | $NHCH_3$ | H |
| $C_2$ | $CH_3$ | $NH_2$ | H |
| $C_{1a}$ | H | $NH_2$ | H |
| $C_{2a}$ | H | $NH_2$ | $CH_3$ |

Gentamicin Sulphate is a mixture of the sulphates of antimicrobial substances produced by *Micromonospora purpurea*.

Gentamicin Sulphate has a potency of not less than 590 µg of gentamicin per mg, calculated on the anhydrous basis.

**Category**. Antibacterial.

**Dose**. By intramuscular or slow intravenous injection, over at least 3 minutes or by intravenous infusion, 2 to 5 mg per kg body weight daily, in divided doses.

**Description**. A white or almost white powder; hygroscopic.

## Identification

*Tests A and B may be omitted if tests C and D are carried out. Test C may be omitted if tests A, B and D are carried out.*

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. The lower layer obtained by shaking together equal volumes of *strong ammonia solution*, *chloroform* and *methanol* and allowing to separate.

*Test solution*. Dissolve 0.5 g of the substance under examination in 100 ml of *water*.

*Reference solution*. A 0.5 per cent w/v solution of *gentamicin sulphate RS*.



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Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with *ethanolic ninhydrin solution* and heat at 110° for 5 minutes. The three principal spots in the chromatogram obtained with the test solution correspond to those in the chromatogram obtained with the reference solution.

B. Dissolve 10 mg in 1 ml of *water* and add 5 ml of a 40 per cent w/v solution of *sulphuric acid*. Heat on a water-bath for 10 minutes, cool and dilute to 25 ml with *water*. When examined in the range 240 nm to 330 nm (2.4.7), the resulting solution shows no absorption maximum.

C. In the test for Composition of gentamicin sulphate, the four principal peaks in the chromatogram obtained with the test solution correspond to the four peaks in the chromatogram obtained with the reference solution.

D. Gives reaction A of sulphates (2.3.1).

## Tests

**Appearance of solution**. A 4.0 per cent w/v solution in *carbon dioxide-free water* (solution A) is clear (2.4.1), and not more intensely coloured than degree 6 of the appropriate range of reference solutions (2.4.1).

**pH** (2.4.24). 3.5 to 5.5, determined in solution A.

**Specific optical rotation** (2.4.22). +107° to +121°, determined in a 10.0 per cent w/v solution.

**Composition of gentamicin sulphate**. Determine by liquid chromatography (2.4.14).

*Test solution*. Add 5 ml of *methanol* and 4 ml of *phthalaldehyde reagent* to 10 ml of a 0.1 per cent w/v solution of the substance under examination in *water*, mix, add sufficient *methanol* to produce 25 ml, heat on a water-bath at 60° for 15 minutes and cool. If the solution is not used immediately, cool to 0° and use within 4 hours.

*Reference solution*. Prepare in the same manner as the test solution but using 10 ml of a 0.1 per cent w/v solution of *gentamicin sulphate RS* in place of the solution of the substance under examination.

Chromatographic system
- a stainless steel column 10 to 12.5 cm x 4.6 to 5 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a solution containing 0.55 per cent w/v of *sodium heptanesulphonate monohydrate* in a mixture of 70 volumes of *methanol*, 25 volumes of *water* and 5 volumes of *glacial acetic acid*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 330 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the resolution between any two peaks is not less than 1.25, the capacity factor determined from the gentamicin $C_1$ peak is between 2 and 7, the column efficiency determined from the gentamicin $C_2$ peak is not less than 1200 theoretical plates, and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution. The elution order is gentamicin $C_1$, gentamicin $C_{1a}$, gentamicin $C_{2a}$, and gentamicin $C_2$. Calculate the content of gentamicin $C_1$, gentamicin $C_{1a}$, gentamicin $C_{2a}$, and gentamicin $C_2$ in the portion of gentamicin sulphate taken by the formula:

$$100 r_f / r_s$$

in which $r_f$ is the peak area response corresponding to the particular gentamicin; and $r_s$ is the sum of the area responses of all four peaks: the content of gentamicin $C_1$ is between 25 per cent and 50 per cent, the content of gentamicin $C_{1a}$ is between 10 per cent and 35 per cent, and the sum of the contents of gentamicin $C_{2a}$ and gentamicin $C_2$ is between 25 per cent and 55 per cent.

**Sulphate**. 32.0 to 35.0 per cent of $SO_4$, calculated on the anhydrous basis, determined by the following method. Dissolve 0.25 g in 100 ml of *distilled water*, adjust the pH to 11 with *strong ammonia solution* and add 10 ml of *0.1 M barium chloride*. Titrate with *0.1 M disodium edetate* using 0.5 mg of *metalphthalein* as indicator; when the colour of the solution begins to change add 50 ml of *ethanol (95 per cent)* and continue the titration until the violet-blue colour disappears. Perform a blank determination and make any necessary correction.

1 ml of *0.1 M barium chloride* is equivalent to 0.009606 g of sulphate, $SO_4$.

**Methanol**. Not more than 1.0 per cent w/w, determined by gas chromatography (2.4.13).

*Test solution (a)*. A 25 per cent w/v solution of the substance under examination.

*Test solution (b)*. A solution containing 25 per cent w/v of the substance under examination and 0.25 per cent v/v of *1-propanol* (internal standard).

*Reference solution*. A solution containing 0.25 per cent v/v of *methanol* and 0.25 per cent v/v of the internal standard.

Chromatographic system
- a column 1.5 m x 4 mm, packed with porous polymer beads (80 to 100 mesh)(such as Porapak Q),
- temperature:
  column. constant at a point between 120° and 140°,
  inlet port and detector. 50° higher than column temperature,
- flow rate. constant at 30 to 40 ml per minute of the carrier gas.



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Calculate the percentage w/w of methanol taking 0.792 g as its weight per ml (2.4.29) at 20°.

**Sulphated ash** (2.3.18). Not more than 1.0 per cent, determined on 0.5 g.

**Water** (2.3.43). Not more than 15.0 per cent, determined on 0.3 g.

**Assay**. Determine by the microbiological assay of antibiotics, Method A (2.2.10), and express the result in µg of gentamicin per mg.

*Gentamicin Sulphate intented for use in the manufacture of parenteral preparations without a further appropriate procedure for removal of bacterial endotoxins complies with the following additional requirement.*

**Bacterial endotoxins** (2.2.3). Not more than 1.67 Endotoxin Units per mg of gentamicin.

**Sterility**. Complies with the test for sterility (2.2.11).

**Storage**. Store protected from moisture. If it is intended for use in the manufacture of parenteral or ophthalmic preparations, the container should be sterile and sealed so as to exclude micro-organisms.

**Labelling**. The label states (1) the potency in terms of µg of gentamicin per mg; (2) whether or not the contents are intended for use in the manufacture of parenteral or ophthalmic preparations.

# Gentamicin Eye Drops

Gentamicin Sulphate Eye Drops

Gentamicin Eye Drops are a sterile solution of Gentamicin Sulphate in Purified Water.

Gentamicin Eye Drops contain not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of gentamicin.

**Usual strength**. 0.3 per cent w/v of gentamicin.

## Identification

A. Determine by thin-layer chromatography (2.4.6), coating the plate with *silica gel G*.

*Mobile phase*. The lower layer obtained by shaking together equal volumes of *strong ammonia solution*, *chloroform* and *methanol* and allowing to separate.

*Test solution*. A volume of the eye drops containing 60 µg of gentamicin.

*Reference solution*. Dissolve 0.1 mg of *gentamicin sulphate RS* in a volume of *water* equivalent to the volume of the eye drops used.

Apply to the plate the specified volumes of each solution. After development, dry the plate in air, spray with *ethanolic ninhydrin solution* and heat at 110° for 5 minutes. The three principal spots in the chromatogram obtained with the test solution correspond to those in the chromatogram obtained with the reference solution.

B. In the test for Composition of gentamicin sulphate, the four principal peaks in the chromatogram obtained with the test solution correspond to the four peaks in the chromatogram obtained with the reference solution.

## Tests

**pH** (2.4.24). 6.5 to 7.5.

**Composition of gentamicin sulphate**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dilute a suitable volume of the eye drops with *water* to contain 0.045 per cent w/v of gentamicin. To 10 ml of the resulting solution add 5 ml of *methanol*, swirl and add 4 ml of *phthalaldehyde reagent*, mix, add sufficient *methanol* to produce 25 ml, heat on a water-bath at 60° for 15 minutes and cool.

*Reference solution*. Prepare in the same manner as the test solution but using 10 ml of a 0.065 per cent w/v solution of *gentamicin sulphate RS* in place of the solution of the preparation under examination.

Chromatographic system

- a stainless steel column 10 to 12.5 cm x 4.6 to 5 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: *0.025 M sodium heptanesulphonate monohydrate* in a mixture of 70 volumes of *methanol*, 25 volumes of *water* and 5 volumes of *glacial acetic acid*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 330 nm,
- injection volume. 5 µl.

If necessary, adjust the methanol content of the mobile phase so that in the chromatogram obtained with the reference solution the retention time of component $C_2$ is 10 to 20 minutes and the peaks are well separated with relative retention times of about 0.13 (reagent), 0.27 (component $C_1$), 0.65 (component $C_{1a}$), 0.85 (component $C_{2a}$) and 1.00 (component $C_2$).

Adjust the sensitivity and the volume of reference solution injected so that the height of the peak due to component $C_1$ is about 75 per cent of the full-scale deflection on the recorder. Plot a horizontal baseline on the chromatogram from the level portion of the curve immediately prior to the reagent peak. Measure the peak height above this baseline for each component. Repeat the procedure with the test solution. The test is not valid unless the resolution between the peaks due to components $C_{2a}$ and $C_2$ is not less than 1.3.



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From the peak heights in the chromatogram obtained with the reference solution and the proportions of the components declared for *gentamicin sulphate RS*, calculate the response factors for components $C_1$, $C_{1a}$, $C_{2a}$ and $C_2$. From these response factors and peak heights in the chromatogram obtained with the test solution, calculate the proportions of components $C_1$, $C_{1a}$, $C_{2a}$ and $C_2$ in the eye drops. The proportion are within the following limits. $C_1$, 25.0 to 50.0 per cent; $C_{1a}$, 10.0 to 35.0 per cent; $C_2 + C_{2a}$, 25.0 to 55.0 per cent.

**Other tests**. Comply with the tests stated under Eye Drops.

**Assay**. Determine by the microbiological assay of antibiotics (2.2.10) on a solution prepared in the following manner.

Dilute a volume of the eye drops containing about 15 mg of gentamicin to 50.0 ml with sterile *phosphate buffer pH 8.0* and dilute 10.0 ml of the resulting solution to 50.0 ml with the same solvent.

Calculate the content of gentamicin in the eye drops, taking each 1000 Units found to be equivalent to 1 mg of gentamicin.

**Labelling**. The label states the quantity of active ingredient in terms of the equivalent amount of gentamicin.

# Gentamicin Injection

Gentamicin Sulphate Injection

Gentamicin Injection is a sterile solution of Gentamicin Sulphate in Water for Injection.

Gentamicin Injection contains not less than 95.0 per cent and not more than 110.0 per cent of the stated amount of gentamicin.

**Usual strengths**. The equivalent of 10 mg per ml and 40 mg per ml of gentamicin.

**Description**. A clear, colourless to pale-yellow solution with a faint odour.

## Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. The lower layer obtained by shaking together equal volumes of *strong ammonia solution*, *chloroform* and *methanol* and allowing to separate.

*Test solution*. A volume of the injection containing 60 µg of gentamicin.

*Reference solution*. Dissolve 0.1 mg of *gentamicin sulphate RS* in a volume of *water* equivalent to the volume of the injection used.

Apply to the plate the specified volumes of each solution. After development, dry the plate in air, spray with *ethanolic ninhydrin solution* and heat at 110° for 5 minutes. The three principal spots in the chromatogram obtained with the test solution correspond to those in the chromatogram obtained with the reference solution.

B. In the test for Composition of gentamicin sulphate, the four principal peaks in the chromatogram obtained with the test solution correspond to the four peaks in the chromatogram obtained with the reference solution.

## Tests

**pH** (2.4.24). 3.0 to 5.0.

**Composition of gentamicin sulphate**. Determine by liquid chromatography (2.4.14).

*Test solution*. Add 5 ml of *methanol* to 10 ml of a solution prepared by diluting a suitable volume of the injection with *water* to contain the equivalent of 0.045 per cent w/v of gentamicin, swirl and add 4 ml of *phthalaldehyde reagent*, mix, add sufficient *methanol* to produce 25 ml, heat on a water-bath at 60° and cool. If the solution is not used immediately, cool at 0° and use within 4 hours.

*Reference solution*. Prepare in the same manner as the test solution but using 10 ml of a 0.065 per cent w/v solution of *gentamicin sulphate RS* in place of the solution of the injection under examination.

Chromatographic system

- a stainless steel column 10 to 12.5 cm x 4.6 to 5 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: *0.025 M sodium heptanesulphonate monohydrate* in a mixture of 70 volumes of *methanol*, 25 volumes of *water* and 5 volumes of *glacial acetic acid*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 330 nm,
- injection volume. 5 µl.

If necessary, adjust the methanol content of the mobile phase so that in the chromatogram obtained with the reference solution the retention time of component $C_2$ is 10 to 20 minutes and the peaks are well separated with relative retention times of about 0.13 (reagent), 0.27 (component $C_1$), 0.65 (component $C_{1a}$), 0.85 (component $C_{2a}$) and 1.00 (component $C_2$).

Adjust the sensitivity and the volume of reference solution injected so that the height of the peak due to component $C_1$ is about 75 per cent of full-scale deflection on the recorder. Plot a horizontal baseline on the chromatogram from the level portion of the curve immediately prior to the reagent peak. Measure the peak height above this baseline for each



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component. Repeat the procedure with the test solution. The test is not valid unless the resolution factor between the peaks due to components $C_{2a}$ and $C_2$ is not less than 1.3.

From the peak heights in the chromatogram obtained with the reference solution and the proportions of the components declared for *gentamicin sulphate RS*, calculate the response factors for components $C_1$, $C_{1a}$, $C_{2a}$ and $C_2$. From these response factors and peak heights in the chromatogram obtained with the test solution, calculate the proportions of components $C_1$, $C_{1a}$, $C_{2a}$ and $C_2$ in the eye drops. The proportions are within the following limits. $C_1$, 25.0 to 50.0 per cent; $C_{1a}$, 10.0 to 35.0 per cent; $C_2 + C_{2a}$, 25.0 to 55.0 per cent.

**Bacterial endotoxins** (2.2.3). Not more than 1.67 Endotoxin Units per mg of gentamicin.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

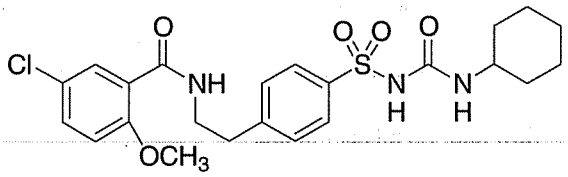
**Assay**. Determine by the microbiological assay of antibiotics, Method A (2.2.10), and express the result in mg of gentamicin per ml.

Calculate the content of gentamicin in the injection, taking each 1000 Units found to be equivalent to 1 mg of gentamicin.

**Labelling**. The label states the strength in terms of the equivalent amount of gentamicin in a suitable dose-volume.

# Glibenclamide

Glyburide



$C_{23}H_{28}ClN_3O_5S$ Mol. Wt. 494.0

Glibenclamide is 1-{4-[2-(5-chloro-2-methoxybenzamido)ethyl]benzenesulphonyl}-3-cyclohexylurea.

Glibenclamide contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{23}H_{28}ClN_3O_5S$, calculated on the dried basis.

**Category**. Hypoglycaemic.

**Dose**. 5 mg daily, adjusted according to response; maximum 15 mg daily, after food.

**Description**. A white or almost white, crystalline powder.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

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

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.01 per cent w/v solution in *0.01 M methanolic hydrochloric acid* shows an absorption maximum at about 300 nm and a less intense maximum at about 275 nm; absorbance at about 300 nm, about 0.63 and at about 275 nm, about 0.29.

C. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

D. Dissolve 20 mg in 2 ml of *sulphuric acid (96 per cent w/w)*; the solution is colourless and exhibits a blue fluorescence in ultraviolet light at 365 nm. Dissolve about 0.1 g of *chloral hydrate* in the solution; within 5 minutes the colour changes to deep yellow and after about 20 minutes a brownish tinge is produced.

## Tests

**Appearance of solution**. A 1.0 per cent w/v solution in *ethanol (95 per cent)*, prepared with the aid of heat, is clear (2.4.1), and colourless (2.4.1).

**Related substances**. Determine by liquid chromatography (2.4.14).

*NOTE—Prepare the solutions immediately before use.*

*Test solution*. Dissolve 25 mg of the substance under examination in 10 ml of *methanol*.

*Reference solution*. Dilute 2.0 ml of the test solution to 100 ml with *methanol*.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (3µm),
- column temperature. 35°,
- mobile phase: A. a mixture of a 20 volumes of 10.2 per cent v/v solution of freshly distilled *triethylamine* adjust the pH to 3.0 with *orthophosphoric acid* and 50 volumes of *acetonitrile*, dilute to 1000 ml with *water*,
  B. a mixture of 20 volumes of mobile phase A, 65 volumes of *water* and 915 volumes of *acetonitrile*,
- a linear gradient programme using the conditions given below,
- flow rate. 0.8 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 10 µl.



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| Time (in min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0- 15 | 45 | 55 |
| 15-30 | 45-5 | 55-95 |
| 30-40 | 5 | 95 |
| 40-41 | 5-45 | 95-55 |
| 41-55 | 45 | 55 |

The relative retention time with reference to glibenclamide for glibenclamide impurity A is about 0.5, for glibenclamide impurity B is about 0.6.

Inject the test solution and the reference solution. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of areas of all the secondary peaks is not more than 4 times the area of peak in the chromatogram obtained with the reference solution (2.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained in the reference solution (0.05 per cent).

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

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.4 g and dissolve in 100 ml of *ethanol (95 per cent)* with the aid of heat; titrate with *0.1 M sodium hydroxide* using 1 ml of *dilute phenolphthalein solution* as indicator until a red colour is obtained.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.04940 g of $C_{23}H_{28}ClN_3O_5S$.

# Glibenclamide Tablets

Glibenclamide Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of glibenclamide, $C_{23}H_{28}ClN_3O_5S$.

**Usual strengths**. 2.5 mg; 5 mg.

## Identification

A. When examined in the range 230 nm to 360 nm (2.4.7), the solution obtained in the Assay shows an absorption maximum at about 300 nm and a less intense maximum at about 275 nm.

B. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

## Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 45 volumes of *chloroform*, 45 volumes of *cyclohexane*, 5 volumes of *glacial acetic acid* and 5 volumes of *ethanol (95 per cent)*.

*Test solution*. Extract a quantity of the powdered tablets containing 20 mg of Glibenclamide with four quantities, each of 5 ml, of a mixture of 20 volumes of *dichloromethane* and 10 volumes of *acetone*, evaporate the combined extracts to dryness at a pressure of 2 kPa and at a temperature not exceeding 40° and dissolve the residue in 4 ml of a mixture of equal volumes of *chloroform* and *methanol*.

*Reference solution (a)*. A 0.5 per cent w/v solution of *glibenclamide RS* in the same solvent mixture.

*Reference solution (b)*. Dilute 2 ml of reference solution (a) to 100 ml with the same solvent mixture.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b).

**Uniformity of content**. Comply with the test stated under Tablets.

Place one tablet in a 50.0 ml volumetric flask and disintegrate with 5 ml of *0.1 M methanolic hydrochloric acid*. Dilute to volume with *0.1 M methanolic hydrochloric acid* and mix well. Centrifuge the solution and measure the absorbance of the resulting solution at the maximum at about 300 nm (2.4.7). Calculate the content of $C_{23}H_{28}ClN_3O_5S$ taking 63 as the specific absorbance at 300 nm.

**Other Tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve a quantity of the powdered tablets containing 5 mg of Glibenclamide with a mixture of 2 ml of *water* and 20 ml of *methanol*.

*Reference solution*. Dissolve 50 mg of *glibenclamide RS* in 50 ml of *methanol*, sonicate for 20 minutes. Dilute 1ml of this solution to 4 ml with *methanol*. To 20 ml of this solution add 2 ml *water* and mix.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm) (such as Spherisorb ODS),
- mobile phase: a mixture of 47 volumes of *acetonitrile* and 53 volumes of a 1.36 per cent w/v solution of *potassium dihydrogen orthophosphate*, previously adjusted to pH 3.0 with *orthophosphoric acid*,



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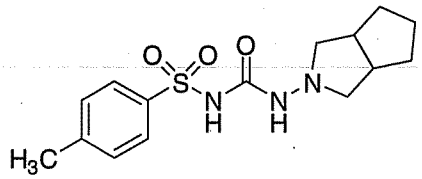
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 300 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{23}H_{28}ClN_3O_5S$ in the tablets.

# Gliclazide



$C_{15}H_{21}N_3O_3S$ Mol. Wt. 323.4

Gliclazide is 1-(hexahydrocyclopenta[*c*]pyrrol-2(1*H*)-yl)-3-[(4-methylphenyl)sulphonyl]urea.

Gliclazide contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{15}H_{21}N_3O_3S$, calculated on the dried basis.

**Category**. Oral hypoglycaemic.

**Description**. A white or almost white powder.

## Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *gliclazide RS* or with the reference spectrum of gliclazide.

## Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. 45 volumes of *acetonitrile* and 55 volumes of *water*.

*Test solution*. Dissolve 50 mg of the substance under examination in 23 ml of *acetonitrile* and dilute to 50.0 ml with *water*.

*Reference solution (a)*. Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Further dilute 5.0 ml of this solution to 50.0 ml with the solvent mixture.

*Reference solution (b)*. Dissolve 5 mg of the substance under examination and 15 mg of *1-(hexahydrocyclopenta[c]pyrrol-2(1H)-yl)-3-[(2-methylphenyl)sulphonyl]urea RS (gliclazide impurity F RS)* in 23 ml of *acetonitrile* and dilute to 50 ml with *water*. Dilute 1 ml of this solution to 20 ml with the solvent mixture.

*Reference solution (c)*. Dissolve 1.0 mg of *gliclazide impurity F RS* in 5 ml of *acetonitrile* and dilute to 10.0 ml with *water*. Dilute 1.0 ml of this solution to 100.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 0.1 volume of *triethylamine*, 0.1 volume of *trifluoroacetic acid*, 45 volumes of *acetonitrile* and 55 volumes of *water*,
- flow rate. 0.9 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume. 20 µl.

Inject reference solution (b). The relative retention time with reference to gliclazide for gliclazide impurity F is about 0.9. The resolution between the peaks due to gliclazide impurity F and gliclazide is not less than 1.8.

Inject reference solution (a), (c) and the test solution. Run the chromatogram twice the retention time of the principal peak. The area of peak corresponding to gliclazide impurity F is not more than the area of peak obtained in the chromatogram with reference solution (c) (0.1 per cent), the area of any other secondary peak is not more than the principle peak in the chromatogram obtained with reference solution (a) (0.1 per cent). The sum of the areas of all other secondary peaks is not more than twice the area of principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent). Ignore any peak with an area 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

**Gliclazide Impurity B**. Determine by liquid chromatography (2.4.14), as described under Related substances.

*Test solution*. Dissolve 0.4 g of the substance under examination in 2.5 ml of *dimethyl sulphoxide* and dilute to 10.0 ml with *water*. Stir for 10 minutes, store at 4° for 30 minutes and filter.

*Reference solution*. Dissolve 20 mg of *2-nitroso-octahydrocyclopenta[c]pyrrole RS (gliclazide impurity B RS)* in 100.0 ml of *dimethyl sulphoxide* . To 1.0 ml of the solution, add 12 ml of *dimethyl sulphoxide* and dilute to 50.0 ml with *water*. To 1.0 ml of this solution, add 12 ml of *dimethyl sulphoxide* and dilute to 50.0 ml with *water*.

Inject 50 µl of the test solution and the reference solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to impurity B is not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (2 ppm).

**Heavy metals** (2.3.13). 1.5 g complies with the limit test for heavy metals, Method B (10 ppm).



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**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.25 per cent, determined on 1.0 g by drying in an oven at 105° for 2 hours.

**Assay**. Weigh accurately about 0.25 g, dissolve in 50 ml of *anhydrous acetic acid* and titrate with *0.1 M perchloric acid,* determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03234 g of $C_{15}H_{21}N_3O_3S$.

## Gliclazide Tablets

Gliclazide Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of gliclazide, $C_{15}H_{21}N_3O_3S$.

**Usual strength**. 80 mg.

### Identification

Shake a quantity of the powdered tablets containing 0.16 g of Gliclazide with 20 ml of *dichloromethane*, centrifuge and evaporate the supernatant liquid to dryness. The residue complies with the following test. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *gliclazide RS* or with the reference spectrum of gliclazide.

### Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *phosphate buffer pH 7.4*,
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter. Measure the absorbance (2.4.7) of the filtrate, suitably diluted if necessary with dissolution medium to obtain a solution containing 12.5 µg per ml of Gliclazide at 226 nm and 290 nm. Correct the absorbance obtained at 226 nm by subtracting the absorbance obtained at 290 nm. Calculate the content of gliclazide, $C_{15}H_{21}N_3O_3S$ in the medium from the absorbances obtained from a solution prepared by dissolving 62 mg of *gliclazide RS* in 20 ml of *methanol*, adding sufficient dissolution medium to produce 1000 ml, dilute 1 ml of this solution to 5 ml with the dissolution medium.

D. Not less than 70 per cent of the stated amount of $C_{15}H_{21}N_3O_3S$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture.* 45 volumes of *acetonitrile* and 55 volumes of *water*.

*Test solution.* Shake a quantity of the powdered tablets containing about 0.8 g of Gliclazide for 1 hour with 200 ml of *acetonitrile*, filter. Dilute 10 ml of the filtrate to 50 ml with a mixture of 1 volume of *acetonitrile* and 2 volumes of *water*.

*Reference solution (a).* Dilute 1 ml of the test solution to 500 ml with the solvent mixture.

*Reference solution (b).* Dissolve 5.0 mg of *gliclazide RS* and 15 mg of *1-(3-azabicyclo[3.3.0]oct-3-yl)-3-o-tolylsulphonylurea RS* in 25 ml of *acetonitrile*, dilute to 50 ml with *water*. Dilute 1 ml of this solution to 20 ml with the solvent mixture.

*Reference solution (c).* Dissolve 8.0 mg of *1-(3-azabicyclo[3.3.0]oct-3-yl)-3-o- tolylsulphonylurea RS* in 25 ml of *acetonitrile*, dilute to 50 ml with *water*. Dilute 1 ml of this solution to 100 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4 mm, packed with endcapped octylsilane bonded to porous silica (4 µm) (such as Superspher 60 RP 8),
- mobile phase: a mixture of 0.1 volume of *triethylamine*, 0.1 volume of *trifluoroacetic acid*, 45 volumes of *acetonitrile* and 55 volumes of *water*,
- flow rate. 0.9 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between gliclazide and 1-(3-azabicyclo[3.3.0]oct-3-yl)-3-o-tolylsulphonylurea is not less than 1.8.

Inject reference solution (a), (c) and the test solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with test solution, the area of peak corresponding to 1-(3-azabicyclo[3.3.0]oct-3-yl)-3-*o*-tolylsulphonylurea is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent); the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent) and the sum of the areas of other secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent). Ignore any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture.* 40 volumes of *acetonitrile* and 60 volumes of *water*.

*Test solution.* Shake a quantity of the powdered tablets containing about 0.8 g of Gliclazide for 1 hour with 200 ml of



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*acetonitrile*, filter. Dilute 10 ml of the filtrate to 200 ml with the solvent mixture.

*Reference solution (a).* Dissolve 40 mg of *gliclazide RS* in 10 ml of *acetonitrile* and dilute to 200 ml with the solvent mixture.

*Reference solution (b).* Dissolve 5 mg of *gliclazide RS* and 15 mg of *1-(3-azabicyclo[3.3.0]oct-3-yl)-3-o-tolylsulphonylurea RS* in 25 ml of *acetonitrile*, dilute to 50 ml with *water*. Dilute 1 ml of this solution to 20 ml with the solvent mixture.

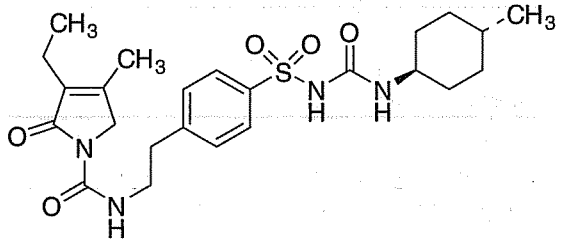
Use chromatographic system as described under Related substances.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to 1-(3-azabicyclo[3.3.0]oct-3-yl)-3-o-tolylsulphonylurea and gliclazide is not less than 1.8.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{15}H_{21}N_3O_3S$ in the tablets.

## Glimepiride



$C_{24}H_{34}N_4O_5S$ Mol. Wt. 490.6

Glimepiride is 1-[[4-[2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido)ethyl]phenyl]sulphonyl]-3-*trans*-(4-methylcyclohexyl)urea.

Glimepiride contains not less than 97.0 per cent and not more than 102.0 per cent of $C_{24}H_{34}N_4O_5S$, calculated on the anhydrous basis.

**Category**. Oral hypoglycaemic.

**Descripiton**. A white or almost white powder.

### Identificaition

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum obtained with that *glimepiride RS* or with the reference spectrum of glimepiride.

### Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*NOTE—Store the solutions at a temperature not exceeding 12° and for not more than 15 hours.*

*Solvent mixture.* 20 volumes of *water* and 80 volumes of *acetonitrile*.

*Test solution.* Dissolve 20 mg of the substance under examination in 100.0 ml of the solvent mixture.

*Reference solution (a).* Dissolve the contents of a vial of *glimepiride for system suitability RS* (containing Glimepiride impurity B, C and D) in 2.0 ml of the test solution.

*Reference solution (b).* Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

*Reference solution (c).* A 0.02 per cent w/v solution of *glimepiride RS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm, packed with endcapped octadecylsilane bonded to porous silica (4 µm),
- mobile phase: a mixture of 50 volumes of a solution prepared by dissolving 0.5 g of *sodium dihydrogen orthophosphate* in 500 ml of *water*, adjusted to pH 2.5 with *orthophosphoric acid* and 50 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 228 nm,
- injection volume. 20 µl.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to glimepiride impurity B and glimepiride impurity C is not less than 4.0. The relative retention time with reference to glimepiride for 3-ethyl-4-methyl-2-oxo-*N*-[2-(4- sulphamoylphenyl)ethyl]-2,3-dihydro-1*H*-pyrrole-1-carboxamide (glimepiride sulphonamide) (glimepiride impurity B) is about 0.2, for methyl [[4-[2-[[(3-ethyl-4-methyl-2-oxo-2, 3-dihydro-1*H*-pyrrol-1-yl)carbonyl]amino]ethyl]phenyl] sulphonyl]carbamate (glimepiride urethane) (glimepiride impurity C) is about 0.3 and for 1-[[3-[2-[[(3-ethyl-4-methyl-2-oxo-2,3-dihydro-1*H*-pyrrol-1-l)carbonyl]amino]ethyl]n phenyl] sulphonyl]-3-(*trans*-4-methylcyclohexyl)urea (glimepiride impurity D) is about 1.1.

Inject reference solution (a), (b) and the test solution. Run the chromatogram 2.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of the peak due to glimepiride impurity B is not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent), the area of the peak due to glimepiride impurity D is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The sum of all the secondary peaks other than glimepiride impurity B is not more than 5 times the area of



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the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

**Impurity A**. Determine by liquid chromatography (2.4.14).

*NOTE—Prepare the solutions immediately before use.*

*Test solution.* Dissolve 10 mg of the substance under examined in 5 ml of *dichloromethane* and dilute to 20.0 ml with the mobile phase.

*Reference solution (a).* Dilute 0.8 ml of the test solution to 100.0 ml with the mobile phase.

*Reference solution (b).* Dissolve 2 mg of *glimepiride RS* (containing glimepiride impurity A) in 1.0 ml of *dichloromethane* and dilute to 4.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.0 mm, packed with silica gel (5 µm),
- mobile phase: a mixture of 1 volume of *anhydrous acetic acid,* 100 volumes of *2-propanol* and 899 volumes of *heptane*,
- flow rate. 0.5 ml per minute,
- spectrophotometer set at 228 nm,
- injection volume.10 µl.

Inject reference solution (b). The test is not valid unless the peak-to-valley ratio is not less than 2.0, where $H_p$ is the height above the baseline of the peak due to impurity A and $H_v$ is the height above the baseline of the lowest point of the curve separating this peak from the peak due to glimepiride. The relative retention time with reference to glimepiride for glimepiride impurity A is about 0.9.

Inject reference solution (a) and the test solution. Run the chromatogram 1.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of the peak due to 1-[[4-[2-[[(3-ethyl-4-methyl-2-oxo-2,3-dihydro-1*H*-pyrrol-1-yl)carbonyl]amino]ethyl]phenyl] sulphonyl]-3-(*cis*-4-methylcyclohexyl)urea (glimepiride impurity A) is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.8 per cent).

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Water** (2.3.43). Not more than 0.5 per cent, determined by dissolving 0.25 g in 5.0 ml of *dimethylformamide*.

**Assay**. Determine by liquid chromatography (2.4.14) as described in the test for Related substances with the following modification.

Inject the test solution and reference solution (c).

Calculate the content of $C_{24}H_{34}N_4O_5S$.

**Storage**. Store protected from moisture, at a temperature not exceeding 30°.

# Glimepiride Tablets

Glimepiride Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of glimepiride, $C_{24}H_{34}N_4O_5S$.

**Usual strengths**. 1 mg; 2 mg.

## Identification

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

## Tests

**Dissolution** (2.5.2).

Apparatus No. 1,

Medium. 900 ml of phosphate buffer pH 7.8 prepared by dissolving 0.58 g of *monobasic potassium phosphate* and 8.86 g of *anhydrous dibasic sodium phosphate* in 1000 ml of *water* and adjusting the pH to 7.8 with dilute *orthophosphoric acid* or *1 M sodium hydroxide,*

Speed and time. 75 rpm and 15 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14), using the chromatographic system as described under Assay, using injection volume. 50 µl.

*Solvent mixture.* 50 volumes of *methanol* and 50 volumes of *water.*

*Test solution.* Dilute the filtrate, if necessary, with the solvent mixture.

*Reference solution.* Dissolve an accurately weighed quantity of *glimepiride RS* in a mixture of 90 volumes of *acetonitrile* and 10 volumes of *water* to obtain a solution having a concentration of 0.125 mg of glimepiride per ml. Dilute 4.0 ml of this solution to 200.0 ml with the dissolution medium. Further dilute 15.0 ml of this solution to 50 ml with the solvent mixture to obtain a final concentration of 0.00075 mg per ml of glimepiride.

Calculate the content of $C_{24}H_{34}N_4O_5S$ in the tablet.

D. Not less than 75.0 per cent of the stated amount of $C_{24}H_{34}N_4O_5S$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*NOTE—Use freshly prepared solution.*

*Solvent mixture.* 90 volumes of *acetonitrile* and 10 volumes of *water*.

*Test solution*. Disperse a quantity of powdered tablets containing about 5 mg of glimepiride in 50 ml of the solvent mixture. Centrifuge and use the clear supernatant.



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*Reference solution (a).* A solution containing 0.004 per cent w/v of *glimepiride RS* and 0.002 per cent w/v each of *glimepiride sulfonamide (glimepiride impurity B RS)* and *glimepiride urethane (glimepiride impurity C RS)* in the solvent mixture. Dilute 5.0 ml of this solution to 50 ml with the solvent mixture.

*Reference solution (b).* Dilute 5.0 ml of reference solution (a) to 100 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (4 µm),
- mobile phase: a mixture of 50 volumes of a solution containing 0.5 g of *monobasic sodium phosphate* in 500 ml of *water*, adjusted to pH 2.1 with *orthophosphoric acid* and 50 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 228 nm,
- injection volume. 10 µl.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to glimepiride impurity B and glimepiride impurity C is not less than 4.0 and the relative standard deviation for replicate injections is not more than 2.0.The relative retention time with reference to glimepiride for glimepiride impurity B is about 0.2 and for glimepiride impurity C is about 0.3.

Inject reference solution (b) and the test solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution the area of the peak corresponding to glimepiride imputiy B is not more than 2.5 per cent of the area of principal peak in the chromatogram obtained with reference solution (b), the area of any other secondary peak is not more than 0.5 per cent of the area of principal peak in the chromatogram obtained with reference solution (b). The sum of all the secondary peaks other than glimepiride impurity B is not more than 1.0 per cent of the area of principal peak in the chromatogram obtained with reference solution (b), the sum of all the secondary peaks is not more than 3.5 per cent of the area of principal peak in the chromatogram obtained with reference solution (b). Ignore any peak with an area less than 0.1 per cent of the area of principal peak in the chromatogram obtained with reference solution (b).

**Uniformity of content**. Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay, using the following solution as the test solution.

*Test solution.* Disperse 1 tablet in 100 ml of the solvent mixture.

Calculate the content of $C_{24}H_{34}N_4O_5S$ in the tablet.

**Assay**. Determine by liquid chromatography (2.4.14).

*NOTE—Use freshly prepared solution.*

*Solvent mixture*. 90 volumes of *acetonitrile* and 10 volumes of *water*.

*Test solution.* Disperse a quantity of powdered tablets containing about 10 mg of Glimepiride in 10 ml of *water,* add 70 ml of *acetonitrile* and sonicate in a water-bath at 20° for 5 to 10 minutes, dilute to 100 ml with *acetonitrile* and filter.

*Reference solution (a).* A solution containing 0.01 per cent w/v of *glimepiride RS* and 0.002 per cent w/v each of *glimepiride impurity B RS* and *glimepiride impurity C RS* in the solvent mixture.

*Reference solution (b).* A 0.01 per cent w/v solution of *glimepiride RS* in the solvent mixture.

Chromatographic system

- a stainless steel column 12.5 cm x 4 mm, packed with octadecylsilane bonded to porous silica (4 µm),
- mobile phase: a mixture of 50 volumes of a solution containing 0.5 g of *monobasic sodium phosphate* in 500 ml of *water*, adjusted to pH 2.1 with *orthophosphoric acid* and 50 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 228 nm,
- injection volume. 10 µl.

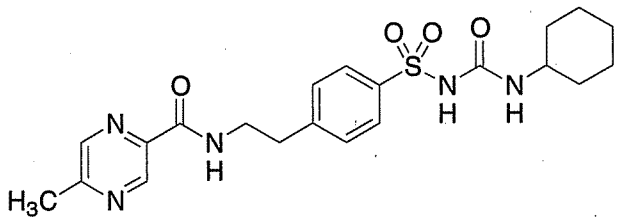
Inject reference solution (a). The test is not valid unless the resolution between the peaks due to glimepiride impurity B and glimepiride impurity C is not less than 1.5 and the relative standard deviation for replicate injections is not more than 2.0. The relative retention time with reference to glimepiride for glimepiride impurity B is about 0.25 and for glimepiride impurity C is about 0.35.

Inject reference solution (b) and the test solution.

Calculate the content of $C_{24}H_{34}N_4O_5S$ in the tablets.

**Storage**. Store protected from moisture, at a temperature not exceeding 30°.

## Glipizide



$C_{21}H_{27}N_5O_4S$ Mol. Wt. 445.5

Glipizide is 1-cyclohexyl-3-[[4-[2-[[(5-methylpyrazine-2-yl)carbonyl]amino]ethyl]phenyl]sulphonyl]urea



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Glipizide contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{21}H_{27}N_5O_4S$, calculated on the dried basis.

**Category**. Oral hypoglycaemic.

**Description**. A white or almost white, crystalline powder.

## Identification

*Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.*

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

B. When examined in the range 220 to 350 nm (2.4.7), a 0.002 per cent solution in *methanol*, shows two maxima, at about 226 nm and 274 nm. The ratio of the absorbance at 226 nm to that at about 274 nm, 2.0 to 2.4 .

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Solvent mixture*. Equal volumes of *methanol* and *methylene chloride*.

*Mobile phase*. A mixture of 25 volumes of *anhydrous formic acid*, 25 volumes of *ethyl acetate* and 50 volumes of *methylene chloride*.

*Test solution*. Dissolve 0.10 g of the substance under examination in 100 ml of solvent mixture.

*Reference solution*. A 0.10 per cent w/v solution of *glipizide RS* in solvent mixture.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in the ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with the reference solution.

## Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 25 mg of the substance under examination in 100.0 ml of the mobile phase.

*Reference solution (a)*. Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

*Reference solution (b)*. A solution containing 0.005 per cent w/v each of *5-methyl-N-[2-(4-sulphamoylphenyl)ethyl] pyrazine-2- carboxamide RS (glipizide impurity A RS)* and *6-methyl-N-[2-(4-sulphamoylphenyl)ethyl]pyrazine-2-carboxamide RS (glipizide impurity D RS)* in the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 17 volumes of *acetonitrile* and 83 volumes of 0.35 per cent w/v solution of *dipotassium hydrogen phosphate*, adjusted to pH 8.0 with *orthophosphoric acid,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 274 nm,
- injection volume. 50 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to glipizide impurity A and glipizide impurity D is not less than 2.0. The relative retention time with reference to glipizide for glipizide impurity A is about 0.3, for glipizide impurity D is about 0.4 and for 1-cyclohexyl-3-[[4-[2-[[(6-methylpyrazin-2- yl)carbonyl]amino]ethyl] phenyl]sulphonyl]urea (glipizide impurity E) is about 1.1.

Inject the test solution and reference solution (a). Run the chromatogram 1.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of the peak due to glipizide impurity A is not more 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent), the area of the peak due to glipizide impurity D and E is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent). The sum of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.4 g, dissolve in 50 ml of *dimethylformamide*, add 0.2 ml of *quinaldine red solution*. Titrate with *0.1 M lithium methoxide* until the colour changes from red to colourless.

1 ml of *0.1 M lithium methoxide* is equivalent to 0.04455 g of $C_{21}H_{27}N_5O_4S$.

**Storage**. Store protected from moisture.

# Glipizide Tablets

Glipizide Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of glipizide, $C_{21}H_{27}N_5O_4S$.



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**Usual strength**. 5 mg.

### Identification

A. Shake a quantity of the powdered tablets containing 25 mg of Glipizide with 10 ml of *dichloromethane* for 5 minutes, filter, dry the filtrate with *anhydrous sodium sulphate*, filter again and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *glipizide RS* or with the reference spectrum of glipizide. .

B. When examined in the range 210 nm to 320 nm (2.4.7), a final solution obtained in the assay shown are absorption maximum at about 226 nm and 274 nm.

### Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17).

*Solvent mixture*. Equal volumes of *dichloromethane* and *methanol*

*Mobile phase*. A mixture of 20 volumes of *ethyl acetate*, 20 volumes of *anhydrous formic acid* and 40 volumes of *dichloromethane*.

*Test solution*. Extract a quantity of powdered tablets containing 0.1 g of Glipizide with four 10 ml quantities of *acetone*, evaporate the combined extracts to dryness under reduced pressure at a temperature not exceeding 30° and dissolve the residue in sufficient of the solvent mixture to produce 5 ml.

*Reference solution (a)*. Dilute 1 volume of test solution to 200 volumes with the solvent mixture.

*Reference solution (b)*. Dilute 1 volume of test solution to 500 volumes with the solvent mixture.

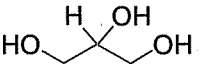
*Reference solution (c)*. A 0.010 per cent w/v solution of glipizide *impurity A RS* (4-[2-(5-methylpyrazine-2-carboxamido) ethyl]benzenesulphonamide) in the solvent mixture.

Apply to the plate 20 µl of each solution. After development, dry the plate in air and examine in the ultraviolet light at 254 nm. Any spot corresponding to glipizide impurity A in the chromatogram obtained with test solution is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.5 per cent). Any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent) and not more than two such spots are more intense than the spot in the chromatogram obtained with reference solution (b) (0.2 per cent).

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 15 mg of Glipizide, dissolve in 30 ml of *methanol* with gentle heating on a water bath, cool and add sufficient *methanol* to produce 50.0 ml. Filter and dilute 5.0 ml of the filtrate to 50.0 ml with *methanol*. Measure the absorbance of the resulting solution at 274 nm (2.4.7). Calculate the content of $C_{21}H_{27}N_5O_4S$ taking 237 as the specific absorbance at 274 nm.

## Glycerin

Glycerol




$C_3H_8O_3$ Mol. Wt. 92.1

Glycerin is propane-1,2,3-triol.

Glycerin contains not less than 98.0 per cent and not more than 101.0 per cent of $C_3H_8O_3$, calculated on the anhydrous basis.

**Category**. Lubricant; laxative; pharmaceutical aid (humectant).

**Description**. A clear, colourless or almost colourless, syrupy liquid; odourless; very hygroscopic.

### Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.*

A. To 5 ml add 1 ml of *water* and mix carefully. The resulting solution complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *glycerin (85 per cent) RS* or with the reference spectrum of glycerin (85 per cent).

B. Mix 1 ml with 0.5 ml of *nitric acid* and superimpose 0.5 ml of *potassium dichromate solution*; a blue ring develops at the interface of the two liquids. Allow to stand for 10 minutes; the blue colour does not diffuse into the lower layer.

C. Heat 1 ml with 2 g of *potassium hydrogen sulphate* in an evaporating dish. Irritant vapours are evolved which blacken filter paper moistened with *alkaline potassium mercuri-iodide solution*.

D. Refractive index (2.4.27). 1.470 to 1.475, determined at 20°.

### Tests

**Appearance of solution**. Dissolve 50 g of the substance under examination in sufficient *carbon dioxide-free water* to produce 100 ml (solution A). Solution A is clear (2.4.1). Dilute 10 ml of solution A to 25 ml with *water*. The solution is colourless (2.4.1).



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**Acidity or alkalinity**. To 50 ml of solution A add 0.5 ml of *phenolphthalein solution*. The solution is colourless and not more than 0.2 ml of *0.1 M sodium hydroxide* is required to produce a pink colour. Reserve the final solution for the test for Ester.

**Heavy metals** (2.3.13). Dissolve 4.0 g in 2 ml of *0.1 M hydrochloric acid* and sufficient *water* to produce 25 ml. The resulting solution complies with the limit test for heavy metals, Method A (5 ppm).

**Iron** (2.3.14). 10.0 g complies with the limit test for iron (4 ppm).

**Chlorides** (2.3.12). 20.0 ml of solution A complies with the limit test for chlorides (25 ppm).

**Sulphates** (2.3.17). 10.0 ml of solution A complies with the limit test for sulphates (30 ppm).

**Aldehydes and reducing substances**. To 7.5 ml of solution A in a glass-stoppered flask add 7.5 ml of *water* and 1 ml of *decolourised pararosaniline solution*, close the flask and allow to stand for 1 hour. Any colour produced is not more intense than that obtained in a standard prepared at the same time and in the same manner but using 7.5 ml of *formaldehyde standard solution (5 ppm $CH_2O$)* in place of solution A. The test is not valid unless the standard solution is pink.

**Ester**. Add *0.1 M sodium hydroxide* to the solution reserved in the test for Acidity or alkalinity until a total of 10.0 ml has been added and boil under a reflux condenser for 5 minutes. Cool, add 0.5 ml of *phenolphthalein solution* and titrate with *0.1 M hydrochloric acid*. Not less than 8.0 ml of *0.1 M hydrochloric acid* is required to decolourise the solution.

**Ethylene glycol, diethylene glycol and related substances**. Determine by gas chromatography (2.4.13).

*Test solution*. A 10.0 per cent w/v solution of the substance under examination.

*Reference solution*. A solution containing 0.05 per cent w/v of the substance under examination, 0.05 per cent w/v of *ethylene glycol* and 0.05 per cent w/v of *diethylene glycol*.

Chromatographic system
- a glass column 2 m x 3 mm, packed with 10 per cent diethylene glycol succinate on acid-washed and silanised, flux-calcinated siliceous earth (such as Chromosorb WHP 80-100 mesh),
- temperature:
  column.200°,
  inlet port and detector. 280°,
- flame ionisation detector,
- flow rate. 30 ml per minute of the carrier gas.

Inject 3 µl or other suitable volume of the test solution. Record the chromatogram adjusting the sensitivity so that the height of the peak due to glycerin is more than 50 per cent of full-scale deflection. Inject the same volume of the reference solution and record the chromatogram. The order of elution is ethylene glycol, diethylene glycol and glycerin.

The test is not valid unless in the chromatogram obtained with the reference solution the resolution factor between the peaks corresponding to diethylene glycol and glycerin is not less than 3.0 and the area of any secondary peak in the chromatogram obtained with the test solution is less than the area of the peak corresponding to diethylene glycol in the chromatogram obtained with the reference solution.

**Sugars**. Heat 10 ml of solution A with 1 ml of *1 M sulphuric acid* on a water-bath for 5 minutes. Add 3 ml of *2 M sodium hydroxide* (carbonate-free), mix and add dropwise 1 ml of freshly prepared *copper sulphate solution*; a clear blue solution is produced. Continue heating on the water-bath for 5 minutes; the solution remains blue and no precipitate is produced.

**Sulphated ash** (2.3.18). Not more than 0.01 per cent, determined on 5.0 g.

**Water** (2.3.43). Not more than 2.0 per cent, determined on 1.5 g.

**Assay**. Weigh accurately about 0.1 g, mix thoroughly with 45 ml of *water*, add 25.0 ml of a 2.14 per cent w/v solution of *sodium periodate* and 1.0 ml of *1 M sulphuric acid*. Allow the mixture to stand protected from light for 15 minutes. Add 5 ml of a 50 per cent w/v solution of *ethylene glycol*, allow to stand protected from light for 20 minutes and titrate with *0.1 M sodium hydroxide* using 0.5 ml of *phenolphthalein solution* as indicator. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of sodium hydroxide required by the test substance.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.00921 g of $C_3H_8O_3$.

**Storage**. Store protected from moisture.

# Glyceryl Monostearate

Monostearin

Glyceryl Monostearate is a mixture of monoglycerides of stearic and palmitic acids, together with variable quantities of di- and triglycerides.

Glyceryl Monostearate contains not less than 35.0 per cent of monoglycerides, calculated as glyceryl monostearopalmitate, $C_{20}H_{40}O_4$, and not more than 7.0 per cent of free glycerin $C_3H_8O_3$, both calculated on the anhydrous basis.



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**Category**. Pharmaceutical aid (emulsifying agent).

**Description**. A white or almost white, hard, waxy mass or unctuous powder or flakes; almost odourless.

### Identification

A. Heat 1 g with 2 g of *potassium bisulphate* in an evaporating dish. Irritant, lachrymatory fumes are evolved which darken filter paper impregnated with *alkaline potassium mercuri-iodide solution*.

B. Heat 2.5 g with 40 ml of *ethanolic potassium hydroxide solution* for 30 minutes on a water-bath under a reflux condenser. Add 30 ml of *water*, evaporate the ethanol, acidify the hot mixture with 15 ml of *dilute hydrochloric acid*, cool and extract with 50 ml of *ether*. Wash the *ether* layer with two quantities, each of 10 ml, of a 20 per cent w/v solution of *sodium chloride*, dry the ether extract over *anhydrous sodium sulphate* and filter. Evaporate the solvent and dry the residue under reduced pressure. Melt the residue and fill one or two capillary tubes (for the *determination of melting range)* and allow to stand for 24 hours in a desiccator. Carry out the determination of melting range by Method II (2.4.21); the residue melts at 54° to 64°.

### Tests

**Acid value** (2.3.23). Not more than 5.0, determined on 0.5 g dissolved in 50 ml of a mixture of equal volumes of *ethanol (95 per cent)* and *ether*.

**Saponification value** (2.3.37). 155 to 170.

**Iodine value** (2.3.28). Not more than 5.0 *(iodine bromide method)*.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 2.0 per cent, determined on 0.5 g dissolved in a mixture of 10 ml of *anhydrous methanol* and 10 ml of anhydrous *chloroform*.

**Assay**. Weigh accurately about 0.4 g and dissolve in 50 ml of *dichloromethane* in a glass-stoppered separating funnel. Add 25 ml of *water* and shake vigorously for 1 minute. Allow the layers to separate (if an emulsion is formed, add a few drops of *glacial acetic acid)*. Repeat the extraction with three further quantities, of 25, 20 and 20 ml, of *water* and reserve the dichloromethane solution (solution A). Filter the combined aqueous extracts through a filter paper moistened with *water*, wash the filter with two quantities, each of 5 ml, of *water* and dilute the combined filtrate and washings to 100.0 ml with *water* (solution B).

*For monoglycerides* — Filter solution A through a cotton wool plug. Wash the separating funnel and the filter with three quantities, each of 5 ml, of *dichloromethane*. Dilute the combined filtrate and washings to 100.0 ml with *dichloromethane*. To 25.0 ml of this solution add 25.0 ml of *periodic-acetic acid solution*, shake cautiously, allow to stand at 25° to 30° for 30 minutes, add 100 ml of *water* and 12 ml of *potassium iodide solution*. Titrate the liberated iodine with *0.1 M sodium thiosulphate* using 1 ml of *starch solution* as indicator. Repeat the determination using 25 ml of *dichloromethane* instead of 25.0 ml of the solution under examination. The difference between the titrations represents the amount of sodium thiosulphate required.

1 ml of *0.1 M sodium thiosulphate* is equivalent to 0.0172 g of monoglycerides, calculated as glyceryl monostearo-palmitate, $C_{20}H_{40}O_4$.

The quantity of *0.1 M sodium thiosulphate* used in the assay is not less than 85 per cent of the quantity of sodium thiosulphate used in the blank assay.

*For free glycerin* — To 50.0 ml of solution B in a 400-ml conical flask fitted with a ground-glass stopper add 25.0 ml of *periodic-acetic acid solution*, shake cautiously, allow to stand at 25° to 30° for 30 minutes, add 100 ml of *water* and 12 ml of *potassium iodide solution*. Titrate the liberated iodine with *0.1 M sodium thiosulphate* using 1 ml of *starch solution* as indicator. Repeat the determination using 50 ml of *water* instead of 50 ml of the solution under examination. The difference between the titrations represents the amount of sodium thiosulphate required.

1 ml of *0.1 M sodium thiosulphate* is equivalent to 0.0023 g of glycerin, calculated as $C_3H_8O_3$.

**Storage**. Store protected from light.

## Concentrated Glyceryl Trinitrate Solution

Concentrated Nitroglycerin Solution

Concentrated Glyceryl Trinitrate Solution is a solution of propane-1,2,3-triol trinitrate in Ethanol (95 per cent).

Concentrated Glyceryl Trinitrate Solution contains not less than 9.0 per cent w/v and not more than 11.0 per cent w/v of $C_3H_5N_3O_9$.

*CAUTION — Undiluted glyceryl trinitrate can be exploded by percussion or excessive heat. Proper precautions should be exercised in handling it and only exceedingly small amounts should be isolated.*

**Category**. Coronary vasodilator

**Dose**. Sublingually (as tablets), 300µg to 1 mg, repeated as required.



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**Description**. A clear, colourless to pale yellow solution.

### Identification

Carry out the procedure described under Related substances but using the following solutions.

*Mobile phase*. *Toluene*.

*Test solution*. Dilute the substance under examination with *acetone* to contain 0.05 per cent w/v of glyceryl trinitrate.

*Reference solution*. Extract one powdered *glyceryl trinitrate tablet 0.5 mg RS* with 1 ml of *acetone* and centrifuge.

The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. To 1 ml add 200 ml of *ether*, evaporate 6 ml of the resulting solution to dryness and dissolve the residue in 0.2 ml of *sulphuric acid* containing a trace of *diphenylamine*; an intense blue colour is produced.

### Tests

**Weight per ml** (2.4.29). 0.830 g to 0.850 g.

**Inorganic nitrates**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H*.

*Mobile phase*. A mixture of 60 volumes of *toluene*, 30 volumes of *acetone* and 15 volumes of *glacial acetic acid*

*Test solution*. The substance under examination.

*Reference solution*. A freshly prepared 0.1 per cent w/v solution of *potassium nitrate* in *ethanol (90 per cent)*.

Apply to the plate 5 µl of each solution. After development, dry the plate in a stream of air and spray with *diphenylamine solution*. Any spot corresponding to potassium nitrate in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 80 volumes of *toluene* and 20 volumes of *ethyl acetate*.

*Test solution*. The substance under examination.

*Reference solution*. Dilute 1 volume of the test solution to 100 volumes with *acetone*.

Apply to the plate 5 µl of each solution. After development, dry the plate in a stream of air and spray with *diphenylamine solution*. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Assay**. Dilute 1.0 ml to 50.0 ml with a 90 per cent v/v solution of *glacial acetic acid* and dilute 10.0 ml of this solution to 100.0 ml with the same solvent. To 1.0 ml of the resulting solution add 2 ml of *phenoldisulphonic acid solution*, mix and allow to stand for 15 minutes. Add 8 ml of *water*, mix well, allow to cool and add slowly, with swirling, 10 ml of *strong ammonia solution*. Cool and dilute to 20.0 ml with *water*. Measure the absorbance of the resulting solution at about 405 nm (2.4.7), using as the blank 1 ml of a 90 per cent v/v solution of *glacial acetic acid* treated in the same manner, beginning at the words "add 2 ml of *phenoldisulphonic acid solution*," Dissolve 0.1335 g of *potassium nitrate* previously dried at 105° in *water* to produce 50.0 ml; to 10.0 ml add sufficient *glacial acetic acid* to produce 100.0 ml. Using 1.0 ml of this solution, repeat the procedure beginning at the words "add 2 ml of *phenoldisulphonic acid solution*,....".

Calculate the content of $C_3H_5N_3O_9$ from the values of the absorbances so obtained.

1 ml of the *potassium nitrate solution* is equivalent to 0.0002 g of $C_3H_5N_3O_9$.

**Storage**. Store protected from light at a temperature between 8° and 15°.

## Glyceryl Trinitrate Tablets

Nitroglycerin Tablets; Trinitrin Tablets

Glyceryl Trinitrate Tablets contain not less than 85.0 per cent and not more than 115.0 per cent of the stated amount of glyceryl trinitrate, $C_3H_5N_3O_9$.

**Usual strengths**. 300 µg; 500 µg; 600 µg.

### Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. *Toluene*.

*Test solution*. Extract a quantity of the powdered tablets containing 0.5 mg of glyceryl trinitrate with 1 ml of *acetone* and centrifuge.

*Reference solution*. Extract one powdered *glyceryl trinitrate tablet 0.5 mg RS* with 1 ml of *acetone* and centrifuge.

Apply to the plate 20 µl of each solution. After development, dry the plate in a stream of air, spray with *diphenylamine solution* and irradiate for 15 minutes with ultraviolet light at 365 nm. Examine the plate in daylight. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.



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B. Extract a quantity of the powdered tablets containing 3 mg of glyceryl trinitrate with 5 ml of *ether* and filter. Evaporate the ether and dissolve the residue in 0.2 ml of *sulphuric acid* containing a trace of *diphenylamine*; an intense blue colour is produced.

### Tests

**Uniformity of content**. Comply with the test stated under Tablets.

Place one tablet in a centrifuge tube containing a few glass beads, add 5 ml of a 90 per cent v/v solution of *glacial acetic acid*, shake for 1 hour and centrifuge. To 1.0 ml of the resulting solution add 2 ml of *phenoldisulphonic acid solution*, mix and allow to stand for 15 minutes. Add 8 ml of *water*, mix well, allow to cool and add slowly, with swirling, 10 ml of *strong ammonia solution*. Cool and dilute to 20.0 ml with *water*. Measure the absorbance of the resulting solution at about 405 nm (2.4.7), using as the blank 1 ml of a 90 per cent v/v solution of *glacial acetic acid* treated in the same manner, beginning at the words "add 2 ml of *phenoldisulphonic acid solution*," Dissolve 0.1335 g of *potassium nitrate* previously dried at 105° in *water* to produce 50.0 ml; to 10.0 ml add sufficient *glacial acetic acid* to produce 100.0 ml. Using 1.0 ml of this solution, repeat the procedure beginning at the words "add 2 ml of *phenoldisulphonic acid solution*,....".

Calculate the content of $C_3H_5N_3O_9$ in the tablet from the values of the absorbances so obtained.

1 ml of the *potassium nitrate solution* is equivalent to 0.0002 g of $C_3H_5N_3O_9$.

*For tablets containing 400 to 600 µg* — Use 1.0 ml of a mixture of equal volumes of the potassium nitrate solution and *glacial acetic acid* in the repeat procedure.

*For tablets containing 200 to 300 µg* — Use 2.0 ml of the resulting solution, prepare the blank with 2.0 ml of a 90 per cent v/v solution of *glacial acetic acid* and use 2.0 ml of a mixture of 3 volumes of *glacial acetic acid* and 1.0 volume of the potassium nitrate solution in the repeat procedure.

*For tablets containing less than 200 µg* — Use 2.0 ml of the resulting solution, measure the absorbance of 2-cm layers, prepare the blank with 2.0 ml of a *90 per cent v/v* solution of *glacial acetic acid* and use 2.0 ml of a mixture of 7 volumes of *glacial acetic acid* and 1.0 volume of the potassium nitrate solution in the repeat procedure.

**Other tests**. Comply with the tests stated under Tablets. The test for Disintegration does not apply.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 1 mg of glyceryl trinitrate, add 5 ml of a 90 per cent v/v solution of *glacial acetic acid*, shake for 1 hour and centrifuge. To 1.0 ml of the resulting solution add 2 ml of *phenoldisulphonic acid solution*, mix and allow to stand for 15 minutes. Add 8 ml of *water*, mix well, allow to cool and add slowly, with swirling, 10 ml of *strong ammonia solution*. Cool and dilute to 20.0 ml with *water*. Measure the absorbance of the resulting solution at about 405 nm (2.4.7), using as the blank 1 ml of a 90 per cent v/v solution of *glacial acetic acid* treated in the same manner, beginning at the words "add 2 ml of *phenoldisulphonic acid solution*," Dissolve 0.1335 g of *potassium nitrate* previously dried at 105° in *water* to produce 50.0 ml; to 10.0 ml add sufficient *glacial acetic acid* to produce 100.0 ml. Using 1.0 ml of this solution, repeat the procedure beginning at the words "add 2 ml of *phenoldisulphonic acid solution*,".

Calculate the content of $C_3H_5N_3O_9$ from the values of the absorbances so obtained.

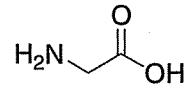
1 ml of the *potassium nitrate solution* is equivalent to 0.0002 g of $C_3H_5N_3O_9$.

**Storage**. Store protected from light and moisture in glass containers of not more than 100 tablets, at a temperature not exceeding 30°. The container should be closed by means of a screw cap lined with aluminium or tin foil. Cotton wool wadding or other additional packing that absorbs glyceryl trinitrate should be avoided.

**Labelling**. The label states that the tablets should be allowed to dissolve slowly in the mouth.

## Glycine

Aminoacetic acid



$C_2H_5NO_2$ Mol. Wt. 75.1

Glycine is 2-aminoethanoic acid.

Glycine contains not less than 98.5 per cent and not more than 101.5 per cent of $C_2H_5NO_2$, calculated on the dried basis.

**Category**. Bladder irrigation agent; pharmaceutical aid.

**Description**. A white, crystalline powder; odourless.

### Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *glycine RS*. Examine the substances as discs prepared using about 1 mg for 0.4 g of *potassium bromide IR*.

B. Dissolve 50 mg in 5 ml of *water*, add 1 ml of *sodium hypochlorite solution (3 per cent Cl)*, boil for 2 minutes, add



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1 ml of *hydrochloric acid* and boil for 4 to 5 minutes. To the resulting solution add 2 ml of *hydrochloric acid* and 1 ml of a 2 per cent w/v solution of *resorcinol*, boil for 1 minute, cool, add 10 ml of *water* and mix. To 5 ml of this solution add 6 ml of *2 M sodium hydroxide*. The resulting solution is violet with a greenish yellow fluorescence. After a few minutes the solution becomes orange and then yellow and the intense fluorescence remains.

### Tests

**Appearance of solution**. A 10.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and not more intensely coloured than reference solution YS7 (2.4.1).

**pH** (2.4.24). 5.9 to 6.3, determined in a 5.0 per cent w/v solution.

**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals, Method A (10 ppm).

**Chlorides** (2.3.12). 2.5 g dissolved in 20 ml of *water* complies with the limit test for chlorides (100 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 2 hours.

**Assay**. Weigh accurately about 0.15 g and dissolve in 100 ml of *anhydrous glacial acetic acid*. Immediately after dissolution titrate with *0.1 M perchloric acid*, using 0.05 ml of *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.00751 g of $C_2H_5NO_2$.

## Glycine Irrigation Solution

Glycine Irrigation Solution is a sterile solution of Glycine in Water for Injections.

Glycine Irrigation Solution contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of $C_2H_5NO_2$. It contains no antimicrobial agent.

**Usual strength**. 1.5 per cent w/v.

**Description**. A clear, colourless solution.

### Identification

A. Evaporate 5 ml to dryness on a water-bath and dry at 105° for one hour. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *glycine RS*

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 70 volumes of *1-propanol* and 30 volumes of *strong ammonia solution*.

*Test solution*. Dilute a suitable volume of the preparation under examination with *water* so that the resulting solution contains 0.25 per cent w/v of Glycine.

*Reference solution*. A 0.25 per cent w/v solution of *glycine RS*.

Apply to the plate 2 µl of each solution. After development, dry the plate at 105° for 10 minutes, spray with *ninhydrin solution* and heat at 105° for 2 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

### Tests

**pH** (2.4.24). 4.5 to 6.5.

**Bacterial endotoxins** (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

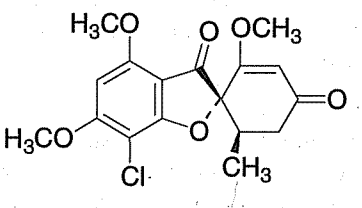
**Assay**. Dilute an accurately measured volume of the preparation under examination containing about 0.15 g of Glycine to 25 ml with *water*. Add 10 ml of *formaldehyde solution*, previously adjusted to a pH of 9.0, and 0.25 ml of a mixed indicator solution prepared by dissolving 75 mg of *phenolphthalein* and 25 mg of *thymol blue* in 100 ml of *ethanol (50 per cent)*. Titrate with *0.1 M sodium hydroxide* until the yellow colour disappears and a faint violet colour appears.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.007507 g of $C_2H_5NO_2$.

**Storage**. Store in single dose containers at a temperature not exceeding 30°.

**Labelling**. The label states (1) Not for Injection; (2) that the solution should not be used if it contains visible particles.

## Griseofulvin




$C_{17}H_{17}ClO_6$ Mol. Wt. 352.8

Griseofulvin is (1′*S*,6′*R*)-7-chloro-2′,4,6-trimethoxy-6′-methylbenzofuran-2-spiro-1-cyclohex-2′-ene-3,4′-dione produced by the growth of certain strains of *Penicillium griseofulvum* or obtained by any other means.



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Griseofulvin contains not less than 97.0 per cent and not more than 102.0 per cent of $C_{17}H_{17}ClO_6$, calculated on the dried basis.

**Category**. Antifungal.

**Dose**. 500 mg to 1 g daily, in divided doses.

**Description**. A white to yellowish white powder, the particles of which are generally upto 5 µm in maximum dimension, although larger particles, which may occasionally exceed 30 µm may be present; almost odourless.

### Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *griseofulvin RS*.

B. Dissolve about 5 mg in 1 ml of *sulphuric acid* and add 5 mg of powdered *potassium dichromate;* a wine-red colour is produced.

C. Melting range (2.4.21). 217° to 224°.

### Tests

**Appearance of solution**. A 7.5 per cent w/v solution in *dimethylformamide* is clear (2.4.1), and not more intensely coloured than reference solution YS4 (2.4.1).

**Acidity**. Suspend 0.25 g in 20 ml of *ethanol (95 per cent)* and titrate with *0.2 M sodium hydroxide* using *phenolphthalein solution* as indicator; not more than 1.0 ml is required to change the colour of the solution.

**Specific optical rotation** (2.4.22). +352° to +364°, determined at 20° in a 1.0 per cent w/v solution in *dimethylformamide*.

**Related substances**. Determine by gas chromatography (2.4.13).

*Test solution (a)*. Dissolve 1.0 g of the substance under examination in 100 ml of *acetone*.

*Test solution (b)*. Dissolve 1.0 g of the substance under examination and 20 mg of *9,10-diphenylanthracene* (internal standard) in 100 ml of *acetone*.

*Reference solution*. A solution containing 0.050 per cent w/v of *griseofulvin RS* and 0.020 per cent w/v of the internal standard in *acetone*.

Chromatographic system

- a glass column 1.0 m x 4 mm, packed with acid-washed diatomaceous support (100 to 200 mesh) coated with 1 per cent w/w of cyanopropylmethyl phenyl silicone fluid,
- temperature:
  column. 250°,
  inlet port and detector. 270°,
- flow rate. 60 ml per minute of the carrier gas.

Continue the chromatography for three times the retention time of griseofulvin.

The chromatogram obtained with test solution (a) shows a peak due to griseofulvin (retention time about 11 minutes) and may show a peak due to dechlorogriseofulvin (retention time about 0.6 times that of griseofulvin) and a peak due to dehydrogriseofulvin (retention time about 1.4 times that of griseofulvin).

Calculate the ratio (r) of the area of the peak due to griseofulvin to that of the peak due to the internal standard in the chromatogram obtained with the reference solution. The ratio of the area of any peak corresponding to dechlorogriseofulvin to that of the peak due to the internal standard in the chromatogram obtained with test solution (b) is less than 0.6r. The ratio of the area of any peak corresponding to dehydrogriseofulvin to that of the peak due to the internal standard in the chromatogram obtained with test solution (b) is less than 0.15r.

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

**Matter soluble in light petroleum**. Not more than 0.2 per cent, determined by the following method. Extract 1 g with 20 ml of *light petroleum (40° to 60°)* by boiling under a reflux condenser for 10 minutes; cool, filter, wash the filter with three quantities, each of 15 ml, of the *light petroleum (40° to 60°)*, evaporate the combined filtrate and washings to dryness, dry the residue at 105° for 1 hour and weigh.

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 80 mg and dissolve in sufficient *ethanol* to produce 200.0 ml. Dilute 2.0 ml to 100.0 ml with *ethanol* and measure the absorbance of the resulting solution at the maximum at about 291 nm (2.4.7). Calculate the content of $C_{17}H_{17}ClO_6$ taking 686 as the specific absorbance at 291 nm.

## Griseofulvin Tablets

Griseofulvin Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of griseofulvin, $C_{17}H_{17}ClO_6$.

**Usual strengths**. 125 mg; 500 mg.

### Identification

A. Extract a quantity of the powdered tablets containing 0.125 g of Griseofulvin with 20 ml of *chloroform*, add 1 g of *anhydrous sodium sulphate*, shake and filter. Evaporate the filtrate to dryness and dry at a pressure not exceeding 0.7 kPa for 1 hour. The residue complies with the following test.



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Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *griseofulvin RS*.

B. Shake a quantity of the powdered tablets containing 80 mg of Griseofulvin with 150 ml of *ethanol (95 per cent)* for 20 minutes. Dilute to 200 ml with *ethanol (95 per cent)* and filter. Dilute 2 ml of the filtrate to 100 ml with *ethanol (95 per cent)*. When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows absorption maxima at about 291 nm and 325 nm, and a shoulder at about 250 nm.

C. Dissolve about 5 mg of the powdered tablets in 1 ml of *sulphuric acid* and add 5 mg of powdered *potassium dichromate;* a wine-red colour is produced.

## Tests

**Related substances**. Determine by gas chromatography (2.4.13).

*Test solution (a)*. Add 60 ml of *chloroform* to a quantity of the powdered tablets containing 50 mg of Griseofulvin, heat at 60° with shaking for 20 minutes, cool and dilute to 100 ml with *chloroform*. Centrifuge and evaporate 20 ml of the clear supernatant liquid to about 1 ml.

*Test solution (b)*. Prepare in the same manner as test solution (a) but adding 1 ml of a 0.1 per cent w/v solution of *9,10-diphenylanthracene* (internal standard) in *chloroform* before diluting to 100 ml with *chloroform*.

*Reference solution*. Dissolve 5 mg of *griseofulvin RS* in *chloroform* and add 2 ml of the internal standard solution and sufficient *chloroform* to produce 200 ml. Evaporate 20 ml of the solution to about 1 ml.

Chromatographic system

- a glass column 1.0 m x 4 mm, packed with acid-washed diatomaceous support (100 to 200 mesh) coated with 1 per cent w/w of cyanopropylmethyl phenyl silicone fluid,
- temperature:
  column. 250°,
  inlet port and detector. 270°,
- flow rate. 60 ml per minute of the carrier gas.

Continue the chromatography for three times the retention time of griseofulvin.

The chromatogram obtained with test solution (a) shows a peak due to griseofulvin (retention time about 11 minutes) and may show a peak due to dechlorogriseofulvin (retention time about 0.6 times that of griseofulvin) and a peak due to dehydrogriseofulvin (retention time about 1.4 times that of griseofulvin).

Calculate the ratio (r) of the area of the peak due to griseofulvin to that of the peak due to the internal standard in the chromatogram obtained with the reference solution. The ratio of the area of any peak corresponding to dechlorogriseofulvin to that of the peak due to the internal standard in the chromatogram obtained with test solution (b) is less than 0.6r. The ratio of the area of any peak corresponding to dehydrogriseofulvin to that of the peak due to the internal standard in the chromatogram obtained with test solution (b) is less than 0.15r.

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of a 4.0 per cent w/v solution of *sodium lauryl sulphate,*
Speed and time. 100 rpm and 60 minutes.

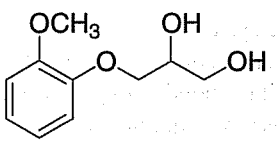
Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted with *methanol (80 per cent)*, at the maximum at about 291 nm (2.4.7). Calculate the content of $C_{17}H_{17}ClO_6$, taking 725 as the specific absorbance at the maximum at about 291 nm.

D. Not less than 70 per cent of the stated amount of $C_{17}H_{17}ClO_6$.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 35 mg of Griseofulvin, add 60 ml of *ethyl acetate*. Centrifuge and transfer two quantities, each of 5 ml, of the clear supernatant liquid into separate 100-ml volumetric flasks. Add 5 ml of *2 M methanolic methanesulphonic acid* to the first flask, allow to stand at 20° for 30 minutes and dilute to 100.0 ml with *methanol* (solution A). Dilute the contents of the second flask to 100.0 ml with *methanol* (solution B). To a third volumetric flask add 5 ml of *2 M methanolic methanesulphonic acid* and dilute to 100.0 ml with *methanol* (solution C). Measure the absorbance of each solution at the maximum at about 266 nm (2.4.7). Calculate the content of $C_{17}H_{17}ClO_6$ from the difference between the absorbance obtained with solution A and the sum of the absorbances obtained with solutions B and C and from the difference obtained by repeating the experiment using 35 mg of *griseofulvin RS* in place of the powdered tablets.

# Guaiphenesin




$C_{10}H_{14}O_4$ Mol. Wt. 198.2

Guaiphenesin is (*RS*)-3-(2-methoxyphenoxy)propane-1,2-diol.



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Guaiphenesin contains not less than 98.0 per cent and not more than 101.5 per cent of $C_{10}H_{14}O_4$, calculated on the dried basis.

**Category**. Expectorant.

**Dose**. For an adult, 200 to 400 mg every four hours; for a child aged 6 to 12 years, 100 to 200 mg every four hours; for a child aged 2 to 6 years, 50 to 100 mg every four hours.

**Description**. A white or almost white, crystalline powder; odourless or with a slight characteristic odour.

## Identification

*Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.*

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

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

C. Melts at 79° to 83° (2.4.21).

## Tests

**Appearance of solution**. A 2.0 per cent w/v solution is clear (2.4.1), and colourless (2.4.1).

**pH** (2.4.24). 5.0 to 7.0, determined in a 1.0 per cent w/v solution.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve 100 mg of the substance under examination in 50.0 ml of *acetonitrile.*

*Reference solution (a).* Dilute 1.0 ml of the test solution to 20.0 ml with *acetonitrile*. Dilute 1.0 ml of this solution to 10.0 ml with *acetonitrile.*

*Reference solution (b).* Dissolve 10 mg of *guaiacol* in 50.0 ml of *acetonitrile*. Dilute 5.0 ml of this solution to 50.0 ml with *acetonitrile.*

*Reference solution (c).* Dissolve 50 mg of *guaiacol* in 50.0 ml of *acetonitrile*. Dilute 5.0 ml of this solution to 50.0 ml with *acetonitrile.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 1 volume of *glacial acetic acid* and 99 volumes of *water*,
  B. *acetonitrile*,
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 276 nm,
- injection volume. 10 µl.

| Time (in min.) | Mobile phase A ( per cent v/v) | Mobile phase B ( per cent v/v) |
|---|---|---|
| 0-32 | 80-50 | 20-50 |
| 32-33 | 50-80 | 50-20 |
| 33-40 | 80 | 20 |

Inject reference solution (c) . The test is not valid unless the resolution between the peaks due to guaiphenesin and guaiphenesin impurity A is not less than 3.0. The relative retention time with reference to guaiphensin for 2-(2-methoxyphenoxy)propane-1,3-diol(B-isomer) (guaiphenesin impurity B) is about 0.9, for 2-methoxyphenol (guaiacol) (guaiphenesin impurity A) is about 1.4, for 1,1'-oxybis[3-(2-methoxyphenoxy)propan-2-ol] (bisether) (guaiphenesin impurity C) is about 3.1, for 1,3-bis(2-methoxyphenoxy)propan-2-ol (guaiphenesin impurity D) is about 3.7.

Inject the test solution and the reference solution. In the chromatogram obtained with the test solution the area of secondary peak corresponding to guaiphenesin impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent), the area of secondary peak corresponding to guaiphenesin impurity B is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). The sum of area of all the secondary peaks other than guaiphenesin impurity B is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

**Heavy metals** (2.3.13). 12 ml of a solution prepared by dissolving 2.0 g in 25 ml of a mixture of 9 volumes of *ethanol (95 per cent)* and 1 volume of *water* complies with the limit test for heavy metals, Method D (25 ppm).

**Chlorides and monochlorohydrins**. To 10 ml of a 2.0 per cent w/v solution, add 2 ml of *2 M sodium hydroxide,* heat on a water-bath for 5 minutes, cool and add 3 ml of *2 M nitric acid.* The resulting solution complies with the limit test for chlorides (2.3.12) using 2.0 ml of *chloride standard solution (25 ppm Cl)*(250 ppm).

**Guaiacol**. To 10 ml of a 2.0 per cent w/v solution add 0.1 ml of *ferric chloride test solution* and allow to stand for 5 minutes. The resulting solution is not more intensely coloured than a mixture of 0.5 ml of CSS, 1.5 ml of FCS, 3.5 ml of CCS and 4.5 ml of a solution of *hydrochloric acid* containing 1 per cent w/v of HCl (2.4.1).



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**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 60° over *phosphorus pentoxide* at a pressure of 1.5 to 2.5 kPa for 3 hours.

**Assay**. Weigh accurately about 50 mg and dissolve in 10 ml of *water*. Add 20 ml of *sodium periodate solution* and allow to stand for 10 minutes. Add 25.0 ml of *sodium arsenite solution* and 1 ml of a 16.6 per cent w/v solution of *potassium iodide*, allow to stand for 10 minutes and titrate with *0.05 M iodine* using 2 ml of *starch solution* as indicator. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of iodine required.

1 ml of *0.05 M iodine* is equivalent to 0.009911 g of $C_{10}H_{14}O_4$.



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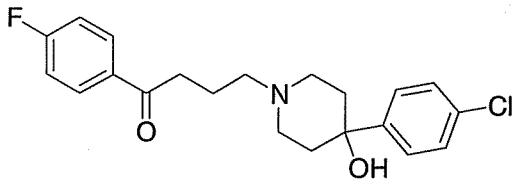


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# Haloperidol




$C_{21}H_{23}ClFNO_2$ Mol. Wt. 375.9

Haloperidol is 4-[4-(4-chlorophenyl)-4-hydroxypiperidino]-4′-fluorobutyrophenone.

Haloperidol contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{21}H_{23}ClFNO_2$, calculated on the dried basis.

**Category**. Antipsychotic.

**Dose**. Orally, 1.5 to 20 mg daily, in divided doses; by intramuscular or intravenous injection, 2 to10 mg.

**Description**. A white to faintly yellowish, amorphous or microcrystalline powder; odourless.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

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

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.0015 per cent w/v solution in a mixture of 90 volumes of *methanol* and 10 volumes of *0.1 M hydrochloric acid* shows an absorption maximum at about 245 nm; absorbance at about 245 nm, about 0.49 to 0.53.

C. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

D. Determine by the oxygen-flask method (2.3.34), using 20 mg of the substance under examination and 5 ml of *1.25 M sodium hydroxide* as the absorbing liquid. When the process is complete, dilute to 10 ml with *water*; the resulting solution complies with the following tests.

(a) Add 0.1 ml to a mixture of 0.1 ml of a freshly prepared *alizarin red S solution* and 0.1 ml of *zirconyl nitrate solution*; the red colour becomes clear yellow.

(b) Acidify 5 ml with *0.5 M sulphuric acid*; the solution gives the reactions of chlorides (2.3.1).

## Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 80 volumes of *chloroform*, 10 volumes of *glacial acetic acid* and 10 volumes of *methanol*.

*Test solution (a)*. Dissolve 0.1 g of the substance under examination in 10 ml of *chloroform*.

*Test solution (b)*. Dilute 5 ml of test solution (a) to 50 ml with *chloroform*.

*Reference solution (a)*. A solution containing 0.005 per cent w/v of the substance under examination in *chloroform*.

*Reference solution (b)*. A 0.1 per cent w/v solution of *haloperidol RS* in *chloroform*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with *dilute potassium iodobismuthate solution*. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (a) shows a distinct and clearly visible spot.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 0.1 kPa for 3 hours.

**Assay**. Weigh accurately about 0.2 g and dissolve in 25 ml of *anhydrous glacial acetic acid*. Titrate with *0.05 M perchloric acid*, using 0.2 ml of *1-naphtholbenzein solution* as indicator and titrating until the colour changes from orange-yellow to yellowish green. Carry out a blank titration.

1 ml of *0.05 M perchloric acid* is equivalent to 0.01879 g $C_{21}H_{23}ClFNO_2$.

**Storage**. Store protected from light.

# Haloperidol Injection

Haloperidol Injection is a sterile solution of Haloperidol in Lactic Acid diluted with Water for Injections.

Haloperidol Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of haloperidol, $C_{21}H_{23}ClFNO_2$.

**Usual strengths**. 5 mg per ml; 10 mg per ml.

## Identification

A. To a volume of the injection containing 20 mg of Haloperidol add 5 ml of *water* and 1 ml of *1 M sodium hydroxide* and



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extract with 10 ml of *chloroform*. Filter the chloroform extract through absorbent cotton, evaporate the filtrate to dryness and dry the residue at 60° at a pressure not exceeding 0.7 kPa. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *haloperidol RS* or with the reference spectrum of haloperidol.

B. When examined in the range 230 nm to 360 nm (2.4.7), the final solution obtained in the Assay shows an absorption maximum only at about 245 nm.

### Tests

**pH** (2.4.24). 2.8 to 3.6.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 80 volumes of *chloroform*, 10 volumes of *glacial acetic acid* and 10 volumes of *methanol*.

*Test solution*. The injection under examination.

*Reference solution (a)*. Dilute 1 volume of the injection to 100 volumes with *methanol*.

*Reference solution (b)*. Dilute 1 volume of the injection to 200 volumes with *methanol*.

Apply to the plate a volume of the injection containing 0.1 mg of Haloperidol and the same volume of the reference solutions. After development, dry the plate in air and spray with *dilute potassium iodobismuthate solution*. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. To an accurately measured volume of the injection containing about 10 mg of Haloperidol add 8 ml of *water* and 10 ml of *1 M hydrochloric acid*. Extract with successive quantities of 25, 25, 10 and 10 ml of *ether*. Wash the combined ether extracts with 10 ml of *water*, combine the aqueous layers and remove the ether using a rotary evaporator. Add sufficient *water* to produce 100.0 ml and dilute 10.0 ml to 100.0 ml with *methanol*. Measure the absorbance of the resulting solution at the maximum at about 245 nm (2.4.7). Calculate the content of $C_{21}H_{23}ClFNO_2$ taking 346 as the specific absorbance at 245 nm.

**Storage**. Store protected from light.

## Haloperidol Oral Solution

Haloperidol Oral Drops; Haloperidol Solution

Haloperidol Oral Solution is a solution of Haloperidol in Purified Water prepared with the aid of Lactic Acid.

Haloperidol Oral Solution contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of haloperidol, $C_{21}H_{23}ClFNO_2$.

**Usual strengths**. 1 mg per ml; 2 mg per ml.

**Description**. A clear, colourless solution.

### Identification

A. To a volume of the oral solution containing 20 mg of Haloperidol, add 1 ml of *1 M sodium hydroxide*, extract with 10 ml of *chloroform*, filter and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *haloperidol RS* treated in the same manner or with the reference spectrum of haloperidol.

B. When examined in the range 230 nm to 360 nm (2.4.7), the final solution obtained in the Assay shows an absorption maximum only at about 245 nm.

### Tests

**pH** (2.4.24). 3.5 to 4.5.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 92 volumes of *dichloromethane*, 8 volumes of *methanol* and 1 volume of *strong ammonia solution*.

*Test solution*. Dilute the oral solution if necessary with *methanol* to contain 0.1 per cent w/v of Haloperidol.

*Reference solution (a)*. Dilute 1 volume of the test solution to 100 volumes with *methanol*.

*Reference solution (b)*. Dilute 1 volume of the test solution to 200 volumes with *methanol*.

Apply to the plate 50 µl of each solution. After development, dry the plate in air and spray with *dilute potassium iodobismuthate solution*. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

**Other tests**. Complies with the tests stated under Oral Liquids.



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**Assay**. To an accurately measured volume of the oral solution containing about 10 mg of Haloperidol add 8 ml of *water* and 10 ml of *1 M hydrochloric acid*. Extract with successive quantities of 25, 25, 10 and 10 ml of *ether*. Wash the combined ether extracts with 10 ml of *water*, combine the aqueous layers and remove the ether using a rotary evaporator. Add sufficient *water* to produce 100.0 ml and dilute 10.0 ml to 100.0 ml with *methanol*. Measure the absorbance of the resulting solution at the maximum at about 245 nm (2.4.7). Calculate the content of $C_{21}H_{23}ClFNO_2$ taking 346 as the specific absorbance at 245 nm.

**Storage**. Store protected from light at a temperature between 15° and 25°.

# Haloperidol Tablets

Haloperidol Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of haloperidol, $C_{21}H_{23}ClFNO_2$.

**Usual strengths**. 1.5 mg; 5 mg; 10 mg.

## Identification

A. To a quantity of the powdered tablets containing 10 mg of Haloperidol add 5 ml of *water* and 1 ml of *1 M sodium hydroxide* and extract with 10 ml of *chloroform*. Filter the chloroform extract through absorbent cotton, evaporate the filtrate to dryness and dry the residue at 60° at a pressure not exceeding 0.7 kPa. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *haloperidol RS* or with the reference spectrum of haloperidol.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

## Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 80 volumes of *chloroform*, 10 volumes of *glacial acetic acid* and 10 volumes of *methanol*.

*Test solution (a)*. Shake a quantity of the powdered tablets containing 10 mg of Haloperidol with 10 ml of *chloroform*, filter, evaporate the filtrate to dryness and dissolve the residue in 1 ml of *chloroform*.

*Test solution (b)*. Dilute 1 volume of test solution (a) to 10 volumes with *chloroform*.

*Reference solution (a)*. Dilute 1 volume of test solution (a) to 200 volumes with *chloroform*.

*Reference solution (b)*. A 0.1 per cent w/v solution of *haloperidol RS* in *chloroform*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with *dilute potassium iodobismuthate solution*. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (a) shows a distinct and clearly visible spot.

**Uniformity of content**. Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14).

*Test solution*. Place one tablet in 10 ml of the mobile phase, shake in an ultrasonic bath for 2 minutes, centrifuge and use the supernatant liquid after diluting suitably with the mobile phase if necessary.

*Reference solution*. A solution containing 0.015 per cent w/v of *haloperidol RS* in the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 5 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 55 volumes of a 1 per cent w/v solution of *ammonium acetate* and 45 volumes of *acetonitrile*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 247 nm,
- injection volume. 20 µl.

Calculate the content of $C_{21}H_{23}ClFNO_2$ in the tablet.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. *For tablets containing more than 2 mg* — Weigh and powder 20 tablets. On the powder determine by liquid chromatography (2.4.14).

*Test solution*. Weigh accurately a quantity of the powder containing about 20 mg of Haloperidol, shake with 60 ml of the mobile phase, place in an ultrasonic bath for 2 minutes, add sufficient quantity of the mobile phase to produce 100.0 ml. Centrifuge and use the supernatant liquid.

*Reference solution*. A solution containing 0.02 per cent w/v of *haloperidol RS* in the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 5 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 55 volumes of a 1 per cent w/v solution of *ammonium acetate* and 45 volumes of *acetonitrile*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 247 nm,
- injection volume. 20 µl.

Calculate the content of $C_{21}H_{23}ClFNO_2$ in the tablets.



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*For tablets containing 2 mg or less* — Use the average of the 10 individual results obtained in the test for Uniformity of content.

**Storage**. Store protected from light.

## Heparin Sodium

Heparin Sodium is the sodium salt of sulphated glycosaminoglycans present as a mixture of heterogeneous molecules varying in molecular weights. It is present in mammalian tissues and is usually obtained from the intestinal mucosa or other suitable tissues of domestic mammals used for food by man. The sourcing of heparin material must be specified in compliance with applicable regulatory requirements. It is purified to retain a combination of activities against different fractions of the blood clotting sequence. It is composed of polymers of alternating derivatives of D-glucosamine (N-sulphated, O-sulphated, or N-acetylated) and uronic acid, L-iduronic acid or D-glucuronic acid) joined by glycosidic linkages. The component activities of the mixture are in ratios corresponding to those shown by the Heparin Sodium reference standard. Some of these components have the property of prolonging the clotting time of blood. This occurs mainly through the formation of a complex of each component with the plasma proteins antithrombin III and heparin cofactor II to potentiate the inactivation of thrombin. Other coagulation proteases in the clotting sequence, such as activated factor X, are also inhibited.

Heparin Sodium intended for use in the manufacture of parenteral preparation contains not less than 150 IU per mg and heparin sodium not intended for the use in the parenteral preparation contains not less than 120 IU per mg, calculated on the dried basis and not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of heparin sodium.

**Category.** Anticoagulant.

**Description.** A white or grayish-white powder; odourless; moderately hygroscopic.

**Identification**

A. It delays the clotting of freshly shed blood.

B. Gives the test A for Sodium (2.3.1).

C. In the test for Oversulphated chondroitin sulphate, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

**Tests**

pH (2.4.24). 5.0 to 7.5, determined in 1.0 per cent w/v solution.

Oversulphated Chondroitin Sulphate (OSCS). Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve 500 mg of the substance under examination in 10.0 ml of water.

*Reference solution (a).* A 2.0 per cent w/v solution of heparin sodium RS in water.

*Reference solution (b).* A 0.02 per cent w/v solution of oversulphated chondroitin RS in reference solution (a).

Chromatographic system

- a stainless steel column 25 cm x 2 mm, packed with a hydroxide- selective, strong anion-exchange resin consisting of a highly cross-linked core of 13 µm micoporous particles having a pore size less than 10 Angstrom units and consisting of ethylvinyl benzene cross-linked with 55 per cent divinylbenzene with a latex coating composed of 85 nm diameter microbeads bonded with alkanol quaternary ammonium ions (6 per cent) (Such as Dionex Ion pac- AS11),
- column temperature. 40°,
- mobile phase: A. a 0.04 per cent w/v solution of sodium dihydrogen phosphate dihydrate in water, adjusted to pH 3.0 with phosphoric acid, filter,
  B. a 14.0 per cent w/v solution of sodium perchlorate in mobile phase A, adjusted to pH 3.0 with phosphoric acid, filter,
- a linear gradient programme using the conditions given below,
- flow rate. 0.22 ml per minute,
- spectrophotometer set at 202 nm,
- injection volume. 10 µl.

| Time (in min) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0 | 80 | 20 |
| 60 | 10 | 90 |
| 61 | 80 | 20 |
| 75 | 80 | 20 |

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to oversulphated chondroitin sulphate and heparin is not less than 1.5. The retention time of heparin is about 30 minutes and of oversulphated chondroitin sulphate is about 50 minutes.

Inject the test solution and reference solution (a). The retention time of the principal peak obtained from the test solution corresponds to the peak obtained from reference solution (a). In the chromatogram obtained with the test solution, no peak corresponding to OSCS is observed.

**Heavy metals** (2.3.13). 0.5 g complies with the limit test for heavy metals, Method B (40 ppm).

**Protein and nucleotidic impurities.** Absorbance of a 0.4 per cent w/v solution at about 260 nm (2.4.7) (for nucleotides) and about 280 nm (for proteins) is not more than 0.2 and 0.15 respectively.

**Nitrogen** (2.3.30). 1.3 to 2.5 per cent, calculated on the dried basis.



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Sulphated ash (2.3.18). 28.0 to 41.0 per cent, determined on 0.2 g.

**Loss on drying** (2.4.19). Not more than 8.0 per cent, determined on 1.0 g by drying in an oven over phosphorous pentaoxide at 60° at a pressure not exceeding 0.7 kPa for 3 hours.

**Assay**. Determine the potency of heparin sodium by comparing the concentration necessary to prevent the clotting of sheep or goat or human plasma with the concentration of the reference solution of heparin sodium necessary to give the same effect under the condition of the following method of assay.

*Test solution*. Dissolve 25 mg of substance under examination, in sufficient saline to produce a concentration of 1 mg per ml, and dilute to a concentration estimated to correspond to that of the reference solution.

*Reference solution*. Determine by preliminary trial, if necessary, approximately the minimum quantity of heparin sodium RS which, when added in 0.8 ml of saline, maintains fluidity in 1 ml of prepared plasma for 1 hour after the addition of 0.2 ml of calcium chloride (1 in 100). This quantity is usually between 1 and 3 Heparin Units. On the day of the assay prepare a reference solution such that it contains, in each 0.8 ml of saline, the above-determined quantity of the reference standard.

*Preparation of plasma*. Collect blood from sheep directly into a vessel containing about 8 per cent of sodium citrate in the proportion of one volume to each 19 volumes of blood to be collected. Mix immediately by gentle agitation and inversion of the vessel. Promptly centrifuge the blood, and pool the separated plasma. To a 1 ml portion of the pooled plasma in a clean test tube add 0.2 ml of calcium chloride (1 in 100), and mix. Consider the plasma suitable for use if a solid clot forms within 5 minutes. To store plasma for future use, subdivide the pooled lot into portions not exceeding 100 ml in volume, and store in the frozen state, preventing even partial thawing prior to use. For use in the assay, thaw the frozen plasma in a water-bath at a temperature not exceeding 37°. Remove particulate matter by straining the thawed plasma through a coarse filter.

*Procedure*. To meticulously clean 13 mm × 100 mm test tubes add graded amounts of the reference solution selecting the amount so that the largest does not exceed 0.8 ml and so that they correspond roughly to a geometric series in which each step is approximately 5 per cent greater than the next lower. To each tube so prepared add sufficient saline to make the total volume 0.8 ml. Add 1.0 ml of prepared plasma to each tube. Then add 0.2 ml of calcium chloride (1 in 100), note the time, immediately insert a suitable stopper in each tube, and mix the contents by inverting three times in such a way that the entire inner surface of the tube is wet.

In the same manner set up a series using the test solution, completing the entire process of preparing and mixing the tubes of both the reference solution and the test solution within 20 minutes after the addition of the prepared plasma. One hour, accurately timed, after the addition of the calcium chloride, determine the extent of clotting in each tube, recognizing three grades (0.25, 0.50, and 0.75) between zero and full clotting (1.0). If the series does not contain 2 tubes graded more than 0.5 and 2 tubes graded less than 0.5, repeat the assay, using appropriately modified reference solution and test solution.

Convert to logarithms the volumes of reference solution used in the successive 5 or 6 tubes that bracket a grade of clotting of 0.5, including at least 2 tubes with a larger and 2 tubes with a smaller grade than 0.5. Number and list the tubes serially, and tabulate for each the grade of clotting observed in each tube. From the log-volumes, x, and separately from their corresponding grades of clotting, y, compute the paired averages xi and yi of Tubes 1, 2, and 3, of Tubes 2, 3, and 4, of Tubes 3, 4, and 5, and, where the series consists of 6 tubes, of Tubes 4, 5, and 6, respectively. If for one of these paired averages the average grade, yi, is exactly 0.50, the corresponding xi is the median log-volume of the reference solution xS. Otherwise, interpolate xS from the paired values of yi, xi and yi+1, xi+1 that fall immediately below and above grade 0.5 as

$$xS = xi + (yi\ 0.5)(xi+1\ xi) / (yi\ yi+1)$$

From the paired data on the tubes of the test solution, compute similarly its median log-volume xU.

The log potency of the test solution is

$$M = xS\ xU + \log R$$

where R = vS /vU is the ratio of the heparin Units (vS) per ml of the reference solution to the mg (vU) of heparin sodium per ml of the test solution.

Heparin Sodium intended for use in the manufacture of parenteral preparation without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

**Bacterial endotoxins** (2.2.3). Not more than 0.03 Endotoxin Unit per Unit of heparin.

Heparin Sodium intended for use in the manufacture of parenteral preparation without a further appropriate sterilization procedure complies with the following additional requirement.

**Sterility** (2.2.11). Complies with the test for sterility.

**Storage**. Store protected from moisture in tightly-closed containers, sealed so as to exclude micro-organisms.

**Labelling**. The label states to indicate the tissue and the animal species from which it is derived, and the number of International Units per miligram.



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# Heparin Injection

Heparin Sodium Injection

Heparin Injection is a sterile solution of Heparin Sodium in Water for Injection. The pH of the solution may be adjusted by the addition of a suitable alkali or acid.

Heparin Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated potency in terms of IU per ml.

**Usual strengths**. 1000 Units per ml; 5000 Units per ml; 10000 Units per ml.

**Description**. A clear, colourless or straw coloured solution, free from turbidity and matter which deposit on standing.

## Identification

A.It delays the clotting of freshly shed blood.

B. Gives reaction A of sodium salts (2.3.1).

## Tests

**pH** (2.4.24). 5.0 to 7.5.

**Bacterial endotoxins** (2.2.3). Not more than 0.03 Endotoxin Unit per Unit of heparin.

**Sterility** (2.2.11). Complies with the test for sterility.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. Determine the potency of heparin sodium by comparing the concentration necessary to prevent the clotting of sheep or goat or human plasma with the concentration of the reference solution of heparin sodium necessary to give the same effect under the condition of the following method of assay.

*Test solution.* Dissolve an accurate weighed quantity containing about 25 mg of heparin sodium, in sufficient saline to produce a concentration of 1 mg per ml, and dilute quantitatively to a concentration estimated to correspond to that of the reference solution.

*Reference solution.* Determine by preliminary trial, if necessary, approximately the minimum quantity of *heparin sodium RS* which, when added in 0.8 ml of saline, maintains fluidity in 1 ml of prepared plasma for 1 hour after the addition of 0.2 ml of *calcium chloride* (1 in 100). This quantity is usually between 1 and 3 Heparin Units. On the day of the assay prepare a reference solution such that it contains, in each 0.8 ml of saline, the above-determined quantity of the reference standard.

*Preparation of plasma.* Collect blood from sheep directly into a vessel containing about 8 per cent of *sodium citrate* in the proportion of one volume to each 19 volumes of blood to be collected. Mix immediately by gentle agitation and inversion of the vessel. Promptly centrifuge the blood, and pool the separated plasma. To a 1 ml portion of the pooled plasma in a clean test tube, add 0.2 ml of *calcium chloride* (1 in 100), and mix. Consider the plasma suitable for use if a solid clot forms within 5 minutes. To store plasma for future use, subdivide the pooled lot into portions not exceeding 100 ml in volume, and store in the frozen state, preventing even partial thawing prior to use. For use in the assay, thaw the frozen plasma in a water bath at a temperature not exceeding 37°. Remove particulate matter by straining the thawed plasma through a coarse filter.

*Procedure.* To meticulously clean 13 mm × 100 mm test tubes add graded amounts of the reference solution selecting the amount so that the largest does not exceed 0.8 ml and so that they correspond roughly to a geometric series in which each step is approximately 5 per cent greater than the next lower. To each tube so prepared add sufficient saline to make the total volume 0.8 ml. Add 1.0 ml of prepared plasma to each tube. Then add 0.2 ml of *calcium chloride* (1 in 100), note the time, immediately insert a suitable stopper in each tube, and mix the contents by inverting three times in such a way that the entire inner surface of the tube is wet.

In the same manner set up a series using the test solution, completing the entire process of preparing and mixing the tubes of both the reference solution and the test solution within 20 minutes after the addition of the prepared plasma. One hour, accurately timed, after the addition of the *calcium chloride*, determine the extent of clotting in each tube, recognizing three grades (0.25, 0.50, and 0.75) between zero and full clotting (1.0). If the series does not contain 2 tubes graded more than 0.5 and 2 tubes graded less than 0.5, repeat the assay, using appropriately modified reference solution and test solution.

Convert to logarithms the volumes of reference solution used in the successive 5 or 6 tubes that bracket a grade of clotting of 0.5, including at least 2 tubes with a larger and 2 tube with a smaller grade than 0.5. Number and list the tubes serially, and tabulate for each the grade of clotting observed in each tube. From the log-volumes, $x$, and separately from their corresponding grades of clotting, $y$, compute the paired averages $xi$ and $yi$ of Tubes 1, 2, and 3, of Tubes 2, 3, and 4, of Tubes 3, 4, and 5, and, where the series consists of 6 tubes, of Tubes 4, 5, and 6, respectively. If for one of these paired averages the average grade, $yi$, is exactly 0.50, the corresponding $xi$ is the median log-volume of the reference solution $xS$. Otherwise, interpolate $xS$ from the paired values of $yi$, $xi$ and $yi+1$, $xi+1$ that fall immediately below and above grade 0.5 as

$$xS = xi + (yi\ 0.5)(xi+1\ xi) / (yi\ yi+1)$$

From the paired data on the tubes of the test solution, compute similarly its median log-volume $xU$.

The log potency of the test solution is



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$$M = xS - xU + \log R$$

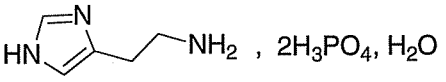
Where $R = vS/vU$ is the ratio of the heparin Units ($vS$) per ml of the reference solution to the mg ($vU$) of heparin sodium per ml of the test solution.

**Storage**. Store at a temperature not exceeding 30°.

**Labelling**. The label states to indicate the volume of the total contents and the potency in terms of Heparin Units per ml, except that single-dose containers may be labeled additionally to indicate the single unit-dose volume and the total number of Heparin Units. Where it is labeled with total content, the label states also that the entire contents are to be used or, if not, any remaining portion is to be discarded. Label it to indicate also the tissue and the animal species from which it is derived.

# Histamine Phosphate

Histamine Acid Phosphate



$C_5H_9N_3,2H_3PO_4,H_2O$ Mol. Wt. 325.2

Histamine Phosphate is 2-(1*H*-imidazol-4-yl)ethylamine diphosphate monohydrate.

Histamine Phosphate contains not less than 98.0 per cent and not more than 101.0 per cent of $C_5H_9N_3,2H_3PO_4$, calculated on the anhydrous basis.

**Category**. Diagnostic aid (gastric secretion indicator).

**Dose**. By subcutaneous injection, 500 µg to 1 mg; after administration of an antihistamine, 5 mg.

**Description**. Colourless, long prismatic crystals; odourless.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *histamine phosphate RS*.

B. In the test for Histidine, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

C. Dissolve 0.1 g in 7 ml of *water* and add 3 ml of *sodium hydroxide solution*. Dissolve 50 mg of *sulphanilic acid* in 10 ml of *water* containing 0.1 ml of *hydrochloric acid* and 0.1 ml of a 10 per cent w/v solution of *sodium nitrite*. On mixing the two solutions a deep red colour is produced.

D. Gives reaction A of phosphates (2.3.1).

## Tests

**Appearance of solution**. Solution A is clear (2.4.1), and not more intensely coloured than reference solution BYS7 (2.4.1).

**pH** (2.4.24). 3.7 to 3.9, determined in a 5.0 per cent w/v solution in *carbon dioxide-free water* prepared from *distilled water* (solution A).

**Histidine**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 75 volumes of *acetonitrile*, 20 volumes of *water* and 5 volumes of *strong ammonia solution*.

*Test solution (a)*. Dissolve 0.5 g of the substance under examination in 10 ml of *water*.

*Test solution (b)*. Dilute 5 ml of test solution to 25 ml with *water*.

*Reference solution (a)*. A 1.0 per cent w/v solution of *histamine phosphate RS*.

*Reference solution (b)*. A solution containing 0.05 per cent w/v of *DL-histidine monohydrochloride*.

*Reference solution (c)*. A mixture of equal volumes of test solution (a) and reference solution (b).

Apply to the plate 1 µl of each solution. After development, dry the plate in a current of air and repeat the development in the same direction. Dry the plate in a current of air, spray with *ninhydrin solution* and heat at 110° for 10 minutes. Any spot corresponding to histidine monohydrochloride in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

**Sulphates** (2.3.17). 3 ml of solution A diluted to 15 ml with *distilled water* complies with the limit test for sulphates (0.1 per cent).

**Water** (2.3.43). 5.0 to 6.2 per cent, determined on 0.3 g.

**Assay**. Weigh accurately about 0.14 g, dissolve in 5 ml of *anhydrous formic acid* and add 20 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01536 g of $C_5H_9N_3,2H_3PO_4$.

**Storage**. Store protected from light.

# Histamine Phosphate Injection

Histamine Acid Phosphate Injection

Histamine Phosphate Injection is a sterile solution of Histamine Phosphate in Water for Injections.

Histamine Phosphate Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of histamine phosphate, $C_5H_9N_3,2H_3PO_4,H_2O$.



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**Usual strength**. 1 mg per ml.

### Identification

A. Evaporate a volume of the injection containing about 2 mg of Histamine Phosphate on a water bath to dryness, dissolve the residue in 0.5 ml of *water*, and add 0.5 ml of *sodium hydroxide*. Add 2 drops of a 10 per cent w/v solution of *sodium nitrite* and 1 ml of a solution prepared by mixing 50 mg of *sulphanilic acid* with 10 ml of *water* containing 2 drops of *hydrochloric acid*; an orange-red colour is produced.

B. To 1 ml of the injection containing not less than 1 mg of Histamine Phosphate (concentrate a larger volume by evaporation, if necessary), add *ammonium molybdate* solution dropwise; a yellow precipitate, which is soluble in *ammonia*, is formed.

### Tests

**pH** (2.4.24). 3.0 to 6.0.

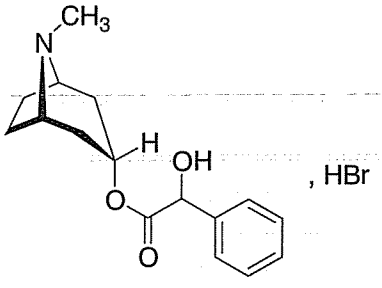
**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. Measure accurately a volume of the injection containing about 10 mg of Histamine Phosphate, transfer to a tared 25 ml centrifuge tube containing a thin glass rod slightly curved at the end, add 0.5 ml of *nitranilic acid solution* with continuous stirring and allow to stand for 15 minutes. Add 10 ml of *ethanol (95 per cent)*, mix and keep at 0° for 3 hours. Centrifuge for 1 minute, dislodge any particles at the surface and again centrifuge for 1 minute. Decant the supernatant liquid and stir the precipitate with 5 ml of ice-cold *ethanol (95 per cent)*. Centrifuge for 2 minutes, decant and repeat the washing with two further quantities, each of 5 ml, of ice-cold *ethanol (95 per cent)* and finally with 5 ml of *ether*. Smear the residue over the inside of the tube by means of the glass rod and dry to constant weight at 130°.

1 g of the residue is equivalent to 0.9529 g of $C_5H_9N_3,2H_3PO_4,H_2O$.

**Storage**. Store protected from light.

## Homatropine Hydrobromide




$C_{16}H_{21}NO_3,HBr$ Mol. Wt. 356.3

Homatropine Hydrobromide is (1*R*,3*r*,5*S*)-3-(*RS*)-mandeloyloxytropane hydrobromide.

Homatropine Hydrobromide contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{16}H_{21}NO_3,HBr$, calculated on the dried basis.

**Category**. Mydriatic; cycloplegic.

**Description**. Colourless crystals or a white, crystalline powder; odourless.

### Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *homatropine hydrobromide RS* or with the reference spectrum of homatropine hydrobromide.

B. Dissolve 50 mg in 1 ml of *water* and add 2 ml of *2 M acetic acid*. Heat, add 4 ml of *picric acid solution* and allow to cool, shaking occasionally. The crystals, after washing with two quantities, each of 3 ml, of iced *water* and drying at 105° melt at 182° to 186° (2.4.21).

C. Dissolve about 10 mg in 1 ml of *water*, add a slight excess of *10 M ammonia* and shake with 5 ml of *chloroform*. Evaporate the chloroform layer to dryness on a water-bath and add 1.5 ml of a 2 per cent w/v solution of *mercuric chloride* in *ethanol (60 per cent)*; a yellow colour develops which becomes red on warming.

D. Gives reaction A of bromides (2.3.1).

### Tests

**Appearance of solution**. A 5.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and not more intensely coloured than reference solution BS8 (2.4.1).

**pH** (2.4.24). 5.0 to 6.5, determined in a 5.0 per cent w/v solution.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 134 volumes of *ethyl acetate*, 33 volumes of *anhydrous formic acid* and 33 volumes of *water*.

*Test solution*. Dissolve 0.4 g of the substance under examination in 10 ml of *methanol (90 per cent)*.

*Reference solution*. Dilute 1 volume of the test solution to 200 volumes with *methanol (90 per cent)*.

Apply to the plate 5 µl of each solution. After development, dry the plate at 105° until the odour of the solvent is no longer detectable, allow to cool and spray with *dilute potassium iodobismuthate solution* until spots appear. Any secondary spot in the chromatogram obtained with the test solution is



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not more intense than the spot in the chromatogram obtained with the reference solution.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent, determined on 0.5 g.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.3 g, dissolve in 20 ml of *anhydrous glacial acetic acid* and add 7 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03563 g of $C_{16}H_{21}NO_3$,HBr.

**Storage**. Store protected from light.

# Homatropine Eye Drops

Homatropine Hydrobromide Eye Drops

Homatropine Eye Drops are a sterile solution of Homatropine Hydrobromide in Purified Water.

Homatropine Eye Drops contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of homatropine hydrobromide, $C_{16}H_{21}NO_3$,HBr.

**Usual strength**. 2 per cent w/v.

## Identification

A. To a volume containing 60 mg of Homatropine Hydrobromide add 3 ml of *dilute ammonia solution*, extract with 15 ml of *chloroform*, dry the chloroform extract over *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *homatropine hydrobromide RS* or with the reference spectrum of homatropine hydrobromide.

B. To the residue obtained in test A, add 1.5 ml of a 2 per cent w/v solution of *mercuric chloride* in *ethanol* (*60 per cent*); a yellow colour is produced which becomes red on gentle warming (distinction from most other alkaloids except atropine and hyoscyamine).

## Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 134 volumes of *ethyl acetate*, 33 volumes of *anhydrous formic acid* and 33 volumes of *water*.

*Test solution*. Use the eye drops, diluted if necessary with water to contain 1 per cent w/v of Homatropine Hydrobromide.

*Reference solution*. Dilute 1 volume of the test solution to 200 volumes with *water*.

Apply to the plate 40 µl of each solution. After development, dry the plate at 105° until the odour of the solvent is no longer detectable, allow to cool and spray with *dilute potassium iodobismuthate solution* until spots appear. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution. After development, dry at 105° until the odour of solvent is no longer detectable, allow to cool and spray with *dilute potassium iodobismuthate solution* until spots appear. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Other tests**. Comply with the tests stated under Eye Drops.

**Assay**. Determine by gas chromatography (2.4.13).

*Test solution (a)*. Add 1 ml of a 2 per cent w/v solution of *atropine sulphate RS* (internal standard) in *methanol* (solution A) and 1 ml of *dilute ammonia solution* to a volume of the eye drops containing about 20 mg of Homatropine Hydrobromide, diluted if necessary to 5.0 ml with *water*. Extract with two quantities, each of 5 ml, of *chloroform*, shake the combined extracts with 1 g of *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness. Dissolve the residue in 10.0 ml of *dichloromethane*. To 1.0 ml of this solution add 0.2 ml of a mixture of 4 volumes of N,O-*bis* (*trimethylsilyl*)-*acetamide* and 1 volume of *trimethyl- chlorosilane*, mix and allow to stand for 30 minutes.

*Test solution (b)*. Prepare in the same manner as test solution (a) but omitting the addition of solution A.

*Reference solution*. Add 1 ml of solution A and 1 ml of *dilute ammonia solution* to 5.0 ml of a 0.4 per cent w/v solution of *homatropine hydrobromide RS* and complete the procedure described under test solution (a) beginning at the words "Extract with two quantities, each of 5 ml, of *chloroform*....".

Chromatographic system

- a glass column 1.5 m x 4 mm, packed with acid-washed, silanised diatomaceous support (80 to 100 mesh) coated with 3 per cent w/w of phenyl methyl silicone fluid (50 per cent phenyl) (such as OV-17),
- temperature:
  column. 220°,
  inlet port and detector. 280°,
- flow rate. 30 ml per minute of the carrier gas.

Calculate the content of $C_{16}H_{21}NO_3$,HBr in the eye drops.

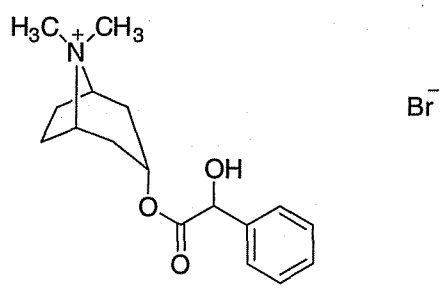


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# Homatropine Methylbromide



$C_{17}H_{24}BrNO_3$ Mol. Wt. 370.3

Homatropine Methylbromide is (1*R*,3*r*,5*S*)-3-[[(2*RS*)-2-hydroxy-2-phenylacetyl]oxy]-8,8-dimethyl-8-azoniabicyclo[3.2.1]octane bromide.

Homatropine Methylbromide contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{17}H_{24}BrNO_3$, calculated on the dried basis.

**Category**. Anticholinegic

**Description**. A white or almost white, crystalline powder or colourless crystals.

## Identification

*Test A may be omitted if tests B and C are carried out. Test B may be omitted if tests A and C are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *homatropine methylbromide RS* or with the reference spectrum of homatropine methylbromide.

B. Dissolve 50 mg in 1 ml of *water* and add 2 ml of *dilute acetic acid*. Heat and add 4 ml of *picric acid solution*, allow to cool, shaking occasionally. Wash the crystals, with two quantities, each of 3 ml of iced *water* and dried at 105°. The residue melts between 132° to 138° (2.4.21).

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

## Tests

**Appearance of solution**. A 5.0 per cent w/v solution in *carbon dioxide-free water* (solution A) is clear (2.4.1) and colourless (2.4.1).

**pH** (2.4.24). 4.5 to 6.5, determined in solution A.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. 18 volumes of *acetonitrile* and 82 volumes of mobile phase A.

*Test solution*. Dissolve 50 mg of the substance under examination in 25.0 ml of the solvent mixture.

*Reference solution (a)*. Dilute 5.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 5.0 ml of the solution to 50.0 ml with the solvent mixture.

*Reference solution (b)*. Dilute 5.0 ml of reference solution (a) to 25.0 ml with the solvent mixture.

*Reference solution (c)*. Dissolve 5.0 mg of *homatropine hydrobromide RS* in the solvent mixture and dilute to 50.0 ml with the solvent mixture. To 10.0 ml of the solution, add 0.5 ml of the test solution and dilute to 100.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- column temperature. 25°,
- mobile phase: A. dissolve 3.4 g of *potassium dihydrogen phosphate* and 5.0 g of *sodium heptanesulphonate monohydrate* in 1000 ml of *water*, and adjust to pH 3.0 with a 33 per cent w/v solution of *orthophosphoric acid*,

  B. a mixture of 40 volumes of mobile phase A and 60 volumes of *acetonitrile*,
- a linear gradient programme using the conditions given below,
- flow rate. 1.4 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 10 µl.

| Time (in min) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0-2 | 70 | 30 |
| 2-15 | 70→30 | 30→70 |
| 15-20 | 30→70 | 70→30 |
| 20-25 | 70 | 30 |

Inject reference solution (c). The test is not valid unless resolution between the peaks due to homatropine methylbromide and homatropine impurity B is not less than 2.5 and tailing factor for the peak due to homatropine methylbromide is not more than 2.5. The relative retention time with respect to homatropine methylbromide for (2*RS*)-2-hydroxy-2-phenylacetic acid (mandelic acid) (homatropine impurity C) is about 0.7, for (1*R*,3*s*,5*s*)-3-[[(2*RS*)-2-hydroxy-2-phenylacetyl]oxy]-8,8-dimethyl-8-azoniabicyclo[3.2.1]oct-6-ene(methyldehydro-homatropine) (homatropine impurity A) is about 0.9, for homatropine (homatropine impurity B) is about 1.2, for (1*R*,2*R*,4*S*,5*S*,7*s*)-7-[[(2*S*)-3-hydroxy-2-phenyl propanoyl]oxy-9,9-dimethyl-3-oxa-9-azoniatricyclo nonane (methylhyoscine) (homatropine impurity D) is about 1.3, for methylatropine (homatropine impurity E) is about 1.4 and for methyl (2*RS*)-2-hydroxy-2-phenylacetate(methyl mandelate) (homatropine impurity F) is about 1.7.



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Inject reference solution (a), (b) and the test solution. In the chromatogram obtained with the test solution the area of secondary peak corresponding to homatropine impurities A and B; for each impurity is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent), The area of the secondary peaks corresponding to homatropine impurities C, D, E and F; for each impurities is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The sum of the areas of all secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore the peak due to the bromide ion which appears close to the principle peak due the solvent. Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.3 g and dissolve in 10 ml of *water*. Titrate with *0.1 M silver nitrate*, determining the end-point potentiometrically (2.4.25), using a silver indicator electrode and a silver- silver chloride reference electrode.

1 ml of *0.1 M silver nitrate* is equivalent to 0.03703 g of $C_{17}H_{24}BrNO_3$.

**Storage**. Store protected from light.

# Homatropine Methylbromide Tablets

Homatropine Methylbromide Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of homatropine methylbromide, $C_{17}H_{24}BrNO_3$.

**Usual strength**. 15 mg.

## Identification

Shake a quantity of powdered tablets containing about 10 mg of Homatropine Methylbromide, with 15 ml of a mixture of equal volumes of *methanol* and *water* for 10 minutes, and filter. Evaporate the filtrate on a steam bath to dryness, and dry at 105° for 1 hour. The residue of homatropine methylbromide so obtained melts between 190° and 198° (2.4.21).

## Tests

**Dissolution** (2.5.2).

Apparatus No. 1,

Medium. 900 ml of *water*,

Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter. Measure the absorbance (2.4.7) of the filtrate, suitably diluted if necessary with dissolution medium at 258 nm. Calculate the content of homatropine methylbromide, $C_{17}H_{24}BrNO_3$ in the medium from the absorbance obtained from a solution of known concentration of *homatropine methylbromide RS*.

D. Not less than 75 per cent of the stated amount of $C_{17}H_{24}BrNO_3$.

**Uniformity of content**.

*Test solution.* Disperse 1 tablet in *water* to obtain a solution of 0.01 per cent w/v of Homatropine Methylbromide.

*Reference solution.* A 0.01 per cent w/v solution of *homatropine methylbromide RS* in *water*.

Transfer 2.0 ml each of the test solution and the reference solution to separate glass-stoppered 50-ml flasks. To each flask, add 0.1 ml of 10 per cent w/v solution of *sodium hydroxide solution* and heat on a water-bath at 80° for 15 minutes, cool. Add 2.0 ml of *0.2 M ceric ammonium sulphate* in *1 M sulphuric acid*, and mix. To each flask, add 20 ml of *n-hexane* and shake for 15 minutes and measure the absorbance at 242 nm (2.4.7).

**Assay**.

*Test solution.* Weigh and finely powder 20 tablets. Shake a quantity of powdered tablets containing about 12.5 mg of Homatropine Methylbromide with 10 ml of *water* for 30 minutes. Filter under reduced pressure through a sintered-glass crucible into a test tube placed in the suction flask under the filtering funnel, and wash under suction with several small portions of *water*. Transfer the contents of the test tube to a 25-ml volumetric flask and dilute with *water*.

*Reference solution.* A 0.05 per cent w/v solution of *homatropine methylbromide RS* in *water*.

Transfer 10.0 ml each of the test solution and the reference solution to separate test tubes, to each add 1 ml of *2.5 M sulphuric acid* and 2 ml of *ammonium reineckate*, shake and allow to stand for 1 hour. Filter through a sintered-glass crucible with suction, using portions of the filtrate to transfer the precipitate completely to the filter, and wash it with three 2-ml portions of ice-cold *water*. Completely dissolve the precipitate by pouring over it 1-ml portions of *acetone* with the application of suction and dilute to 10 ml with *acetone* and measure the absorbance at 525 nm (2.4.7).

Calculate the content of $C_{17}H_{24}BrNO_3$ in the tablets.

**Storage**. Store protected from light.



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# Hyaluronidase

Hyaluronidase is a material containing enzymes, which depolymerise the mucopolysaccharide, hyaluronic acid. It may be prepared from the testes and semen of mammals and purified by fractional precipitation so as to remove inert material and to which hydrolysed gelatin or a suitable non-protein stabilising agent may be added. The product is freeze-dried in single dose containers, which are sealed so as to exclude micro-organisms.

Hyaluronidase contains not less than 300 Units per mg, calculated on the dried basis. It may contain a suitable stabilizer.

**Category**. Depolymerising enzyme used as spreading factor.

**Dose**. By subcutaneous or intramuscular injection, 1500 Units either mixed with the injection fluid (if compatible) or injected into the site before injection is administered.

**Description**. A white or yellowish-white, fluffy powder.

## Identification

A. A solution containing the equivalent of 100 Units in 1 ml of *saline solution* depolymerises an equal volume of a 1 per cent w/v solution of *sodium hyaluronate* at 20° in 1 minute as shown by a pronounced decrease in viscosity. This action is destroyed by heating the initial solution at 100° for 30 minutes.

B. A solution containing the equivalent of 1 Unit in 0.2 ml of *saline solution* when injected intracutaneously into experimental animals together with a suitable indicator shows a spreading activity when compared with a control solution.

## Tests

**Appearance of solution**. A 1.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and not more than faintly yellow.

**pH** (2.4.24). 4.5 to 7.5, determined in a 0.3 per cent w/v solution in *carbon dioxide-free water*.

**Light absorption**. Dissolve a quantity containing 1500 Units in sufficient *carbon dioxide-free water* to produce 5.0 ml and measure the absorbance of the resulting solution at about 260 nm and 280 nm; absorbance at about 260 nm, not more than 0.42 and at about 280 nm, not more than 0.60 (2.4.7).

**Bacterial endotoxins** (2.2.3). Not more than 0.2 Endotoxin Unit per unit of hyaluronidase.

**Sterility** (2.2.11). Complies with the test for sterility.

**Assay**. The potency of hyaluronidase is determined by comparing its effects against those of the Standard Preparation.

### Standard Preparation

The Standard Preparation is the 1st International Standard for Hyaluronidase, bovine, established in 1955, consisting of dried bovine testicular hyaluronidase diluted with lactose (supplied in ampoules containing 10 tablets of 20 mg each; each tablet contains approximately 200 Units).

*Test solution*. Dissolve a suitable quantity of the preparation under examination by adding cold *diluent* for *hyaluronidase solutions*. Dilute the solution with cold diluent for *hyaluronidase solutions* so that the absorbances of the dilutions being assayed will fall on the upper linear part of the reference curve prepared as follows.

To each of 12 test-tubes (100 mm x 16 mm) add 0.50 ml of *hyaluronate solution* and, respectively and in duplicate, 0.5, 0.4, 0.3, 0.2, 0.1 and 0.0 ml of *diluent* for *hyaluronidase solutions*. If quantities of the solution of the standard solution other than those indicated below are used, change the quantities of *diluent* for *hyaluronidase solutions* accordingly. At intervals of 30 seconds add to the tubes 0.0, 0.1, 0.2, 0.3, 0.4 and 0.5 ml of the solution of the standard solution, respectively and in duplicate, making the final volume in each tube 1.0 ml, mixing the contents by shaking gently and placing each tube in a water-bath maintained at 37.0° ± 0.2°. After exactly 30 minutes, remove each tube in order from the water-bath at intervals of 30 seconds and immediately add 4.0 ml of *serum solution*. Shake and allow to stand at room temperature for 30 minutes. Shake again and measure the absorbance at about 640 nm (2.4.7). Repeat the operation using 0.50 ml of *phosphate-buffered saline* in place of the *hyaluronate solution* and make any necessary corrections. Prepare a reference curve by plotting the mean of the corrected absorbance for each level against the potency.

*Standard solution*. Dissolve one tablet of the Standard Preparation, accurately weighed, in sufficient cold *diluent for hyaluronidase solutions* to give a solution of known concentration containing about 1.5 Units per ml. This solution should be prepared immediately before use.

To each of 6 test-tubes (100 mm x 16 mm) add 0.50 ml of *hyaluronate solution* and sufficient *diluent* for *hyaluronidase solutions* so that the final volume in each tube after the addition of the solution of the preparation being examined is 1.0 ml. At intervals of 30 seconds add to each tube sufficient of the solution of the preparation being examined so that the tubes contain about 0.3, 0.5 and 0.7 Units, respectively and in duplicate, shaking each tube gently and continuing as described under test solution, beginning at the words "placing each tube in a water-bath..."

**Storage**. Store protected from moisture at a temperature not exceeding 15°.

**Labelling**. The label states (1) the total number of Units in the container; (2) the name of any added stabilising agent; (3) that the preparation is not intended for intravenous injection.



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# Hyaluronidase Injection

Hyaluronidase Injection is a sterile material consisting of Hyaluronidase with or without excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

*The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).*

**Storage**. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Hyaluronidase Injection contains not less than 90.0 per cent and not more than 115.0 per cent of the stated number of Units of hyaluronidase activity.

**Usual strength**. 1500 Units.

**Description**. A white or yellowish-white powder.

*The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.*

## Identification

A. A solution containing the equivalent of 100 Units in 1 ml of *saline solution* depolymerises an equal volume of a 1 per cent w/v solution of *sodium hyaluronate* at 20° in 1 minute as shown by a pronounced decrease in viscosity. This action is destroyed by heating the initial solution at 100° for 30 minutes.

B. A solution containing the equivalent of 1 Unit in 0.2 ml of *saline solution* when injected intracutaneously into experimental animals together with a suitable indicator shows a spreading activity when compared with a control solution.

## Tests

**pH** (2.4.24). 4.5 to 7.5, determined in a 0.3 per cent w/v solution in *carbon dioxide-free water*.

**Appearance of solution**. A 1.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and not more than faintly yellow.

**Bacterial endotoxins** (2.2.3). Not more than 0.2 Endotoxin Unit per unit of hyaluronidase.

**Assay**. The potency of hyaluronidase is determined by comparing its effects against those of the Standard Preparation.

### Standard Preparation

The Standard Preparation is the 1st International Standard for Hyaluronidase, bovine, established in 1955, consisting of dried bovine testicular hyaluronidase diluted with lactose (supplied in ampoules containing 10 tablets of 20 mg each; each tablet contains approximately 200 Units).

*Test solution*. Dissolve the contents of a container by adding cold *diluent* for *hyaluronidase solutions*. Dilute the solution with cold *diluent for hyaluronidase solutions* so that the absorbances of the dilutions being assayed will fall on the upper linear part of the reference curve prepared as follows.

To each of 12 test-tubes (100 mm x 16 mm) add 0.50 ml of *hyaluronate solution* and, respectively and in duplicate, 0.5, 0.4, 0.3, 0.2, 0.1 and 0.0 ml of *diluent for hyaluronidase solutions*. If quantities of the solution of the standard solution other than those indicated below are used, change the quantities of *diluent for hyaluronidase solutions* accordingly. At intervals of 30 seconds add to the tubes 0.0, 0.1, 0.2, 0.3, 0.4 and 0.5 ml of the solution of the standard solution, respectively and in duplicate, making the final volume in each tube 1.0 ml, mixing the contents by shaking gently and placing each tube in a water-bath maintained at 37.0° ± 0.2°. After exactly 30 minutes, remove each tube in order from the water-bath at intervals of 30 seconds and immediately add 4.0 ml of *serum solution*. Shake and allow to stand at room temperature for 30 minutes. Shake again and measure the absorbance at about 640 nm (2.4.7). Repeat the operation using 0.50 ml of *phosphate-buffered saline* in place of the *hyaluronate solution* and make any necessary corrections. Prepare a reference curve by plotting the mean of the corrected absorbance for each level against the potency.

*Standard solution*. Dissolve one tablet of the Standard Preparation, accurately weighed, in sufficient cold *diluent for hyaluronidase solutions* to give a solution of known concentration containing about 1.5 Units per ml. This solution should be prepared immediately before use.

To each of 6 test-tubes (100 mm x 16 mm) add 0.50 ml of *hyaluronate solution* and sufficient *diluent for hyaluronidase solutions* so that the final volume in each tube after the addition of the solution of the preparation being examined is 1.0 ml. At intervals of 30 seconds add to each tube sufficient of the solution of the preparation being examined so that the tubes contain about 0.3, 0.5 and 0.7 Units, respectively and in duplicate, shaking each tube gently and continuing as described under test solution, beginning at the words "placing each tube in a water-bath..."

**Labelling**. The label states (1) the total number of Units contained in it; (2) the nature of any added stabilising agent; (4) that the injection should be used immediately after preparation; (5) that the preparation is not intended for intravenous injection.

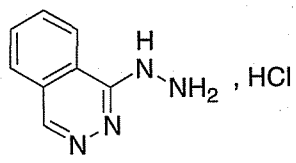


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# Hydralazine Hydrochloride



$C_8H_8N_4,HCl$ Mol. Wt. 196.6

Hydralazine Hydrobromide is phthalazin-1-ylhydrazine hydrochloride.

Hydralazine Hydrochloride contains not less 98.5 per cent and not more than 101.0 per cent of $C_8H_8N_4,HCl$, calculated on the dried basis.

**Category**. Vasodilator; antihypertensive.

**Dose**. Orally, 25 to 50 mg twice daily. By slow intravenous injection, 5 to 20 mg over 20 minutes; may be repeated after about 30 minutes.

**Description**. A white or almost white, crystalline powder.

## Identification

*Test A may be omitted if tests B, C, D and E are carried out. Tests B, C and D may be omitted if tests A and E are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *hydralazine hydrochloride RS* or with the reference spectrum of hydralazine hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution shows absorption maxima at about 240 nm, 260 nm, 305 nm and 315 nm and their absorbances are about 0.55, 0.55, 0.27 and 0.22 respectively.

C. Dissolve 0.5 g in a mixture of 8 ml of *2 M hydrochloric acid* and 100 ml of *water*. Add 2 ml of *sodium nitrite solution*, allow to stand for 10 minutes and filter. The precipitate, after washing with *water* and drying at 105°, melts at 209° to 212° (2.4.21).

D. To a solution of about 10 mg in 2 ml of *water* add 2 ml of a 2 per cent w/v solution of *2-nitrobenzaldehyde* in *ethanol (95 per cent);* an orange precipitate is obtained.

E. A 5 per cent w/v solution gives the reactions of chlorides (2.3.1).

## Tests

**Appearance of solution**. A 0.4 per cent w/v solution is clear (2.4.1), and not more intensely coloured than reference solution GYS4 (2.4.1).

**pH** (2.4.24). 3.5 to 4.2, determined in a 2.0 per cent w/v solution.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution (a)*. Dissolve 25 mg of the substance under examination in sufficient quantity of the mobile phase to make 50 ml.

*Test solution (b)*. Dilute 1 ml of test solution (a) to 100 ml with the mobile phase.

*Reference solution (a)*. Dilute 5 ml of test solution (b) to 25 ml with the mobile phase.

*Reference solution (b)*. Dissolve 25 mg of *phthalazine* in sufficient quantity of the mobile phase to make 50 ml and dilute 4 ml of this solution to 100 ml with the mobile phase.

*Reference solution (c)*. Mix 4 ml of test solution (a) and 10 ml of reference solution (b) and dilute to 100 ml with the mobile phase.

*The solutions should be used within 8 hours of preparation.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with porous spherical particles of finely-divided silica gel chemically bonded to nitrile groups (10 µm),
- mobile phase: a mixture of 22 volumes of *acetonitrile* and 78 volumes of a solution containing 1.44 g of *sodium dodecyl sulphate* and 0.75 g of *tetrabutylammonium bromide* per litre adjusted to pH 3.0 with *0.05 M sulphuric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 20 µl.

Inject test solution (b) and adjust the sensitivity of the detector so that the height of the principal peak in the chromatogram is not less than 70 per cent of the full scale of the recorder. When the chromatograms are recorded in the prescribed conditions, the retention time of hydralazine is about 10 to 12 minutes. If necessary, adjust the concentration of acetonitrile in the mobile phase.

Inject test solution (a) and continue the chromatography for 3 times the retention time of hydralazine. Inject reference solution (a). The area of any secondary peak in the chromatogram obtained with test solution (a) is not greater than the area of the peak in the chromatogram obtained with reference solution (a).

The test is not valid unless (a) the chromatogram obtained with reference solution (c) shows two principal peaks and the resolution between the peaks is not less than 2.5 and (b) the principal peak in the chromatogram obtained with reference solution (a) has a signal-to-noise ratio of at least 3.

**Hydrazine**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 90 volumes of *toluene* and 10 volumes of *ethanol (95 per cent)*.



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*Test solution*. Dissolve 0.12 g of the substance under examination in 4 ml of *water* and 4 ml of a 15 per cent w/v solution of *salicylaldehyde* in *methanol* and 0.2 ml of *hydrochloric acid*. Mix and let it stand at a temperature not exceeding 25° for 2 to 4 hours to allow complete sedimentation of the precipitate. Add 4 ml of toluene, shake vigorously and centrifuge. Transfer the supernatant liquid to a 100-ml separating funnel, separate the toluene layer and shake vigorously, each time for 3 minutes, with two quantities, each of 20 ml, of a 20 per cent w/v solution of *sodium metabisulphite* and with two quantities, each of 50 ml, of *water*. Separate the toluene layer and use it as the test solution.

*Reference solution*. Prepare at the same time and in the same manner as described for the test solution using 1 ml of a solution prepared by dissolving 12 mg of *hydrazine sulphate* in sufficient quantity of *2 M hydrochloric acid* to make 100 ml and diluting 1 ml of this solution to 100 ml with the same solvent and 3 ml of *water*.

Apply to the plate 20 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in air and examine in ultraviolet light at 365 nm. Any spot in the chromatogram obtained with the test solution showing a yellow fluorescence is not more intense than the spot in the chromatogram obtained with the reference solution.

**Heavy metals** (2.3.13). Moisten the residue obtained in the test for Sulphated ash with 2 ml of *hydrochloric acid*, evaporate to dryness and dissolve the residue in sufficient *water* to produce 20 ml. 12 ml of the resulting solution complies with the limit test for heavy metals, Method D (20 ppm). Use *lead standard solution (2 ppm Pb)* to prepare the standard

**Sulphated ash** (2.3.18). Not more than 0.1 per cent, determined on 2.0 g.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa.

**Assay**. Weigh accurately about 0.1 g and dissolve in a mixture of 25 ml of water and 35 ml of *hydrochloric acid*. Titrate with *0.05 M potassium iodate*, determining the end-point potentiometrically (2.4.25) and using a calomel reference electrode and a platinum indicator electrode.

1 ml of *0.05 M potassium iodate* is equivalent to 0.009832 g of $C_8H_8N_4$,HCl.

**Storage**. Store protected from light.

# Hydralazine Injection

## Hydralazine Hydrochloride Injection

Hydralazine Injection is a sterile material consisting of Hydralazine Hydrochloride with or without auxiliary substances. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use. For intravenous infusion, the injection should be diluted with an appropriate volume of a suitable diluent.

*The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).*

**Storage**. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Hydralazine Injection contains not less than 98.0 per cent and not more than 114.0 per cent of the stated amount of hydralazine hydrochloride, $C_8H_8N_4$,HCl.

**Usual strength**. 20 mg.

**Description**. A white or almost white powder; very hygroscopic.

*The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.*

### Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *hydralazine hydrochloride RS* or with the reference spectrum of hydralazine hydrochloride.

B. Give the reactions of chlorides (2.3.1).

### Tests

**pH** (2.4.24). 3.5 to 4.2, determined in a 2.0 per cent w/v solution.

**Appearance of solution**. A 2.0 per cent w/v solution is not more opalescent than opalescence standard OS2 (2.4.1). A 2.0 per cent w/v solution in *0.01 M hydrochloric acid* is not more intensely coloured than reference solution GYS6 (2.4.1).

**Hydrazine**. Determine by thin-layer chromatography (2.4.17), using a silica gel 60-precoated plate.

*Mobile phase*. The upper layer obtained by shaking together 80 volumes of *hexane*, 20 volumes of *strong ammonia solution* and 20 volumes of *ethyl acetate*.

*Test solution*. Dissolve the contents of a container in sufficient *0.1 M methanolic hydrochloric acid* to produce a solution containing 0.5 per cent w/v of Hydralazine Hydrochloride. To 2.0 ml add 1.0 ml of a 2 per cent w/v solution of *salicylaldehyde* in *methanol* and 0.1 ml of *hydrochloric acid*, centrifuge and decant the supernatant liquid.

*Reference solution*. Prepare in the same manner, but using 2.0 ml of a 0.00025 per cent w/v solution of *hydrazine sulphate*



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in *0.1 M methanolic hydrochloric acid* in place of the solution of the substance under examination.

Apply to the plate 40 µl of each solution. After development, dry the plate in air and spray with *dimethylaminobenzaldehyde reagent*. In the chromatogram obtained with the test solution, any spot corresponding to hydrazine is not more intense than the spot in the chromatogram obtained with the reference solution.

**Assay**. Determine the weight of the contents of 10 containers. Dissolve 0.1 g of the mixed contents of the 10 containers in a mixture of 25 ml of *water* and 35 ml of *hydrochloric acid*. Titrate with *0.05 M potassium iodate*, determining the end-point potentiometrically (2.4.25) and using a calomel reference electrode and a platinum indicator electrode.

1 ml of *0.05 M potassium iodate* is equivalent to 0.009832 g of $C_8H_8N_4,HCl$.

**Storage**. Store protected from light at a temperature not exceeding 30°.

**Labelling**. The label states that solutions containing glucose should not be used in the preparation of an intravenous infusion.

# Hydrochloric Acid

Concentrated Hydrochloric Acid

HCl Mol. Wt. 36.5

Hydrochloric Acid contains not less than 35.0 per cent w/w and not more than 38.0 percent w/w of HCl.

**Category**. Pharmaceutical aid (acidifying agent).

**Description**. A clear, colourless, fuming liquid; odour, pungent.

## Identification

A. When added to *potassium permanganate,* chlorine is evolved.

B. Gives the reactions of chlorides (2.3.1).

## Tests

**Arsenic** (2.3.10). Mix 10.0 g with 40 ml of *water* and 1 ml of *stannous chloride solution AsT*; the resulting solution complies with the limit test for arsenic (1 ppm).

**Heavy metals** (2.3.13). Evaporate 3.5 ml to dryness on a water-bath, add 2 ml of *dilute acetic acid* to the residue and add *water* to make 25 ml. The resulting solution complies with the limit test for heavy metals, Method A (5 ppm).

**Bromide and iodide**. Dilute 5 ml with 10 ml of *water*, add 1 ml of *chloroform* and, dropwise with constant shaking, *chlorinated lime solution*; the chloroform layer does not become brown or violet.

**Free chlorine**. To 60 ml add 50 ml of *carbon dioxide-free water,* 1 ml of a 10 per cent w/v solution of *potassium iodide* and 0.5 ml of *starch solution* and allow to stand in the dark for 2 minutes. Any blue colour produced disappears on the addition of 0.2 ml of *0.01 M sodium thiosulphate.*

**Sulphite**. Dilute 1 ml with 10 ml of *water* and add 0.25 ml of *barium chloride solution* and 1 ml of *0.01 M iodine*; the colour of the iodine is not completely discharged.

**Sulphates** (2.3.17). Mix 6.5 ml with 10 mg of *sodium bicarbonate,* evaporate to dryness on a water-bath and dissolve the residue in 15 ml of *distilled water.* The resulting solution complies with the limit test for sulphates (20 ppm).

**Residue on evaporation**. Not more than 0.01 per cent, determined on 100 g.

**Assay**. Weigh accurately about 2.0 g, add 30 ml of *water*, mix and titrate with *1 M sodium hydroxide* using *methyl red solution* as indicator.

1 ml of *1 M sodium hydroxide* is equivalent to 0.03646 g of HCl.

**Storage**. Store in stoppered containers of glass or any other inert material at a temperature not exceeding 30°.

# Dilute Hydrochloric Acid

Dilute Hydrochloric Acid is prepared by mixing 274 g of Hydrochloric Acid and 726 g of Purified Water.

Dilute Hydrochloric Acid contains not less than 9.5 per cent and not more than 10.5 per cent w/w of HCl.

**Description**. A clear, colourless liquid.

## Identification

A. When added to *potassium permanganate,* chlorine is evolved.

B. Gives the reactions of chlorides (2.3.1).

## Tests

**Arsenic** (2.3.10). Mix 20.0 g with 20 ml of *water* and 1 ml of *stannous chloride AsT*; the resulting solution complies with the limit test for arsenic (0.5 ppm).

**Heavy metals** (2.3.13). Dissolve the residue obtained in the test for Residue on evaporation in 1 ml of *2 M hydrochloric acid*, dilute to 25 ml with *water;* to 2.5 ml of the resulting solution add 2 ml of *dilute acetic acid* and add *water* to make 25 ml. The resulting solution complies with the limit test for heavy metals, Method A (2 ppm).



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**Free chlorine**. To 60 ml add 50 ml of *carbon dioxide-free water*, 1 ml of a 10 per cent w/v solution of *potassium iodide* and 0.5 ml of *starch solution* and allow to stand in the dark for 2 minutes. Any blue colour produced disappears on the addition of 0.2 ml of *0.01 M sodium thiosulphate*.

**Residue on evaporation**. Not more than 0.01 per cent, determined on 100 g.

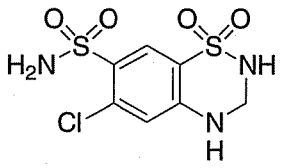
**Sulphates** (2.3.17). Mix 26 ml with 10 mg of *sodium bicarbonate*, evaporate to dryness on a water-bath and dissolve the residue in 15 ml of *distilled water*. The resulting solution complies with the limit test for sulphates (5 ppm).

**Assay**. Weigh accurately about 6.0 g, add 30 ml of *water*, mix and titrate with *1 M sodium hydroxide* using *methyl red solution* as indicator.

1 ml of *1 M sodium hydroxide* is equivalent to 0.03646 g of HCl.

**Storage**. Store in stoppered containers of glass or any other inert material at a temperature not exceeding 30°.

# Hydrochlorothiazide



$C_7H_8ClN_3O_4S_2$ Mol. Wt. 297.7

Hydrochlorothiazide is 6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulphonamide 1,1-dioxide.

Hydrochlorothiazide contains not less than 98.0 per cent and not more than 102.0 per cent of $C_7H_8ClN_3O_4S_2$, calculated on the dried basis.

**Category**. Diuretic.

**Dose**. 25 to 100 mg.

**Description**. A white or almost white, crystalline powder; odourless.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B and D may be omitted if tests A and C are carried out.*

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

B. When examined in the range 230 nm to 300 nm (2.4.7), a 0.001 per cent w/v solution in *0.01 M sodium hydroxide* shows an absorption maximum only at about 273 nm; absorbance at about 273 nm, 0.5 to 0.54. When examined in the range 300 nm to 360 nm, a 0.005 per cent w/v solution in *0.01 M sodium hydroxide* shows an absorption maximum at 323 nm; absorbance at about 323 nm, 0.45 to 0.48.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase. Ethyl acetate.*

*Test solution*. Dissolve 0.5 g of the substance under examination in 100 ml of *acetone*.

*Reference solution*. A 0.5 per cent w/v solution of *hydrochlorothiazide RS* in *acetone*.

Apply to the plate 4 µl of each solution. After development, dry the plate in a current of air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

D. Heat gently about 1 mg with 2 ml of a freshly prepared 0.05 per cent w/v solution of *chromotropic acid sodium salt* in a cooled mixture of 7 volumes of *water* and 13 volumes of *sulphuric acid;* a violet colour develops.

## Tests

**Acidity or alkalinity**. Shake 0.5 g of the powdered substance under examination with 25 ml of *water* for 2 minutes and filter. To 10 ml of the filtrate add 0.2 ml of *0.01 M sodium hydroxide* and 0.15 ml of *methyl red solution*. The solution is yellow and not more than 0.4 ml of *0.01 M hydrochloric acid* is required to change the colour of the solution to red.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 85 volumes of *ethyl acetate* and 15 volumes of *2-propanol*.

*Test solution*. Dissolve 0.2 g of the substance under examination in 10 ml of *acetone*.

*Reference solution*. A 0.01 per cent w/v solution of the substance under examination in *acetone*.

Apply to the plate 5 µl of each solution. After development, dry the plate in a current of air until the odour of the solvent is no longer detectable and spray with *ethanolic sulphuric acid (10 per cent);* use about 10 ml for a plate (200 mm x 200 mm) and spray in small portions, allowing the solvent to evaporate each time and avoiding excessive wetting. Heat to 105° for 30 minutes and immediately place above, but not in, 10 ml of a saturated solution of *sodium nitrite* in a glass tank. Carefully add 0.5 ml of *sulphuric acid* to the sodium nitrite solution and allow to stand in the closed tank for 15 minutes. Remove the plate, heat it in a ventilated oven at 40° for 15 minutes, spray with three quantities, each of 5 ml, of a freshly prepared 0.5 per



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cent w/v solution of *N-(1-naphthyl)ethylenediamine dihydrochloride* in *ethanol (95 per cent)* and examine by transmitted light. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Chlorides** (2.3.12). Dissolve 1.0 g in 25 ml of *acetone* and dilute to 30 ml with *water*. 7.5 ml of the resulting solution complies with the limit test for chlorides (250 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.12 g and dissolve in 50 ml of *anhydrous pyridine*. Titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.01488 g of $C_7H_8ClN_3O_4S_2$.

# Hydrochlorothiazide Tablets

Hydrochlorothiazide Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of hydrochlorothiazide, $C_7H_8ClN_3O_4S_2$.

**Usual strengths**. 25 mg; 50 mg.

## Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase. Ethyl acetate.*

*Test solution*. Triturate a quantity of the powdered tablets containing 10 mg of Hydrochlorothiazide with 10 ml of *acetone* and filter.

*Reference solution*. A 0.1 per cent w/v solution of *hydrochlorothiazide RS* in *acetone*.

Apply to the plate 5 µl of each solution. After development, dry the plate in a current of air, examine in ultraviolet light at 254 nm and then spray with *ethanolic sulphuric acid (20 per cent)*, heat at 105° for 30 minutes and immediately expose to nitrous fumes in a closed tank for 15 minutes (the nitrous fumes may be generated by adding *7 M sulphuric acid* dropwise to a solution containing 10 per cent w/v of *sodium nitrite* and 3 per cent w/v of *potassium iodide*). Place the plate in a current of warm air for 15 minutes and spray with a 0.5 per cent w/v solution of *N-(1-naphthyl)ethylenediamine dihydrochloride* in *ethanol (95 per cent)*. Examine the plate again. By each method of visualisation the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

## Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 85 volumes of *ethyl acetate* and 15 volumes of *2-propanol*.

*Test solution*. Shake a quantity of the powdered tablets containing 50 mg of Hydrochlorothiazide with 50 ml of *acetone*, filter, evaporate the filtrate to dryness and dissolve the residue in 10 ml of *acetone*.

*Reference solution*. Dilute 1 volume of the test solution to 100 volumes with *acetone*.

Apply to the plate 5 µl of each solution. After development, dry the plate in a current of air until the odour of the solvent is no longer detectable and spray with *ethanolic sulphuric acid (10 per cent);* use about 10 ml for a plate (200 mm x 200 mm) and spray in small portions, allowing the solvent to evaporate each time and avoiding excessive wetting. Heat to 105° for 30 minutes and immediately place above, but not in, 10 ml of a saturated solution of *sodium nitrite* in a glass tank. Carefully add 0.5 ml of *sulphuric acid* to the *sodium nitrite solution* and allow to stand in the closed tank for 15 minutes. Remove the plate, heat it in a ventilated oven at 40° for 15 minutes, spray with three quantities, each of 5 ml, of a freshly prepared 0.5 per cent w/v solution of *N-(1-naphthyl)ethylenediamine dihydrochloride* in *ethanol (95 per cent)* and examine by transmitted light. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the solution and filter promptly through a membrane filter disc with an average pore diameter not greater than 1.0 mm. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate with *0.1 M hydrochloric acid*. Measure the absorbance of the resulting solution at the maximum at about 272 nm (2.4.7). Calculate the content of $C_7H_8ClN_3O_4S_2$ taking 644 as the specific absorbance at 272 nm.

D. Not less than 60 per cent of the stated amount of $C_7H_8ClN_3O_4S_2$.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 20 mg of Hydrochlorothiazide, add 50 ml of *0.1 M sodium hydroxide*, shake for 20 minutes and dilute to 100.0 ml with *0.1 M sodium hydroxide*. Mix, filter, dilute 5.0 ml of the filtrate to 100.0 ml with *water* and measure the absorbance of the resulting



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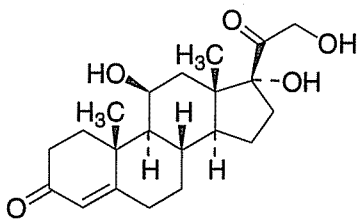
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solution at the maximum at about 273 nm (2.4.7). Calculate the content of $C_7H_8ClN_3O_4S_2$ taking 520 as the specific absorbance at 273 nm.

# Hydrocortisone

Cortisol



$C_{21}H_{30}O_5$ Mol. Wt. 362.5

Hydrocortisone is 11β,17α,21-trihydroxypregn-4-ene-3,20-dione.

Hydrocortisone contains not less than 96.0 per cent and not more than 104.0 per cent of $C_{21}H_{30}O_5$, calculated on the dried basis.

**Category**. Adrenocortical steroid.

**Dose**. Orally, in the treatment of adrenocortical insufficiency, 20 to 30 mg daily, in divided doses; by intramuscular injection or by slow intravenous injection or infusion, 100 to 500 mg, 3 to 4 times in 24 hours or as required.

**Description**. A white to practically white, crystalline powder.

## Identification

*Test A may be omitted if tests B and C are carried out. Test C may be omitted if tests A and B are carried out.*

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

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase (a)*. A mixture of 77 volumes of *dichloromethane*, 15 volumes of *ether*, 8 volumes of *methanol* and 1.2 volumes of *water*.

*Mobile phase (b)*. A mixture of 80 volumes of *ether*, 15 volumes of *toluene* and 5 volumes of *1-butanol* saturated with *water*.

*Solvent mixture*. A mixture of 90 volumes of *chloroform* and 10 volumes of *methanol*.

*Test solution*. Dissolve 0.25 g of the substance under examination in 100 ml with solvent mixture.

*Reference solution*. A 0.25 per cent w/v solution of *hydrocortisone RS* in the same solvent mixture.

Apply to the plate 2 µl of each solution. Develop the chromatograms successively with each mobile phase. After both developments, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution. Spray the plate with *ethanolic sulphuric acid (20 per cent)*, heat at 120° for 10 minutes or until spots appear and allow to cool. Examine the chromatograms in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution is similar in colour in daylight, fluorescence in ultraviolet light at 365 nm, position and size to the principal spot in the chromatogram obtained with the reference solution.

C. To 2 ml of a 0.1 per cent w/v solution in *ethanol (95 per cent)* add 2 ml of *sulphuric acid*; an intense yellow colour is produced with a green fluorescence, which is particularly intense in ultraviolet light at 365 nm. Add the solution to 10 ml of *water* and mix; the fluorescence in ultraviolet light at 365 nm does not disappear.

## Tests

**Specific optical rotation** (2.4.22). +150° to +156°, determined in a 1.0 per cent w/v solution in *dioxan*.

**Light absorption** (2.4.7). A 0.001 per cent w/v solution in *ethanol (95 per cent)* exhibits a maximum at about 240 nm; absorbance at about 240 nm, between 0.42 and 0.45.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 25 mg of the substance under examination in sufficient *tetrahydrofuran* to produce 10 ml.

*Reference solution (a)*. Dissolve 2 mg of *hydrocortisone RS* and 2 mg of *prednisolone RS* in the mobile phase and dilute to 100 ml with the mobile phase.

*Reference solution (b)*. Dilute 1 ml of the test solution to 100 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with base deactivated end-capped octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 220 ml of *tetrahydrofuran* and 700 ml of *water*, allowed to equilibrate, diluted to 1000 ml with *water* and mixed again,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Equilibrate the column with the mobile phase for about 30 minutes.

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference



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solution (b) is not less than 50 per cent of the full scale of the recorder.

Inject reference solution (a). The retention times are: prednisolone, about 14 minutes and hydrocortisone about 15.5 minutes. The test is not valid unless the resolution between the peaks corresponding to prednisolone and hydrocortisone is at least 2.2. If necessary, adjust the concentration of *tetrahydrofuran* in the mobile phase.

Inject separately the solvent mixture of the test solution as a blank, the test solution and reference solution (b). Continue the chromatography of the test solution for four times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak other than the principal peak, is not greater than half the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent): the sum of the areas of all the peaks other than the principal peak is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent). Ignore any peak obtained with the blank and any peak with an area less than 0.05 times that of the principal peak in the chromatogram obtained with reference solution (b).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

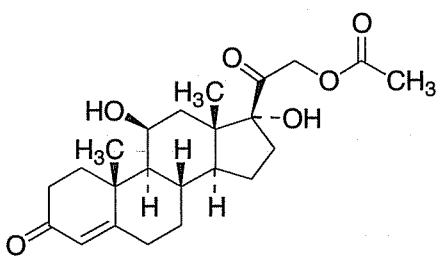
**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

**Assay**. Weigh accurately about 0.1 g, dissolve in sufficient *ethanol* to produce 100.0 ml. Dilute 2.0 ml of this solution to 100.0 ml with *ethanol* and mix. Measure the absorbance of the resulting solution (2.4.7) at the maximum at 241.5 nm. Calculate the content of $C_{21}H_{30}O_5$ taking 440 as the specific absorbance at 241.5 nm.

**Storage**. Store protected from light.

## Hydrocortisone Acetate

Cortisol Acetate




$C_{23}H_{32}O_6$ Mol. Wt. 404.5

Hydrocortisone Acetate is 11β,17α-dihydroxy-3,20-dioxopregn-4-en-21-yl acetate.

Hydrocortisone Acetate contains not less than 96.0 per cent and not more than 104.0 per cent of $C_{23}H_{32}O_6$, calculated on the dried basis.

**Category**. Adrenocortical steroid.

**Dose**. By intra-articular injection or local infiltration, 5 to 50 mg.

**Description**. A white or almost white, crystalline powder, odourless.

### Identification

*Test A may be omitted if tests B, C, and D are carried out. Tests C and D may be omitted if tests A and B are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *hydrocortisone acetate RS* or with the reference spectrum of hydrocortisone acetate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. Add a mixture of 1.2 volumes of *water* and 8 volumes of *methanol* to a mixture of 15 volumes of *ether* and 77 volumes of *dichloromethane*.

*Test solution (a)*. Dissolve 25 mg of the substance under examination in *methanol* and dilute to 5 ml with the same solvent. Use this solution to prepare test solution (b). Dilute 2 ml of the solution to 10 ml with *dichloromethane*.

*Test solution (b)*. Transfer 2 ml of the solution obtained in preparing test solution (a) to a 15-ml glass tube with a glass or plastic stopper. Add 10 ml of *saturated methanolic potassium hydrogen carbonate solution* and immediately pass a stream of *nitrogen* through the solution for 5 minutes. Stopper the tube. Heat in a water-bath at 45° protected from light for 2½ hours. Allow to cool.

*Reference solution (a)*. Prepare in the same manner as test solution (a) but using *hydrocortisone acetate RS* in place of the substance under examination.

*Reference solution (b)*. Prepare in the same manner as test solution (b) but using 2 ml of the solution obtained in preparing reference solution (a) in place of the solution obtained in preparing test solution (a).

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in each of the chromatograms obtained with the test solutions is similar to the principal spot in the chromatogram obtained with the corresponding reference solution. Spray with *ethanolic sulphuric acid (20 per cent v/v)* and heat at 120° for 10 minutes or until the spots appear. Allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in each of the chromatograms



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obtained with the test solutions is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm, position and size to that in the chromatogram obtained with the corresponding reference solution. The principal spots in the chromatograms obtained with test solution (b) and reference solution (b) have an $R_f$ value distinctly lower than that of the principal spots in the chromatograms obtained with test solution (a) and reference solution (a).

C. To 2 ml of a 0.1 per cent w/v solution in *ethanol (95 per cent)* add 2 ml of *sulphuric acid* and mix; an orange colour is produced with a green fluorescence which is particularly intense under ultraviolet light at 365 nm. Add the solution to 10 ml of *water* and mix; the fluorescence under ultraviolet light at 365 nm does not disappear.

D. Gives the reaction of acetyl groups (2.3.1).

### Tests

**Specific optical rotation** (2.4.22). +158° to +167°, determined in a 1.0 per cent w/v solution in *dioxan*.

**Light absorption** (2.4.7). A 0.001 per cent w/v solution in *ethanol (95 per cent)* shows an absorption maximum at about 240 nm; absorbance at about 240 nm, between 0.38 and 0.40.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 25 mg of the substance under examination in sufficient *methanol* to produce 10 ml.

*Reference solution (a)*. Dissolve 2 mg of *hydrocortisone acetate RS* and 2 mg of *cortisone acetate RS* in the mobile phase and dilute to 100 ml with the mobile phase.

*Reference solution (b)*. Dilute 1 ml of the test solution to 100 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with base deactivated endcapped octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 400 ml of *acetonitrile* and 550 ml of *water*, allowed to equilibrate, diluted to 1000 ml with *water* and mixed again,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Equilibrate the column with the mobile phase for about 30 minutes.

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (b) is not less than 50 per cent of the full scale of the recorder.

Inject reference solution (a). The retention times are: hydrocortisone acetate, about 10 minutes and cortisone acetate about 12 minutes. The test is not valid unless the resolution between the peaks corresponding to hydrocortisone acetate and cortisone acetate is at least 4.2. If necessary, adjust the concentration of acetonitrile in the mobile phase.

Inject separately the test solution and reference solution (b). Continue the chromatography of the test solution for 2.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution: the area of any peak other than the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and not more than one such peak has an area greater than half the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent); the sum of the areas of all the peaks other than the principal peak is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent). Ignore any peak due to the solvent and any peak with an area less than 0.05 times that of the principal peak in the chromatogram obtained with reference solution (b).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 2.0 g by drying in an oven at 105° for 3 hours.

**Assay**. Weigh accurately about 0.1 g, dissolve in sufficient *ethanol* to produce 100.0 ml. Dilute 2.0 ml of this solution to 100.0 ml with *ethanol* and mix. Measure the absorbance of the resulting solution (2.4.7) at the maximum at 241.5 nm. Calculate the content of $C_{23}H_{32}O_6$ taking 395 as the specific absorbance at 241.5 nm.

**Storage**. Store protected from light.

## Hydrocortisone Eye Ointment

Hydrocortisone Acetate Eye Ointment; Cortisol Acetate Eye Ointment

Hydrocortisone Eye Ointment contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of hydrocortisone acetate, $C_{23}H_{32}O_6$.

**Usual strength**. 2.5 per cent w/w.

### Identification

Boil 2 g with 20 ml of *methanol,* shake, cool to 0° for 30 minutes, filter and evaporate the filtrate to dryness. The residue complies with the following tests.

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Solvent mixture*. A mixture of 90 volumes of *acetone* and 10 volumes of *formamide*.



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*Mobile phase*. A mixture of 30 volumes of *toluene* and 10 volumes of *chloroform*.

*Test solution*. Dissolve 25 mg of the substance under examination in 10 ml of the solvent mixture.

*Reference solution (a)*. Dissolve 25 mg of *hydrocortisone RS* in 10 ml of the solvent mixture.

*Reference solution (b)*. Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v)*. Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

B. Dissolve about 1 mg in 1 ml of *sulphuric acid* and mix; an orange colour is produced with a green fluorescence, which is particularly intense under ultraviolet light at 365 nm. Add the solution to 10 ml of *water* and mix; the fluorescence under ultraviolet light at 365 nm does not disappear.

C. Dissolve 10 mg in 1 ml of *methanol*, warm and add 1 ml of *potassium cupri-tartrate solution*; an orange-red precipitate is slowly formed.

### Tests

**Other tests**. Complies with the tests stated under Eye Ointments.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh accurately a quantity of the ointment containing about 10 mg of Hydrocortisone Acetate, shake with 20.0 ml of *methanol* for 30 minutes and centrifuge. To 10.0 ml of the clear, supernatant layer add sufficient *methanol* to produce 50.0 ml.

*Reference solution*. A solution containing 0.01 per cent w/v of *hydrocortisone acetate RS* in *methanol*.

Chromatographic system
- a stainless steel column 30 cm x 3.9 mm, packed with porous silica particles (5 µm),
- mobile phase: a mixture of 425 volumes of *butyl chloride* saturated with *water*, 70 volumes of *tetrahydrofuran*, 35 volumes of *methanol* and 30 volumes of *glacial acetic acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Calculate the content of $C_{23}H_{32}O_6$ in the ointment.

**Storage**. Store protected from light at a temperature not exceeding 30°.

## Hydrocortisone Acetate Injection

Cortisol Acetate Injection

Hydrocortisone Acetate Injection is a sterile suspension of a very fine powder of Hydrocortisone Acetate in Water for Injections or Sodium Chloride Injection containing suitable dispersing agents.

Hydrocortisone Acetate Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of hydrocortisone acetate, $C_{23}H_{32}O_6$.

**Usual strength**. 25 mg per ml.

### Identification

Filter a volume containing 50 mg of Hydrocortisone Acetate through a sintered-glass filter, wash the residue with four quantities, each of 5 ml, of *water*, dissolve in 20 ml of *chloroform*, wash the chloroform solution with four quantities, each of 10 ml, of *water*, discard the washings, filter the chloroform solution through a plug of cotton and evaporate the filtrate to dryness. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *hydrocortisone acetate RS* or with the reference spectrum of hydrocortisone acetate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Solvent mixture*. A mixture of 90 volumes of *acetone* and 10 volumes of *formamide*.

*Mobile phase*. A mixture of 30 volumes of *toluene* and 10 volumes of *chloroform*.

*Test solution*. Dissolve 25 mg of the substance under examination in 10 ml of the solvent mixture.



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*Reference solution (a)*. Dissolve 25 mg of *hydrocortisone acetate RS* in 10 ml of the solvent mixture.

*Reference solution (b)*. Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v)*. Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

### Tests

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections)

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. To an accurately measured volume of the injection containing about 50 mg of Hydrocortisone Acetate add 70 ml of *methanol*, shake to produce a clear solution and dilute to 100.0 ml with *methanol*. Dilute 10.0 ml of the resulting solution to 20.0 ml with *water*.

*Reference solution*. Dissolve 25.0 mg of *hydrocortisone acetate RS* in 50 ml of *methanol* and add sufficient *water* to produce 100.0 ml.

Chromatographic system

- a stainless steel column 10 cm x 5 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Spherisorb ODS 1),
- mobile phase: 50 per cent v/v solution of *methanol*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume. 20 µl.

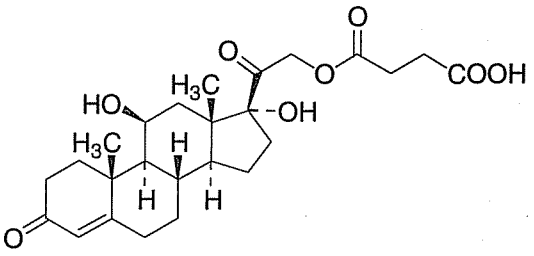
Calculate the content of $C_{23}H_{32}O_6$ in the injection.

**Storage**.Store protected from light.

**Labelling**. The label states (1) that the contents are meant for local injection only; (2) that the container should be gently shaken before a dose is withdrawn; (3) the names of the dispersing agents used, if any.

## Hydrocortisone Hemisuccinate

Hydrocortisone Hydrogen Succinate; Cortisol Hydrogen Succinate




$C_{25}H_{34}O_8$ Mol. Wt. 462.6

Hydrocortisone Hemisuccinate is 11β,17α -dihydroxy-3, 20-dioxopregn-4-en-21-yl hydrogen succinate.

Hydrocortisone Hemisuccinate contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{25}H_{34}O_8$, calculated on the dried basis.

**Category**. Adrenocortical steroid.

**Dose**. By intravenous injection, the equivalent of 100 to 500 mg of hydrocortisone 3 to 4 times in 24 hours or as required.

**Description**. A white or almost white, crystalline powder; hygroscopic.

### Identification

*Test A may be omitted if tests B, C and D are carried out. Tests C and D may be omitted if tests A and B are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Dry the substances before use at 105° for 3 hours and examine them as discs. Compare the spectrum with that obtained with *hydrocortisone hemisuccinate RS* or with the reference spectrum of hydrocortisone hemisuccinate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. Add a mixture of 1.2 volumes of *water* and 8 volumes of *methanol* to a mixture of 15 volumes of *ether* and 77 volumes of *dichloromethane*.

*Test solution (a)*. Dissolve 25 mg of the substance under examination in *methanol* and dilute to 5 ml with the same solvent. Use this solution to prepare test solution (b). Dilute 2 ml of the solution to10 ml with *dichloromethane*.

*Test solution (b)*. Transfer 2 ml of the solution obtained in preparing test solution (a) to a 15-ml glass tube with a glass or plastic stopper. Add 10 ml of a 0.8 g per litre solution of *sodium hydroxide* in *methanol* and immediately pass a stream of *nitrogen* through the solution for 5 minutes. Stopper the tube.



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Heat in a water-bath at 45° protected from light for 30 minutes. Allow to cool.

*Reference solution (a)*. Prepare in the same manner as test solution (a) but using *hydrocortisone hemisuccinate RS* in place of the substance under examination.

*Reference solution (b)*. Prepare in the same manner as test solution (b) but using 2 ml of the solution obtained in preparing reference solution (a) in place of the solution obtained in preparing test solution (a).

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in each of the chromatograms obtained with the test solutions is similar to the principal spot in the chromatogram obtained with the corresponding reference solution. Spray with *ethanolic sulphuric acid (20 per cent v/v)* and heat at 120° for 10 minutes or until the spots appear. Allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in each of the chromatograms obtained with the test solutions is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm, position and size to that in the chromatogram obtained with the corresponding reference solution. The principal spots in the chromatograms obtained with test solution (b) and reference solution (b) have an $R_f$ value distinctly higher than that of the principal spots in the chromatograms obtained with test solution (a) and reference solution (a).

C. Add about 2 mg to 2 ml of *sulphuric acid;* a yellow to brownish red colour develops with a green fluorescence that is particularly intense when examined in ultraviolet light at 365 nm.

D. About 30 mg gives the reaction of esters (2.3.1).

## Tests

**Appearance of solution.** A 2.0 per cent w/v solution in *sodium bicarbonate solution* is clear (2.4.1).

**Light absorption** (2.4.7). A 0.001 per cent w/v solution in *ethanol (95 per cent)* shows an absorption maximum at about 240 nm; absorbance at about 240 nm, between 0.34 and 0.36.

**Specific optical rotation** (2.4.22). +147° to +153°, determined in a 1.0 per cent w/v solution in *ethanol*.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 25 mg of the substance under examination in sufficient *methanol* to produce 10 ml.

*Reference solution (a)*. Dissolve 2 mg of *hydrocortisone hemisuccinate RS* and 2 mg of *dexamethasone RS* in the mobile phase and dilute to 100 ml with the mobile phase.

*Reference solution (b)*. Dilute 1 ml of the test solution to100 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with base deactivated end-capped octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 330 ml of *acetonitrile* and 600 ml of *water* and 1.0 ml of *phosphoric acid*, allowed to equilibrate, diluted to 1000 ml with *water* and mixed again,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Equilibrate the column with the mobile phase for about 30 minutes.

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (b) is not less than 50 per cent of the full scale of the recorder.

Inject reference solution (a). The retention times are: dexamethasone, about 12.5 minutes and hydrocortisone hemisuccinate about 15 minutes. The test is not valid unless the resolution between the peaks corresponding to dexamethasone and hydrocortisone hemisuccinate is at least 5.0. If necessary, adjust the concentration of acetonitrile in the mobile phase.

Inject separately the test solution and reference solution (b). Continue the chromatography for twice the retention time of the principal peak. In the chromatogram obtained with the test solution: the area of any peak other than the principal peak, is not greater than half the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent); the sum of the areas of all the peaks other than the principal peak is not greater than 0.75 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.75 per cent). Ignore any peak due to the solvent and any peak with an area less than 0.05 times that of the principal peak in the chromatogram obtained with reference solution (b).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 4.0 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.1 g, dissolve in sufficient *ethanol* to produce 100.0 ml. Dilute 2.0 ml of this solution to 100.0 ml with *ethanol* and mix. Measure the absorbance of the resulting solution (2.4.7) at the maximum at about 241.5 nm. Calculate the content of $C_{25}H_{34}O_8$ taking 353 as the specific absorbance at 241.5 nm.

**Storage**. Store protected from light and moisture.



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# Hydrocortisone Sodium Succinate Injection

Cortisol Sodium Succinate Injection

Hydrocortisone Sodium Succinate Injection is a sterile material made from Hydrocortisone Hemisuccinate with the aid of a suitable alkali such as Sodium Hydroxide or Sodium Carbonate. It may contain suitable buffering agents. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

*The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).*

**Storage**. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Hydrocortisone Sodium Succinate Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of hydrocortisone, $C_{21}H_{30}O_5$.

*The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.*

**Usual strengths**. The equivalent of 100 mg and 500 mg of hydrocortisone.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with the reference spectrum of hydrocortisone sodium succinate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A freshly prepared mixture of 1 volume of *anhydrous formic acid*, 10 volumes of *absolute ethanol* and 150 volumes of *dichloromethane*.

*Test solution*. A 0.1 per cent w/v solution of the contents of the sealed container in a mixture of 1 volume of *methanol* and 9 volumes of *dichloromethane*.

*Reference solution (a)*. A 0.1 per cent w/v solution of *hydrocortisone sodium succinate RS* in the same solvent mixture.

*Reference solution (b)*. A solution containing 0.1 per cent w/v each of *hydrocortisone hemisuccinate RS* and *methylprednisolone hydrogen succinate RS* in the same solvent mixture.

Apply separately to the plate 5 µl of each solution. After development, dry the plate in air until the solvents have evaporated, spray with *ethanolic sulphuric acid (20 per cent)*, heat at 120° for 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. By each method of visualization the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two spots that may not be completely separated.

## Tests

**pH** (2.4.24). 6.5 to 8.0, determined in a solution containing the equivalent of 5.0 per cent w/v of hydrocortisone.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. A mixture of equal volumes of *acetonitrile* and *water*.

*Test solution*. Dissolve a sufficient quantity of the contents of the sealed container in a solvent mixture to produce a solution containing the equivalent of 0.25 per cent w/v of hydrocortisone.

*Reference solution (a)*. Dilute 2 volumes of the test solution to 100 volumes with solvent mixture.

*Reference solution (b)*. Dilute a 0.035 per cent w/v solution of *hydrocortisone RS* in *acetonitrile* with solvent mixture.

*Reference solution (c)*. Dilute a solution containing 0.04 per cent w/v each of *hydrocortisone hemisuccinate RS* and *dexamethasone RS* with solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 330 ml of *acetonitrile* and 600 ml of *water* and 1 ml of *phosphoric acid*, allowed to equilibrate, diluted to 1000 ml with *water* and mixed again,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Equilibrate the column with the mobile phase for about 30 minutes.

Inject reference solution (c). The test is not valid unless in the chromatogram obtained the resolution between the peaks corresponding to dexamethasone and hydrocortisone hemisuccinate is at least 5.0.

Inject the test solution. Allow the chromatography to proceed for twice the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any peak corresponding ro hydrocortisone is not greater than the area of the peak in the chromatogram obtained with reference solution (b) (7 per cent) and the area of any other secondary



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peak is not greater than the area of the peak in the chromatogram obtained with reference solution (a) (2 per cent).

**Assay**. Determine the weight of the contents of 10 containers. Dissolve the mixed contents of the 10 containers in sufficient *water* to produce a solution containing the equivalent of 0.001 per cent w/v of hydrocortisone. Measure the absorbance of the resulting solution at the maximum at about 248 nm (2.4.7). Calculate the content of $C_{21}H_{30}O_5$ taking 449 as the specific absorbance at 248 nm.

**Storage**. Store protected from moisture in single dose containers at a temperature not exceeding 30°.

**Labelling**. The label states (1) the strength in terms of the equivalent amount of hydrocortisone; (2) that the prepared solution should be used only if it is clear; (3) that the solution should be used immediately after preparation.

## Hydrogen Peroxide Solution (20 Vol)

$H_2O_2$ Mol. Wt. 34.0

Hydrogen Peroxide Solution (6 per cent); Dilute Hydrogen Peroxide Solution

Hydrogen Peroxide Solution (20 Vol) is an aqueous solution of hydrogen peroxide. It may contain a suitable stabilising agent.

Hydrogen Peroxide Solution (20 Vol) contains not less than 5.0 per cent w/v and not more than 7.0 per cent w/v of $H_2O_2$, corresponding to about 20 times its volume of available oxygen.

**Category**. Antiseptic; deodorant.

**Description**. A clear, colourless liquid; odourless. It decomposes in contact with oxidisable organic matter and with certain metals and also if allowed to become alkaline.

### Identification

A. To 1 ml add 0.2 ml of *1 M sulphuric acid* and 0.25 ml of *0.02 M potassium permanganate*; the solution becomes colourless with evolution of gas.

B. Shake 0.05 ml with 2 ml of *1 M sulphuric acid*, 2 ml of *ether* and 0.05 ml of *potassium chromate solution*; the ether layer becomes blue.

### Tests

**Acidity**. To 10 ml add 20 ml of *water* and 0.25 ml of *methyl red solution*. Not less than 0.2 ml and not more than 1.0 ml of *0.1 M sodium hydroxide* is required to change the colour of the solution.

**Organic stabilizers**. Shake 20 ml with successive quantities of 10, 5 and 5 ml of *chloroform*. Evaporate the combined chloroform extracts at a temperature not exceeding 25° at a pressure of 2 kPa and dry in a desiccator. The residue weighs not more than 10 mg (500 ppm).

**Non-volatile matter**. Place 10 ml in a platinum dish and allow to stand until effervescence ceases. Evaporate the solution on a water-bath and dry the residue at 105°; the residue weighs not more than 20 mg.

**Assay**. To 1.0 ml add 20 ml of *1 M sulphuric acid* and titrate with *0.02 M potassium permanganate*.

1 ml of *0.02 M potassium permanganate* is equivalent to 0.001701 g of $H_2O_2$ or 0.56 ml of oxygen.

**Storage**. Store protected from light in containers resistant to hydrogen peroxide at a temperature not exceeding 30°. If the solution does not contain a stabilising agent, it should be stored in a refrigerator (2° to 8°). It should not be stored for long periods.

**Labelling**. The label states whether or not the solution contains a stabilising agent.

## Hydrogen Peroxide Solution (100 Vol)

Hydrogen Peroxide Solution (27 per cent); Strong Hydrogen Peroxide Solution

Hydrogen Peroxide Solution (100 Vol) is an aqueous solution of hydrogen peroxide. It may contain a suitable stabilising agent.

Hydrogen Peroxide Solution (100 Vol) contains not less than 26.0 per cent w/w and not more than 28.0 per cent w/w of $H_2O_2$, corresponding to about 100 times its volume of available oxygen.

**Category**. Antiseptic; deodrant.

**Description**. A clear, colourless liquid; odourless. It decomposes vigorously in contact with oxidisable organic matter and with certain metals and also if allowed to become alkaline.

### Identification

A. To 1 ml add 0.2 ml of *1 M sulphuric acid* and 0.25 ml of *0.02 M potassium permanganate*; the solution becomes colourless with evolution of gas.

B. Shake 0.05 ml with 2 ml of *1 M sulphuric acid*, 2 ml of *ether* and 0.05 ml of *potassium chromate solution*; the ether layer becomes blue.

### Tests

**Acidity**. Dilute 10 ml with 100 ml of *water* and add 0.25 ml of *methyl red solution*. Not less than 0.05 ml and not more than 0.5 ml of *0.1 M sodium hydroxide* is required to change the colour of the solution.



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**Organic stabilisers**. Shake 20 ml with successive quantities of 10, 5 and 5 ml of *chloroform*. Evaporate the combined chloroform extracts at a temperature not exceeding 25° at a pressure of 2 kPa and dry in a desiccator. The residue weighs not more than 10 mg (500 ppm).

**Non-volatile matter**. Place 10 ml in a platinum dish and allow to stand until effervescence ceases. Evaporate the solution on a water-bath and dry the residue at 105°; the residue weighs not more than 20 mg.

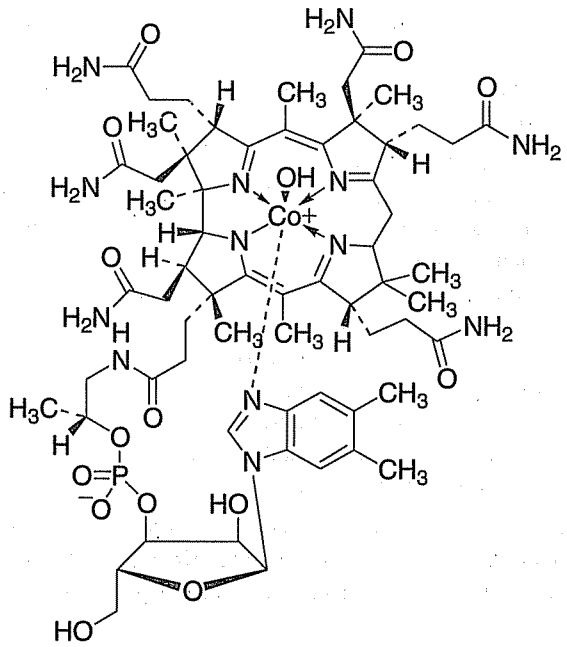
**Assay**. Dilute about 1.0 g to 100.0 ml with *water*. To 10.0 ml of the resulting solution add 20 ml of *1 M sulphuric acid* and titrate with *0.02 M potassium permanganate*.

1 ml of *0.02 M potassium permanganate* is equivalent to 0.001701 g of $H_2O_2$ or 0.56 ml of oxygen.

**Storage**. Store protected from light in containers resistant to hydrogen peroxide at a temperature not exceeding 30°. If the solution does not contain a stabilising agent, it should be stored in a refrigerator (2° to 8°). It should not be stored for long periods.

**Labelling**. The label states whether or not the solution contains a stabilising agent.

# Hydroxocobalamin



$C_{62}H_{89}CoN_{13}O_{15}P$ Mol. Wt. 1346.4

Hydroxocobalamin is Coα-[α-(5,6-dimethylbenzimidazolyl)]-Coβ-hydroxocobamide. It occurs either as aquocobalamin chloride (Coα-[α-(5,6-dimethylbenzimidazolyl)]-ICoβ-aquocobamide chloride) or as aquocobalamin sulphate.

Hydroxocobalamin contains not less than 96.0 per cent and not more than 102.0 per cent of aquocobalamin chloride, $C_{62}H_{89}CoN_{13}O_{15}P,HCl$, or of aquocobalamin sulphate, $C_{124}H_{178}Co_2N_{26}O_{30}P_2,H_2SO_4$, both calculated on the dried basis.

**Category**. Vitamin $B_{12}$ analogue used as haematopoietic.

**Dose**. In the treatment of megaloblastic anaemia, by intramuscular injection, 1 to 2 mg, in divided doses, in the first week; subsequent doses, 250 µg weekly until the blood count is normal; maintenance dose, 1 mg every two months.

**Description**. Dark red crystals or a crystalline powder; very hygroscopic. Some decomposition may occur on drying.

## Identification

A. Measure the absorbance of the solution used in the Assay at the maxima at about 274 nm, 351 nm and 525 nm (2.4.7); ratios of the absorbances at about 274 nm and 525 nm to that at about 351 nm, about 0.8 and about 0.3 respectively.

B. Fuse 1 mg of the substance with 50 mg of *potassium hydrogen sulphate*, cool, break up the mass, add 3 ml of *water* and boil until dissolved. Add 0.05 ml of *phenolphthalein solution* and sufficient *5 M sodium hydroxide* to produce a faint pink colour. Add 0.5 g of *sodium acetate*, 0.5 ml of *1 M acetic acid* and 0.5 ml of a 0.2 per cent w/v solution of *nitroso R salt*; a red or orange-red colour is produced immediately. Add 0.5 ml of *hydrochloric acid* and boil for 1 minute; the red colour persists.

C. Gives the reactions of chlorides or of sulphates, as the case may be (2.3.1).

## Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Use freshly prepared solutions and protect them from bright light.*

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

*Reference solution (a).* Dilute 5 ml of the test solution to 100 ml with the mobile phase.

*Reference solution (b).* Dilute 1 ml of the test solution to 10 ml with the mobile phase. Dilute 1 ml of this solution to 100 ml with the mobile phase.

*Reference solution (c).* Dissolve 25 mg of the substance under examination in 10 ml of *water*, warming if necessary. Allow to cool and add 1 ml of a 20 g/l solution of *chloramine T* and 0.5 ml of *0.05 M hydrochloric acid*. Dilute to 25 ml with *water*. Shake and allow to stand for 5 minutes. Inject immediately.



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Chromatographic system
- a stainless steel column 25 cm x 4 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 19.5 volumes of *methanol* and 80.5 volumes of a solution containing 15 g per litre of *citric acid* and 8.1g of *disodium hydrogen phosphate*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 351 nm,
- injection volume. 20 µl.

Inject reference solution (c). The test is not valid unless the chromatogram obtained shows three principal peaks and the resolution between each pair of adjacent peaks is at least 3.0.

Inject reference solution (b). The chromatogram obtained shows one principal peak with a signal-to-noise ratio of at least 5.

Inject the test solution and reference solution (a). Continue the chromatography for four times the retention time of the principal peak in the chromatogram obtained with reference solution (a). In the chromatogram obtained with the test solution, the sum of the areas of any peaks apart from the principal peak is not greater than the area of the principal peak in the chromatogram obtained with reference solution (a) (5 per cent). Ignore any peak whose area is less than that of the principal peak in the chromatogram obtained with reference solution (b).

**Loss on drying** (2.4.19). 8 to 12 per cent (aquocobalamin chloride) and 8 to 16 per cent (aquocobalamin sulphate), determined on 1.0 g by drying in an oven at 100° at a pressure not exceeding 0.7 kPa.

**Assay**. *Protect the solutions from light throughout the Assay.*

Weigh accurately about 25 mg and dissolve in sufficient of a solution containing 0.8 per cent v/v of *glacial acetic acid* and 1.09 per cent w/v of *sodium acetate* to produce 1000 ml. Measure the absorbance of the resulting solution at the maximum at about 351 nm (2.4.7). Calculate the content of $C_{62}H_{89}CoN_{13}O_{15}P$,HCl, or of $C_{124}H_{178}Co_2N_{26}O_{30}P_2$,$H_2SO_4$, taking 190 or 188 respectively, as the specific absorbance at 351 nm.

**Storage**. Store protected from light and moisture at a temperature not exceeding 30°.

**Labelling**. The label states whether the contents are aquocobalamin chloride or aquocobalamin sulphate.

# Hydroxocobalamin Injection

Hydroxocobalamin Injection is a sterile solution of Hydroxocobalamin in Water for Injections containing sufficient Acetic Acid, Hydrochloric Acid or Sulphuric acid to adjust the pH to about 4.

Hydroxocobalamin Injection contains not less than 95.0 per cent and not more than 110.0 per cent of the stated amount of anhydrous hydroxocobalamin, $C_{62}H_{89}CoN_{13}O_{15}P$.

**Usual strengths**. 500 µg per ml; 1 mg per ml.

## Identification

Measure the absorbance at about 351 nm and 361 nm; ratio of the absorbance at about 361 nm to that at about 351 nm, about 0.65 (2.4.7).

## Tests

**pH** (2.4.24). 3.8 to 5.5.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Use freshly prepared solutions and protect them from bright light.*

*Test solution*. Dilute the injection with the mobile phase, if necessary, to obtain a solution having a concentration of 0.1 per cent w/v of hydroxocobalamin.

*Reference solution (a).* Dilute 1 ml of the test solution to 20 ml with the mobile phase.

*Reference solution (b).* Dilute 1 ml of reference solution (a) to 100 ml with the mobile phase.

*Reference solution (c).* Add 0.2 ml of a freshly prepared 2 per cent w/v solution of *chloramine T* and 0.1 ml of *0.05 M hydrochloric acid* to a volume of the injection containing the equivalent of 5 mg of hydroxocobalamin, dilute to 10 ml with *water*, shake, allow to stand for 5 minutes and inject immediately.

Chromatographic system
- a stainless steel column 25 cm × 4 mm, packed with octylsilane bonded to porous silica (5 µm) (such as Lichrosorb 100 CH 8/11),
- mobile phase: a mixture of 19.5 volumes of *methanol* and 80.5 volumes of a solution containing 1.5 per cent w/v of *citric acid* and 0.81per cent w/v of *disodium hydrogen orthophosphate*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 351 nm,
- injection volume. 20 µl.

Inject reference solution (c). The test is not valid unless the chromatogram obtained shows three principal peaks and the resolution between each pair of adjacent peaks is not less than 3.0.

Inject reference solution (b). The chromatogram obtained shows one principal peak with a signal-to-noise ratio of not less than 5.

Inject the test solution and reference solution (a). In the chromatogram obtained with the test solution the sum of the



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areas of any secondary peaks is not greater than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (10 per cent). Ignore any peak the area of which is less than that of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

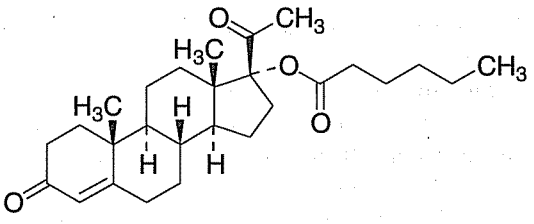
**Assay**. Dilute an accurately measured volume of the injection containing about 4 mg of anhydrous hydroxocobalamin to 200.0 ml with a solution containing 0.8 per cent v/v of *glacial acetic acid* and 1.09 per cent w/v of *sodium acetate* and measure the absorbance of the resulting solution at the maximum at about 351 nm (2.4.7). Calculate the content of $C_{62}H_{89}CoN_{13}O_{15}P$ taking 195 as the specific absorbance at 351 nm.

**Storage**. Store protected from light.

**Labelling**. The label states the strength in terms of the equivalent amount of anhydrous hydroxocobalamin.

# Hydroxyprogesterone Hexanoate

Hydroxyprogesterone Caproate



$C_{27}H_{40}O_4$ Mol. Wt. 428.6

Hydroxyprogesterone Hexanoate is 3,20-dioxopregn-4-en-17α-yl hexanoate

Hydroxyprogesterone Hexanoate contains not less than 97.0 per cent and not more than 103.0 per cent of $C_{27}H_{40}O_4$, calculated on the dried basis.

**Category**. Progestogen.

**Dose**. By intramuscular injection, 250 to 500 mg weekly during first half of pregnancy.

**Description**. A white or almost white, crystalline powder; odourless or almost odourless.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *hydroxyprogesterone hexanoate RS* or with the reference spectrum of hydroxyprogesterone hexanoate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Solvent mixture*. A mixture of 90 volumes of *acetone* and 10 volumes of *1,2-propane diol*.

*Mobile phase*. A mixture of equal volumes of *cyclohexane* and *light petroleum (40° to 60°)*.

*Test solution*. Dissolve 25 mg of the substance under examination in 10 ml of the same solvent mixture.

*Reference solution (a)*. Dissolve 25 mg of *hydroxyprogesterone hexanoate RS* in 10 ml of the same solvent mixture.

*Reference solution (b)*. Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v)*. Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

C. Dissolve 1 mg in 1 ml of *sulphuric acid* and allow to stand for 2 minutes; a faint yellow colour is produced which, on the addition of 0.5 ml of *water*, changes first to green, then to red and finally to reddish-violet with a blue fluorescence.

D. Heat 50 mg with 2 ml of *0.5 M ethanolic potassium hydroxide* in a water-bath for 5 minutes. Add 3 ml of *water*, evaporate the ethanol, add 2 ml of *sulphuric acid (50 per cent)* and heat on a water-bath; the odour of hexanoic acid is produced.

## Tests

**Acidity**. Dissolve 0.2 g in 25 ml of *ethanol* previously neutralised to *bromothymol blue solution* and titrate immediately with *0.01 M sodium hydroxide* until a faint blue colour is produced. Not more than 1.0 ml of *0.01 M sodium hydroxide* is required.



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**Specific optical rotation** (2.4.22). +44.0° to +49.0°, determined in a 2.0 per cent w/v solution in *dioxan*.

**Related foreign steroids**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254*.

*Mobile phase*. A mixture of equal volumes of *cyclohexane* and *ethyl acetate*.

*Test solution*. Dissolve 0.1 g of the substance under examination in 10 ml of *chloroform*.

*Reference solution*. Dilute 1 ml of the test solution to 100 ml with *chloroform*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 2.0 g by drying over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 24 hours.

**Assay**. Weigh accurately about 60 mg and dissolve in sufficient *ethanol* to produce 100.0 ml. Dilute 5.0 ml to 250.0 ml with *ethanol* and measure the absorbance of the resulting solution at the maximum at about 240 nm (2.4.7). Calculate the content of $C_{27}H_{40}O_4$ taking 395 as the specific absorbance at 240 nm.

**Storage**. Store protected from light.

# Hydroxyprogesterone Injection

Hydroxyprogesterone Caproate Injection; Hydroxyprogesterone Hexanoate Injection

Hydroxyprogesterone Injection is a sterile solution of Hydroxyprogesterone Hexanoate in a suitable ester, in a suitable fixed oil or in any mixture of fixed oils.

Hydroxyprogesterone Hexanoate Injection contains not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of hydroxyprogesterone hexanoate, $C_{27}H_{40}O_4$.

**Usual strength**. 250 mg per ml.

## Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254*.

*Mobile phase*. A mixture of equal volumes of *cyclohexane* and *ethyl acetate*.

*Test solution*. Dilute the injection with *chloroform* to give a solution containing 1.0 per cent w/v of Hydroxyprogesterone Hexanoate.

*Reference solution*. A solution containing 1 per cent w/v of *hydroxyprogesterone hexanoate RS* in *chloroform*.

Apply to the plate 1µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution. Ignore any spots due to the vehicle.

B. Dissolve a volume of the injection containing 0.1 g of Hydroxyprogesterone Hexanoate in 10 ml of *light petroleum (40° to 60°)* and extract with three quantities, each of 10 ml, of a mixture of 7 volumes of *glacial acetic acid* and 3 volumes of *water*. Wash the combined extracts with 10 ml of *light petroleum (40 ° to 60°)*, dilute with *water* until the solution becomes turbid and allow to stand in ice for about 2 hours until a white precipitate is produced. The precipitate, after washing with *water*, melts at about 120° (2.4.21).

## Tests

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. To a quantity of the injection containing about 0.125 g of Hydroxyprogesterone Hexanoate add sufficient *chloroform* to produce 100.0 ml. Dilute 5.0 ml to 100.0 ml with *chloroform*; to 5.0 ml add 10 ml of *isoniazid solution* and sufficient *methanol* to produce 20.0 ml. Allow to stand for 45 minutes and measure the absorbance of the resulting solution at the maximum at about 380 nm (2.4.7), using as the blank 5 ml of *chloroform* treated in the same manner. Calculate the content of $C_{27}H_{40}O_4$ from the absorbance obtained by repeating the operation using a 0.00625 per cent w/v solution of *hydroxyprogesterone hexanoate RS* in *chloroform* and beginning at the words "to 5.0 ml add..."

**Storage**. Store protected from light.

**Labelling**. The label states that the preparation is intended for intramuscular injection only.

# Hydroxypropyl Cellulose

Cellulose, 2-Hydroxypropyl Ether; Hyprolose

Hydroxypropylcellulose is a cellulose having some of the hydroxyl groups in the form of the 2-hydroxypropyl ether. It may contain not more than 0.6 per cent of silica ($SiO_2$). The various grades commercially available are distinguished by a number indicative of the apparent viscosity in millipascal seconds of a 2 per cent w/w solution measured at 20°.



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**Category**. Pharmaceutical aid (tablet excipient; suspending agent).

**Description**. A white or yellowish white powder; practically odourless; hygroscopic after drying.

### Identification

A. With constant stirring add a quantity equivalent to 1 g of the dried substance into 50 ml of *carbon dioxide-free water* previously heated to 90°. Allow to cool, dilute to 100 ml with *carbon dioxide-free water* and continue stirring until solution is complete (solution A). Heat 10 ml of solution A on a water-bath with stirring. At temperatures above 40° the solution becomes cloudy or a flocculent precipitate is formed. On cooling, the solution becomes clear.

B. To 10 ml of solution A add 0.3 ml of *2 M acetic acid* and 2.5 ml of a 10 per cent w/v solution of *tannic acid*; a yellowish white, flocculent precipitate is produced which dissolves in *6 M ammonia*.

C. Without heating completely dissolve 0.2 g in 15 ml of a 70 per cent w/w solution of *sulphuric acid*, pour the solution with stirring into 100 ml of iced *water*. In a test-tube kept in ice, mix thoroughly 1 ml of the solution with 8 ml of *sulphuric acid*, added dropwise. Heat in a water-bath for exactly 3 minutes and cool immediately in ice. When the mixture is cool, carefully add 0.6 ml of a solution containing 3 g of *ninhydrin* in 100 ml of a 4.55 per cent w/v solution of *sodium metabisulphite*, mix well and allow to stand at 25°; a pink colour is produced immediately which becomes violet within 100 minutes.

D. Place 1 ml of solution A on a glass plate. After evaporation of the *water* a thin film is produced.

### Tests

**Appearance of solution**. Solution A is not more opalescent than opalescence standard OS3 (2.4.1), and not more intensely coloured than reference solution YS6 (2.4.1).

**pH** (2.4.24). 5.0 to 8.5, determined in solution A.

**Apparent viscosity**. 75 to 140 per cent of the stated value, determined by the following method. Weigh accurately a quantity equivalent to 2.0 g of the dried substance and add, with constant stirring, to 50 ml of *water* previously heated to 90°. Allow to cool, dilute to 100 ml with *water* and continue stirring until solution is complete. Adjust the weight of the solution to 100 g and centrifuge the solution to expel any trapped air. Determine the viscosity, Method C, at 20° using a shear rate of 10 $s^{-1}$ (2.4.28).

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

**Chlorides** (2.3.12). Dilute 5.0 ml of solution A to 15 ml with *water*. The resulting solution complies with the limit test for chlorides (0.5 per cent).

**Silica**. Not more than 0.6 per cent, determined by the following method. To the residue obtained in the test for Sulphated ash add sufficient *ethanol (95 per cent)* to moisten the residue completely. Add 6 ml of *hydrofluoric acid* in small portions. Evaporate to dryness at 95° to 105° taking care to avoid loss by spurting. Cool and rinse the walls of the platinum crucible with 6 ml of *hydrofluoric acid*. Add 0.5 ml of *sulphuric acid* and evaporate to dryness. Progressively increase the temperature, ignite at 900°, allow to cool in a desicator and weigh. The difference between the weight of the residue obtained in the test for Sulphated ash and the weight of the final residue is equal to the amount of silica in the substance under examination.

**Sulphated ash** (2.3.18). Not more than 1.6 per cent, determined on 1.0 g in a platinum crucible.

**Loss on drying** (2.4.19). Not more than 7.0 per cent, determined on 0.5 g by drying in an oven at 105°.

**Storage**. Store protected from moisture.

**Labelling**. The label states the apparent viscosity in millipascal seconds of a 2 per cent w/w solution. For products of low viscosity the label also states the concentration of the solution to be used and the apparent viscosity in millipascal seconds.

## Hydroxypropylmethylcellulose

Cellulose, 2-Hydroxypropylmethyl Ether; Hypromellose

Hydroxypropylmethylcellulose is a cellulose having some of the hydroxyl groups in the form of the methyl ether and some in the form of the 2-hydroxypropyl ether. The various grades commercially available are distinguished by a number indicative of the apparent viscosity in millipascal seconds of a 2 per cent w/v solution measured at 20°.

**Category**. Treatment of tear deficiency; pharmaceutical aid (tablet excipient; suspending agent).

**Description**. A white or yellowish white, fibrous or granular powder; almost odourless; hygroscopic after drying.

### Identification

A. With constant stirring add a quantity containing 1 g of the dried substance into 50 ml of *carbon dioxide-free water* previously heated to 90°. Allow to cool, dilute to 100 ml with *carbon dioxide-free water* and continue stirring until solution is complete (solution A). Heat 10 ml of solution A in a water-bath with stirring. At temperatures above 50° the solution becomes cloudy or a flocculent precipitate is formed. On cooling, the solution becomes clear or slightly opalescent.



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B. To 10 ml of solution A add 10 ml of *1 M sodium hydroxide* or *1 M hydrochloric acid*; in either case the mixture remains stable.

C. To 10 ml of solution A add 0.3 ml of *2 M acetic acid* and 2.5 ml of a 10 per cent w/v solution of *tannic acid*; a yellowish white, flocculent precipitate is produced which dissolves in *6 M ammonia*.

D. Without heating completely dissolve 0.2 g in 15 ml of a 70 per cent w/w solution of *sulphuric acid*, pour the solution with stirring into 100 ml of iced *water*. In a test-tube kept in ice, mix thoroughly 1 ml of the solution with 8 ml of *sulphuric acid*, added dropwise. Heat in a water-bath for exactly 3 minutes and cool immediately in ice. When the mixture is cool, carefully add 0.6 ml of a solution containing 3 g of *ninhydrin* in 100 ml of a 4.55 per cent w/v solution of *sodium metabisulphite*, mix well and allow to stand at 25°; a pink colour is produced immediately which becomes violet within 100 minutes.

E. Place 1 ml of solution A on a glass plate. After evaporation of the *water* a thin film is produced.

### Tests

**pH** (2.4.24). 5.5 to 8.0, determined in solution A.

**Appearance of solution**. Solution A is not more opalescent than opalescence standard OS3 (2.4.1), and not more intensely coloured than reference solution YS6 (2.4.1).

**Apparent viscosity**. 75 to 140 per cent of the stated value, determined by the following method. Weigh accurately a quantity equivalent to 2.0 g of the dried substance and add, with constant stirring, to 50 ml of *water* previously heated to 90°. Allow to cool, dilute to 100 ml with *water* and continue stirring until solution is complete. Adjust the weight of the solution to 100 g and centrifuge the solution to expel any trapped air. Determine the viscosity, Method C, at 20° using a shear rate of 10 $s^{-1}$ (2.4.28).

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

**Chlorides** (2.3.12). Dilute 5.0 ml of solution A to 15 ml with *water*. The resulting solution complies with the limit test for chlorides (0.5 per cent).

**Sulphated ash** (2.3.18). Not more than 3.0 per cent.

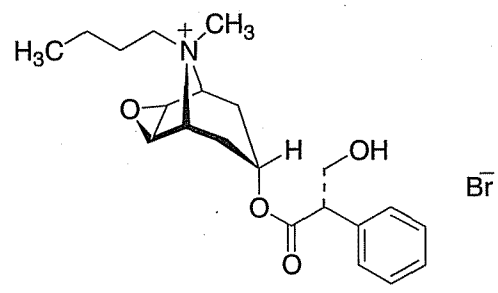
**Loss on drying** (2.4.19). Not more than 10.0 per cent, determined on 0.5 g by drying in an oven at 105°.

**Storage**. Store protected from moisture.

**Labelling**. The label states the apparent viscosity in millipascal seconds of a 2 per cent w/v solution.

## Hyoscine Butylbromide

Scopolamine Butylbromide




$C_{21}H_{30}BrNO_4$ Mol. Wt. 440.4

Hyoscine Butylbromide is (1*S*,3*s*,5*R*,6*R*,7*S*,8*r*)-6,7-epoxy-8-butyl-3-[(*S*)-tropoyloxy]tropanium bromide.

Hyoscine Butylbromide contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{21}H_{30}BrNO_4$, calculated on the dried basis.

**Category**. Parasympatholytic.

**Dose**. Orally, 20 mg four times daily; by intramuscular or intravenous injection, 20 mg, repeated after 30 minutes if necessary.

**Description**. A white or almost white, crystalline powder; odourless or almost odourless.

### Identification

*Test A may be omitted if tests B, C and D are carried out. Tests C and D may be omitted if tests A and B are carried out.*

A. Determine by infrared absorption spectrphotometry (2.4.6). Compare the spectrum with that obtained with *hyoscine butylbromide RS* or with the reference spectrum of hyoscine butylbromide.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.15 per cent w/v solution in *0.01 M hydrochloric acid* shows absorption maxima at about 252 nm, 257 nm and 264 nm and a less well-defined maximum at about 247 nm; absorbance at about 252 nm, about 0.50, at about 257 nm, about 0.67 and at about 264 nm, about 0.50.

C. To 1 mg add 0.2 ml of *fuming nitric acid* and evaporate to dryness on a water-bath. Dissolve the residue in 2 ml of *acetone* and add 0.1 ml of a 3 per cent w/v solution of *potassium hydroxide* in *methanol*; a violet colour is produced.

D. Gives the reactions of bromides (2.3.1).

### Tests

**Appearance of solution**. A 5.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and colourless (2.4.1).



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**pH** (2.4.24). 5.5 to 6.5, determined in a 10.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). –18.0° to –20.0°, determined in a 5.0 per cent w/v solution.

**Apo-compounds**. Ratio of the absorbance (2.4.7) of a 0.1 per cent w/v solution in *0.01 M hydrochloric acid* at the maximum at about 247 nm to that at the maximum at about 264 nm is not more than 0.94.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *microcrystalline cellulose*.

*Mobile phase*. The upper layer obtained by shaking together 50 volumes of *1-butanol*, 25 volumes of *water* and 5 volumes of *anhydrous formic acid*.

*Test solution*. Dissolve 0.2 g of the substance under examination in 10 ml of *methanol (50 per cent)*.

*Reference solution (a)*. A solution containing 0.004 per cent w/v of the substance under examination in *methanol (50 per cent)*.

*Reference solution (b)*. A solution containing 0.002 per cent w/v of *hyoscine hydrobromide RS* in *methanol (50 per cent)*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with *dilute potassium iodobismuthate solution*. In the chromatogram obtained with the test solution any spot corresponding to hyoscine hydrobromide is not more intense than the spot in the chromatogram obtained with reference solution (b) and any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (a).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 2.5 per cent, determined on 0.5 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.6 g, dissolve in 30 ml of *anhydrous glacial acetic acid* and add 5 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, using *1-naphtholbenzein solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04404 g of $C_{21}H_{30}BrNO_4$.

**Storage**. Store at a temperature not exceeding 30° protected from light and moisture.

# Hyoscine Butylbromide Injection

Scopolamine Butylbromide Injection

Hyoscine Butylbromide Injection is a sterile solution of Hyoscine Butylbromide in Water for Injections.

Hyoscine Butylbromide Injection contains not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of hyoscine butylbromide, $C_{21}H_{30}BrNO_4$.

**Usual strength**. 20 mg per ml.

## Identification

Evaporate to dryness a volume of the injection containing 0.1 g of Hyoscine Butylbromide, shake the residue with 20 ml of *chloroform*, filter, evaporate the filtrate to dryness and triturate the residue with 5 ml of *acetonitrile*. Evaporate to dryness and dry the residue at 50° at a pressure not exceeding 0.7 kPa for 1 hour. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *hyoscine butylbromide RS* or with the reference spectrum of hyoscine butylbromide.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.15 per cent w/v solution in *0.01 M hydrochloric acid* shows absorption maxima at about 252 nm, 257 nm and 264 nm and a less well-defined maximum at about 247 nm; absorbance at about 252 nm, about 0.50, at about 257 nm, about 0.67 and at about 264 nm, about 0.50.

C. To 1 mg add 0.2 ml of *fuming nitric acid* and evaporate to dryness on a water-bath. Dissolve the residue in 2 ml of *acetone* and add 0.1 ml of a 3 per cent w/v solution of *potassium hydroxide* in *methanol*; a violet colour is produced.

## Tests

**pH** (2.4.24). 3.7 to 5.5.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *microcrystalline cellulose*.

*Mobile phase*. The upper layer obtained by shaking together 50 volumes of *1-butanol*, 25 volumes of *water* and 5 volumes of *anhydrous formic acid*.

*Test solution*. The injection diluted if necessary with *methanol (50 per cent)* to contain 2 per cent w/v of Hyoscine Butylbromide.

*Reference solution (a)*. Dilute 1 volume of the test solution to 20 volumes with *methanol (50 per cent)*.

*Reference solution (b)*. A solution containing 0.002 per cent w/v of *hyoscine hydrobromide RS* in *methanol (50 per cent)*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with *dilute potassium iodobismuthate solution*. In the chromatogram obtained with the test solution any spot corresponding to hyoscine hydrobromide is not more intense than the spot in the chromatogram obtained with reference solution (b) and any



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other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (a).

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. Dilute a volume of the injection containing about 60 mg of Hyoscine Butylbromide to 50.0 ml with *water* and dilute 5.0 ml of this solution to 200.0 ml with *water*. To 10.0 ml of the resulting solution add 10 ml of *water*, 15 ml of *dichloromethane*, 15 ml of a 0.01 per cent w/v solution of *hexanitrodiphenylamine* in *dichloromethane* and 5 ml of *5 M sodium hydroxide* and shake for 2 minutes. Allow the layers to separate and reserve the organic layer. Extract the aqueous layer with successive quantities, each of 5 ml, of *dichloromethane* until no further colour is extracted from the aqueous layer. Add the dichloromethane extracts to the reserved organic layer, filter through absorbent cotton, add sufficient *dichloromethane* to produce 50.0 ml and measure the absorbance of the resulting solution at the maximum at about 420 nm (2.4.7), using as the blank a solution prepared by repeating the procedure without the injection. Calculate the content of $C_{21}H_{30}BrNO_4$ from the absorbance obtained by repeating the procedure using 10.0 ml of a 0.003 per cent w/v solution of *hyoscine butylbromide RS* and beginning at the words "add 10 ml of *water*....".

**Storage**. Store at a temperature not exceeding 30° protected from light and moisture.

# Hyoscine Butylbromide Tablets

Scopolamine Butylbromide Tablets

Hyoscine Butylbromide Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of hyoscine butylbromide, $C_{21}H_{30}BrNO_4$.

**Usual strength**. 10 mg.

## Identification

Shake a quantity of the powdered tablets containing 50 mg of Hyoscine Butylbromide with 20 ml of *chloroform*, filter, evaporate the filtrate to dryness and triturate the residue with 5 ml of *acetonitrile*. Evaporate to dryness and dry the residue at 50° at a pressure not exceeding 0.7 kPa for 1 hour. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *hyoscine butylbromide RS* or with the reference spectrum of hyoscine butylbromide.

B. When examined in the range 230 nm to 360 nm, a 0.15 per cent w/v solution in *0.01 M hydrochloric acid* shows absorption maxima at about 252 nm, 257 nm and 264 nm and a less well-defined maximum at about 247 nm; absorbance at about 252 nm, about 0.50, at about 257 nm, about 0.67 and at about 264 nm, about 0.50 (2.4.7).

C. To 1 mg add 0.2 ml of *fuming nitric acid* and evaporate to dryness on a water-bath. Dissolve the residue in 2 ml of *acetone* and add 0.1 ml of a 3 per cent w/v solution of *potassium hydroxide* in *methanol*; a violet colour is produced.

## Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *microcrystalline cellulose*.

*Mobile phase*. The upper layer obtained by shaking together 50 volumes of *1-butanol*, 25 volumes of *water* and 5 volumes of *anhydrous formic acid*.

*Test solution*. Shake a quantity of the powdered tablets containing 40 mg of Hyoscine Butylbromide with 2 ml of *methanol (50 per cent)* and filter.

*Reference solution (a)*. Dilute 1 volume of the test solution to 500 volumes with *methanol (50 per cent)*.

*Reference solution (b)*. A solution containing 0.002 per cent w/v of *hyoscine hydrobromide RS* in *methanol (50 per cent)*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with *dilute potassium iodobismuthate solution*. In the chromatogram obtained with the test solution any spot corresponding to hyoscine hydrobromide is not more intense than the spot in the chromatogram obtained with reference solution (b) and any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (a).

**Uniformity of content**. Comply with the test stated under Tablets.

Shake one tablet with 100 ml of *water* for 30 minutes, add sufficient *water* to produce 250.0 ml and filter. To 10.0 ml of the resulting solution add 10 ml of *water*, 15 ml of *dichloromethane*, 15 ml of a 0.01 per cent w/v solution of *hexanitrodiphenylamine* in *dichloromethane* and 5 ml of *5 M sodium hydroxide* and shake for 2 minutes. Allow the layers to separate and reserve the organic layer. Extract the aqueous layer with successive quantities, each of 5 ml, of *dichloromethane* until no further colour is extracted from the aqueous layer. Add the dichloromethane extracts to the reserved organic layer, filter through absorbent cotton, add sufficient *dichloromethane* to produce 50.0 ml and measure the absorbance of the resulting solution at the maximum at about 420 nm (2.4.7), using as the blank a solution prepared by repeating the procedure without the injection. Calculate the content of $C_{21}H_{30}BrNO_4$ from the absorbance obtained by repeating the procedure using 10.0 ml of a 0.003 per cent w/v solution of *hyoscine*



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*butylbromide RS* and beginning at the words "add 10 ml of *water*....".

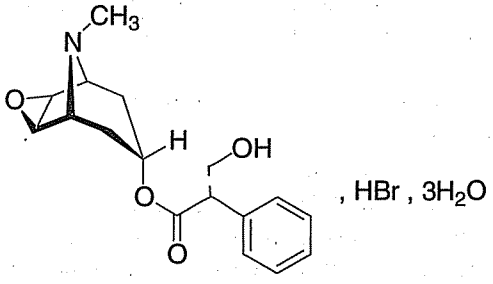
**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 3 mg of Hyoscine Butylbromide and shake with 50 ml of *water* for 30 minutes, add sufficient *water* to produce 100.0 ml and filter. To 10.0 ml of the resulting solution add 10 ml of *water*, 15 ml of *dichloromethane*, 15 ml of a 0.01 per cent w/v solution of *hexanitrodiphenylamine* in *dichloromethane* and 5 ml of *5 M sodium hydroxide* and shake for 2 minutes. Allow the layers to separate and reserve the organic layer. Extract the aqueous layer with successive quantities, each of 5 ml, of *dichloromethane* until no further colour is extracted from the aqueous layer. Add the dichloromethane extracts to the reserved organic layer, filter through absorbent cotton, add sufficient *dichloromethane* to produce 50.0 ml and measure the absorbance of the resulting solution at the maximum at about 420 nm (2.4.7), using as the blank a solution prepared by repeating the procedure without the injection. Calculate the content of $C_{21}H_{30}BrNO_4$ from the absorbance obtained by repeating the procedure using 10.0 ml of a 0.003 per cent w/v solution of *hyoscine butylbromide RS* and beginning at the words "add 10 ml of *water*....".

**Storage**. Store at a temperature not exceeding 30° protected from light and moisture.

# Hyoscine Hydrobromide

Scopolamine Hydrobromide




$C_{17}H_{21}NO_4,HBr,3H_2O$ Mol. Wt. 438.3

Hyoscine Hydrobromide is (1*S*,3*s*,5*R*,6*R*,7*S*)-6,7-epoxytropan-3-yl(*S*)-tropate hydrobromide trihydrate.

Hyoscine Hydrobromide contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{17}H_{21}NO_4,HBr$, calculated on the anhydrous basis.

**Category**. Parasympatholytic.

**Dose**. Orally, in prevention of motion sickness, 300 µg 30 minutes before commencing journey and repeated every 6 hours, if required; by subcutaneous or intramuscular injection, for preanesthetic medication, 200 to 600 µg.

**Description**. Colourless crystals or a white, crystalline powder; odourless; efflorescent.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *hyoscine hydrobromide RS*.

B. To about 1 mg add 0.2 ml of *fuming nitric acid* and evaporate to dryness on a water-bath. Dissolve the residue in 2 ml of *acetone* and add 0.1 ml of a 3 per cent w/v solution of *potassium hydroxide* in *methanol*; a violet colour is produced.

C. Gives the reaction of alkaloids (2.3.1).

D. Gives the reactions of bromides (2.3.1).

## Tests

**pH** (2.4.24). 4.0 to 5.5, determined in a 5.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). –24.0° to –27.0°, determined in a 5.0 per cent w/v solution.

**Apohyoscine**. Dissolve 0.1 g in sufficient *0.01 M hydrochloric acid* to produce 100 ml and measure the absorbance (2.4.7) at about 245 nm; absorbance, about 0.36, calculated on the anhydrous basis (0.5 per cent).

**Related substances and decomposition products**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 50 volumes of *chloroform*, 30 volumes of *acetone*, 10 volumes of *methanol* and 2 volumes of *strong ammonia solution*.

*Test solution*. Dissolve 0.2 g of the substance under examination in 10 ml of *methanol*.

*Reference solution (a)*. Dilute 1 ml of the test solution to 100 ml with *methanol*.

*Reference solution (b)*. Dilute 25 ml of reference solution (a) to 50 ml with *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate at 105° for 15 minutes, allow to cool and spray with *dilute potassium iodobismuthate solution* until spots appear. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the



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chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b). Ignore any yellow spot remaining on the line of application.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). 10.0 to 13.0 per cent, determined on 0.2 g.

**Assay**. Weigh accurately about 0.4 g, dissolve in 10 ml of *anhydrous glacial acetic acid*, warming if necessary, cool the solution and add 20 ml of *dioxan*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03843 g of $C_{17}H_{21}NO_4,HBr$.

**Storage**. Store protected from light and moisture in well-filled containers of small capacity in a refrigerator at a temperature not exceeding 15°.

# Hyoscine Hydrobromide Injection

Scopolamine Hydrobromide Injection

Hyoscine Hydrobromide Injection is a sterile solution of Hyoscine Hydrobromide in Water for Injection.

Hyoscine Hydrobromide Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of hyoscine hydrobromide, $C_{17}H_{21}NO_4,HBr,3H_2O$.

**Usual strength**. 400 mg per ml.

## Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 50 volumes of *chloroform*, 40 volumes of *acetone* and 10 volumes of *diethylamine*.

*Test solution*. Evaporate a volume of the injection containing 5 mg of Hyoscine Hydrobromide to dryness on a water-bath, triturate the residue with 1 ml of *ethanol (95 per cent)*, allow to stand and use the supernatant liquid.

*Reference solution*. A solution containing 0.5 per cent w/v of *hyoscine hydrobromide RS* in *ethanol (95 per cent)*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air, heat it at 105° for 20 minutes, allow to cool and spray with *dilute potassium iodobismuthate solution*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the chromatogram obtained with test solution (b) shows a peak with the same retention time as the peak derived from hyoscine hydrobromide in the chromatogram obtained with the reference solution.

C. Evaporate a suitable volume to dryness. To 1 ml of a 1 per cent w/v solution of the residue add 1 ml of *5 M ammonia*, shake with *chloroform* and evaporate the chloroform solution to dryness on a water-bath. To the residue add 1.5 ml of a 2 per cent w/v solution of *mercuric chloride* in *ethanol (60 per cent)*; a white precipitate is produced which dissolves on warming (distinction from atropine and hyoscyamine).

D. Gives reaction A of bromides (2.3.1).

## Tests

**pH** (2.4.24). 4.0 to 6.0.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. Determine by gas chromatography (2.4.13).

*Test solution (a)*. Add 1 of a 0.3 per cent w/v solution of *atropine sulphate RS* (internal standard) in *methanol* (solution A) and 1 ml of *5 M ammonia* to a volume of the injection containing about 5 mg of Hyoscine Hydrobromide, dilute if necessary to 15 ml with *water*. Extract with two quantities, each of 10 ml, of *chloroform*, shake the combined extracts with 2 g of *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness. Dissolve the residue in 2.0 ml of *dichloromethane*. To 1.0 ml of this solution add 0.2 ml of a mixture of 4 volumes of *N,O-bis(trimethylsilyl)acetamide* and 1 volume of *trimethylchlorosilane*, mix and allow to stand for 30 minutes.

*Test solution (b)*. Prepare in the same manner as test solution (a) but omitting the addition of solution A.

*Reference solution*. Add 1 ml of solution A and 1 ml of *5 M ammonia* to 15.0 ml of a 0.033 per cent w/v solution of *hyoscine hydrobromide RS* and complete the procedure described under test solution (a) beginning at the words "Extract with two quantities,....".

Chromatographic system

- a glass column 1.5 m x 4 mm, packed with acid-washed, diatomaceous support (80 to 100 mesh) coated with 3 per cent w/w phenyl methyl silicone fluid (50 per cent phenyl),
- temperature:
  column.230°,
  inlet port and detector. 280°,
- flow rate. 30 ml per minute of the carrier gas.

Calculate the content of $C_{17}H_{21}NO_4,HBr,3H_2O$ in the injection.

**Storage**. Store at a temperature not exceeding 15°, protected from light.



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# Hyoscine Hydrobromide Tablets

Scopolamine Hydrobromide Tablets

Hyoscine Hydrobromide Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of hyoscine hydrobromide, $C_{17}H_{21}NO_4,HBr,3H_2O$.

**Usual strengths**. 300 mg; 600 mg.

## Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 50 volumes of *chloroform*, 40 volumes of *acetone* and 10 volumes of *diethylamine*.

*Test solution*. Shake a quantity of the powdered tablets containing 10 mg of Hyoscine Hydrobromide with 2 ml of *ethanol (95 per cent)* and centrifuge.

*Reference solution*. A solution containing 0.5 per cent w/v of *hyoscine hydrobromide RS* in *ethanol (95 per cent)*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air, heat it at 105° for 20 minutes, allow to cool and spray with *dilute potassium iodobismuthate solution*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the chromatogram obtained with test solution (b) shows a peak with the same retention time as the peak derived from hyoscine hydrobromide in the chromatogram obtained with the reference solution.

C. Extract a quantity of the powdered tablets containing 1 mg of Hyoscine Hydrobromide with 5 ml of *ethanol (95 per cent)*, filter and evaporate the filtrate to dryness on a water-bath. Cool, add 0.2 ml of *fuming nitric acid* and again evaporate to dryness on a water-bath; a yellow residue is produced. To the cooled residue add 2 ml of *acetone* and 0.2 ml of a 3 per cent w/v solution of *potassium hydroxide* in *methanol;* a deep violet colour is produced. (Atropine and hyoscyamine also yield this reaction; the reaction is masked by other alkaloids).

D. The powdered tablets give reaction A of bromides (2.3.1).

## Tests

**Uniformity of content**. Comply with the test stated under Tablets.

*For tablets containing 600 µg of Hyoscine Hydrobromide—*

*Test solution (a)*. Powder one tablet and triturate with 5 ml of *0.1 M hydrochloric acid*. Add 1.0 ml of a 0.0375 per cent w/v solution of *atropine sulphate RS* (internal standard) in *methanol* (solution A), extract with two quantities, each of 5 ml, of *chloroform* and discard the chloroform extracts. Add 1 ml of *5 M ammonia*. Extract with two quantities, each of 5 ml, of *chloroform*, shake the combined extracts with 1 g of *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness. Dissolve the residue in 0.5 ml of a mixture of 20 volumes of *dichloromethane*, 4 volumes of *N,O-bis(trimethylsilyl) acetamide* and 1 volume of *trimethylchlorosilane*, mix and allow to stand for 30 minutes.

*Test solution (b)*. Prepare in the same manner as test solution (a) but omitting the addition of solution A.

*Reference solution*. Add 1 ml of a 0.0375 per cent w/v solution of *atropine sulphate RS* (internal standard) in *methanol* (solution A) and 1 ml of *5 M ammonia* to 5.0 ml of a 0.012 per cent w/v solution of *hyoscine hydrobromide RS* and complete the procedure described under test solution (a) beginning at the words "extract with two quantities,...".

Carry out the procedure described in the Assay. Calculate the content of $C_{17}H_{21}NO_4,HBr,3H_2O$ in the tablet.

*For tablets containing less than 600 µg of Hyoscine Hydrobromide* — Use the same procedure but with correspondingly smaller concentrations of *hyoscine hydrobromide RS* and *atropine sulphate RS*.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by gas chromatography (2.4.13).

*Test solution (a)*. Shake a quantity of the powdered tablets containing about 5 mg of Hyoscine Hydrobromide with 10 ml of *0.1 M hydrochloric acid*. Add 1 ml of a 0.3 per cent w/v solution of *atropine sulphate RS* (internal standard) in *methanol* (solution A), extract with two quantities, each of 10 ml, of *chloroform* and discard the chloroform extracts. Add 1 ml of *5 M ammonia*. Extract with two quantities, each of 10 ml, of *chloroform*, shake the combined extracts with 2 g of *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness. Dissolve the residue in 2.0 ml of *dichloromethane*. To 1.0 ml of this solution add 0.2 ml of a mixture of 4 volumes of *N,O-bis(trimethylsilyl)acetamide* and 1 volume of *trimethylchlorosilane*, mix and allow to stand for 30 minutes.

*Test solution (b)*. Prepare in the same manner as test solution (a) but omitting the addition of solution A.

*Reference solution*. Add 1 ml of solution A and 1 ml of *5 M ammonia* to 15.0 ml of a 0.033 per cent w/v solution of *hyoscine hydrobromide RS* and complete the procedure described under test solution (a) beginning at the words "Extract with two quantities,"

Chromatographic system

- a glass column 1.5 m x 4 mm, packed with acid-washed, diatomaceous support (80 to 100 mesh) coated with 3 per cent w/w phenyl methyl silicone fluid (50 per cent phenyl),
- temperature:
  column.230°,



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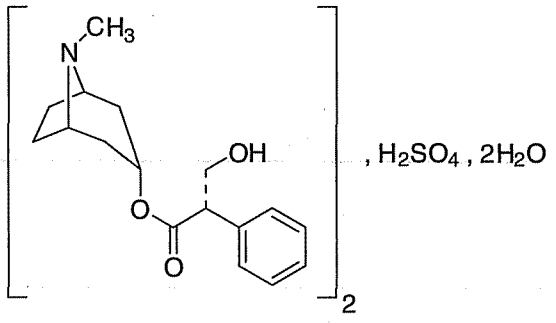


inlet port and detector. 280°,
- flow rate. 30 ml per minute of the carrier gas.

Calculate the content of $C_{17}H_{21}NO_4,HBr,3H_2O$ in the tablets.

**Storage**. Store at a temperature not exceeding 15°, protected from light.

# Hyoscyamine Sulphate




$(C_{17}H_{23}NO_3)_2,H_2SO_4,2H_2O$ Mol. Wt. 712.9

Hyoscyamine Sulphate is bis (1*R*,3*r*,5*S*)- 8-methyl-8-azabicyclo[3.2.1]oct-3-yl (2*S*)-3-hydroxy-2-phenylpropionate] sulphate dihydrate.

Hyoscyamine Sulphate contains not less than 98.0 per cent and not more than 101.0 per cent of $(C_{17}H_{23}NO_3)_2,H_2SO_4,2H_2O$, calculated on the anhydrous basis.

**Category**. Anticholinergic.

**Description**. A white or almost white, crystalline powder or colourless needles.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.*

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

B. To 0.5 ml of solution A, add 2 ml of *dilute acetic acid* and heat. To the hot solution add 4 ml of *picric acid solution*, allow to cool, shaking occasionally. Collect the crystals, wash with 2 quantities, each of 3 ml, of iced *water* and dry at 105°. The crystal melts between 164° to 168°(2.4.21).

C. To about 1 mg of test solution add 0.2 ml of *fuming nitric acid* and evaporate to dryness on a water-bath and dissolve the residue in 2 ml of *acetone*, add 0.2 ml of a 3.0 per cent w/v solution of *potassium hydroxide* in *methanol*; a violet colour develops.

D. Gives reaction A of sulphates (2.3.1).

## Tests

**Appearance of solution**. A 5.0 per cent w/v solution in *water* (solution A) is clear (2.4.1) and not more intensely coloured than reference solution BYS6 (2.4.1).

**pH** (2.4.24). 4.5 to 6.2, determined in 2.0 per cent w/v solution in *carbon dioxide-free water*.

**Specific optical rotation** (2.4.22). -24.0° to -29.0°, determined in solution A.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 60 mg of the substance under examination to 50.0 ml with mobile phase A. Further dilute 10.0 ml of the solution to 50.0 ml with mobile phase A.

*Reference solution (a)*. Dilute 5.0 ml of the test solution to 100.0 ml with mobile phase A. Further dilute 5.0 ml of this solution to 50.0 ml with mobile phase A.

*Reference solution (b)*. Dilute 5.0 ml of reference solution (a) to 25.0 ml with mobile phase A.

*Reference solution (c)*. Dissolve 5 mg of *(1R,3r,5S)-8-azabicyclo[3.2.1]oct-3-yl(2S)-3-hydroxy-2-phenyl-propanoate(norhyoscyamine) RS (hyoscyamine impurity E RS)* in the 20.0 ml of test solution. Dilute 5.0 ml of this solution to 25.0 ml with mobile phase A.

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- column temperature. 25°,
- mobile phase: A. dissolve 3.5 g of *sodium dodecyl sulphate* in 606 ml of a 0.7 per cent w/v solution of *potassium dihydrogen phosphate* previously adjusted to pH 3.3 with *0.05 M orthophosphoric acid* and mix with 320 ml of *acetonitrile*,
  B. *acetonitrile*,
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 10 µl.

| Time (in min) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0 - 2 | 95 | 5 |
| 2 - 20 | 95 → 70 | 5 → 30 |
| 20 - 22 | 70 → 30 | 30 → 70 |
| 22 - 25 | 95 | 5 |

Inject reference solution (c). The relative retention time for (2*RS*)-3-hydroxy-2-phenylpropanoic acid (DL-tropic acid) (hyoscyamine impurity A) is about 0.2, for (1*R*,3*S*,5*R*,6*RS*)-6-



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hydroxy-8-methyl-8-azabicyclo[3.2.1]oct-3-yl (2*S*)-3-hydroxy-2-phenylpropanoate(7-hydroxyhyoscyamine) (hyoscyamine impurity B) is about 0.67, for (1*S*,3*R*,5*S*,6*RS*)-6-hydroxy-8-methyl-8-azabicyclo[3.2.1]oct-3-yl (2*S*)-3-hydroxy-2-phenylpropanoate (6-hydroxyhyoscyamine) (hyoscyamine impurity C) is about 0.72, hyoscine (hyoscyamine impurity D) is about 0.8, for (1*R*,3*r*,5*S*)-8- azabicyclo[3.2.1]oct-3-yl (2*S*)-3-hydroxy-2-phenylpropanoate(norhyoscyamine) (hyoscyamine impurity E) is about 0.9, for (1*R*,3*r*,5*S*)-8-methyl-8- azabicyclo[3.2.1]oct-3-yl (2*R*)-2-hydroxy-3-phenyl propanoate (littorine) (hyoscyamine impurity F) is about 1.1 and for (1*R*,3*r*,5*S*)-8-methyl-8- azabicyclo[3.2.1]oct-3-yl 2-phenylprop-2-enoate (apoatropine) (hyoscyamine impurity G ) is about 1.8. The test is not valid unless resolution between the peaks due to hyoscyamine and hyoscyamine impurity E is not less than 2.5. Multiply the peak areas of the impurities by the correction factor for calculating the contents, for impurity A is 0.3; impurity G is 0.6.

Inject reference solution (a), (b) and the test solution. In the chromatogram obtained with the test solution, the area of secondary peak corresponding to impurity E is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent). The area of the secondary peaks corresponding to hyoscyamine impurities A, B, C, D, F, G, for each impurity, is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The sum of the areas of all secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). 2.0 to 5.5 per cent, determined on 0.5 g.

**Assay**. Weigh accurately about 0.5 g and dissolve in 25 ml of *anhydrous acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.0677 g of $C_{34}H_{48}N_2O_{10}S$.

**Storage**. Store protected from light and moisture.

# Hyoscyamine Injection

## Hyoscyamine Sulphate Injection

Hyoscyamine Injection is a sterile solution of Hyoscyamine Sulphate in Water for Injections.

Hyoscyamine Injection contains not less than 93.0 per cent and not more than 107.0 per cent of the stated amount of hyoscyamine sulphate, $(C_{17}H_{23}NO_3)_2,H_2SO_4,2H_2O$.

**Usual strengths**. 0.5 mg per ml; 2.0 mg per ml.

### Identification

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

B. After evaporation to dryness, complies with the test for sulphates (2.3.1).

### Tests

**pH** (2.4.24). 3.0 to 6.5.

**Bacterial Endotoxin test** (2.2.3). Not more than 714.3 Endotoxin Units per mg of hyoscyamine sulphate.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injection).

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Dilute a volume of injection containing about 1 mg of Hyoscyamine Sulphate to 200 ml with *0.01 M hydrochloric acid.*

*Reference solution (a).* A 0.016 per cent w/v solution of *hyoscyamine sulphate RS* in *0.01 M hydrochloric acid.*

*Reference solution (b).* Dilute 3.0 ml of reference solution (a) to 100 ml with *0.01 M hydrochloric acid.*

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with phenyl groups chemically bonded to porous silica (5 µm),
- mobile phase: a mixture of 1800 volumes of the buffer solution, prepared by dissolving 13.6 g of *monobasic potassium phosphate* in 1800 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid*, dilute to 2000 ml with *water*, add 0.3 volume of *triethylamine* and 200 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 205 nm,
- injection volume. 50 µl.

Inject reference solution (b). The test is not valid unless the tailing factor is not more than 1.8 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (b) and the test solution.

Calculate the content of $(C_{17}H_{23}NO_3)_2,H_2SO_4,2H_2O$ in the injection.

**Storage**. Store in single-dose or multiple-dose containers, preferably of Type I glass at controlled room temperature.



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# Hyoscyamine Oral Solution

Hyoscyamine Sulphate Oral Solution

Hyoscyamine Oral Solution is a mixture consisting of Hyoscyamine sulphate with buffering agents and other excipients. It contains suitable flavouring agents. It is filled in a sealed container.

*The oral solution is constituted by dispersing the contents of the sealed container in the specified volume of water just before use.*

Hyoscyamine Oral Solution contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of hyoscyamine sulphate, $(C_{17}H_{23}NO_3)_2,H_2SO_4,2H_2O$.

**Usual strength**. 50 mg per ml.

**Storage**. Store the constituted solution in a refrigerator 2° to 8°. Discard any unused portion after 30 days of reconstitution.

*The contents of the sealed container comply with the requirements stated under Oral Liquids and with the following requirements.*

## Identification

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

## Tests

**pH** (2.4.24). 3.0 to 6.5, determined in the reconstituted solution.

**Other tests**. Complies with the tests stated under Oral Liquids.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh accurately a quantity of the constituted solution containing 0.5 mg of Hyoscyamine Sulphate and dissolve in 100.0 ml of *0.01 M hydrochloric acid.*

*Reference solution (a).* A 0.016 per cent w/v solution of *hyoscyamine sulphate RS* in *0.01 M hydrochloric acid.*

*Reference solution (b).* Dilute 3.0 ml of reference solution (a) to 100 ml with *0.01 M hydrochloric acid.*

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with phenyl groups chemically bonded to porous silica (5 µm),
- mobile phase: a mixture of 90 volumes of the buffer solution prepared by dissolving 13.6 g of *monobasic potassium phosphate* in 1800 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid*, dilute to 2000 ml with *water*, add 0.3 volume of *triethylamine* and 10 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 205 nm,
- injection volume. 50 µl.

Inject reference solution (b). The test is not valid unless the tailing factor is not more than 1.8 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (b) and the test solution.

Calculate the content of $C_{17}H_{23}NO_3)_2,H_2SO_4,2H_2O$ in oral solution.

# Hyoscyamine Tablets

Hyoscyamine Sulphate Tablets

Hyoscyamine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of hyoscyamine sulphate, $(C_{17}H_{23}NO_3)_2,H_2SO_4,2H_2O$.

**Usual strength**. 0.375 mg.

## Identification

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

B. Gives reaction A of sulphates (2.3.1).

## Tests

**Uniformity of content.** Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay, using the following solution as the test solution.

*Test solution.* Disperse 1 Tablet in 25 ml of *0.01 M hydrochloric acid.*

Calculate the content of $(C_{17}H_{23}NO_3)_2,H_2SO_4,2H_2O$.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh and powder 20 Tablets. Disperse a quantity of powdered tablets containing about 0.125 mg of Hyoscyamine Sulphate in 25 ml of *0.01 M hydrochloric acid.*

*Reference solution (a).* A 0.016 per cent w/v solution of *hyoscyamine sulphate RS* in *0.01 M hydrochloric acid.*

*Reference solution (b).* Dilute 3.0 ml of reference solution (a) to 100 ml with *0.01 M hydrochloric acid.*

*Tropic acid solution.* A 0.0003 per cent w/v solution of tropic acid in *0.01 M hydrochloric acid.*

*Reference solution (c).* To 3.0 ml of the reference solution (a), add 4.0 ml of the tropic acid solution and dilute to 100 ml with *0.01 M hydrochloric acid.*



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Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with phenyl groups bonded to porous silica (4 µm),
- mobile phase: a mixture of 0.3 volume of *triethylamine,* 1800 volumes of the buffer solution prepared by dissolving about 13.6 g of *monobasic potassium phosphate* in 1800 ml of *water*, adjust the pH to 3.0 with *orthophosphoric acid,* dilute to 2000 ml with *water* and 200 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 205 nm,
- injection volume. 50 µl.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to hyoscyamine and tropic acid is not less than 1.5 and the tailing factor is not more than 1.8. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (b) and the test solution.

Calculate the content of $(C_{17}H_{23}NO_3)_2,H_2SO_4,2H_2O$ in the tablets.



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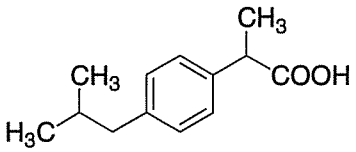


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# Ibuprofen



$C_{13}H_{18}O_2$ Mol. Wt. 206.3

Ibuprofen is (*RS*)-2-(4-isobutylphenyl)propionic acid.

Ibuprofen contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{13}H_{18}O_2$, calculated on the dried basis.

**Category**. Anti-inflammatory; analgesic.

**Dose**. 600 mg to 1.2 g daily, in divided doses, after food.

**Description**. A white or almost white, crystalline powder or colourless crystals; odour, slight.

## Identification

*Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.*

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

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.05 per cent w/v solution in *0.1 M sodium hydroxide* shows absorption maxima at about 264 nm and 272 nm, and a shoulder at about 258 nm. The ratio of the absorbance at about 264 nm to that at the shoulder at about 258 nm is 1.20 to 1.30. The ratio of the absorbance at the maximum at about 272 nm to that at the shoulder at about 258 nm is 1.00 to 1.10.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H*.

*Mobile phase*. A mixture of 75 volumes of *n-hexane*, 25 volumes of *ethyl acetate* and 5 volumes of *glacial acetic acid*.

*Test solution*. Dissolve 0.5 g of the substance under examination in 100 ml of *dichloromethane*.

*Reference solution*. A 0.5 per cent w/v solution of *ibuprofen RS* in *dichloromethane*.

Apply to the plate 5 µl of each solution. After development, dry the plate at 120° for 30 minutes, lightly spray the plate with a 1 per cent w/v solution of *potassium permanganate* in *1 M sulphuric acid*, heat at 120° for 20 minutes and examine in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

## Tests

**Appearance of solution**. A 10.0 per cent w/v solution in *ethanol (95 per cent)* is clear (2.4.1), and colourless (2.4.1).

**Optical rotation** (2.4.22). +0.05° to –0.05°, determined in a 2.5 per cent w/v solution in *methanol*.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 20 mg of the substance under examination in 2 ml of *acetonitrile* and add sufficient of the mobile phase to produce 10 ml.

*Reference solution (a)*. Dilute 1 ml of the test solution to 100 ml with the mobile phase.

*Reference solution (b)*. Dissolve 20 mg of *ibuprofen RS* in 2 ml of *acetonitrile*, add 1 ml of 0.006 per cent w/v solution of *2-(4-butylphenyl)propionic acid RS* in *acetonitrile* and add sufficient mobile phase to produce 10 ml.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 600 volumes of *water*, 340 volumes of *acetonitrile* and 0.5 volume of *phosphoric acid* diluted to 1000 volumes with *water* after equilibration,
- flow rate. 2 ml per minute,
- spectrophotometer set at 214 nm,
- injection volume. 20 µl.

Adjust the sensitivity so that the height of the principal peak in the chromatogram obtained with reference solution (a) is about 80 per cent of full-scale deflection on the recorder. Record the chromatogram for 1.5 times the retention time of the principal peak. Equilibrate the column with the mobile phase for about 45 minutes before starting the chromatography.

The retention time of ibuprofen is about 20 minutes. In the chromatogram obtained with reference solution (b) measure the height (*a*) of the peak due to 2-(4-butylphenyl) propionic acid and the height (*b*) of the lowest point of the curve separating this peak from that due to ibuprofen. The test is not valid unless *a* is greater than 1.5*b*. If necessary, adjust the concentration of acetonitrile in the mobile phase to obtain the required resolution. Verify the repeatability by making five separate injections of 20 µl of reference solution (a). The test is not valid unless the relative standard deviation of the area of the principal peak is less than 2.0 per cent.

In the chromatogram obtained with the test solution, the area of any peak corresponding to 2-(4-butylphenyl) propionic acid is not greater than that of the peak due to 2-(4-butylphenyl) propionic acid in the chromatogram obtained with reference solution (b), the area of any other secondary peak is not greater than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) and the sum of the areas of any such peaks is not greater than 0.7 times the area of the principal peak in the chromatogram obtained with reference solution (a). Ignore any peak the area of which is less than



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0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a).

**Impurity F**. Determine by gas chromatography (2.4.13).

*Methylating solution*. Dilute 1 ml of *N,N-dimethylformamide dimethyl acetal* and 1ml of *pyridine* to 10 ml with *ethyl acetate*.

*Test solution*. Weigh about 50 mg of the substance under examination into a sealable vial, dissolve in 1.0 ml of *ethyl acetate*, add 1 ml of the methylating solution, seal and heat at 100° in a block heater for 20 minutes, cool. Remove the reagents under a stream of nitrogen at room temperature. Dissolve the residue in 5 ml of *ethyl acetate*.

Chromatographic system
- a glass column 25 m x 0.53 mm, packed with fused silica coated with macrogol 20000 (film thickness 2 µm),
- temperature:
  column 150°,
  injector port 200° and detector port 250°,
- flame ionization detector,
- flow rate. 5 ml per minute using nitrogen as the carrier gas.

Inject the test solution. Run the chromatogram twice the retention time of the principal peak. The relative retention time with reference to ibuprofen for 3-[4-(2methylpropyl)phenyl] propionic acid (ibuprofen impurity F) is about 1.5. The area of the peak corresponding to ibuprofen impurity F is not more than 0.1 per cent of the principal peak.

**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying over *phosphorus pentoxide* at a pressure not exceeding 0.1 kPa.

**Assay**. Weigh accurately about 0.4 g, dissolve in 100 ml of *ethanol (95 per cent)* and titrate with *0.1 M sodium hydroxide* using 0.2 ml of *phenolphthalein solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.02063 g of $C_{13}H_{18}O_2$.

# Ibuprofen Cream

Ibuprofen Cream contains Ibuprofen in a suitable basis.

Ibuprofen Cream contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of ibuprofen, $C_{13}H_{18}O_2$.

**Usual strength**. 15 per cent w/w.

## Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H*.

*Mobile phase*. A mixture of 5 volumes of *anhydrous acetic acid*, 25 volumes of *ethyl acetate* and 75 volumes of *n-hexane*.

*Test solution*. Shake a quantity of the cream containing 50 mg of Ibuprofen with 10 ml of *dichloromethane* for 5 minutes and filter.

*Reference solution*. A 0.5 per cent w/v solution of *ibuprofen RS* in *dichloromethane*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate at 120° for 30 minutes, lightly spray the plate with a 1 per cent w/v solution of *potassium permanganate* in *1 M sulphuric acid*, heat at 120° for 20 minutes and examine under ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

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

## Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Shake a quantity of the cream containing 0.1 g of Ibuprofen with 25 ml of *methanol* for 10 minutes, dilute to 50 ml with *methanol* and filter.

*Reference solution (a)*. Dilute 1 ml of the test solution to 100 ml with *methanol*.

*Reference solution (b)*. Dissolve 50 mg of *ibuprofen RS* in 2.5 ml of a 0.006 per cent w/v solution of *2-(4-butylphenyl) propionic acid RS* in *methanol* and dilute to 25 ml with *methanol*.

Chromatographic system
- a stainless steel column 15 cm × 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm) (such as Spherisorb ODS 2),
- mobile phase: a mixture of 0.5 volume of *orthophosphoric acid*, 340 volumes of *acetonitrile* and 660 volumes of *water*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 214 nm,
- injection volume. 20 µl.

Adjust the sensitivity so that the height of the principal peak in the chromatogram obtained with reference solution (a) is



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70 to 90 per cent of full-scale deflection on the recorder. Run the chromatogram for 1.5 times the retention time of the principal peak.

Inject reference solution (b). Measure the height (a) of the peak due to 2-(4-butylphenyl)-propionic acid and the height (b) of the lowest point of the curve separating this peak from that due to ibuprofen. The test is not valid unless (a) is more than 1.5(b).

Inject reference solution (a), (b) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to 2-(4-butylphenyl)-propionic acid is not more than that of the peak due to 2-(4- butylphenyl)propionic acid in the chromatogram obtained with reference solution (b), the area of any other secondary peak is not more than 0.3 times the area of the principal peak in the chromatogram obtained with the reference solution (a) (0.3 per cent) and the sum of the areas of other secondary peaks is not more than 0.7 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.7 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

**Other tests**. Complies with the tests stated under Creams.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Shake a quantity of the cream containing 50 mg of ibuprofen with 25 ml of the mobile phase for 10 minutes, decant the solution into a 50 ml graduated flask, rinse the original flask with two 10 ml quantities of the mobile phase, dilute the combined solution and rinsings to 50 ml with the mobile phase and filter.

*Reference solution*. A 0.1 per cent w/v solution of *ibuprofen RS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (10 µm) (such as Nucleosil C18),
- mobile phase: a mixture of 0.3 volumes of *orthophosphoric acid*, 24.7 volumes of *water* and 75 volumes of *methanol*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 264 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{13}H_{18}O_2$ in the cream.

# Ibuprofen Gel

Ibuprofen Gel is a solution of Ibuprofen in a suitable water-miscible base.

Ibuprofen Gel contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of ibuprofen, $C_{13}H_{18}O_2$.

**Usual strength**. 15 per cent w/w.

## Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H*.

*Mobile phase*. A mixture of 5 volumes of *anhydrous acetic acid*, 25 volumes of *ethyl acetate* and 75 volumes of *n-hexane*.

*Test solution*. Weigh accurately a quantity containing about 50 mg of Ibuprofen and transfer to a separating funnel with the aid of 10 ml of *dichloromethane*. Shake vigorously for 5 minutes and filter.

*Reference solution*. A 0.5 per cent w/v solution of *ibuprofen RS* in *dichloromethane*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 10 cm. After development, dry the plate at 120° for 30 minutes, lightly spray the plate with a 1 per cent w/v solution of *potassium permanganate* in *1 M sulphuric acid*, heat at 120° for 20 minutes and examine under ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

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

## Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Disperse a quantity of the substance under examination containing 0.1 g of Ibuprofen in 25 ml of warm *methanol*, cool and dilute to 50 ml with *methanol*.

*Reference solution (a)*. Dilute 1 ml of the test solution to 100 ml with *methanol*.

*Reference solution (b)*. Dissolve 50 mg of *ibuprofen RS* in 2.5 ml of a 0.006 per cent w/v solution of *2-(4-butylphenyl)-propionic acid RS* in *methanol* and dilute to 25 ml with *methanol*.

Chromatographic system
- a stainless steel column 15 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),



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- mobile phase: a mixture of 0.5 volume of *orthophosphoric acid*, 340 volumes of *acetonitrile* and 660 volumes of *water*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 214 nm,
- injection volume. 20 µl.

Equilibrate the column with the mobile phase for 45 minutes.

Inject reference solution (b). Measure the height (a) of the peak due to 2-(4-butylphenyl) propionic acid and the height (b) of the lowest point of the curve separating this peak from that due to ibuprofen.

The test is not valid unless *a* is greater than 1.5*b*. If necessary, adjust the concentration of acetonitrile in the mobile phase to obtain the required resolution. Adjust the sensitivity so that the height of the principal peak in the chromatogram obtained with reference solution (a) is 70 to 90 per cent of full-scale deflection on the recorder. Record the chromatogram for 1.5 times the retention time of the principal peak.

Inject reference solution (a), (b) and the test solution. In the chromatogram obtained with the test solution the area of any peak corresponding to 2-(4-butylphenyl) propionic acid is not more than that of the peak due to 2-(4- butylphenyl)-propionic acid in the chromatogram obtained with reference solution (b) (0.3 per cent), the area of any other secondary peak is not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent) and the sum of the areas of other secondary peaks is not more than 0.7 times the area of the principal peak in the chromatogram obtained with the reference solution (a) (0.7 per cent). Ignore any peak the area of which is less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Disperse a quantity of the substance under examination containing 50 mg of Ibuprofen with 50 ml of warm *methanol* for 10 minutes, cool and add sufficient *methanol* to produce 100 ml. Dilute 10 ml of this solution to 20 ml with the mobile phase.

*Reference solution*. Dilute 10 ml of a solution containing 0.05 per cent w/v of *ibuprofen RS* in *methanol* to 20 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 3 volumes of *orthophosphoric acid*, 247 volumes of *water* and 750 volumes of *methanol*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 264 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{13}H_{18}O_2$ in the gel.

# Ibuprofen Tablets

Ibuprofen Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of ibuprofen, $C_{13}H_{18}O_2$. The tablets are coated.

**Usual strengths**. 200 mg; 400 mg; 600 mg.

## Identification

A. Extract a quantity of the powdered tablets containing 0.5 g of Ibuprofen with 20 ml of *acetone*, filter and evaporate the filtrate to dryness in a current of air without heating. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ibuprofen RS* or with the reference spectrum of ibuprofen.

B. The residue obtained in test A, after recrystallisation from *light petroleum (40° to 60°)*, melts at about 75° (2.4.21).

## Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *phosphate buffer pH 7.2*,
Speed and time. 100 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate. Dilute a suitable volume of the filtrate with the same solvent. Measure the absorbance of the resulting solution at the maximum at about 221 nm (2.4.7). Calculate the content of $C_{13}H_{18}O_2$.

D. Not less than 50 per cent of the stated amount of $C_{13}H_{18}O_2$.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H*.

*Mobile phase*. A mixture of 75 volumes of *n-hexane*, 25 volumes of *ethyl acetate* and 5 volumes of *glacial acetic acid*.

*Test solution*. Extract a quantity of the powdered tablets containing 0.2 g of Ibuprofen with three quantities, each of 10 ml, of *chloroform*, filter, evaporate the combined filtrate to about 1 ml and add sufficient *chloroform* to produce 2 ml.



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*Reference solution*. Dilute 1 volume of the test solution to 100 volumes with *chloroform*.

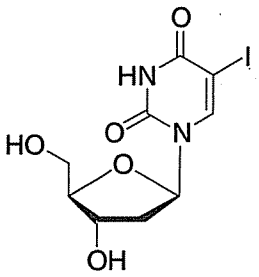
Apply to the plate 5 µl of each solution. After development, dry the plate in air, lightly spray the plate with a 1 per cent w/v solution of *potassium permanganate* in *1 M sulphuric acid*, heat at 120° for 20 minutes and examine in ultraviolet light at 365 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution. Ignore any spot with an $R_f$ value relative to ibuprofen of about 1.2.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.5 g of Ibuprofen, extract with 60 ml of *chloroform* for 15 minutes and filter through a sintered-glass crucible of porosity 3. Wash the residue with three quantities, each of 10 ml, of *chloroform* and gently evaporate the filtrate just to dryness in a current of air. Dissolve the residue in 100 ml of *ethanol (95 per cent)*, previously neutralized to *phenolphthalein solution*, and titrate with *0.1 M sodium hydroxide* using *phenolphthalein solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.02063 g of $C_{13}H_{18}O_2$.

# Idoxuridine




$C_9H_{11}IN_2O_5$ Mol. Wt. 354.1

Idoxuridine is 2'-deoxy-5-iodouridine.

Idoxuridine contains not less than 98.0 per cent and not more than 101.0 per cent of $C_9H_{11}IN_2O_5$, calculated on the dried basis.

**Category**. Antiviral for topical use.

**Description**. A white or almost white, crystalline powder; odourless.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *idoxuridine RS* or with the reference spectrum of idoxuridine. Examine the substances as dispersions containing 1 mg in 0.3 g of *potassium bromide IR*.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.004 per cent w/v solution in *0.01 M sodium hydroxide* shows an absorption maximum only at about 279 nm; absorbance at about 279 nm, about 0.65.

C. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (c).

D. Heat about 5 mg in a test-tube over a naked flame; a violet vapour is evolved.

## Tests

**Appearance of solution**. A 1.0 per cent w/v solution in *1 M sodium hydroxide* is clear (2.4.1), and colourless (2.4.1).

**pH** (2.4.24). 5.5 to 6.5, determined in a 0.1 per cent w/v solution.

**Specific optical rotation** (2.4.22). +28.0° to +32.0°, determined in a 1.0 per cent w/v solution in *1 M sodium hydroxide*.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 50 volumes of *2-propanol*, 40 volumes of *chloroform* and 10 volumes of *strong ammonia solution*.

*Test solution (a)*. Dissolve 0.4 g of the substance under examination in 10 ml of a mixture of 5 volumes of *methanol* and 1 volume of *strong ammonia solution*.

*Test solution (b)*. Dilute 1 ml of test solution (a) to 10 ml with the same solvent mixture.

*Reference solution (a)*. Dilute 5 ml of test solution (b) to 100 ml with the same solvent mixture.

*Reference solution (b)*. A solution containing 0.02 per cent w/v each of *5-iodouracil RS* and *2-deoxyuridine RS* in the same solvent mixture.

*Reference solution (c)*. A solution containing 0.4 per cent w/v of *idoxuridine RS* in the same solvent mixture.

Apply to the plate 5 µl of each solution. After development, dry the plate in a current of cold air and repeat the development. After removal of the plate following the second development, dry it in a current of cold air and examine in ultraviolet light at 254 nm. The spots due to 5-iodouracil and 2'-deoxyuridine in the chromatogram obtained with reference solution (b) are more intense than any corresponding spots in the chromatogram obtained with test solution (a). Any other secondary spot in the chromatogram obtained with test



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solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a).

**Iodide**. Not more than 0.1 per cent, determined by the following method. Dissolve 0.25 g in 25 ml of *0.1 M sodium hydroxide*, 5 ml of *dilute hydrochloric acid* and sufficient *water* to produce 50 ml, allow to stand for 10 minutes and filter. To 25 ml of the filtrate add 5 ml of *hydrogen peroxide solution (10 vol)* and 10 ml of *chloroform* and shake. Any pink colour produced in the organic layer is not more intense than that obtained by repeating the procedure using 1 ml of a 0.033 per cent w/v solution of *potassium iodide* in place of the substance under examination.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 60° over *phosphorus pentoxide* at a pressure of 1.5 to 2.5 kPa.

**Assay**. Weigh accurately about 0.3 g, dissolve in 50 ml of *dimethylformamide* and titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.03541 g of $C_9H_{11}IN_2O_5$.

**Storage**. Store protected from light.

## Idoxuridine Eye Drops

Idoxuridine Eye Drops are a sterile solution of Idoxuridine in Purified Water.

Idoxuridine Eye Drops contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of idoxuridine, $C_9H_{11}IN_2O_5$.

**Usual strength**. 0.1 per cent w/v.

### Identification

A. Dilute a suitable volume with *0.01 M sodium hydroxide* to produce a solution containing 0.003 per cent w/v of Idoxuridine. When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum only at about 279 nm.

B. In the Assay, the chromatogram obtained with the reference solution (a) shows a peak that corresponds to the peak due to idoxuridine in the chromatogram obtained with the test solution.

### Tests

**Other tests**. Comply with the tests stated under Eye Drops.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Add 2 ml of a 10 per cent v/v solution of *ethanol (95 per cent)* to 15.0 ml of a solution prepared by diluting an accurately measured volume of the eye drops with *water* if necessary to give a final concentration of 0.1 per cent w/v of Idoxuridine (solution A) and dilute to 20.0 ml with *water*.

*Reference solution (a)*. Shake 0.1 g of *idoxuridine RS* with 50 ml of *water* until dissolved and then dilute to 100.0 ml with *water*. To 15.0 ml of this solution add 2.0 ml of a solution prepared by diluting 10 ml of a 1.2 per cent w/v of *sulphathiazole* (internal standard) in *ethanol (95 per cent)* to 100 ml with *water* (solution B), and dilute to 20.0 ml with *water*.

*Reference solution (b)*. Add 2.0 ml of solution B to 15.0 ml of solution A and dilute to 20.0 ml with *water*.

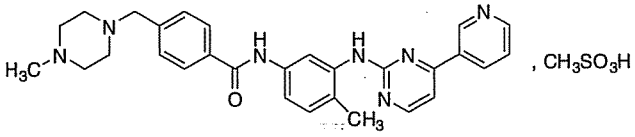
Chromatographic system
- a stainless steel column 30 cm x 4 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 87 volumes of *water* and 13 volumes of *methanol*,
- flow rate. 1.7 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Calculate the content of $C_9H_{11}IN_2O_5$ in the eye drops.

**Storage**. Store at a temperature not exceeding 30°. The eye drops should not be allowed to freeze.

**Labelling**. The label states that the eye drops should not be used for continuous periods of treatment exceeding 21 days.

## Imatinib Mesylate




$C_{29}H_{31}N_7O,CH_3SO_3H$ Mol. Wt. 589.7

Imatinib Mesylate is 4-[(4-methyl-1-piperazinyl)methyl]-*N*-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]phenyl] benzamide methanesulphonate.

Imatinib Mesylate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{29}H_{31}N_7O,CH_3SO_3H$, calculated on the anhydrous basis.

**Category**. Anticancer.

**Description**. A white to off white, crystalline powder.

*CAUTION — Imatinib Mesylate is cytotoxic; extra care required to prevent inhaling particles and exposing the skin to it.*



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### Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *imatinib mesylate RS* or with the reference spectrum of imatinib mesylate.

### Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve 50 mg of the substance under examination in 50.0 ml of the mobile phase.

*Reference solution.* Dilute 1.0 ml of test solution to 100.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecysilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 40 volumes of 1 per cent w/v solution of *ammonium acetate* and 60 volumes of *acetonitrile,*
- flow rate. 0.7 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the theoritical plates is not less than 2000 and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent). The sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (2.0 per cent). Ignore any peak with an area less than 0.1 times the area of the peak in the chromatogram obtained with the reference solution (0.1 per cent).

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

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Water** (2.3.43). Not more than 1.0 per cent, determined on 1.0 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve 50 mg of the substance under examination in 50.0 ml of the mobile phase. Dilute 5 ml of the solution to 50 ml with the mobile phase.

*Reference solution.* A 0.01 per cent w/v solution of *imatinib mesylate RS* with the mobile phase.

Use the chromatographic system as described under Related substances.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{29}H_{31}N_7O,CH_3SO_3H$.

**Storage**. Store protected from light.

## Imatinib Capsules

Imatinib Mesylate Capsules

Imatinib Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of stated amount of imatinib, $C_{29}H_{31}N_7O$.

**Usual strength**. 100 mg.

### Identification

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

### Tests

**Other tests**. Comply with the tests stated under Capsules.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Disperse a quantity of mixed contents of 20 capsules containing about 20 mg of imatinib with 100.0 ml of the mobile phase.

*Reference solution.* A 0.02 per cent w/v solution of *imatinib mesylate RS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 40 volumes of 1 per cent *ammonium acetate* and 60 volumes of *acetonitrile,*
- flow rate. 0.7 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, the theoritical plates is not less than 1500 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{29}H_{31}N_7O$ in the capsules.

**Storage**. Store protected from light and moisture.

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

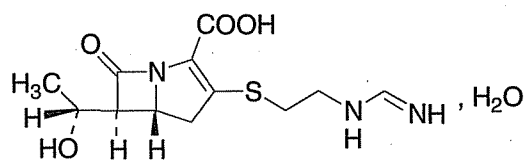


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# Imipenem



$C_{12}H_{17}N_3O_4S,H_2O$ Mol. Wt. 317.4

Imipenem is (5*R*,6*S*)-6-[(*R*)-1-hydroxyethyl]-3-[[2-[ (imino-methyl)amino]ethyl]sulphanyl]-7-oxo-1-azabi-cyclo[3.2.0]hept-2-ene-2-carboxylic acid monohydrate.

Imipenem contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{12}H_{17}N_3O_4S$, calculated on the anhydrous basis.

**Category**. Antibacterial.

**Description**. A white to almost white or pale yellow powder.

## Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *imipenem monohydrate RS* or with the reference spectrum of imipenem monohydrate.

## Tests

**Appearance of solution**. A 1.0 per cent w/v solution in *phosphate buffer pH 7.0* is not more opalescent than opalescence standard OS2 (2.4.1) and not more intensely coloured than reference solution BYS6 (2.4.1).

**pH** (2.4.24). 4.5 to 7.0 determined in 0.5 per cent w/v solution in *water*.

**Specific optical rotation** (2.4.22). + 84.0° to + 89.0°, determined in a 0.5 per cent w/v solution in *phosphate buffer pH 7*.

**Related substances**. Determine by liquid chromatography (2.4.14).

*NOTE –Keep the solutions in an ice-bath and use within 8 hours of preparation.*

*Solvent mixture.* 0.7 volume of *acetonitrile* and 99.3 volumes of a 1.35 per cent w/v solution of *dipotassium hydrogen phosphate,* adjusted to pH 6.8 with *orthophosphoric acid.*

*Test solution.* Dissolve 40 mg of the substance under examination in 100.0 ml of the solvent mixture.

*Reference solution (a).* A 0.04 per cent w/v solution of *imipenem RS* in the solvent mixture.

*Reference solution (b).* Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture.

*Reference solution (c).* Heat 20 ml of the test solution, previously adjusted to pH 10.0 with *sodium hydroxide solution* at 80° for 5 minutes (in situ preparation of impurity A).

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 0.7 volumes of *acetonitrile* and 99.3 volumes of a 0.87 per cent w/v solution of *dipotassium hydrogen phosphate,* adjust the pH to 7.3 with *orthophosphoric acid,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (c). The relative retention time with reference to imipenem for imipenem impurity A is about 0.8 and the resolution between the peaks due to imipenem impurity A and imipenem is not less than 3.5.

Inject the test solution and reference solution (b). Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of peak corresponding to imipenem impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). The area of any other secondary peak is not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent). The sum of the areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

**Sulphated ash** (2.3.18). Not more than 0.2 per cent, determined on 1.0 g.

**Water** (2.3.43). 5.0 per cent to 8.0 per cent, determined on 0.2 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*NOTE –Keep the solutions in an ice-bath and use within 8 hours of preparation.*

*Test solution.* Dissolve about 40 mg of the substance under examination in 100 ml of the mobile phase.

*Reference solution.* A 0.04 per cent w/v solution of *imipenem monohydrate RS* in the mobile phase.

Chromatographic system
- a stainless steel column 30 cm × 3.9 mm, packed with octadecylsilane bonded to porous silica (10 µm) (such as Bondapak C18),
- mobile phase: a solution prepared by dissolving 54 mg of *monobasic potassium phosphate* in 360 ml of *water*,



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adjusted to pH 6.8 with *0.5 M sodium hydroxide or 0.5 M orthophosphoric acid*, dilute to 400 ml with *water* and filter,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 300 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 600 theoretical plates, and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_{12}H_{17}N_3O_4S$.

*Imipenem intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.*

**Bacterial Endotoxins** (2.2.3). Not more than 0.17 Endotoxin Unit per mg of imipenem.

*Imipenem intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.*

**Sterility** (2.2.11). Complies with the test for sterility.

**Storage**. Store in an airtight container in a refrigerator (2° to 8°).

**Labelling**. The label states, where applicable, that the substance is sterile and is free from bacterial endotoxins.

# Imipenem and Cilastatin Injection

Imipenem and Cilastatin Injection is a sterile mixture of Imipenem, Cilastatin Sodium, and Sodium Bicarbonate.

The injection is constituted as per the labelling requirements.

*The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).*

**Storage**. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Imipenem and Cilastatin Injection contains not less than 90.0 per cent and not more than 115.0 per cent of the stated amounts of imipenem, $C_{12}H_{17}N_3O_4S$ and cilastatin, $C_{16}H_{26}N_2O_5S$.

*The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.*

## Identification

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

## Tests

**pH** (2.4.24). 6.5 to 8.5, when constituted as directed on the label.

**Bacterial endotoxins** (2.2.3). Not more than 0.17 Endotoxin Unit per mg of imipenem and not more than 0.17 Endotoxin Unit per mg of cilastatin.

**Sterility** (2.2.11). Complies with the test for sterility.

**Loss on drying** (2.4.19). Not more than 3.5 per cent, determined on 0.1 g, by drying in an oven at 60° for 3 hours at a pressure not exceeding 0.7 kPa.

**Assay**. Determine by liquid chromatography (2.4.14).

*NOTE – Prepare the following solutions immediately before use.*

*Test solution*. Determine the weight of the contents of 10 containers. Weigh accurately a quantity of the mixed contents of the 10 containers containing about 50 mg of Imipenem dissolve in *buffer solution pH 6.8* and dilute to 100.0 ml with the same solution.

*Reference solution (a)*. A 0.05 per cent w/v solution of *imipenem monohydrate RS* in *buffer solution pH 6.8*.

*Reference solution (b)*. A 0.05 per cent w/v solution of *cilastatin ammonium RS* in *buffer solution pH 6.8*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature 50°,
- mobile phase: dissolve 2.0 g of *sodium 1-hexane-sulphonate* in 800 ml of *buffer solution pH 6.8*, adjust the pH to 6.8 with *0.5 M sodium hydroxide* or *0.5 M phosphoric acid*, dilute to 1000 ml with *buffer solution pH 6.8* and filter,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Inject reference solution (a). The test is not valid unless the tailing factor is not more than 1.5, the column efficiency is not less than 600 theoretical plates, and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and reference solutions (a) and (b).

Calculate the contents of $C_{12}H_{17}N_3O_4S$ and $C_{16}H_{26}N_2O_5S$ in the injection.

**Storage**. Store protected from moisture, in a single dose or multiple dose container.



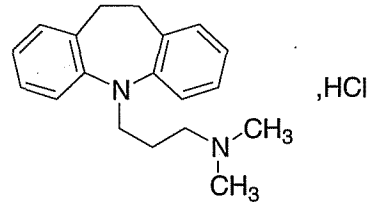
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**Labelling.** The label states that the suspension obtained when constituted as directed in the labelling is for intramuscular injection only.

## Imipramine Hydrochloride



$C_{19}H_{24}N_2,HCl$ Mol. Wt. 316.9

Imipramine Hydrochloride is 10,11-dihydro-5*H*-dibenz[*b,f*]azepine-5-(dimethylaminopropyl) hydrochloride.

Imipramine Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{19}H_{24}N_2,HCl$, calculated on the dried basis.

**Category.** Antidepressant.

**Dose.** 50 to 150 mg daily, in divided doses.

**Description.** A white or slightly yellow, crystalline powder; almost odourless.

### Identification

*Test A may be omitted if tests B, C, D and E are carried out. Tests B, C and D may be omitted if tests A and E are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *imipramine hydrochloride RS* or with the reference spectrum of imipramine hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.002 per cent w/v solution in *0.01 M hydrochloric acid* shows an absorption maximum only at about 250 nm and a shoulder at about 270 nm; absorbance at about 250 nm, about 0.52.

C. Dissolve 5 mg in 2 ml of *nitric acid;* an intense blue colour is produced.

D. Dissolve 50 mg in 3 ml of *water* and add 1 drop of a 2.5 per cent w/v solution of *quinhydrone* in *methanol*; no red colour is produced within 15 minutes.

E. 20 mg gives reaction A of chlorides (2.3.1).

### Tests

**Appearance of solution.** Triturate with a glass rod 3.0 g with 20 ml of *carbon dioxide-free water* and dilute to 30 ml with the same solvent (solution A). Solution A is clear (2.4.1). Immediately after preparation dilute the solution with an equal volume of *water*. The resulting solution is not more intensely coloured than reference solution BYS6 (2.4.1).

**pH** (2.4.24). 3.6 to 5.0, determined in solution A immediately after preparation.

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase.* A mixture of 55 volumes of *ethyl acetate*, 35 volumes of *glacial acetic acid*, 5 volumes of *hydrochloric acid* and 5 volumes of *water*.

*NOTE –Prepare the following solutions immediately before use.*

*Test solution.* Dissolve 0.25 g of the substance under examination in 10 ml of *methanol*.

*Reference solution (a).* A 0.005 per cent w/v solution of the substance under examination in *methanol*.

*Reference solution (b).* A 0.005 per cent w/v solution of *iminodibenzyl RS* in *methanol*.

Apply to the plate 10 µl of each solution. After development, remove the plate, allow the solvent to evaporate for 5 minutes, spray with a 0.5 per cent w/v solution of *potassium dichromate* in *sulphuric acid (20 per cent)* and examine immediately. In the chromatogram obtained with the test solution any spot corresponding to iminodibenzyl is not more intense than the spot in the chromatogram obtained with reference solution (b) and any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (a).

**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay.** Weigh accurately about 0.5 g, dissolve in 50 ml of *chloroform*, add 10 ml of *mercuric acetate solution* and titrate with *0.1 M perchloric acid*, using 0.5 ml of *metanil yellow solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03169 g of $C_{19}H_{24}N_2,HCl$.

**Storage.** Store protected from light.

## Imipramine Tablets

Imipramine HydrochlorideTablets

Imipramine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of imipramine hydrochloride, $C_{19}H_{24}N_2,HCl$. The tablets are coated.



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**Usual strengths**. 10 mg; 25 mg.

### Identification

Triturate a quantity of the powdered tablets containing about 0.25 g of Imipramine Hydrochloride with 10 ml of *chloroform*, filter, evaporate the filtrate to low bulk, add *ether* until a turbidity is produced, and allow to stand. The precipitate, after recrystallisation from *acetone*, melts at about 172° (2.4.21), and complies with the following tests.

A. Dissolve 5 mg in 2 ml of *nitric acid;* an intense blue colour is produced.

B. Dissolve 50 mg in 3 ml of *water* and add 1 drop of a 2.5 per cent w/v solution of *quinhydrone* in *methanol*; no red colour is produced within 15 minutes.

C. 20 mg gives reaction A of chlorides (2.3.1).

### Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 55 volumes of *ethyl acetate*, 35 volumes of *glacial acetic acid*, 5 volumes of *hydrochloric acid* and 5 volumes of *water*.

*Prepare the following solutions immediately before use.*

*Test solution*. Shake a quantity of the powdered tablets containing 0.2 g of Imipramine Hydrochloride with three quantities, each of 10 ml, of *chloroform*, filter the combined chloroform extracts, evaporate to dryness and dissolve the residue in 10 ml of *methanol*.

*Reference solution (a)*. Dilute 3 volumes of the test solution to 1000 volumes with *methanol*.

*Reference solution (b)*. A 0.006 per cent w/v solution of *iminodibenzyl RS* in *methanol*.

Apply to the plate 10 µl of each solution. After development, remove the plate, allow the solvent to evaporate for 5 minutes, spray with a 0.5 per cent w/v solution of *potassium dichromate* in *sulphuric acid (20 per cent)* and examine immediately. In the chromatogram obtained with the test solution any spot corresponding to iminodibenzyl is not more intense than the spot in the chromatogram obtained with reference solution (b) and any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (a).

**Uniformity of content**. *(For tablets containing 10 mg or less)*.

Comply with the test stated under Tablets.

Powder one tablet, shake with 25 ml of *0.1 M hydrochloric acid* for 30 minutes, add sufficient *0.1 M hydrochloric acid* to produce 100.0 ml and filter. Dilute 10.0 ml of the filtrate to 50.0 ml with *0.1 M hydrochloric acid* and measure the absorbance of the resulting solution at the maximum at about 250 nm (2.4.7). Calculate the content of $C_{19}H_{24}N_2$,HCl in the tablet taking 264 as the specific absorbance at 250 nm.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 25 mg of Imipramine Hydrochloride, shake with 75 ml of *0.1 M hydrochloric acid* for 30 minutes, dilute to 100.0 ml with the same solvent and filter through a sintered-glass filter. Dilute 10.0 ml to 100.0 ml with *0.1 M hydrochloric acid* and measure the absorbance of the resulting solution at the maximum at about 250 nm (2.4.7). Calculate the content of $C_{19}H_{24}N_2$, HCl taking 264 as the specific absorbance at 250 nm.

## Indapamide Tablets

Indapamide Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of indapamide hemihydrate, $C_{16}H_{16}ClN_3O_3S,½H_2O$.

**Usual Strengths**. 1.5 mg; 2.5 mg.

### Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 20 volumes of *acetone* and 80 volumes of *toluene*.

*Test solution*. Shake a quantity of the powdered tablets containing 50 mg of Indapamide with 10.0 ml of *acetone* for 15 minutes and filter.

*Reference solution*. A 0.5 per cent w/v solution of *indapamide RS* in *acetone*.

Apply to the plate 20 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in air and examine under ultraviolet light at 254 nm. Spray with a solution prepared by mixing 10 volumes of *potassium iodobismuthate solution* and 20 volumes of *glacial acetic acid* and diluting to 100 volumes with *water* and examine again. Finally spray the plate with a 5.0 per cent w/v solution of *sodium nitrite* in a mixture of equal volumes of *water* and *ethanol (95 per cent)* and examine again. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

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



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## Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 500 ml of *0.1 M hydrochloric acid,*
Speed and time. 100 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate (2.4.7), suitably diluted with *0.1 M hydrochloric acid* at 240 nm and at 275 nm using *0.1M hydrochloric acid* as blank. Prepare a reference solution by diluting 1 ml of a 0.1 per cent w/v solution of *indapamide RS* in *methanol* to 200 ml with *0.1 M hydrochloric acid* and measure the absorbance of this solution at 240 nm and at 275 nm using *0.1 M hydrochloric acid* as blank. Calculate the content of $C_{16}H_{16}ClN_3O_3S,½H_2O$, in the medium using the differences in absorbances at 240 nm and at 275 nm.

1 mg of $C_{16}H_{16}ClN_3O_3S$ is equivalent to 1.0246 mg of $C_{16}H_{16}ClN_3O_3S,½H_2O$.

D. Not less than 75.0 per cent of the stated amount of $C_{16}H_{16}ClN_3O_3S,½H_2O$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*NOTE—Prepare the solutions protected from light.*

*Test solution*. Shake a quantity of 10 whole tablets with 70 ml of *ethanol (95 per cent)*, mix mechanically until the tablets have disintegrated and continue mixing for 2 hours. Add sufficient *ethanol (95 per cent)* to produce 100 ml, mix and centrifuge. Dilute the supernatant liquid with the mobile phase to produce a solution containing 0.005 per cent w/v solution of Indapamide.

*Reference solution (a).* Dilute 1.0 ml of the test solution to 10.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

*Reference solution (b).* Dilute 1.0 ml of 0.00025 per cent w/v solution of *indapamide impurity B RS* in *ethanol (95 per cent)* to 10.0 ml with the mobile phase.

*Reference solution (c).* Dilute 1.0 ml of 0.00025 per cent w/v solution of *4- chloro-3-sulfamoylbenzoic acid* in *ethanol (95 per cent)* to 10.0 ml with the mobile phase.

*Reference solution (d).* Mix 1.0 ml of the test solution, 1.0 ml of a 0.00025 per cent w/v solution of *4-chloro-N-(2-methyl-1H-indol-1-yl]-3-sulphamoylbenzamide RS (indapamide impurity B RS)* in *ethanol (95 per cent)*, 1.0 ml of a 0.00025 per cent w/v solution of *4-chloro-3-sulfamoylbenzoic acid* in *ethanol (95 per cent)* and 7.0 ml of the mobile phase.

Chromatographic system
- a stainless steel column 15 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Nuclosil C18),
- mobile phase: a mixture of 6 volumes of 5.0 per cent w/v solution of *sodium dodecyl sulphate* and 3.0 per cent v/v of *glacial acetic acid*, 10 volumes of *triethylamine*, 20 volumes of *butanol*, 310 volumes of *acetonitrile* and 690 volumes of *water*, adjust the pH to 3.0 with *orthophosphoric acid,*
- flow rate. 1.6 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume. 20 µl.

Inject reference solution (d). The relative retention time with reference to indapamide for indapamide impurity B is about 1.7 and for 4-chloro-3-sulfamoylbenzoic acid is about 0.3.

Inject reference solution (a), (b), (c) and the test solution. In the chromatogram obtained with the test solution the area of peak corresponding to indapamide impurity B is not more than the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent), the area of peak corresponding to 4-chloro-3-sulfamoylbenzoic acid is not more than 0.4 times the area of the peak in the chromatogram obtained with reference solution (c) (0.2 per cent) and the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent). The sum of the areas of all other secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent).

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*NOTE—Prepare the solutions protected from light.*

*Test solution*. Shake a quantity of 10 whole tablets with 70 ml of *ethanol (95 per cent)* and continue mixing for 2 hours. Add sufficient *ethanol (95 per cent)* to produce 100 ml, mix and centrifuge. Dilute the supernatant liquid with the mobile phase to produce a solution containing 0.005 per cent w/v solution of Indapamide.

*Reference solution.* Dilute 1.0 ml of a 0.025 per cent w/v solution of *indapamide RS* in *ethanol (95 per cent)* to 5.0 ml with the mobile phase.

Use the chromatographic system as described under Related substances.

Calculate the content of $C_{16}H_{16}ClN_3O_3S,½H_2O$ in the tablets.

1 mg of $C_{16}H_{16}ClN_3O_3S$ is equivalent to 1.0246 mg of $C_{16}H_{16}ClN_3O_3S,½H_2O$.

**Storage**. Store protected from light.

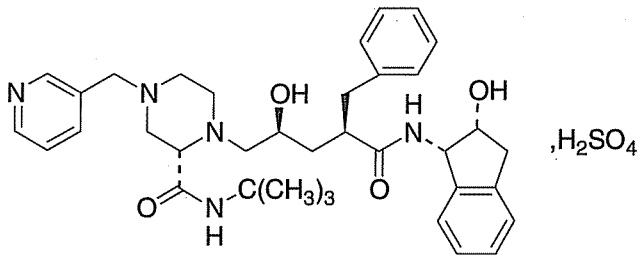


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# Indinavir Sulphate



$C_{36}H_{47}N_5O_4, H_2SO_4$, Mol. Wt. 711.9

Indinavir Sulphate is [1-(1*S*,2*R*),5-(2*S*)]-2,3,5-trideoxy-*N*-(2,3-dihydro-2-hydroxy-1*H*-inden-1-yl)-5-[2-[{1,1-dimethylethyl)amino}carbonyl]-4-(3-pyridinylmethyl)-1-piperazinyl]-2-(phenylmethyl)-D-*erythro*-pentonamide sulphate.

Indinavir Sulphate contains not less than 98.5 per cent and not more than 101.5 per cent of $C_{36}H_{47}N_5O_4,H_2SO_4$, calculated on the anhydrous and ethanol-free basis.

**Category**. Antiretroviral.

**Dose**. 800 mg twice daily.

**Description**. A white or almost white powder; hygroscopic.

## Identification

A. When examined in the range 200 nm to 300 nm (2.4.7), a 0.005 per cent w/v solution in *water* shows an absorption maximum at about 260nm.

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

## Tests

**pH** (2.4.24). 2.8 to 3.2, determined in a 1.0 per cent w/v solution in *carbon dioxide-free water*.

**Specific optical rotation** (2.4.22). +122° to +129°, determined at about 365 nm in a 1.0 per cent w/v solution in *water*, calculated on the anhydrous and ethanol-free basis.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 0.1 g of the substance under examination in 100 ml of the mobile phase.

*Reference solution (a)*. A 0.0001 per cent w/v solution of *indinavir RS* in the mobile phase.

*Reference solution (b)*. Transfer 15 mg of *indinavir RS* to a 50-ml volumetric flask and add 0.1 ml of *5 M hydrochloric acid*. Keep this solution for about 1 hour at room temperature and make up to volume with the mobile phase.

Chromatographic system

- a stainless steel column 20 cm x 4.6 mm, packed with base deactivated octylsilane bonded to porous silica (5 µm),
- column temperature. 40°,
- mobile phase: a filtered and degassed mixture of 40 volumes of *acetonitrile* and 60 volumes of a solution containing 0.37 per cent w/v of *sodium citrate* and 0.16 per cent w/v of *citric acid*, the pH of which has been adjusted to 5.0 with *1 M sodium hydroxide* or *1 M phosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume. 50 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to indinavir and any impurity at a relative retention time of about 1.4 is not less than 2.

Inject reference solution (a). The test is not valid unless the capacity factor for indinavir peak is not less than 2.0, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 5 per cent.

Inject the test solution. Calculate the content of each impurity in the chromatogram obtained with the test solution by comparing with the area of the principal peak obtained with reference solution (a). The content of any individual impurity is not more than 0.1 per cent and the sum of all impurities is not more than 0.5 per cent.

**Monoethyl sulphate content**. Determine by liquid chromatography (2.4.14).

*NOTE—Prepare the following solutions freshly.*

*Test solution*. Dissolve about 0.125 g of the substance under examination in 25 ml of *water* and filter through a fine porosity membrane.

*Reference solution (a)*. Weigh and transfer about 65 mg of *potassium monoethyl sulphate* to a 50-ml volumetric flask, dissolve and dilute to volume with *water*. Dilute 5 ml of this solution to 50 ml with *water*.

*Reference solution (b)*. Dilute 5 ml of the test solution to 50 ml with *water*. Further dilute 25 ml of this solution to 100 ml with *water* and filter through a fine porosity membrane.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with rigid, spherical styrene-divinylbenzenecopolymer (5 to 10 µm),
- mobile phase: a mixture of 10 volumes of *acetone* and 90 volumes of a buffer prepared by dissolving 0.25 g each of *sodium carbonate* and *sodium bicarbonate* in 1000 ml of *water*,



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- flow rate. 0.5 ml per minute,
- a conductivity detector,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the relative standard deviation of replicate injections is not more than 2.0 per cent and the tailing factor for monoethyl sulphate anion peak is not more than 2.0.

Inject the test solution. Calculate the content of monoethyl sulphate present from the declared content of monoethyl sulphate present in *potassium monoethyl sulphate*.

1 mg of potassium monoethyl sulphate corresponds to 0.77 mg of monoethyl sulphate. The content of monoethyl sulphate is not more than 0.05 per cent w/w.

**Ethanol**. 5.0 to 8.0 per cent, calculated on the anhydrous basis, determined by the following method.

Determine by gas chromatography (2.4.13).

*Test solution*. Add 5 ml of a 1.5 per cent w/v solution of *n-propanol* (internal standard) to 1.0 g of the substance under examination in a 25-ml volumetric flask and dilute to volume with *water*.

*Reference solution*. A 0.1 per cent w/v solution of *ethanol*. Transfer 5 ml of this solution and 5 ml of the internal standard to a 25-ml volumetric flask and make up to volume with *water*.

Chromatographic system
- a stainless steel column 1.8 m x 2 mm, packed with ethylvinyl benzene divinyl benzene copolymer, mesh size 80/100,
- temperature:
  inlet port. 130° maintained at a temperature of 240°,
- flame ionisation detector,
- flow rate. 20 ml per minute of the carrier gas (nitrogen).

Separately inject 1 µl of the test solution and the reference solution. Calculate the ethanol content by comparing the ratio of the area of the peak corresponding to ethanol and the area of the internal standard peak in the chromatogram obtained with the test solution with that of the reference solution.

**Sulphate**.13.2 to 14.4 per cent w/w, calculated on the anhydrous and ethanol-free basis, determined by the following method. Weigh accurately about 0.5 g, dissolve in 50 ml of *dimethylformamide* and titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.0048 g of sulphate.

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

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 1.5 per cent, determined on 0.2 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh accurately about 60 mg of the substance under examination in 100.0 ml of the mobile phase.

*Reference solution*. A 0.06 per cent w/v solution of *indinavir RS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with base deactivated octylsilane bonded to porous silica (5 µm),
- column temperature. 40°,
- mobile phase: a filtered and degassed mixture of 45 volumes of *acetonitrile* and 55 volumes of a buffer prepared by dissolving 3 g of *phosphoric acid* and 1.7 ml of *dibutylamine* in 900 ml of *water*, adjusting the pH to 6.5 with *1 M sodium hydroxide* and making up the volume to 1000.0 ml with *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency determined from the indinavir peak is not less than 4000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_{36}H_{47}N_5O_4$, $H_2SO_4$.

1 mg of indinavir corresponds to 1.16 mg of indinavir sulphate.

**Storage**. Store protected from light.

# Indinavir Capsules

## Indinavir Sulphate Capsules

Indinavir Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of indinavir, $C_{36}H_{47}N_5O_4$.

**Usual strength**. The equivalent of 400 mg of indinavir.

### Identification

A. Shake a quantity of the contents of the capsules containing 0.1 g of Indinavir Sulphate with 80 ml of *water*, dilute to 100 ml with *water* and filter. Dilute 5 ml of the filtrate to 100 ml with *water*. When examined in the range 200 nm to 300 nm (2.4.7), the resulting solution shows an absorption maximum at about 260 nm.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to indinavir in the chromatogram obtained with the reference solution.



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## Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Mix well the contents of 20 capsules and shake a quantity of the mixed contents containing about 50 mg of indinavir with about 60 ml of a solution prepared by mixing 40 volumes of *acetonitrile* and 60 volumes of *0.05 M dipotassium hydrogen phosphate*, the pH of which is adjusted to 7.5 with *dilute phosphoric acid* (solution A) in a 100 ml volumetric flask, mix with the aid of ultrasound for 10 minutes, dilute to volume with solution A and filter.

*Reference solution (a)*. Weigh accurately a quantity of *indinavir sulphate RS* containing about 50 mg of indinavir in a 100-ml volumetric flask, dissolve and dilute to volume with solution A. Dilute 1 ml of this solution to 100 ml with solution A.

*Reference solution (b)*. Dissolve a quantity of *indinavir sulphate RS* containing about 50 mg of indinavir and 5 mg of *indinavir 4-epimer RS* and dilute to 100 ml with solution A.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: filtered and degassed mixtures of *acetonitrile* and *0.05 M dipotassium hydrogen phosphate*, adjusting the pH of the solution to 7.5 with *dilute phosphoric acid*,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 260 nm,
- injection volume. 20 µl.

| Time (in min) | Phosphate buffer (pH 7.5) (per cent v/v) | Acetonitrile (per cent v/v) |
|---|---|---|
| 0 | 80 | 20 |
| 3 | 80 | 20 |
| 5 | 65 | 35 |
| 11 | 65 | 35 |
| 17 | 30 | 70 |
| 20 | 30 | 70 |
| 21 | 80 | 20 |
| 25 | 80 | 20 |

Inject reference solution (b). The test is not valid unless the column efficiency determined from the indinavir peak is not less than 10,000 theoretical plates, the tailing factor is not more than 1.5 and the resolution between indinavir and indinavir 4-epimer peaks is not less than 1.5.

Inject reference solution (a) and the test solution. Examine the chromatogram obtained with reference solution (a) for any extraneous peaks and ignore the corresponding peaks observed in the chromatogram obtained with the test solution.

Any secondary peak observed in the chromatogram obtained with the test solution should not be more than 1.0 per cent and the sum of the areas of all the secondary peaks should not be more than 2.5 per cent when calculated by percentage area normalisation.

**Dissolution** (2.5.2).

Apparatus No. 1,

Medium. 900 ml of a buffer prepared by dissolving 21 g of *citric acid* in 880 ml of *water*, adjusting the pH to 3.8 with a 50 per cent w/v solution of *sodium hydroxide* and making up to 1000 ml with *water*,

Speed and time. 50 rpm and 30 minutes. Use sinkers.

Withdraw a suitable volume of the medium and filter through a membrane filter disc with an average pore diameter not greater than 1.0 µm. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate if necessary, with the same solvent. Measure the absorbance of the resulting solution at the maximum at about 260 nm (2.4.7). Calculate the content of indinavir, $C_{36}H_{47}N_5O_4$, in the medium from the absorbance obtained from a solution of known concentration of *indinavir sulphate RS* in the same solvent.

D. Not less than 75 per cent of the stated amount of $C_{36}H_{47}N_5O_4$.

**Other tests**. Comply with the tests stated under Capsules.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Mix well the contents of 20 capsules and shake a quantity of the mixed contents containing about 200 mg of indinavir with about 60 ml of the mobile phase, mix with the aid of ultrasound for 10 minutes, dilute to 100.0 ml with the mobile phase, mix and filter. Dilute 10.0 ml of the filtrate to 100.0 ml with the mobile phase.

*Reference solution*. Weigh accurately a quantity of *indinavir sulphate RS* containing about 50 mg of indinavir in a 50 ml volumetric flask, dissolve and dilute to volume with the mobile phase. Dilute 10.0 ml of this solution to 50.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with base deactivated octylsilane bonded to porous silica (5 µm),
- mobile phase: a filtered and degassed mixture of 45 volumes of *acetonitrile* and 60 volumes of *0.05 M dipotassium hydrogen phosphate*, adjusted to pH 7.5 with *orthophosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume. 20 µl.



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Inject the reference solution. The test is not valid unless the column efficiency determined from the indinavir peak is not less than 6000 theoretical plates, the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

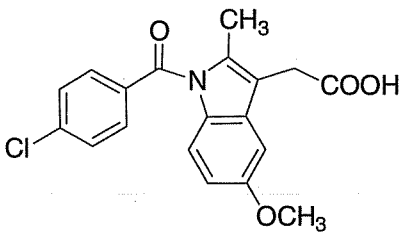
Inject alternately the test solution and the reference solution.

Calculate the content of $C_{36}H_{47}N_5O_4$ in the capsules.

**Storage**. Store protected from moisture at a temperature not exceeding 30°.

# Indomethacin

Indometacin




$C_{19}H_{16}ClNO_4$ Mol. Wt. 357.8

Indomethacin is 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-indol-3-ylacetic acid.

Indomethacin contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{19}H_{16}ClNO_4$, calculated on the dried basis.

**Category**. Antiinflammatory; analgesic.

**Dose**. Orally, 50 to 200 mg daily, in divided doses, with food. As suppositories, 100 mg at night and in the morning if required. Maximum combined oral and rectal dose, 150 to 200 mg daily.

**Description**. A white to pale yellow, crystalline powder; odourless or almost odourless.

## Identification

*Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *indomethacin RS* or with the reference spectrum of indomethacin. Examine the substances in the solid state without recrystallisation.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.0025 per cent w/v solution in a mixture of 90 volumes of *methanol* and 10 volumes of *1 M hydrochloric acid* shows an absorption maximum only at about 320 nm; absorbance at about 320 nm, about 0.45.

C. Dissolve 0.1 g in 10 ml of *ethanol (95 per cent)*, heating gently if necessary. To 0.1 ml add 2 ml of a freshly prepared mixture of 1 volume of a 25 per cent w/v solution of *hydroxylamine hydrochloride* and 3 volumes of *2 M sodium hydroxide*. Add 2 ml of *2 M hydrochloric acid* and 1 ml of *ferric chloride solution* and mix; a violet-pink colour develops.

## Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with a suspension of *silica gel HF254* in a 4.68 per cent w/v solution of *sodium dihydrogen phosphate*.

*Mobile phase*. A mixture of 70 volumes of *ether* and 30 volumes of *light petroleum (50° to 70°)*.

*NOTE—Prepare the following solutions immediately before use.*

*Test solution*. Dissolve 0.2 g of the substance under examination in 10 ml of *methanol*.

*Reference solution*. A 0.01 per cent w/v solution of the substance under examination in *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.45 g, dissolve in 75 ml of *acetone* and titrate under nitrogen with carbonate-free *0.1 M sodium hydroxide* using 0.2 ml of *phenolphthalein solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.03578 g of $C_{19}H_{16}ClNO_4$.

**Storage**. Store protected from light.

# Indomethacin Capsules

Indomethacin Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of indomethacin, $C_{19}H_{16}ClNO_4$.

**Usual strength**. 25 mg.



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### Identification

A. Shake a quantity of the contents of the capsules containing 0.1 g of Indomethacin with 5 ml of *chloroform*, filter and evaporate the filtrate to dryness. Dry the residue at 60° at a pressure not exceeding 0.7 kPa for 1 hour. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *indomethacin RS* or with the reference spectrum of indomethacin.

B. When examined in the range 230 nm to 360 nm (2.4.7), the final solution obtained in the Assay shows an absorption maximum only at about 320 nm.

C. Mix a quantity of the contents of the capsules containing 25 mg of Indomethacin with 2 ml of *water* and add 2 ml of *2 M sodium hydroxide*; a bright yellow colour is produced which fades rapidly.

### Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with a suspension of *silica gel HF254* in a 4.68 per cent w/v solution of *sodium dihydrogen phosphate*.

*Mobile phase*. A mixture of 70 volumes of *ether* and 30 volumes of *light petroleum (50° to 70°)*.

*NOTE—Prepare the following solutions immediately before use*.

*Test solution*. Shake a quantity of the contents of the capsules containing 0.1 g of Indomethacin with 5 ml of *chloroform*, filter and use the filtrate.

*Reference solution*. Dilute 1 volume of the test solution to 200 volumes with *chloroform*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 750 ml of a freshly prepared mixture of 1 volume of *phosphate buffer pH 7.2* and 4 volumes of *water*,
Speed and time. 100 rpm and 20 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate. Dilute the filtrate suitably with the medium and measure the absorbance of the resulting solution at the maximum at about 320 nm (2.4.7). Calculate the content of $C_{19}H_{16}ClNO_4$ in the medium from the absorbance obtained from a solution of known concentration of *indomethacin RS* in the same medium.

D. Not less than 80 per cent of the stated amount of $C_{19}H_{16}ClNO_4$.

**Other tests**. Comply with the tests stated under Capsules.

**Assay**. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 50 mg of Indomethacin, add 10 ml of *water* and allow to stand for 10 minutes, with occasional swirling. Add 75 ml of *methanol*, shake well, add sufficient *methanol* to produce 100.0 ml and filter if necessary. To 5.0 ml of the filtrate add sufficient of a mixture of equal volumes of *methanol* and *phosphate buffer pH 7.2* to produce 100.0 ml. Measure the absorbance of the resulting solution at the maximum at about 320 nm (2.4.7). Calculate the content of $C_{19}H_{16}ClNO_4$ taking 193 as the specific absorbance at 320 nm.

**Storage**. Store protected from moisture.

## Indomethacin Suppositories

Indomethacin Suppositories contain Indomethacin in a suitable suppository basis.

Indomethacin Suppositories contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of indomethacin, $C_{19}H_{16}ClNO_4$.

**Usual strength**. 100 mg.

### Identification

A. Dissolve a quantity of the powdered suppositories containing 0.1 g of Indomethacin as completely as possible in 50 ml of hot *water*, filter, wash the residue with hot *water* and allow to dry in air. Dissolve the residue in 5 ml of *chloroform* and evaporate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *indomethacin RS* or with the reference spectrum of indomethacin.

B. Shake a quantity containing 25 mg of Indomethacin with 5 ml of *water* until the base dissolves; a white suspension is produced. Add 2 ml of *2 M sodium hydroxide*; a bright yellow colour is produced which fades rapidly.

### Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*NOTE—Prepare the following solutions freshly*.

*Test solution (a)*. Powder or cut into small pieces a suitable number of suppositories, dissolve a quantity containing 0.1 g of Indomethacin in sufficient *methanol* to produce 50 ml.



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*Reference solution (a)*. Dilute 3 volumes of test solution (a) to 100 volumes with the mobile phase.

Chromatographic system

- a stainless steel column 30 cm x 4 mm, packed with octadecylsilane bonded to porous silica (10 µm) (such as Bondapack C18),
- mobile phase: a mixture of 60 volumes of *methanol* and 40 volumes of 0.2 per cent v/v solution of *phosphoric acid*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 320 nm,
- injection volume. 20 µl.

The sum of the areas of any secondary peaks that elute before the principal peak in the chromatogram obtained with test solution (a) is not greater than the area of the peak in the chromatogram obtained with reference solution (a).

Repeat the procedure but using the following freshly prepared solutions and a detection wavelength of about 240 nm.

*Test solution (b)*. Dilute 10 volumes of test solution (a) to 20 volumes with the mobile phase.

*Reference solution (b)*. A solution containing 0.001 per cent w/v of *4-chlorobenzoic acid* in the mobile phase.

In the chromatogram obtained with test solution (b) the sum of the areas of any secondary peaks that elute before the principal peak, other than those determined in test solution (a), is not greater than the area of the peak in the chromatogram obtained with reference solution (b).

The column efficiency, determined using the principal peak in the chromatogram obtained with reference solution (a), should be not less than 7500 theoretical plates per metre.

**Disintegration** (2.5.1). Use a weighed suppository and *phosphate buffer pH 6.8* in place of *water* and operate the apparatus for 90 minutes. At the end of this period remove the suppository, dry with filter paper and weigh. Repeat the operation with two further weighed suppositories. Not less than 75 per cent of each suppository is dissolved.

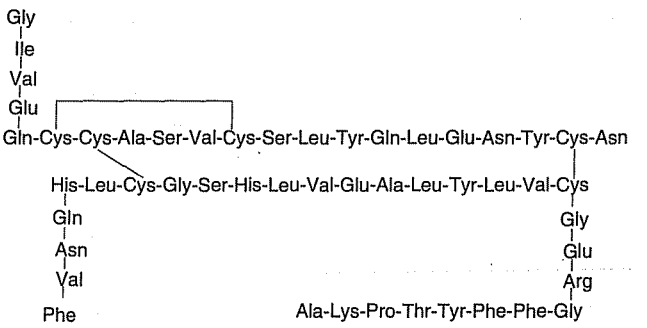
**Other tests**. Comply with the tests stated under Suppositories.

**Assay**. Weigh 10 suppositories and powder or cut into small pieces. Weigh accurately a quantity of the powder or small pieces containing about 0.1 g of Indomethacin, add 50 ml of *methanol*, shake until the dispersion is complete and, if necessary, filter. To 2.0 ml add sufficient of a mixture of equal volumes of *methanol* and *phosphate buffer pH 7.2* to produce 100.0 ml. Measure the absorbance of the resulting solution at the maximum at about 320 nm (2.4.7). Calculate the content of $C_{19}H_{16}ClNO_4$ taking 193 as the specific absorbance at 320 nm.

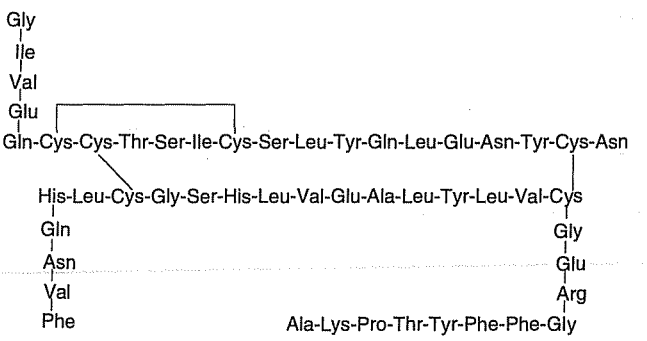
**Storage**. Store protected from moisture at a temperature not exceeding 30°.

# Insulin

Crystalline Insulin




$C_{256}H_{381}N_{65}O_{76}S_6$ (porcine) Mol. Wt. 5777.6




$C_{254}H_{377}N_{65}O_{75}S_6$ (bovine) Mol. Wt. 5733.5

Insulin is the specific natural antidiabetic principle obtained from the pancreas of either the pig or the ox and purified.

Insulin contains not less than 26.5 Units per mg of porcine insulin, $C_{256}H_{381}N_{65}O_{76}S_6$, or of bovine insulin, $C_{254}H_{377}N_{65}O_{75}S_6$, as appropriate, calculated on the dried basis.

**Category**. Hypoglycaemic.

**Dose**. By subcutaneous, intramuscular or intravenous injection or intravenous infusion, in accordance with the needs of the patient.

**Description**. A white or almost white powder.

## Identification

A. In the Assay, the principal peak due to insulin in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the appropriate reference solution.

B. Complies with the test for peptide mapping (2.3.47).



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### Tests

**Light absorption** (2.4.7). Absorbance of a 0.05 per cent w/v solution in *0.01 M hydrochloric acid* at the maximum at about 276 nm, 0.48 to 0.56.

**Other tests**. Complies with the tests for Impurities with molecular masses greater than that of insulin, Related proteins and Total zinc stated under Insulin Preparations.

**Sulphated ash** (2.3.18). Not more than 2.5 per cent, calculated on the dried basis, determined on 0.2 g.

**Loss on drying** (2.4.19). Not more than 10.0 per cent, determined on 0.2 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa.

**Assay**. Determine as described under Assay of Insulins (2.3.46).

*Test solution*. Dissolve a suitable quantity of the substance under examination in *0.01 M hydrochloric acid* to obtain a concentration of 4.0 mg per ml.

*Insulin intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.*

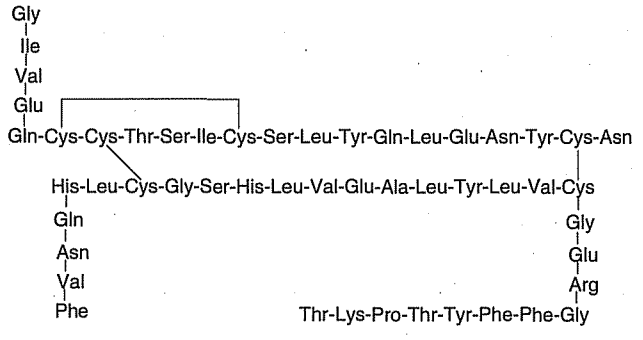
**Bacterial endotoxins** (2.2.3). Not more than 20 Endotoxin Units per mg.

**Storage**. Store protected from light, at a temperature not exceeding –20° until released by the manufacturer. When thawed, it should be stored in a refrigerator (2° to 8°) and used for the manufacture of preparations within a short period of time.

**Labelling**. The label states (1) the animal source or sources of the insulin; (2) where applicable, that the material is free from bacterial endotoxins; (3) the storage conditions.

*NOTE — 0.0345 mg of porcine or 0.0342 mg of bovine insulin is equivalent to 1 Unit of insulin.*

## Human Insulin




$C_{257}H_{383}N_{65}O_{77}S_6$ Mol. Wt. 5808.0

Human insulin is a 2-chain peptide having the structure of the antidiabetic hormone produced by the human pancreas. It is produced either by enzymatic modification and suitable purification of insulin obtained from the pancreas of the pig or by a method based on recombinant DNA (rDNA).

Human insulin contains not less than 27.5 Units per mg of human insulin, $C_{257}H_{383}N_{65}O_{77}S_6$, calculated on the dried basis.

**Description**. A white or almost white powder.

### Identification

A. In the Assay, the principal peak due to insulin in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (b) or (c), as appropriate.

B. Complies with the test for peptide mapping (2.3.47).

### Tests

**Light absorption** (2.4.7). Absorbance of a 0.05 per cent w/v solution in *0.01 M hydrochloric acid* at the maximum at about 276 nm, 0.48 to 0.56.

**Total zinc**. Not more than 1.0 per cent, calculated on the dried basis, determined by the following method. To 5 ml of a 0.5 per cent w/v solution in *0.01 M hydrochloric acid* add 10 ml of *alkaline borate buffer pH 9.0*, 3 ml of *zincon solution* and sufficient *water* to produce 50 ml. Allow to stand for 1 hour and measure the absorbance of the resulting solution at about 620 nm, using as the blank a solution prepared by treating 5 ml of *water* instead of the substance being examined in a similar manner. Calculate the content of zinc from the *absorbance* obtained by repeating the procedure using a suitable aliquot of a mixture of 4 volumes of *zinc sulphate solution* and 6 volumes of *water*.

**Other tests**. Complies with the tests for Impurities with molecular masses greater than that of insulin, Related proteins and Total zinc stated under Insulin Preparations.

**Sulphated ash** (2.3.18). Not more than 2.5 per cent, calculated on the dried basis, determined on 0.2 g.

**Loss on drying** (2.4.19). Not more than 10.0 per cent, determined on 0.2 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa.

**Assay**. Determine as described under Assay of Insulins (2.3.46).

*Test solution*. Dissolve a suitable quantity of the substance under examination in *0.01 M hydrochloric acid* to obtain a concentration of 4.0 mg per ml.

*Human Insulin intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.*



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**Bacterial endotoxins** (2.2.3). Not more than 10 Endotoxin Units per mg.

**Storage**. Store protected from light, at a temperature not exceeding –18° until released by the manufacturer. When thawed, it should be stored in a refrigerator (2° to 8°) and used for the manufacture of preparations within a short period of time.

**Labelling**. The label states (1) whether the material is produced by enzymatic modification of porcine insulin or by rDNA technology; (2) where applicable, that the material is free from bacterial endotoxins; (3) the storage conditions.

*NOTE— 0.0347 mg of human insulin is equivalent to 1 Unit of insulin.*

## Insulin Injection

Neutral Insulin; Neutral Insulin Injection; Soluble Insulin; Plain Insulin

Insulin Injection is a neutral, sterile solution of bovine, porcine or human insulin.

Insulin Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated number of Units of Insulin.

**Usual strengths**. 40 Units per ml; 80 Units per ml.

**Description**. A colourless liquid, free from turbidity and foreign matter; during storage, traces of a very fine sediment may be deposited.

### Identification

In the chromatograms obtained in the Assay, the position of the peak due to insulin in the chromatogram obtained with the test solution corresponds to that of the principal peak obtained with the appropriate reference solution.

### Tests

**pH** (2.4.24). 6.8 to 7.8.

**Total zinc**. Not more than 40 µg per 100 Units of insulin, determined by either of the methods stated under Insulin Preparations.

*Test solution*. Dilute a volume of the gently shaken preparation under examination containing 200 Units to 25.0 ml with *water*. Dilute if necessary to a suitable concentration with *water*.

**Other tests**. Complies with the tests stated under Insulin Preparations.

**Assay**. Determine as described under Assay of Insulins (2.3.46).

*Test solution*. Add 4 µl of *6 M hydrochloric acid* per millilitre of the injection to obtain a clear solution. For a preparation containing more than 100 IU per ml, an additional dilution with *0.01 M hydrochloric acid* is necessary to avoid overloading the column. Dilute a suitable volume of the injection with *0.01 M hydrochloric acid* to obtain a concentration of 40 Units per ml.

**Storage**. Store in multiple dose containers in a refrigerator (2° to 8°). It should not be allowed to freeze.

**Labelling**. The label states (1) whether the preparation is acidified or neutral; (2) the strength in terms of the number of Units per ml; (3) the animal source or sources of the insulin; (4) that the preparation should not be allowed to freeze; (5) the storage conditions.

## Biphasic Insulin Injection

Biphasic Insulin Injection is a sterile suspension of crystals containing bovine insulin in a solution of porcine insulin.

Biphasic Insulin Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated number of Units of Insulin.

**Usual strength**. 40 Units per ml (30: 70).

**Description**. A white suspension. When examined under a microscope, the majority of the particles appear as rhombohedral crystals, with a maximum dimension of the crystals greater than 10 µm but rarely exceeding 40 µm.

### Identification

In the chromatograms obtained in the Assay the position of the peaks due to the two insulins in the chromatogram obtained with the test solution correspond to those of the principal peaks in the chromatogram obtained with the appropriate reference solution.

### Tests

**pH** (2.4.24). 6.6 to 7.2.

**Total zinc**. 26.0 µg to 37.5 µg per 100 Units of insulin, determined by either of the methods stated under Insulin Preparations.

**Insulin in the supernatant**. 22.0 per cent to 28.0 per cent of insulin in solution, determined as stated under Insulin Preparations.

**Other tests**. Complies with the tests stated under Insulin Preparations.

**Assay**. Determine as described under Assay of Insulins (2.3.46).

*Test solution*. To 10 ml of the preparation under examination add 40 µl of *5 M hydrochloric acid*, mix well, allow to stand for



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1 hour to ensure solution of the sediment and dilute with *0.03 M hydrochloric acid* to obtain a solution containing 40 Units per ml.

## Biphasic Isophane Insulin Injection

Biphasic Isophane Insulin Injection is a sterile buffered suspension of either porcine or human insulin, complexed with protamine sulphate or any other suitable protamine, in a solution of insulin of the same species.

It is prepared by the procedure described under Insulin Preparations. It is produced by mixing, in defined ratios, soluble insulin injection and isophane insulin injection.

Biphasic Isophane Insulin Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated number of Units of Insulin.

**Usual strengths**. 40 Units per ml (30:70); 100 Units per ml (30: 70); 40 Units per ml (50: 50); 100 Units per ml (50:50).

**Description**. A white suspension which on standing deposits a white sediment and leaves a colourless or almost colourless supernatant liquid; the sediment is readily resuspended by gently shaking. When examined under a microscope, the particles are seen to be rod-shaped crystals, the majority with a maximum dimension greater than 1 µm but rarely exceeding 60 µm, free from large aggregates.

### Identification

In the chromatograms obtained in the Assay the position of the peak due to insulin in the chromatogram obtained with the test solution corresponds to that of the principal peak in the chromatogram obtained with the appropriate reference solution.

### Tests

**pH** (2.4.24). 6.9 to 7.5.

**Total zinc**. Not more than 40 µg per 100 Units of insulin, determined by either of the methods stated under Insulin Preparations.

**Other tests**. Complies with the tests stated under Insulin Preparations, except Insulin in the supernatant.

**Assay**. Determine as described under Assay of Insulins (2.3.46).

*Test solution*. To 10 ml of the preparation under examination add 40 µl of *5 M hydrochloric acid*, mix well, allow to stand for 1 hour to ensure solution of the sediment and dilute with *0.03 M hydrochloric acid* to obtain a solution containing 40 Units per ml.

**Labelling**. The label states the ratio of soluble insulin injection to isophane insulin injection used in the manufacture of biphasic isophane insulin injection.

## Isophane Insulin Injection

Isophane Insulin; Isophane Insulin (NPH)

Isophane Insulin injection is a sterile suspension of bovine, porcine or human insulin, complexed with protamine sulphate or another suitable protamine.

It is prepared by the procedure described under Insulin Preparations. The amount of protamine is based on the known isophane ratio and is not less than the equivalent of 0.3 mg and not more than the equivalent of 0.6 mg of protamine sulphate for each 100 Units of insulin in the insulin-protamine complex. It contains sodium phosphate as a buffering agent.

Isophane Insulin Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated number of Units of Insulin.

**Usual strength**. 40 Units per ml.

**Description**. A white suspension which on standing deposits a white sediment and leaves a colourless or almost colourless supernatant liquid; the sediment is readily resuspended by gently shaking. When examined under a microscope, the particles are seen to be rod-shaped crystals, the majority with a maximum dimension greater than 1 µm but rarely exceeding 60 µm, free from large aggregates.

### Identification

In the chromatograms obtained in the Assay the position of the peak due to insulin in the chromatogram obtained with the test solution corresponds to that of the principal peak in the chromatogram obtained with the appropriate reference solution.

### Tests

**pH** (2.4.24). 6.9 to 7.5.

**Total zinc**. Not more than 40 µg per 100 Units of insulin, determined by either of the methods stated under Insulin Preparations.

**Other tests**. Complies with the tests stated under Insulin Preparations.

**Assay**. Determine as described under Assay of Insulins (2.3.46).

*Test solution*. To 10 ml of the preparation under examination add 40 µl of *5 M hydrochloric acid*, mix well, allow to stand for 1 hour to ensure solution of the sediment and dilute with *0.03 M hydrochloric acid* to obtain a solution containing 40 Units per ml.



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**Storage**. Store in multiple dose containers in a refrigerator (2° to 8°). It should not be allowed to freeze.

**Labelling**. The label states (1) the strength in terms of the number of Units per ml; (2) the animal source or sources of the insulin; (3) that the preparation should not be allowed to freeze; (4) that the container should be gently shaken before a dose is withdrawn; (5) the storage conditions.

## Insulin Zinc Suspension

I.Z.S; Insulin Zinc Suspension (Mixed); I.Z.S (Mixed); Insulin Lente

Insulin Zinc Suspension is a sterile, buffered suspension of Insulin in the form of a complex obtained by the addition of zinc chloride to insulin in a manner such that the insulin is in a form insoluble in water. It may be prepared by mixing aseptically about 3 volumes of Insulin Zinc Suspension (Amorphous) and about 7 volumes of Insulin Zinc Suspension (Crystalline) and distributing the mixture aseptically into sterile containers which are then sealed so as to exclude micro-organisms.

Insulin Zinc Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated number of Units of Insulin.

**Usual strengths**. 40 Units per ml; 80 Units per ml.

**Description**. A white suspension which on standing deposits a white sediment and leaves an almost colourless supernatant liquid. The sediment is readily resuspended on gentle shaking. When examined under a microscope, the majority of the particles in the suspension are seen as rhombohedral crystals, with a maximum dimension greater than 10 mm but rarely exceeding 40 mm; a considerable number of particles have no uniform shape and do not exceed 2 mm in maximum dimension.

### Identification

In the Assay, the principal peak due to insulin in the chromatogram obtained with test solution has a retention time similar to that of the principal peak in the chromatogram obtained with reference solution (a), (b) or (c) as appropriate.

### Tests

**pH** (2.4.24). 6.9 to 7.5.

**Total zinc**. Not more than 0.0095 per cent w/v (for preparations containing 40 Units per ml) and not more than 0.014 per cent w/v (for preparations containing 80 Units per ml), determined by the following method. Take a volume of the well-shaken suspension containing 200 Units of insulin and add 1 ml of *0.1M hydrochloric acid*, 10 ml of *alkaline borate buffer pH 9.0*, 1 ml of *0.1M sodium hydroxide*, 2 ml of a 0.0009 per cent w/v solution of *trypsin* in *0.01M hydrochloric acid*. Mix, allow to stand for 10 minutes and add 3 ml of *zincon solution* and sufficient *water* to produce 50 ml. Allow to stand for 1 hour and measure the absorbance of the resulting solution at about 620 nm (2.4.7), using as the blank a solution prepared by treating 5 ml of *water* instead of the substance under examination in a similar manner. Calculate the content of zinc from the absorbance obtained by repeating the procedure using a suitable aliquot of a mixture of 4 volumes of *zinc sulphate solution* and 6 volumes of *water*.

**Zinc in solution**. Not more than 70 per cent of the total zinc (for preparations containing 40 Units per ml) and not more than 55 per cent of the total zinc (for preparations containing 80 Units per ml), determined by the method described in the test for Total zinc, using 1 ml of the clear supernatant liquid obtained by centrifuging and beginning at the words "add 1 ml of *0.1M hydrochloric acid*,.....".

**Insulin extractable with buffered acetone solution**. 27 per cent to 40 per cent, determined by the following method. Centrifuge a volume containing 400 Units and reject the supernatant liquid. Suspend the residue in 3.3 ml of *water*, add 6.6 ml of *buffered acetone solution*, stir for 3 minutes and again centrifuge. Transfer the supernatant liquid as completely as possible to a long-necked, round-bottomed flask, add 0.3 g of *nitrogen-free mercuric oxide*, 3 g of *anhydrous sodium sulphate*, and 6 ml of *nitrogen-free sulphuric acid*, heat over a low flame until the liquid is colourless and boil for a further 30 minutes. Allow to cool, dilute carefully with *water*, add 1 g of *zinc powder*, shake and allow to stand for 10 minutes. Add an excess of *sodium hydroxide solution*, immediately connect the flask to an ammonia distillation apparatus, mix the contents and distil the liberated ammonia into 20 ml of *0.005 M sulphuric acid* prepared with *carbon dioxide-free water*. Rinse the condenser tube into the flask containing the distillate, add sufficient *carbon dioxide-free water* to produce a total volume of about 50 ml and titrate the excess of sulphuric acid with *0.01 M sodium hydroxide* to pH 6.0, using a glass electrode. Centrifuge a further volume containing 400 Units and reject the supernatant liquid. Dissolve the residue in 10 ml of a 5 per cent w/v solution of *nitrogen-free sulphuric acid*, transfer to a long-necked, round-bottomed flask, and repeat the procedure described above beginning at the words "add 0.3 g of *nitrogen-free mercuric oxide*,.....". Calculate the percentage of insulin extractable with the buffered acetone solution from the formula $100A/B$, where A is the volume of *0.005 M sulphuric acid* used in the first determination and B is the volume used in the second determination.



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The result of the test is not valid unless in carrying out the first determination omitting the insulin preparation, not more than 0.2 ml of *0.005 M sulphuric acid* is required.

**Insulin in solution**. Determine by liquid chromatography (2.4.14).

*Test solution. For preparations containing 100 Units per ml* Centrifuge a well-shaken suspension of the preparation under examination, transfer 2.0 ml of the supernatant liquid to a 5 ml volumetric flask, dilute to volume with *0.03 M hydrochloric acid* and mix well.

*Reference solution (a)*. Prepare as test solution but using 2.5 ml of the supernatant liquid in place of 2.0 ml.

*Reference solution (b)*. Use 5 ml of the supernatant liquid.

Reference solution (c). Weigh accurately 4.5 mg of *bovine insulin RS* into a 100-ml volumetric flask containing 50 ml of *0.025 M hydrochloric acid*, dissolve by shaking for 5 minutes, dilute to volume with *0.025 M hydrochloric acid* and mix to obtain a solution containing approximately 1 Unit per ml.

Use the chromatographic system as described in the Assay.

The area of the peak due to insulin in the chromatogram obtained with test solution, reference solution (a) or reference (b), as the case may be, is not more than that of the principal peak in the chromatogram obtained with reference solution (c).

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. To 10 ml of the preparation under examination add 40 ml of *5 M hydrochloric acid*, mix well, allow to stand for 1 hour to ensure solution of the sediment and dilute with *0.03 M hydrochloric acid* to obtain a solution containing 20 Units per ml.

*Reference solution (a)*. A 0.08 per cent w/v of *bovine insulin RS* in *0.025 M hydrochloric acid*.

*Reference solution (b)*. A 0.08 per cent w/v of *porcine insulin RS* in *0.025 M hydrochloric acid*.

*Reference solution (c)*. A solution containing 0.04 per cent w/v of *bovine insulin RS* and 0.04 per cent w/v of *porcine insulin RS* in *0.025 M hydrochloric acid* for a preparation containing both bovine and pork insulin.

*Reference solution (d)*. A 0.04 per cent w/v each of *human insulin RS* and *porcine insulin RS* in *0.025 M hydrochloric acid*.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm) (Such as Ultrasphere ODS),
- column temperature. 45°,
- mobile phase. a mixture of 72.5 volumes of *0.1 M sodium dihydrogen phosphate* adjusted to pH 2.0 with *phosphoric acid* and 27.5 volumes of *acetonitrile*,
- flow rate 1 ml per minute,
- spectrophotometer set at 214 nm,
- injection volume. 50 µl.

The test is not valid unless the resolution factor between the peaks corresponding to human insulin and porcine insulin is at least 1.2 in the chromatogram obtained with reference solution (d). If necessary, adjust the concentration of *acetonitrile* in the mobile phase by slight decrease or increase until the required resolution is obtained. In the chromatogram obtained with reference solution (d) the two principal peaks, in order of emergence, are due to human insulin and porcine insulin and any smaller peaks appearing immediately following each of the principal peaks are due to the corresponding monodesamido derivatives.

Inject either of reference solutions (a), (b) and (c), as appropriate, alternatively six times. The test is not valid unless the relative standard deviation of the area of the principal peak is not more than 2 per cent.

Inject the test solution. If necessary, make further adjustments in the composition of the mobile phase so that the antimicrobial preservatives present in test solution are well separated from insulin and show shorter retention times. A small reduction in the concentration of acetonitrile increases the retention time of insulin peak relatively more than those of the preservatives. If necessary, after having carried out the chromatography of a solution, wash the column with a mixture of equal volumes of *acetonitrile* and *water* for a sufficient time in order to elute any interfering substances before injecting the next solution.

Calculate the content of insulin from the area of the peaks due to the bovine, porcine or human insulin and that of any peak due to the monodesamido derivative of the insulin from the declared content of insulin in *bovine insulin RS, porcine insulin RS* or *human insulin RS*, as appropriate. For preparations containing both bovine and porcine insulin use the sum of the areas of both the bovine and porcine insulin peaks and of any peak due to the desamido derivative of either insulin.

**Storage**. Store in multiple dose containers at a temperature between 2° and 8°. It should not be allowed to freeze.

**Labelling**. The label states (1) the strength in terms of the number of Units per ml; (2) the animal source or sources of the insulin; (3) that the preparation should not be allowed to freeze; (4) that the container should be gently shaken before a dose is withdrawn; (5) the storage conditions.



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# Insulin Zinc Suspension (Amorphous)

Amorph. I.Z.S.; Prompt Insulin Zinc Suspension

Insulin Zinc Suspension (Amorphous) is a sterile, buffered suspension of Insulin in the form of a complex obtained by the addition of zinc chloride to insulin in a manner such that the solid phase of the suspension is amorphous. It may be prepared by adding aseptically to crystalline insulin having a potency not less than 23 Units per mg, calculated on the dried basis, a suitable quantity of zinc chloride, an appropriate amount of a suitable substance to render the preparation isotonic with blood and a sufficient quantity of a suitable bactericide. It is distributed aseptically into sterile containers which are then sealed so as to exclude micro-organisms.

Insulin Zinc Suspension (Amorphous) contains not less than 90.0 per cent and not more than 110.0 per cent of the stated number of Units of Insulin.

**Category**. Hypoglycaemic.

**Dose**. By subcutaneous injection, in accordance with the needs of the patient.

**Usual strengths**. 40 Units per ml; 80 Units per ml.

**Description**. A white suspension which on standing deposits a white sediment and leaves an almost colourless supernatant liquid. The sediment is readily resuspended on gentle shaking. When examined under a microscope, the particles in the suspension are seen to have no uniform shape and rarely exceed 2 mm in maximum dimension.

## Identification

In the Assay, the principal peak due to insulin in the chromatogram obtained with test solution has a retention time similar to that of the principal peak in the chromatogram obtained with reference solution (a), (b) or (c) as appropriate.

## Tests

**pH** (2.4.24). 6.9 to 7.5.

**Total zinc**. Not more than 0.0095 per cent w/v (for preparations containing 40 Units per ml) and not more than 0.014 per cent w/v (for preparations containing 80 Units per ml), determined by the following method. Take a volume of the well-shaken suspension containing 200 Units of insulin and add 1 ml of *0.1 M hydrochloric acid*, 10 ml of *alkaline borate buffer pH 9.0*, 1 ml of *0.1 M sodium hydroxide*, 2 ml of a 0.0009 per cent w/v solution of *trypsin* in *0.01 M hydrochloric acid*. Mix, allow to stand for 10 minutes and add 3 ml of *zincon solution* and sufficient *water* to produce 50 ml. Allow to stand for 1 hour and measure the absorbance of the resulting solution at about 620 nm (2.4.7), using as the blank a solution prepared by treating 5 ml of *water* instead of the substance under examination in a similar manner. Calculate the content of zinc from the absorbance obtained by repeating the procedure using a suitable aliquot of a mixture of 4 volumes of *zinc sulphate solution* and 6 volumes of *water*.

**Zinc in solution**. Not more than 70 per cent of the total zinc (for preparations containing 40 Units per ml) and not more than 55 per cent of the total zinc (for preparations containing 80 Units per ml), determined by the method described in the test for Total zinc, using 1 ml of the clear supernatant liquid obtained by centrifuging and beginning at the words "add 1 ml of *0.1 M hydrochloric acid*,.....".

**Insulin in solution**. Determine by liquid chromatography (2.4.14).

*Test solution*. (for preparations containing 100 Units per ml) centrifuge a well-shaken suspension of the preparation under examination, transfer 2.0 ml of the supernatant liquid to a 5 ml volumetric flask, dilute to volume with *0.03 M hydrochloric acid* and mix well.

*Reference solution (a)*. Prepare as test solution but using 2.5 ml of the supernatant liquid in place of 2.0 ml.

*Reference solution (b)*. Use 5 ml of the supernatant liquid.

*Reference solution (c)*. Weigh accurately 4.5 mg of *bovine insulin RS* into a 100-ml volumetric flask containing 50 ml of *0.025 M hydrochloric acid*, dissolve by shaking for 5 minutes, dilute to volume with *0.025 M hydrochloric acid* and mix to obtain a solution containing approximately 1 Unit per ml.

Use the chromatographic system as described in the Assay.

The area of the peak due to insulin in the chromatogram obtained with test solution, reference solution (a) or (b), as the case may be, is not more than that of the principal peak in the chromatogram obtained with reference solution (c).

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. To 10 ml of the preparation under examination add 40 ml of *5 M hydrochloric acid*, mix well, allow to stand for 1 hour to ensure solution of the sediment and dilute with *0.03 M hydrochloric acid* to obtain a solution containing 20 Units per ml.

*Reference solution (a)*. A 0.08 per cent w/v of *bovine insulin RS* in *0.025 M hydrochloric acid*.

*Reference solution (b)*. A 0.08 per cent w/v of *porcine insulin RS* in *0.025 M hydrochloric acid*.

*Reference solution (c)*. A solution containing 0.04 per cent w/v of *bovine insulin RS* and 0.04 per cent w/v of *porcine*



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*insulin RS* in *0.025 M hydrochloric acid* for a preparation containing both bovine and pork insulin.

*Reference solution (d).* A 0.04 per cent w/v each of *human insulin RS* and *porcine insulin RS* in *0.025 M hydrochloric acid.*

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- column. temperature 45°,
- mobile phase. a mixture of 72.5 volumes of *0.1 M sodium dihydrogen phosphate* adjusted to pH 2.0 with *phosphoric acid* and 27.5 volumes of *acetonitrile,*
- flow rate 1 ml per minute.
- spectrophotometer set at 214 nm,
- injection volume. 50 µl.

The test is not valid unless the resolution factor between the peaks corresponding to human insulin and porcine insulin is at least 1.2 in the chromatogram obtained with reference solution (d). If necessary, adjust the concentration of *acetonitrile* in the mobile phase by slight decrease or increase until the required resolution is obtained. In the chromatogram obtained with reference solution (d) the two principal peaks, in order of emergence, are due to human insulin and porcine insulin and any smaller peaks appearing immediately following each of the principal peaks are due to the corresponding monodesamido derivatives.

Inject either of reference solutions (a), (b) and (c), as appropriate, alternatively six times. The test is not valid unless the relative standard deviation of the area of the principal peak is not more than 2 per cent.

Inject the test solution. If necessary, make further adjustments in the composition of the mobile phase so that the antimicrobial preservatives present in test solution are well separated from insulin and show shorter retention times. A small reduction in the concentration of acetonitrile increases the retention time of insulin peak relatively more than those of the preservatives. If necessary, after having carried out the chromatography of a solution, wash the column with a mixture of equal volumes of *acetonitrile* and *water* for a sufficient time in order to elute any interfering substances before injecting the next solution.

Calculate the content of insulin from the area of the peaks due to the bovine, porcine or human insulin and that of any peak due to the monodesamido derivative of the insulin from the declared content of insulin in *bovine insulin RS, porcine insulin RS* or *human insulin RS*, as appropriate. For preparations containing both bovine and porcine insulin use the sum of the areas of both the bovine and porcine insulin peaks and of any peak due to the desamido derivative of either insulin.

**Storage.** Store in multiple dose containers at a temperature between 2° and 8°. It should not be allowed to freeze.

**Labelling.** The label states (1) the strength in terms of the number of Units per ml; (2) the animal source or sources of the insulin; (3) that the preparation should not be allowed to freeze; (4) that the container should be gently shaken before a dose is withdrawn; (5) the storage conditions.

# Insulin Zinc Suspension (Crystalline)

Cryst. I.Z.S.; Extended Insulin Zinc Suspension

Insulin Zinc Suspension (Crystalline) is a sterile, buffered suspension of Insulin in the form of a complex obtained by the addition of zinc chloride to insulin in a manner such that the insulin is in the form of crystals insoluble in water. It may be prepared by adding aseptically to crystalline insulin having a potency not less than 23 Units per mg, calculated with reference to the dried substance, a suitable quantity of zinc chloride, an appropriate amount of a suitable substance to render the preparation isotonic with blood and a sufficient quantity of a suitable bactericide. The solution is partially neutralised to allow crystallisation to occur and the pH of the crystalline suspension is adjusted to about 7.2. The suspension is distributed aseptically into sterile containers which are then sealed so as to exclude micro-organisms.

Insulin Zinc Suspension (Crystalline) contains not less than 90.0 per cent and not more than 110.0 per cent of the stated number of Units of Insulin.

**Category.** Hypoglycaemic.

**Dose.** By subcutaneous injection, in accordance with the needs of the patient.

**Usual strengths.** 40 Units per ml; 80 Units per ml.

**Description.** A white suspension which on standing deposits a white sediment and leaves an almost colourless supernatant liquid. The sediment is readily resuspended on gentle shaking. When examined under a microscope, the particles in the suspension are seen to be rhombohedral crystals, the majority having a maximum dimension greater than 10 mm but rarely exceeding 40 mm.

## Identification

In the Assay, the principal peak due to insulin in the chromatogram obtained with test solution has a retention time similar to that of the principal peak in the chromatogram obtained with reference solution (a), (b) or (c) as appropriate.



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## Tests

**pH** (2.4.24). 6.9 to 7.5.

**Total zinc**. Not more than 0.0095 per cent w/v (for preparations containing 40 Units per ml) and not more than 0.014 per cent w/v (for preparations containing 80 Units per ml), determined by the following method. Take a volume of the well-shaken suspension containing 200 Units of insulin and add 1 ml of *0.1 M hydrochloric acid*, 10 ml of *alkaline borate buffer pH 9.0*, 1 ml of *0.1 M sodium hydroxide*, 2 ml of a 0.0009 per cent w/v solution of *trypsin* in *0.01 M hydrochloric acid*. Mix, allow to stand for 10 minutes and add 3 ml of *zincon solution* and sufficient *water* to produce 50 ml. Allow to stand for 1 hour and measure the absorbance of the resulting solution at about 620 nm (2.4.7), using as the blank a solution prepared by treating 5 ml of *water* instead of the substance under examination in a similar manner. Calculate the content of zinc from the absorbance obtained by repeating the procedure using a suitable aliquot of a mixture of 4 volumes of *zinc sulphate solution* and 6 volumes of *water*.

**Zinc in solution**. Not more than 70 per cent of the total zinc (for preparations containing 40 Units per ml) and not more than 55 per cent of the total zinc (for preparations containing 80 Units per ml), determined by the method described in the test for Total zinc, using 1 ml of the clear supernatant liquid obtained by centrifuging and beginning at the words "add 1 ml of *0.1 M hydrochloric acid,.....*".

**Insulin extractable with buffered acetone solution**. Not more than 15 per cent, determined by the following method. Centrifuge a volume containing 400 Units and reject the supernatant liquid. Suspend the residue in 3.3 ml of *water*, add 6.6 ml of *buffered acetone solution*, stir for 3 minutes and again centrifuge. Transfer the supernatant liquid as completely as possible to a long-necked, round-bottomed flask, add 0.3 g of *nitrogen-free mercuric oxide*, 3 g of *anhydrous sodium sulphate*, and 6 ml of *nitrogen-free sulphuric acid*, heat over a low flame until the liquid is colourless and boil for a further 30 minutes. Allow to cool, dilute carefully with *water*, add 1 g of *zinc powder*, shake and allow to stand for 10 minutes. Add an excess of *sodium hydroxide solution*, immediately connect the flask to an ammonia distillation apparatus, mix the contents and distil the liberated ammonia into 20 ml of *0.005 M sulphuric acid* prepared with *carbon dioxide-free water*. Rinse the condenser tube into the flask containing the distillate, add sufficient *carbon dioxide-free water* to produce a total volume of about 50 ml and titrate the excess of sulphuric acid with *0.01 M sodium hydroxide* to pH 6.0, using a glass electrode. Centrifuge a further volume containing 400 Units and reject the supernatant liquid. Dissolve the residue in 10 ml of a 5 per cent w/v solution of *nitrogen-free sulphuric acid*, transfer to a long-necked, round-bottomed flask, and repeat the procedure described above beginning at the words "add 0.3 g of *nitrogen-free mercuric oxide*,.....". Calculate the percentage of insulin extractable with the buffered acetone solution from the formula $100A/B$, where A is the volume of *0.005 M sulphuric acid* used in the first determination and B is the volume used in the second determination.

The result of the test is not valid unless in carrying out the first determination omitting the insulin preparation, not more than 0.2 ml of *0.005 M sulphuric acid* is required.

**Insulin in solution**. Determine by liquid chromatography (2.4.14).

*Test solution. For preparations containing 100 Units per ml.* Centrifuge a well-shaken suspension of the preparation under examination, transfer 2.0 ml of the supernatant liquid to a 5 ml volumetric flask, dilute to volume with *0.03 M hydrochloric acid* and mix well.

*Reference solution (a)*. Prepare as the test solution but using 2.5 ml of the supernatant liquid in place of 2.0 ml.

*Reference solution (b)*. Use 5 ml of the supernatant liquid.

*Reference solution (c)*. Weigh accurately 4.5 mg of *bovine insulin RS* into a 100-ml volumetric flask containing 50 ml of *0.025 M hydrochloric acid*, dissolve by shaking for 5 minutes, dilute to volume with *0.025 M hydrochloric acid* and mix to obtain a solution containing approximately 1 Unit per ml.

Use the chromatographic system as described in the Assay.

The area of the peak due to insulin in the chromatogram obtained with test solution, reference solution (a) or reference (b), as the case may be, is not more than that of the principal peak in the chromatogram obtained with reference solution (c).

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. To 10 ml of the preparation under examination add 40 ml of *5 M hydrochloric acid*, mix well, allow to stand for 1 hour to ensure solution of the sediment and dilute with *0.03 M hydrochloric acid* to obtain a solution containing 20 Units per ml.

*Reference solution (a)*. A 0.08 per cent w/v of *bovine insulin RS in 0.025 M hydrochloric acid*.

*Reference solution (b)*. A 0.08 per cent w/v of *porcine insulin RS* in *0.025 M hydrochloric acid*.

*Reference solution (c)*. A solution containing 0.04 per cent w/v of *bovine insulin RS* and 0.04 per cent w/v of *porcine insulin RS* in *0.025 M hydrochloric acid* for a preparation containing both bovine and pork insulin.

*Reference solution (d)*. A 0.04 per cent w/v each of *human insulin RS* and *porcine insulin RS* in *0.025 M hydrochloric acid*.



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Chromatographic system
- a stainless steel column 25 cm × 4.6 mm packed with octadecylsilane chemically bonded to porous silica (5 µm),
- column temperature. 45°,
- mobile phase. a mixture of 72.5 volumes of *0.1 M sodium dihydrogen phosphate* adjusted to pH 2.0 with *phosphoric acid* and 27.5 volumes of *acetonitrile,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 214 nm,
- injection volume. 50 µl.

The test is not valid unless the resolution factor between the peaks corresponding to human insulin and porcine insulin is at least 1.2 in the chromatogram obtained with reference solution (d). If necessary, adjust the concentration of *acetonitrile* in the mobile phase by slight decrease or increase until the required resolution is obtained. In the chromatogram obtained with reference solution (d) the two principal peaks, in order of emergence, are due to human insulin and porcine insulin and any smaller peaks appearing immediately following each of the principal peaks are due to the corresponding monodesamido derivatives.

Inject either of reference solutions (a), (b) and (c), as appropriate, alternatively six times. The test is not valid unless the relative standard deviation of the area of the principal peak is not more than 2 per cent.

Inject the test solution. If necessary, make further adjustments in the composition of the mobile phase so that the antimicrobial preservatives present in test solution are well separated from insulin and show shorter retention times. A small reduction in the concentration of acetonitrile increases the retention time of insulin peak relatively more than those of the preservatives. If necessary, after having carried out the chromatography of a solution, wash the column with a mixture of equal volumes of *acetonitrile* and *water* for a sufficient time in order to elute any interfering substances before injecting the next solution.

Calculate the content of insulin from the area of the peaks due to the bovine, porcine or human insulin and that of any peak due to the monodesamido derivative of the insulin from the declared content of insulin in *bovine insulin RS, porcine insulin RS* or *human insulin RS*, as appropriate. For preparations containing both bovine and porcine insulin use the sum of the areas of both the bovine and porcine insulin peaks and of any peak due to the desamido derivative of either insulin.

**Storage**. Store in multiple dose containers at a temperature between 2° and 8°. It should not be allowed to freeze.

**Labelling**. The label states (1) the strength in terms of the number of Units per ml; (2) the animal source or sources of the insulin; (3) that the preparation should not be allowed to freeze; (4) that the container should be gently shaken before a dose is withdrawn; (5) the storage conditions.

# Invert Sugar Injection

Invert Sugar Injection is a sterile solution of a mixture of equal amounts of Dextrose and Fructose in Water for Injections, or an equivalent sterile solution produced by the hydrolysis of Sucrose in Water for Injections. It contains no antimicrobial agent.

Invert Sugar Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the labelled amount of $C_6H_{12}O_6$.

**Usual strengths**. 5, 10, and 20 per cent w/v.

**Description**. A clear, colourless or faintly straw-coloured solution.

## Identification

To 1 ml add 0.05 ml of *potassium cupri-tartrate solution*; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.

## Tests

**pH** (2.4.24). 3.0 to 6.5.

**5-Hydroxymethylfurfural and Related substances**. Dilute a volume containing 1.0 g of invert sugar to 500.0 ml with *water* and measure the absorbance (2.4.7) of the resulting solution at the maximum at about 284 nm; absorbance at about 284 nm, not more than 0.25.

**Heavy metals** (2.3.13). A solution prepared by evaporating a volume containing 4.0 g of invert sugar to 10 ml and adding 2 ml of *dilute acetic acid* and sufficient *water* to produce 25 ml complies with the limit test for heavy metals, Method A (5 ppm).

**Chlorides** (2.3.12). 2.0 ml of the injection complies with the limit test for chlorides (120 ppm).

**Bacterial endotoxins** (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

### Completeness of inversion

*NOTE — Invert Sugar Injection that is produced by mixing Dextrose and Fructose is exempt from this test.*

Determine by liquid chromatography (2.4.14).

*Test solution*. Transfer an accurately measured volume of the preparation under examination containing about 2.5 g of invert



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sugar to a 100-ml volumetric flask, dilute to volume with *water* and mix.

*Reference solution*. Prepare a solution in *water* containing known concentrations of about 0.25 mg of sucrose and about 12.5 mg of dextrose per ml.

Chromatographic system
- a stainless steel column 30 cm x 7.8 mm, packed with a strong cation-exchange resin consisting of sulphonated cross-linked styrene-divinylbenzene copolymer in the calcium form (9 µm),
- mobile phase: *water*,
- flow rate. 1 ml per minute,
- column temperature. 40°,
- refractive index detector,
- injection volume. 20 µl.

Inject the reference solution. The sucrose elutes first and the peak is baseline separated from the dextrose peak. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution and measure the responses for the sucrose peak. Calculate the content of sucrose in the volume taken of the preparation under examination. Not more than 1.5 per cent of the quantity of invert sugar in the volume taken of the preparation under examination, based on the value stated on the label, is found.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. Transfer 50.0 ml of *cupri-tartaric solution* into a 400 ml beaker, add 48 ml of *water*, mix and add 2.0 ml of the preparation under examination that has been diluted quantitatively with *water*, if necessary, to a 5.0 per cent concentration. Cover the beaker with a watch glass, heat the solution, regulating the heat so that boiling begins in 4 minutes and continue boiling for 2 minutes. Filter the hot solution at once through a tared porcelain filtering crucible, wash the precipitate with water maintained at 60°, then with 10 ml of *ethanol (95 per cent)*. Dry at 105° to constant weight. Carry out a blank determination and make any necessary correction. The corrected weight of the precipitate so obtained is not less than 204.0 mg and not more than 224.4 mg.

**Storage**. Store in single dose containers at a temperature not exceeding 30°.

**Labelling**. The label states (1) whether it is produced by hydrolysis of Sucrose or by mixing Dextrose and Fructose; (2) the strength as the percentage w/v of invert sugar; (3) total osmolar concentration in mOsmol per litre; (4) that the injection should not be used if it contains visible particles.

# Invert Sugar and Sodium Chloride Injection

Sodium Chloride and Invert Sugar Intravenous Infusion

Invert Sugar and Sodium Chloride Injection is a sterile solution of a mixture of equal amounts of Dextrose and Fructose in Water for Injections to which the required amount of Sodium Chloride is added. Invert sugar may be prepared by acid hydrolysis of Sucrose.

Invert Sugar and Sodium Chloride Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amounts of sodium chloride, NaCl, and invert sugar, $C_6H_{12}O_6$. It contains no antimicrobial agent.

**Usual strengths**. Injections containing the following amounts of Sodium Chloride, NaCl, and invert sugar, $C_6H_{12}O_6$.

| per cent w/v of Sodium Chloride (NaCl) | per cent w/v of invert sugar ($C_6H_{12}O_6$) |
|---|---|
| 0.45 | 5 |
| 0.45 | 10 |
| 0.90 | 5 |
| 0.90 | 10 |

**Description**. A clear, colourless or faintly straw-coloured solution.

## Identification

A. To 1 ml add 0.05 ml of *potassium cupri-tartrate solution*; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.

B. Gives reaction A of chlorides and reaction B of sodium salts (2.3.1).

## Tests

**pH** (2.4.24). 3.0 to 6.5.

**5-Hydroxymethylfurfural and Related substances**. Dilute a volume containing 1.0 g of invert sugar to 500.0 ml with *water* and measure the absorbance (2.4.7) of the resulting solution at the maximum at about 284 nm; absorbance at about 284 nm, not more than 0.25.

**Completeness of inversion**

*NOTE — Invert Sugar and Sodium Chloride Injection that is produced by mixing Dextrose and Fructose is exempt from this test.*

Determine by liquid chromatography (2.4.14).

*Test solution*. Transfer an accurately measured volume of the preparation under examination containing about 2.5 g of invert



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sugar to a 100-ml volumetric flask, dilute to volume with *water* and mix.

*Reference solution*. Prepare a solution in *water* containing known concentrations of about 0.25 mg of sucrose and about 12.5 mg of dextrose per ml.

Chromatographic system

- a stainless steel column 30 cm x 7.8 mm, packed with a strong cation-exchange resin consisting of sulphonated cross-linked styrene-divinylbenzene copolymer in the calcium form (9 µm),
- mobile phase: filtered and degassed *water*,
- flow rate. 1.0 ml per minute,
- column temperature. constant at about 40°,
- refractive index detector,
- injection volume. 20 µl.

Inject the reference solution. The sucrose elutes first and the peak is baseline separated from the dextrose peak. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution and measure the responses for the sucrose peak. Calculate the content of sucrose in the volume taken of the preparation under examination. Not more than 1.5 per cent of the quantity of invert sugar in the volume taken of the preparation under examination, based on the value stated on the label, is found.

**Heavy metals** (2.3.13). A solution prepared by evaporating a volume containing 4.0 g of invert sugar to 10 ml and adding 2 ml of *dilute acetic acid* and sufficient *water* to produce 25 ml complies with the limit test for heavy metals, Method A (5 ppm).

**Bacterial endotoxins** (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. *For sodium chloride* — Titrate an accurately measured volume containing about 0.1 g of Sodium Chloride with *0.1 M silver nitrate* using *potassium chromate solution* as indicator.

1 ml of *0.1 M silver nitrate* is equivalent to 0.005844 g of NaCl.

*For invert sugar* — Transfer 50.0 ml of *cupri-tartaric solution* into a 400-ml beaker, add 48 ml of *water*, mix and add 2.0 ml of the preparation under examination that has been diluted quantitatively with *water*, if necessary, to a 5.0 per cent concentration. Cover the beaker with a watch glass, heat the solution, regulating the heat so that boiling begins in 4 minutes and continue boiling for 2 minutes. Filter the hot solution at once through a tared porcelain filtering crucible, wash the precipitate with water maintained at 60°, then with 10 ml of *ethanol (95 per cent)*. Dry at 105° to constant weight. Carry out a blank determination and make any necessary correction. The corrected weight of the precipitate so obtained is not less than 204.0 mg and not more than 224.4 mg.

**Storage**. Store in single dose containers at a temperature not exceeding 30°.

**Labelling**. The label states (1) whether it is produced by hydrolysis of Sucrose or by mixing Dextrose and Fructose; (2) the strength as the percentage w/v of sodium chloride and invert sugar; (3) total osmolar concentration in mOsmol per litre; (4) approximate concentrations, in millimoles per litre, of the sodium and chloride ions; (5) that the injection should not be used if it contains visible particles.

# Iodine

$I_2$ Mol. Wt. 253.8

Iodine contains not less than 99.5 per cent and not more than 100.5 per cent of $I_2$.

**Category**. Antiseptic; used in the treatment of thyroid deficiency.

**Description**. Greyish violet brittle plates or small crystals with a metallic sheen; odour, irritant. It volatalises slowly at room temperature.

## Identification

A. When heated gently it gives violet vapours which condense forming a bluish-black crystalline sublimate.

B. A saturated solution yields a blue colour in the presence of *starch solution* which disappears when the solution is heated and reappears when it is cooled.

## Tests

**Bromides and chlorides**. Not more than 250 ppm, determined by the following method. Triturate 3.0 g with 20 ml of *water*, filter, wash the filter, dilute the filtrate to 30 ml with *water* and add 1 g of *zinc powder*. When the solution is decolorised, filter and wash the filter with sufficient *water* to produce 40 ml of filtrate. To 10 ml of this solution add 3 ml of *10 M ammonia* and 6 ml of *silver nitrate solution*, filter, wash the filter with *water* and dilute to 20 ml with *water*. To 10 ml of the filtrate add 1.5 ml of *nitric acid*. After 1 minute any opalescence produced is not more intense than that produced in a solution prepared at the same time by mixing 10.75 ml of *water*, 0.25 ml of *0.01 M hydrochloric acid*, 0.2 ml of *2 M nitric acid* and 0.3 ml of *silver nitrate solution*.

**Non-volatile matter**. Not more than 0.1 per cent, determined by heating 1.0 g in a porcelain dish on a water-bath until the iodine has volatilised and drying the residue at 105°.



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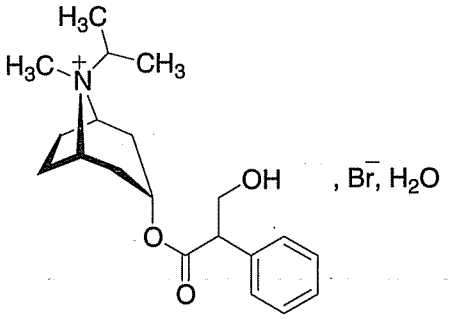


**Assay**. Weigh accurately about 0.2 g, transfer to a flask containing 1 g of *potassium iodide* and 2 ml of *water*, add 1 ml of *2 M acetic acid*, dissolve completely and add 50 ml of *water*. Titrate with *0.1 M sodium thiosulphate* using *starch solution* as indicator.

1 ml of *0.1 M sodium thiosulphate* is equivalent to 0.01269 g of I.

**Storage**. Store in ground-glass-stoppered containers or in earthenware containers with waxed bungs.

## Ipratropium Bromide




$C_{20}H_{30}BrNO_3,H_2O$ Mol. Wt. 430.4

Ipratropium Bromide is 3α-Hydroxy-8-isopropyl-1α*H*,5α*H*-tropanium bromide (*RS*)-tropate monohydrate.

Ipratropium Bromide contains not less than 99.0 per cent and not more than 100.5 per cent of $C_{20}H_{30}BrNO_3,H_2O$, calculated on the anhydrous basis.

**Category**. Anticholinergic; bronchodilator.

**Description**. A white or almost white crystalline powder.

### Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ipratropium bromide RS* or with the reference spectrum of ipratropium bromide.

### Tests

**pH** (2.4.24). 5.0 to 7.5, determined in a 1.0 per cent w/v solution in *carbon dioxide-free water*.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 100 mg of the substance under examination in 10 ml of the mobile phase.

*Reference solution*. A 0.001 per cent w/v solution of *ipratropium bromide RS* in the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (4 µm),
- mobile phase: a mixture of 87 volumes of *buffer solution pH 5.5* prepared by dissolving 1.24 g of sodium *dihydrogen phosphate* and 0.17 g of *tetrapropyl ammonium chloride* in 87 ml of *water* and adjusting pH to 5.5 with 18 per cent w/v solution of *disodium hydrogen phosphate*, and 13 volumes of *methanol*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.5.

Inject the test solution. Any individual impurity is not more than 0.5 per cent and the sum of all the impurities is not more than 1.0 per cent.

**Water** (2.3.43). Not more than 5.0 per cent, determined on 0.5 g.

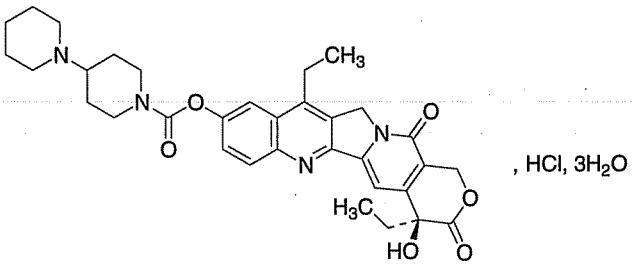
**Sulphated Ash** (2.3.18). Not more than 0.1 per cent.

**Assay**. Weigh accurately about 0.35 g of the substance under examination, dissolve in 50 ml of *water* and add 3 ml of *dilute nitric acid*. Titrate with *0.1 M silver nitrate*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M silver nitrate* is equivalent to 0.04124 g of $C_{20}H_{30}BrNO_3$.

**Storage**. Store protected from light and moisture.

## Irinotecan Hydrochloride Trihydrate




$C_{33}H_{38}N_4O_6,HCl,3H_2O$ Mol. Wt. 677.2

Irinotecan Hydrochloride Trihydrate is (4*S*)-4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo-1*H*-pyrano[3′,4′:6,7]indolizino[1,2-*b*]quinolin-9-yl [1,4′-bipiperidine]-1′-carboxylate hydrochloride trihydrate.

Irinotecan Hydrochloride Trihydrate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{33}H_{38}N_4O_6,HCl$, calculated on the anhydrous basis.



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**Category**. Anticancer.

**Description**. A pale yellow to yellow crystalline powder.

*CAUTION — Irinotecan Hydrochloride Trihydrate is potentially cytotoxic. Great care should be taken in handling the powder and preparing solutions.*

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *irinotecan hydrochloride trihydrate RS* or with the reference spectrum of irinotecan hydrochloride trihydrate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

## Tests

**Appearance of solution**. A 1.0 per cent w/v solution is clear (2.4.1) and not more intensely coloured than the reference solution GYS3 (2.4.1).

**pH** (2.4.24). 3.0 to 5.0, determined in 1.0 per cent w/v solution in *water*.

**Specific optical rotation** (2.4.22). + 60.0° to + 73.0°, determined in 1.0 per cent w/v solution.

**Light absorption**. Dissolve 0.1 g of the substance under examination in 10 ml of *methanol*, prepare the solution immediately before use. The absorbance of the resulting solution, at the maximum at about 430 nm (2.4.7) is not more than 0.17.

**Related substances**. Determine by liquid chromatography (2.4.14).

*NOTE — Use freshly prepared solutions.*

*Solvent mixture*. Dissolve 6.41 g of *disodium hydrogen phosphate dihydrate* and 0.73 g *1-heptane sulphonic acid sodium* in 720 ml of *water*. Adjust the pH to 3.0 with *orthophosphoric acid* and dilute to 1000 ml with *acetonitrile*.

*Test solution*. Dissolve 40 mg of the substance under examination in 100.0 ml of the solvent mixture.

*Reference solution (a)*. A 0.04 per cent w/v solution of *irinotecan hydrochloride trihydrate RS* in the solvent mixture.

*Reference solution (b)*. Dilute 1.0 ml of reference solution (a) to 100.0 ml with the solvent mixture.

*Reference solution (c)*. Dissolve 20 mg of *irinotecan hydrochloride trihydrate RS* in 10 ml of 0.04 per cent w/v solution of *7-ethyl-10-hydroxycamptothecin RS* in *N,N,dimethylformamide* and dilute to 50.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. dissolve 8.90 g of *disodium hydrogen phosphate dihydrate* and 1.01 g of *1- heptane sulphonic acid sodium* in 900 ml of *water*. Adjust the pH to 3.0 with *orthophosphoric acid* and dilute to 1000 ml with *water* and filter,
  B. *acetonitrile*,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

| Time (in min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0 | 78 | 22 |
| 5 | 78 | 22 |
| 28 | 67 | 33 |
| 40 | 67 | 33 |
| 45 | 78 | 22 |
| 46 | 78 | 22 |

The system is not valid unless the resolution between 7-ethyl-10-hydroxycamptothecin and irinotecan hydrochloride trihydrate is not less than 3.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.2 times the area of the peak in the chromatogram obtained with reference solution (b) (0.2 per cent) and the sum of the areas of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with reference solution (b) (1.0 per cent).

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method A (20 ppm).

**Total Chloride**. 5.0 per cent to 5.5 per cent, Weigh accurately about 500 mg of the substance under examination, dissolve in 10 ml of *methanol*, add 20 ml of *water* and 20 ml of *glacial acetic acid* and titrate with *0.1 M silver nitrate solution*, using eosin yellow solution as indicator. Carry out a blank titration.

1 ml of *0.1 M silver nitrate* is equivalent to 0.003545 g of chloride.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). 8.0 per cent to 9.0 per cent, determined on 0.1 g.

**Microbial contamination** (2.2.9). The total aerobic microbial count does not exceed 100 cfu per g. It meets the requirements of the tests for the absence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella species*, and *Escherichia coli*.



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**Bacterial endotoxins** (2.2.3). Not more than 0.275 Endotoxin Unit per mg of irinotecan hydrochloride trihydrate.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture.* Dissolve 6.41 g of *disodium hydrogen phosphate dihydrate* and 0.73 g *1-heptane sulphonic acid sodium* in 720 ml of *water.* Adjust the pH to 3.0 with *orthophosphoric acid*, dilute to 1000 ml with *acetonitrile*, filter.

*Test solution.* Dissolve 40 mg of the substance under examination in 100.0 ml of the solvent mixture.

*Reference solution.* A 0.04 per cent w/v solution of *irinotecan hydrochloride trihydrate RS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. dissolve 8.90 g of *disodium hydrogen phosphate dihydrate* and 1.01 g of *1-heptane sulphonic acid sodium* in 900 ml of *water*. Adjust the pH to 3.0 with *orthophosphoric acid* and dilute to 1000 ml with *water,*

  B. *acetonitrile*,
- flow rate.1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

| Time (in min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0 | 78 | 22 |
| 5 | 78 | 22 |
| 28 | 67 | 33 |
| 40 | 67 | 33 |
| 45 | 78 | 22 |
| 46 | 78 | 22 |

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{33}H_{38}N_4O_6$,HCl.

**Storage**. Store protected from light, at a temperature not exceeding 25°.

# Irinotecan Injection

Irinotecan Hydrochloride Injection

Irinotecan Injection is a sterile solution of Irinotecan Hydrochloride Trihydrate in *water*.

Irinotecan Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of irinotecan hydrochloride trihydrate, $C_{33}H_{38}N_4O_6$,HCl,$3H_2O$.

**Usual strength**. 20 mg per ml.

**Description**. A light yellow coloured solution, free from visible particles.

## Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. It gives the reaction of chlorides (2.3.1).

## Tests

**Appearance of solution**. Prepare a solution equivalent to1.0 per cent of the substance in *water.* The solution is clear or not more intensely coloured than the reference solution *GYS3* (2.4.1).

**pH** (2.4.24). 3.0 to 3.8.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution.* Dilute a measured volume containing 40 mg of Irinotecan Hydrochloride Trihydrate to 100 ml with mobile phase.

*Reference solution (a).* A 0.04 per cent w/v solution of *irinotecan hydrochloride trihydrate RS* in the mobile phase.

*Reference solution (b).* Dilute 1 ml of reference solution (a) to 100 ml with mobile phase.

*Reference solution (c).* Dissolve 20 mg of *irinotecan hydrochloride trihydrate RS* in 10 ml of 0.04 per cent w/v solution of *7-ethyl-10-hydroxycamptothecin RS* in *N,N,dimethyl-formamide* and dilute to 50.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 72 volumes of *buffer solution* prepared by dissolving 1.1 g of *1-heptane sulphonic acid sodium monohydrate* and 7.1 g of *disodium hydrogen orthophosphate anhydrous* in 1000 ml *water*, adjusted to pH 3.0 with *orthophosphoric acid* and 28 volumes of *acetonitrile*, filter.
- flow rate.1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Inject reference solution (c). The test is not valid unless the resolution between 7-ethyl-10-hydroxycamptothecin and irinotecan hydrochloride trihydrate is not less than 3.0.



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Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.2 times the area of the peak in the chromatogram obtained with the reference solution (b) (0.2 per cent) and the sum of areas of all the secondary peaks is not more than 1.2 times the area of the peak in the chromatogram obtained with the reference solution (b) (1.2 per cent).

**Bacterial endotoxins** (2.2.3). Not more than 0.556 Endotoxin Unit per mg of irinotecan hydrochloride trihydrate.

**Sterility** (2.2.11).Complies with the test for sterility.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Accurately measured volume of the injection containing 40 mg of *irinotecan hydrochloride trihydrate,* diluted to 100.0 ml with mobile phase.

*Reference solution.* A 0.04 per cent w/v solution of *irinotecan hydrochloride trihydrate RS* in the mobile phase.

Use chromatographic system as described under Related substances.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.4. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{33}H_{38}N_4O_6,HCl, 3H_2O$ in the injection.

**Storage**. Store protected from light, at a temperature not exceeding 25°.

# Iron and Ammonium Citrate

Ferric Ammonium Citrate

Iron and Ammonium Citrate is a complex of ammonium ferric citrate.

Iron and Ammonium Citrate contains not less than 20.5 per cent and not more than 22.5 per cent of iron, Fe.

**Category**. Haematinic.

**Dose**. 1 to 3 g.

**Description**. Thin, transparent, dark red scales or granules or a brownish red powder; odourless; deliquescent in moist air and is affected by light.

## Identification

A. Ignite 0.1 g gently and dissolve the residue in 5 ml of *hydrochloric acid*; the solution gives the reactions of ferric salts (2.3.1).

B. Warm 50 mg with *5 M sodium hydroxide*; ammonia is evolved and the solution gives the reactions of citrates (2.3.1).

## Tests

**Arsenic** (2.3.10). Mix 2.5 g with 1.5 g of *anhydrous sodium carbonate*, add 10 ml of *bromine solution* and mix thoroughly. Evaporate to dryness on a water-bath, gently ignite and dissolve the cooled residue in 20 ml of *brominated hydrochloric acid* and 10 ml of *water*. Transfer to a small flask, add sufficient *stannous chloride AsT* to remove the yellow colour, connect to a condenser and distill 22 ml. To the distillate add 40 ml of *water* and 0.15 ml of *stannous chloride AsT*. The resulting solution complies with the limit test for arsenic (4 ppm).

**Lead**. Dissolve 2.0 g in 20 ml of *hydrochloric acid* and 8 ml of *water*, add 0.5 ml of *nitric acid*, heat just to boiling, cool, transfer to a separating funnel and extract with three quantities, each of 20 ml, of *ether*. If the acid solution is still more than faintly yellow, extract with an additional 20 ml of *ether*. Reject the ether extracts. Transfer the acid solution to a narrow-necked flask, rinse the separating funnel with 5 ml of *water* and add the rinsings to the flask. Heat to remove the dissolved ether, cool, make alkaline with *ammonia solution*, add 1 ml of *potassium cyanide solution*, dilute to 50 ml with *water* and add 0.1 ml of *sodium sulphide solution*. Any colour produced is not more intense than that produced by mixing 10 ml of *hydrochloric acid,* 0.5 ml of *nitric acid* and 6 ml of *lead standard solution (10 ppm Pb)*, making alkaline with *ammonia solution* and adding 1 ml of *potassium cyanide solution* and 0.1 ml of *sodium sulphide solution* (30 ppm).

**Zinc**. Dissolve 2.0 g in a mixture of 20 ml of *hydrochloric acid* and 8 ml of *water*, add 0.5 ml of *nitric acid,* heat just to boiling, cool and extract with three quantities, each of 20 ml, of *ether*. If the acid solution is still more than faintly yellow, repeat the extraction with an additional 20 ml of *ether*. Reject the ether extracts. Warm the acid solution on a water-bath to remove dissolved ether, cool and add sufficient *water* to produce 100 ml. To 10 ml add 1 g of *citric acid* and 0.1 g of *resorcinol,* neutralise with *dilute ammonia solution,* using *thymol blue solution* as indicator, and shake for 1 minute with two successive quantities, each of 20 ml, of *dithizone solution*. To the combined extracts add 10 ml of *0.1 M hydrochloric acid,* shake for 1 minute, separate the acid layer and wash it with 2 ml of *chloroform*. To the acid layer add 3 ml of *1 M hydrochloric acid* and 20 ml of *ammonium chloride solution* and adjust the volume to 50 ml with *water*. Add 1 ml of *potassium ferrocyanide solution* and allow to stand for 15 minutes. Any turbidity produced is not more than that produced by the addition of 1 ml of *potassium ferrocyanide solution* to a freshly prepared mixture of 1.0 ml of *zinc standard solution (10 ppm Zn)*, 4 ml of *1 M hydrochloric acid*, 20 ml of *ammonium chloride solution* and sufficient *water* to produce 50 ml (50 ppm).



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**Free ferric compounds**. A 1.0 per cent w/v solution gives no blue precipitate with *potassium ferrocyanide solution* unless acidified with *hydrochloric acid*.

**Chlorides** (2.3.12). Dissolve 0.1 g in 5 ml of *water* and boil with 2 ml of *nitric acid*; the solution complies with the limit test for chlorides (0.25 per cent).

**Sulphates** (2.3.17). 10 ml of a 0.5 per cent w/v solution complies with the limit test for sulphates (0.3 per cent).

**Assay**. Weigh accurately about 0.5 g, dissolve in a mixture of 15 ml of *water* and 1 ml of *sulphuric acid* and warm until the dark brown colour becomes yellow. After cooling the solution to 15° add dropwise *0.02 M potassium permanganate* till a pink colour persisting for 5 seconds is obtained. Add 15 ml of *hydrochloric acid* and 2 g of *potassium iodide*, allow to stand for 3 minutes, add about 60 ml of *water* and titrate with *0.1 M sodium thiosulphate* using *starch solution* as indicator.

1 ml of *0.1 M sodium thiosulphate* is equivalent to 0.005585 g of Fe.

**Storage**. Store protected from light and moisture.

# Iron Dextran Injection

Iron Dextran Injection is a sterile colloidal solution containing a complex of ferric hydroxide with dextrans of average molecular weight between 5000 and 7500.

Iron Dextran Injection contains not less than 4.75 per cent and not more than 5.25 per cent w/v of iron and not less than 17.0 per cent and not more than 23.0 per cent w/v of dextrans.

**Category**. Haematinic.

**Dose**. To be determined by the physician as per needs of the patient and in accordance with the manufacturer's dosage schedules.

**Description**. A dark brown solution.

## Identification

A. To 0.2 ml, previously diluted to 5 ml with *water*, add *5 M ammonia*; no precipitate is produced.

B. Mix 1 ml with 100 ml of *water*. To 5 ml of this solution add 0.1 ml of *hydrochloric acid*, boil for 30 seconds, cool rapidly, add 2 ml of *strong ammonia solution* and 5 ml of *hydrogen sulphide solution*, boil to remove hydrogen sulphide, cool and filter. Boil 5 ml of the filtrate with 5 ml of *potassium cupri-tartrate solution*; the solution remains greenish in colour and no precipitate is produced. Boil a further 5 ml of the filtrate with 0.5 ml of *hydrochloric acid* for 5 minutes, cool, add 2.5 ml of *5 M sodium hydroxide* and 5 ml of *potassium cupri-tartrate solution* and boil again; a reddish precipitate is produced.

C. To 1 ml add 20 ml of *water* and 5 ml of *hydrochloric acid* and boil for 5 minutes. Cool, add an excess of *strong ammonia solution* and filter. Wash the precipitate with *water*, dissolve in the minimum volume of *2 M hydrochloric acid* and add sufficient *water* to produce 20 ml. The resulting solution gives reaction B of ferric salts (2.3.1).

## Tests

**pH** (2.4.24). 5.2 to 6.5.

**Arsenic** (2.3.10). To 10.0 ml in a round-bottomed, long-necked flask add 20 ml of *water* and 20 ml of *nitric acid* and heat until the vigorous evolution of brown fumes ceases. Cool, add 20 ml of *sulphuric acid* and heat again until fumes are evolved, adding *nitric acid* dropwise from time to time until oxidation is complete. Cool, add 60 ml of *water*, bring to boil and continue boiling until the volume of liquid is reduced to about 40 ml. Cool and dilute to 100 ml with *water*. Reserve a portion of the solution (solution A) for the test for Heavy metals. Boil gently 40 ml of this solution until the volume is reduced to about 15 ml, cool and add 15 ml of *stannous chloride solution AsT*. Connect to a condenser and distil 15 ml into 25 ml of *water*. To the distillate add 0.2 ml of *bromine solution*, remove the excess of bromine with a few drops of *stannous chloride solution AsT* and add 20 ml of *water*. The resulting solution complies with the limit test for arsenic. Use 0.8 ml of *arsenic standard solution (10 ppm As)* to prepare the standard (2 ppm).

**Heavy metals** (2.3.13). To 16.0 ml of solution A reserved in the test for Arsenic add 50 ml of *hydrochloric acid* and extract with four quantities, each of 20 ml, of *isobutyl acetate*, discarding the extracts. Evaporate the acid solution to dryness and dissolve the residue in 20 ml of *water*. The resulting solution complies with the limit test for heavy metals, Method D (25 ppm). Use *lead standard solution (2 ppm Pb)* to prepare the standard.

**Copper**. To 5.0 ml add 5 ml of *nitric acid* and heat until the vigorous evolution of brown fumes ceases. Cool, add 2 ml of *sulphuric acid* and heat again until fumes are evolved, adding *nitric acid* dropwise at intervals until oxidation is complete. Cool, add 25 ml of *hydrochloric acid*, warm to dissolve, cool and extract with four quantities, each of 25 ml, of *isobutyl acetate*, discarding the extracts. Evaporate the acid solution to dryness, adding *nitric acid* dropwise if charring occurs. Dissolve the residue in 10 ml of *1 M hydrochloric acid*, reserving a portion (solution B) for the test for Zinc. To 1 ml add 25 ml of *water* and 1 g of *citric acid*, make alkaline to *litmus paper* with *5 M ammonia*, dilute to 50 ml with *water*, add 1 ml of *sodium diethyldithiocarbamate solution* and allow to stand for 5 minutes. Any colour produced is not more intense than that produced by treating in the same manner a mixture of 3 ml of *copper standard solution (10 ppm Cu)* and 1 ml of *1 M hydrochloric acid* beginning at the words "add 25 ml of *water*....." (60 ppm).



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**Zinc**. To 5.0 ml of solution B reserved in the test for Copper add 15 ml of *1 M sodium hydroxide*, boil, filter, wash the residue with *water* and dilute the combined filtrate and washings to 25 ml with *water*. To 5 ml add 5 ml of *1 M hydrochloric acid* and 2 g of *ammonium chloride*, dilute to 50 ml with *water*, add 1 ml of freshly prepared *dilute potassium ferrocyanide solution* and allow to stand for 20 minutes. Any opalescence produced is not more than that produced when 1 ml of freshly prepared *dilute potassium ferrocyanide solution* is added to a solution prepared from 3 ml of *zinc standard solution (25 ppm Zn)*, 3 ml of *1 M sodium hydroxide*, 6 ml of *1 M hydrochloric* acid and 2 g of *ammonium chloride* diluted to 50 ml with *water* and allowed to stand for 20 minutes (150 ppm).

**Chlorides**. To 5.0 ml add 75 ml of *water* and 0.05 ml of *nitric acid* and titrate immediately with *0.1 M silver nitrate*, determining the end-point potentiometrically (2.4.25). 6.8 to 9.6 ml of *0.1 M silver nitrate* is required.

**Iron absorption**. Prepare a site over the semitendinosus muscle of one leg of each of two rabbits, each weighing between 1.5 and 2.5 kg, by clipping the fur and disinfecting the exposed skin. Inject each site with a dose of 0.4 ml per kg of body weight in the following manner. Place the needle in the distal end of the semitendinosus muscle at an angle such as to ensure that the full length of the needle is used, then pass it through the sartorius and vastus medialis muscles. House the rabbits separately. Sacrifice the rabbits after 7 days and remove the legs into which the injections were made. Carefully dissect the muscles and examine the site of injection. The muscle is only lightly stained and no heavy black deposit of unabsorbed iron compounds or leakage along fascial planes is observed.

Skin the leg, dissect the flesh from the bone and cut into small pieces. Transfer the pieces to a 1000-ml beaker, add 75 ml of *2 M sodium hydroxide* and sufficient *water* to submerge them, cover the beaker with a watch glass and boil until most of the solid matter has disintegrated. Cool cautiously, add 50 ml of *sulphuric acid*, heat the mixture almost to boiling and add carefully 10 ml of *fuming nitric acid* about 1 ml at a time, until no charring occurs when the excess of nitric acid has been boiled off. Cool, add 175 ml of *water*, boil until solution is complete, cool and dilute to 250.0 ml with *water*. To 5.0 ml of the solution add 3 ml of *sulphuric acid*, heat to fuming and complete the oxidation by adding small quantities of *nitric acid* until the solution is colourless. Cool, add 20 ml of *water*, boil for 3 minutes and add 10 ml of *ammonium citrate solution*, 10 ml of *ammonium thioglycollate solution* followed by *dilute ammonia solution* dropwise until the iron colour is fully developed. Add 1 ml excess of *dilute ammonia solution* and sufficient *water* to produce 100.0 ml. Measure the absorbance of the resulting solution at about 530 nm (2.4.7). For the reference solution, add 10 ml of *ammonium citrate solution*, 10 ml of *ammonium thioglycollate solution* and the same quantities of *dilute ammonia solution* as used above to 20 ml of *water*, and dilute to 100.0 ml. Measure the absorbance of this solution at about 530 nm (2.4.7). From the difference between the absorbances, calculate the amount of Fe present in the legs from a reference curve prepared by treating suitable aliquots of a solution of *ferric ammonium sulphate* containing 0.01 per cent w/v of Fe by the above procedure beginning at the words "add 10 ml of *ammonium citrate solution,.....*".

Repeat the determination of Fe on the corresponding legs into which injection was not made beginning at the words "Carefully dissect the muscles....". From the difference between the two amounts of Fe, calculate the proportion of injected iron, as Fe, remaining in the leg tissues. Not more than 20 per cent of the injected iron remains.

**Abnormal toxicity**. Inject 0.10 ml into a tail vein of each of 10 mice; not more than 3 mice die within 5 days of injection. If more than 3 mice die within 5 days, repeat the test on another group of 20 mice. Not more than 10 of the 30 mice used in the combined tests die within 5 days of injection.

**Bacterial endotoxins** (2.2.3). Not more than 0.50 Endotoxin Unit per mg of iron.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. *For iron* — Weigh accurately about 2.0 g, add 10 ml of *water* and 5 ml of *sulphuric acid* and stir for several minutes. Allow to stand for 5 minutes, cool and dilute to 50 ml with *water*. Prepare a suitable zinc amalgam by covering 300 g of zinc shots with a 2 per cent w/v solution of *mercuric chloride* and stir for 10 minutes. Decant the solution, wash the residue three times with *water* and transfer it to a column (30 cm × 18 mm) fitted with a sintered-glass disc (porosity No. 0). Activate the zinc amalgam by passing through the column 200 ml of *sulphuric acid (5 per cent v/v)*. Pass the prepared solution slowly through the column and wash successively with 50 ml of *water*, four quantities, each of 25 ml, of *sulphuric acid (5 per cent v/v)* and 50 ml of *water*. Titrate the combined eluates with *0.1 M ceric ammonium sulphate* using *ferroin solution* as indicator.

1 ml of *0.1 M ceric ammonium sulphate* is equivalent to 0.005585 g of Fe.

Determine the weight per ml of the injection (2.4.29), and calculate the percentage w/v of Fe.

*For dextrans* — Weigh accurately about 1.0 g, dilute to 500.0 ml with *water*, dilute 10.0 ml of this solution to 100.0 ml with *water*, transfer 3.0 ml of the resulting solution to a test-tube and cool to 0°. Add, to form a lower layer, 6.0 ml of a solution prepared and maintained at 0° containing 0.2 per cent w/v of *anthrone* in a mixture of 19 volumes of *sulphuric acid* and 1 volume of *water*, mix and immediately heat on a water-bath for 5 minutes. Cool and measure the absorbance of the



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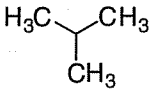
resulting solution at about 625 nm (2.4.7). Repeat the operation using 3.0 ml of *water* in place of the dilution of the injection. From the difference between the absorbances, calculate the content of dextrose present using a calibration curve prepared by treating suitable amounts of *dextrose* in the same manner.

1 g of dextrose is equivalent to 0.94 g of dextrans.

Determine the weight per ml of the injection (2.4.29), and calculate the percentage w/v of dextrans.

**Labelling**. The label states the strength in terms of the equivalent amount of iron, Fe, in a suitable dose-volume.

# Isobutane



$C_4H_{10}$ Mol. Wt. 58.1

Isobutane is 2-methylpropane.

Isobutane contains not less than 95.0 per cent of $C_4H_{10}$.

**Category**. Pharmaceutical aid.

*CAUTION—Isobutane is highly flammable and explosive.*

## Identification

Determine by infrared absorption spectrophotometry (2.4.6), the solution shows absorption maxima at wavelength of 3.4 µm, 6.8 µm, 7.2 µm, 8.5 µm and 10.9 µm.

## Tests

**Water** (2.3.43). Not more than 0.001 per cent with the following modifications (a) provide the closed-system titrating vessel with an opening through which passes a coarse-porosity gas dispersion tube connected to a sample cylinder; (b) dilute the reagent with *anhydrous methanol* to give a water equivalence factor of between 0.2 and 1.0 mg per ml, age this diluted solution for not less than 16 hours before sanitation; (c) obtain a 100 g sample as directed under inhalation preparation, and introduce the sample into the titration vessel through the gas dispersion tube at a rate of about 100 ml of gas per minute.

**High-boiling residues**. Not more than 5 µg per ml.

Prepare a cooling coil from copper tubing (about 6 mm outside diameter × about 6.1 m long) to fit into a vacuum-jacketed flask. Immerse the cooling coil in a mixture of dry ice and *acetone* in a vacuum-jacketed flask, and connect one end of the tubing to the propellant sample cylinder. Carefully open the sample cylinder valve, flush the cooling coil with about 50 ml of the propellant, and discard this portion of liquefied propellant. Continue delivering liquefied propellant from the cooling coil, and collect it in a previously chilled 1000-ml sedimentation cone until the cone is filled to the 1000-ml mark. Allow the propellant to evaporate, using a warm water bath maintained at about 40° to reduce evaporating time. When all of the liquid has evaporated, rinse the sedimentation cone with two 50-ml portions of *pentane*, and combine the rinsings in a tared 150-ml evaporating dish. Transfer 100 ml of the *pentane* solvent to a second tared 150-ml evaporating dish, place both evaporating dishes on a water bath, evaporate to dryness, and heat the dishes in an oven at 100° for 60 minutes. Cool the dishes in a desiccator, and weigh. Repeat the heating for 15-minute periods until successive weighings are within 0.1 mg, and calculate the weight of the residue obtained from the propellant as the difference between the weights of the residues in the two evaporating dishes.

**Acidity of residue**. Add 10 ml of *water* to the residue obtained in the test for High boiling residues, mix by swirling for about 30 seconds, add 2 drops of *methyl orange solution*, insert the stopper in the tube, and shake vigorously; no pink or red color appears in the aqueous layer.

**Assay**. Determine by gas chromatography (2.4.13).

*Test solution.* Connect one Isobutane cylinder to the chromatograph through a suitable sampling valve and a flow control valve downstream from the sampling valve. Flush the liquid sample through the sampling valve, taking care to avoid entrapment of gas or air in the sampling valve.

Chromatographic system

- an aluminum column 6 m x 3 mm, packed with 10 per cent of liquid phase G30 (Tetraethylene glycol dimethyle ether) on non-acid-washed support S1C ( A support prepared from crushed firebrick and calcined or burned with a clay binder above 900° with subsequent acid-wash. It may be silanized),
- temperature:
  column.33°,
- a thermal-conductivity detector,
- flow rate. 50 ml per minute using nitrogen as carrier gas.

Inject the test solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 1.0.

Inject 2 µl of the reference solution and the test solution.

Calculate the percentage purity by dividing 100 times the Isobutane response by the sum of all of the responses in the chromatogram.

**Storage**. Store protected from moisture and prevent exposure to excessive heat.

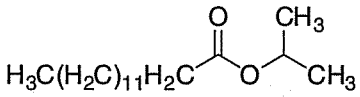


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# Isopropyl Myristate



$C_{17}H_{34}O_2$ Mol. Wt. 270.5

Isopropyl Myristate is isopropyl tetradecanoate.

Isopropyl Myristate contains not less than 90.0 per cent of $C_{17}H_{34}O_2$.

**Category**. Pharmaceutical aid.

**Description**. A clear, colourless, oily liquid.

## Identification

*Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.*

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. Superpose 2 ml of a 0.1 per cent w/v solution in *ethanol (95 per cent)* on a freshly prepared solution of 20 mg of *dimethylaminobenzaldehyde* in 2.0 ml of *sulphuric acid*. After 2 minutes, a yellowish-red colour appears at the junction of the 2 liquids and gradually becomes red.

C. Complies with the test for saponification value (2.3.37).

## Tests

**Appearance of solution**. A 10.0 per cent w/v solution in *methanol* is clear (2.4.1), and not more intensely coloured than reference solution YS7 (2.4.1).

**Refractive index** (2.4.27). 1.434 to 1.437.

**Relative density** (2.4.29). About 0.853.

**Acid value** (2.3.23). Not more than 1.0.

**Saponification value** (2.3.37). 202 to 212.

**Iodine value** (2.3.28). Not more than 1.0.

**Total ash** (2.3.19). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 0.1 per cent, determined on 5.0 g.

**Assay**. Determine by gas chromatography (2.4.13).

*Internal standard solution*. Dissolve about 50 mg of *tricosane* in 250.0 ml of *heptane*.

*Test solution*. Dissolve about 20 mg of the substance under examination in 100.0 ml of the internal standard solution.

*Reference solution*. A 0.02 per cent w/v solution of *isopropyl tetradecanoate RS* in internal standard solution.

Chromatographic system

- a fused silica column 50 m x 0.2 mm, packed with poly(cyanopropyl) siloxane (film thickness 0.2 µm),
- temperature:
  column. 185°,
  inlet port and detector at 250°,
- flame ionization detector,
- flow rate. 1 ml per minute using nitrogen as a carrier gas.

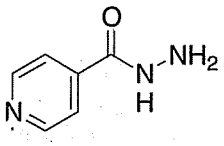
Inject 2 µl of the reference solution and the test solution.

Calculate the content of $C_{17}H_{34}O_2$.

**Storage**. Store protected from light.

# Isoniazid

Isonicotinylhydrazid; INH



$C_6H_7N_3O$ Mol. Wt. 137.1

Isoniazid is isonicotinic acid hydrazide.

Isoniazid contains not less than 98.0 per cent and not more than 101.0 per cent of $C_6H_7N_3O$, calculated on the dried basis.

**Category**. Antituberculosis.

**Dose**. 300 mg daily or upto 1 g twice weekly.

**Description**. Colourless crystals or a white, crystalline powder; odourless.

## Identification

*Test A may be omitted if tests B and C are carried out. Test B may be omitted if tests A and C are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *isoniazid RS* or with the reference spectrum of isoniazid.

B. Dissolve 0.1 g in 2 ml of *water*, add a warm solution of 0.1 g of *vanillin* in 10 ml of *water*, allow to stand and scratch the inside of the container with a glass rod; a yellow precipitate is produced. The precipitate after recrystallisation from 5 ml of *ethanol (70 per cent)* and drying at 105° melts at 226° to 231° (2.4.21).

C. Melts at 170° to 174° (2.4.21).

## Tests

**Appearance of solution**. A 5.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and not more intensely coloured than reference solution BYS7 (2.4.1).



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**pH** (2.4.24). 6.0 to 8.0, determined in a 5.0 per cent w/v solution.

**Hydrazine**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 50 volumes of *ethyl acetate*, 20 volumes of *acetone*, 20 volumes of *methanol* and 10 volumes of *water*.

*Test solution*. Dissolve 1 g of the substance under examination in sufficient of a mixture of equal volumes of *acetone* and *water* to produce 10 ml.

*Reference solution*. Dissolve 50 mg of *hydrazine sulphate* in 50 ml of *water* and dilute to 100 ml with *acetone*; to 10 ml of this solution add 0.2 ml of the test solution and dilute to 100 ml with a mixture of equal volumes of *acetone* and *water*.

After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution. Spray with *dimethylaminobenzaldehyde reagent* and examine in daylight. The additional spot (due to hydrazine) in the chromatogram obtained with the reference solution is more intense than any corresponding spot in the chromatogram obtained with the test solution.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 50 mg of the substance under examination in 100 ml of *water*.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 96 volumes of a solution prepared by dissolving 1.4 g *disodium hydrogen phosphate* and 1 ml of *triethylamine* to 1000 ml with *water*, adjusting the pH to 6.0 with *orthophosphoric acid* and 4 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume. 20 µl.

Inject the test solution. Any individual impurity is not more than 0.2 per cent and the sum of all impurities found is not more than 1.0 per cent.

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve about 25.0 mg of the substance under examination in 50.0 ml of *water*. Dilute 5.0 ml of this solution to 25.0 ml with *water*.

*Reference solution*. Dissolve 25.0 mg of the *isoniazid RS* in 50.0 ml of *water*. Dilute 5.0 ml of this solution to 25.0 ml with *water*.

Use the chromatographic system described under the test for Related substances.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_6H_7N_3O$.

**Storage**. Store protected from light.

# Isoniazid Tablets

Isonicotinylhydrazid Tablets; INH Tablets

Isoniazid Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of isoniazid, $C_6H_7N_3O$.

**Usual strengths**. 50 mg; 100 mg; 300 mg.

## Identification

A. Shake a quantity of the powdered tablets containing 0.1 g of Isoniazid with 10 ml of *ethanol (95 per cent)* for 15 minutes, centrifuge and decant the supernatant liquid. Extract the residue with two further quantities, each of 10 ml, of *ethanol (95 per cent)* and evaporate the combined extracts to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *isoniazid RS* or with the reference spectrum of isoniazid.

B. Shake a quantity of the powdered tablets containing 1 mg of Isoniazid with 50 ml of *ethanol (95 per cent)* and filter. To 5 ml of the filtrate add 0.1 g of *borax* and 5 ml of a 5 per cent w/v solution of *1-chloro-2,4-dinitrobenzene* in *ethanol (95 per cent)*, evaporate to dryness on a water-bath and continue heating for a further 10 minutes. To the residue add 10 ml of *methanol* and mix; a reddish purple colour is produced.

## Tests

**Related substances**. Determine by liquid chromatography (2.4.14).



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*Test solution.* Weigh and powder 20 tablets. Weigh a quantity of the powdered tablets containing 50 mg of Isoniazid, dissolve in 100 ml of the mobile phase.

Use the chromatographic system described under Assay.

Inject the test solution. Any individual impurity is not more than 1.0 per cent and the sum of all impurities found is not more than 2.0 per cent.

**Dissolution** (2.5.2).

Apparatus. No. 1,
Medium. 900 ml of *water,*
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first 1 ml of the filtrate. Dilute suitably with *water* and measure the absorbance of the resulting solution at the maximum at about 263 nm (2.4.7). Calculate the content of $C_6H_7N_3O$ taking 307 as the specific absorbance at 263 nm.

D. Not less than 80 per cent of the stated amount of $C_6H_7N_3O$.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 25.0 mg of Isoniazid and dissolve in 50.0 ml of the mobile phase. Dilute 5.0 ml of this solution to 25.0 ml with the mobile phase.

*Reference solution.* A 0.01 per cent w/v solution of the *isoniazid RS* in the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Intersil ODS-3),
- mobile phase: mix 96 volumes of a solution prepared by dissolving 1.4 g *disodium hydrogen orthophosphate anhydrous* and 1.0 ml of *triethylamine* to 1000 ml with *water* and adjusting the pH to 6.0 with *orthophosphoric acid*, and 4 volumes of *acetonitrile* and filter,
- flow rate. 1 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the column efficiency is not less than 2000 theoretical plates. The relative standard deviation for replicate injections is not more than 2.0 per cent.

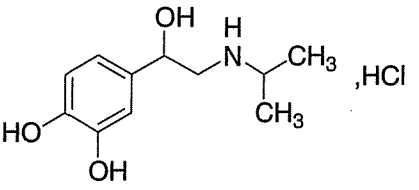
Inject alternately the test solution and the reference solution.

Calculate the content of $C_6H_7N_3O$ in the tablets.

**Storage**. Store protected from light.

# Isoprenaline Hydrochloride

Isoproterenol Hydrochloride




$C_{11}H_{17}NO_3,HCl$ Mol. Wt. 247.7

Isoprenaline Hydrochloride is (*RS*)-1-(3,4-dihydroxyphenyl)-2-isopropylaminoethanol hydrochloride.

Isoprenaline Hydrochloride contains not less than 98.0 per cent and not more than 101.5 per cent of $C_{11}H_{17}NO_3,HCl$, calculated on the dried basis.

**Category**. Sympathomimetic.

**Dose**. By intravenous infusion, 0.5 to 2 µg per minute, usually administered after dilution with Dextrose Injection.

**Description**. A white or almost white, crystalline powder; almost odourless. Gradually darkens on exposure to air and light; even in the absence of light, it is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures. Aqueous solutions become pink to brownish on standing exposed to air and almost immediately after being made alkaline.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *isoprenaline hydrochloride RS* or with the reference spectrum of isoprenaline hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.005 per cent w/v solution shows an absorption maximum only at about 280 nm; absorbance at about 280 nm, about 0.5.

C. To 2 ml of a freshly prepared 1 per cent w/v solution add 0.1 ml of *ferric chloride test solution*; an emerald-green colour is produced which, on the gradual addition of *sodium bicarbonate solution*, changes first to blue and then to red.

D. Gives the reactions of chlorides (2.3.1).

## Tests

**Isoprenalone**. Absorbance of a 0.2 per cent w/v solution in *0.005 M sulphuric acid* at about 310 nm, not more than 0.15 (2.4.7).



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**Sulphates** (2.3.17). Dissolve 0.5 g in 100 ml of *water*; 15 ml of the resulting solution complies with the limit test for sulphates (0.2 per cent).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 4 hours.

**Assay**. Weigh accurately about 0.5 g, dissolve in 50 ml of *anhydrous glacial acetic acid* with the aid of the minimum of heat and titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02477 g of $C_{11}H_{17}NO_3$,HCl.

**Storage**. Store protected from light.

## Isoprenaline Injection

Isoprenaline Hydrochloride Injection; Isoproterenol Injection; Isoproterenol Hydrochloride Injection

Isoprenaline Injection is a sterile solution of Isoprenaline Hydrochloride in Water for Injections. It may contain suitable stabilising agents.

Isoprenaline Hydrochloride Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of isoprenaline hydrochloride, $C_{11}H_{17}NO_3$,HCl.

**Usual strength**. 200 µg per ml.

**Description**. A colourless or very pale yellow solution.

### Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 50 volumes of *ethyl acetate*, 30 volumes of *2-propanol*, 16 volumes of *water* and 4 volumes of *strong ammonia solution*.

*Test solution*. Use the injection, diluted if necessary with sufficient *methanol (80 per cent)* to produce a solution containing 0.02 per cent w/v of isoprenaline hydrochloride.

*Reference solution*. A solution containing 0.02 per cent w/v of *isoprenaline hydrochloride RS* in *methanol (80 per cent)*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air until the odour of the solvent is no longer detectable, place it for a few minutes in an atmosphere saturated with *diethylamine* and spray with *diazotised nitroaniline solution*. The chromatogram obtained with the test solution exhibits an elongated zone corresponding to that obtained with the reference solution.

B. To 2 ml add 0.1 ml of *ferric chloride test solution*; an emerald-green colour develops which, on gradual addition of *sodium bicarbonate solution*, changes to blue and then to red.

### Tests

**pH** (2.4.24). 2.5 to 3.0.

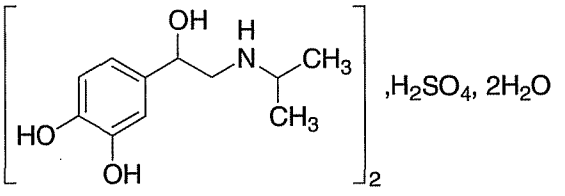
**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. To an accurately measured volume containing about 5 mg of Isoprenaline Hydrochloride add sufficient *water* to produce 50.0 ml. To 20.0 ml add 0.5 ml of *ferrous sulphate-citrate solution* and 2 ml of *glycine buffer solution*, mix and allow to stand for 20 minutes. Add sufficient *water* to produce 25.0 ml, mix and measure the absorbance of the resulting solution at the maximum at about 540 nm (2.4.7). Calculate the content of $C_{11}H_{17}NO_3$,HCl from the absorbance obtained by repeating the determination using 2.0 ml of a 0.1 per cent w/v solution of *isoprenaline hydrochloride RS* in place of the substance under examination.

**Storage**. Store protected from light at a temperature not exceeding 30°.

## Isoprenaline Sulphate

Isoproterenol Sulphate




$(C_{11}H_{17}NO_3)_2,H_2SO_4,2H_2O$ Mol. Wt. 556.6

Isoprenaline Sulphate is (*RS*)-1-(3,4-dihydroxyphenyl)-2-isopropylaminoethanol sulphate dihydrate.

Isoprenaline Sulphate contains not less than 98.0 per cent and not more than 102.0 per cent of $(C_{11}H_{17}NO_3)_2,H_2SO_4$, calculated on the anhydrous basis.

**Category**. Sympathomimetic.

**Dose**. 5 to 20 mg daily.

**Description**. A white or almost white, crystalline powder.

### Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.*



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A. Dissolve 0.5 g in 1.5 ml of *water*, add 3.5 ml of *2-propanol* scratch the walls of the container with a glass rod to induce crystallisation, collect the crystals and dry over *phosphorus pentoxide* at 60° at a pressure of 1.5 to 2.5 kPa. The crystals comply with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *isoprenaline sulphate RS* treated in the same manner.

B. To 1 ml of a 1 per cent w/v solution add 0.1 ml of *ferric chloride test solution*; an emerald-green colour is produced which, on gradual addition of *sodium bicarbonate solution*, changes first to blue and then to red.

C. To 5 ml of a freshly prepared 1 per cent w/v solution add 0.15 ml of *silver nitrate solution*; a greyish precipitate is produced on standing for 10 minutes and the solution becomes pink.

D. A 10 per cent w/v solution gives the reaction of sulphates (2.3.1).

### Tests

**Appearance of solution**. A freshly prepared 10.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and not more intensely coloured than reference solution YS6 (2.4.1).

**pH** (2.4.24). 4.0 to 5.5, determined in a freshly prepared 1.0 per cent w/v solution in *carbon dioxide-free water*.

**Isoprenalone**. Absorbance of a 0.2 per cent w/v solution in *0.005 M sulphuric acid* at about 310 nm, not more than 0.2 (2.4.7).

**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals, Method A (10 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). 5.0 to 7.5 per cent determined on 0.2 g.

**Assay**. Weigh accurately about 0.8 g, dissolve in 40 ml of *anhydrous glacial acetic acid*, warming gently if necessary and titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.05206 g of $(C_{11}H_{17}NO_3)_2,H_2SO_4$.

**Storage**. Store protected from light.

## Isoprenaline Tablets

Isoprenaline Sulphate Tablets; Isoproterenol Tablets; Isoproterenol Sulphate Tablets

Isoprenaline Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of isoprenaline sulphate, $(C_{11}H_{17}NO_3)_2,H_2SO_4,2H_2O$.

**Usual strength**. 10 mg.

### Identification

A. Extract a quantity of the powdered tablets containing about 50 mg of Isoprenaline Sulphate with 5 ml of *water* and filter. Reserve the filtrate for test C. To 1 ml of the filtrate add 0.1 ml of *ferric chloride test solution*; an emerald-green colour is produced which, on gradual addition of *sodium bicarbonate solution*, changes first to blue and then to red.

B. Extract a quantity of the powdered tablets containing about 50 mg of Isoprenaline Sulphate with 5 ml of *water* and filter. To the filtrate add 0.15 ml of *silver nitrate solution*; a greyish precipitate is produced on standing for 10 minutes and the solution becomes pink.

C. To 2 ml of the filtrate reserved from test A add 0.5 ml of *dilute hydrochloric acid* and 0.5 ml of *barium chloride solution*; a white precipitate is formed.

### Tests

**Uniformity of content**. Comply with the test stated under Tablets.

Crush one tablet and shake with 50 ml of *water* for 15 minutes. Add sufficient *water* to produce 100.0 ml, mix and filter. To 20.0 ml of the filtrate add 0.5 ml of *ferrous sulphate-citrate solution* and 2 ml of *glycine buffer solution* and allow to stand for 20 minutes. Dilute to 25.0 ml with *water* and mix. Measure the absorbance of the resulting solution at the maximum at about 540 nm (2.4.7). Calculate the content of $(C_{11}H_{17}NO_3)_2,H_2SO_4,2H_2O$ in the tablet from the absorbance obtained by repeating the determination using 2.0 ml of a 0.1 per cent w/v solution of *isoprenaline sulphate RS* in place of the substance under examination.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.1 g of Isoprenaline Sulphate and shake with 50 ml of *water* for 15 minutes. Add sufficient *water* to produce 100.0 ml, mix and filter. Dilute 20.0 ml of the filtrate to 200.0 ml with *water*. To 20.0 ml of the resulting solution add 0.5 ml of *ferrous sulphate-citrate solution* and 2 ml of *glycine buffer solution* and allow to stand for 20 minutes. Dilute to 25.0 ml with *water* and mix. Measure the absorbance of the resulting solution at the maximum at about 540 nm (2.4.7). Calculate the content of $(C_{11}H_{17}NO_3)_2,H_2SO_4,2H_2O$ from the absorbance obtained by repeating the determination using 2.0 ml of a 0.1 per cent w/v solution of *isoprenaline sulphate RS* in place of the substance under examination.

**Storage**. Store protected from light.



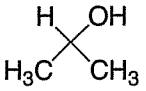
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# Isopropyl Alcohol

2-Propanol; Propan-2-ol



$C_3H_8O$ Mol. Wt. 60.1

Isopropyl Alcohol is propan-2-ol.

**Category**. Pharmaceutical aid (solvent).

**Description**. A clear, colourless liquid; odour, characteristic and spirituous; flammable.

## Identification

A. Mix 1 ml of a 10 per cent v/v solution with 2 ml of *mercuric sulphate solution* and heat just to boiling; a white or yellowish white precipitate is produced.

B. Gently heat 1 ml with 4 ml of *dilute potassium dichromate solution* and 1 ml of *sulphuric acid;* acetone, recognisable by its odour, is evolved.

## Tests

**Acidity or alkalinity**. Gently boil 25 ml for 5 minutes with 25 ml of *carbon dioxide-free water* and cool, taking precautions to exclude carbon dioxide. Not more than 0.06 ml of *0.1 M sodium hydroxide* is required to make the resulting solution alkaline to *phenolphthalein solution*.

**Distillation range** (2.4.8). Not less than 95.0 per cent v/v distils between 81° and 83°.

**Refractive index** (2.4.27). 1.377 to 1.378, determined at 20°.

**Weight per ml** (2.4.29). 0.782 g to 0.786 g, determined at 20°.

**Aldehydes and ketones**. Mix in a cylinder 25 ml with 25 ml of *water* and 50 ml of *hydroxylamine solution*, allow to stand for 5 minutes and titrate with *0.1 M sodium hydroxide* until the colour is the same as that of a mixture of 50 ml of *hydroxylamine solution* and 50 ml of *water* contained in a similar cylinder, each being viewed down the vertical axis of the cylinder. Not more than 2.0 ml of *0.1 M sodium hydroxide* is required.

**Benzene and related substances**. Determine by gas chromatography (2.4.13).

*Test solution*. The substance under examination.

*Reference solution (a)*. A 0.1 per cent v/v solution of *2-butanol reagent* in the substance under examination.

*Reference solution (b)*. A solution containing 0.1 per cent v/v of each of *2-butanol reagent* and *1-propanol* in the substance under examination.

*Reference solution* (c). A 0.0002 per cent v/v solution of *benzene* in the substance under examination.

Chromatographic system
- a glass column 1.8 m x 2 mm, packed with acid-washed diatomaceous support (80 to 100 mesh) coated with 15 per cent w/w of *polyethylene glycol 400*,
- temperature:
  column.50°,
  inlet port.150°,
- flow rate. 30 ml per minute of the carrier gas,
- flame ionisation detector at 200°.

Inject separately 2 µl of each of the test solution and reference solution (a). The chromatogram obtained with the test solution shows no peak with retention time similar to the peak due to 2-butanol (retention time relative to isopropyl alcohol, about 1.5) obtained with reference solution (a). Inject 2 µl of reference solution (b) and adjust the sensitivity of the system so that the heights of the peaks due to 2-butanol and 1-propanol in the chromatogram obtained with reference solution (b) are not less than 50 per cent of the full scale of the recorder. The test is not valid unless the resolution between the peaks due to 2-butanol and 1-propanol in the chromatogram obtained with reference solution (b) is at least 1.2.

Inject alternately 2 µl each of the test solution and reference solution (c). The area of any peak due to benzene in the chromatogram obtained with the test solution is not greater than the difference between the area of the peak due to benzene in the chromatogram obtained with reference solution (c) and that of the peak due to benzene in the chromatogram obtained with the test solution.

In the chromatogram obtained with reference solution (a) the sum of areas of any peaks other than the principal peak and the peaks due to 2-butanol is not greater than 3 times the area of the peak due to 2-butanol (0.3 per cent).

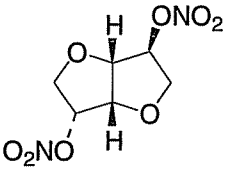
**Non-volatile matter**. Not more than 0.002 per cent w/v, determined by evaporating 100 ml on a water-bath and drying the residue at 105°.

**Water-insoluble matter**. Mix 1 volume with 19 volumes of *water;* no opalescence is produced.

**Water** (2.3.43). Not more than 0.5 per cent, determined on 5 g.

# Diluted Isosorbide Dinitrate

Diluted Sorbide Dinitrate; Diluted Sorbide Nitrate



$C_6H_8N_2O_8$ Mol. Wt. 236.1



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Diluted Isosorbide Dinitrate is a dry mixture of 1,4:3, 6-dianhydro-D-glucitol 2,5-dinitrate with Lactose, Mannitol or other suitable inert diluent. It may contain a suitable stabilising agent.

Diluted Isosorbide Dinitrate contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of isosorbide dinitrate, $C_6H_8N_2O_8$ and usually contains 20 per cent to 50 per cent of isosorbide dinitrate.

**Category**. Antianginal.

**Dose**. In angina the equivalent of isosorbide dinitrate, sublingually, 5 to 10 m; orally, 30 to 120 mg daily, in divided doses.

**Description**. A fine, white, crystalline powder; odourless or almost odourless.

*CAUTION — Undiluted isosorbide dinitrate is a powerful explosive and can be exploded with percussion or excessive heat. Proper precautions must be taken in handling it and only exceedingly small amounts should be isolated.*

## Identification

A. Shake a quantity containing 50 mg of isosorbide dinitrate with 15 ml of *acetone* for 2 minutes. Filter, evaporate the filtrate to dryness at a temperature not exceeding 35° and dry the residue over *phosphorus pentoxide* at a pressure of 0.7 kPa for 16 hours. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *diluted isosorbide dinitrate RS* treated in the same manner or with the reference spectrum of isosorbide dinitrate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase. Toluene.*

*Test solution.* Extract a quantity containing 2 mg of isosorbide dinitrate with 1 ml of *ether* and centrifuge.

*Reference solution.* Prepare in the same manner as the test solution but using *diluted isosorbide dinitrate RS* in place of the substance under examination.

Apply to the plate 20 µl of each solution. After development, dry the plate in a current of air, spray with a 1 per cent w/v solution of *diphenylamine* in *methanol* and expose for 15 minutes to ultraviolet light at 254 and 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

C. Extract a quantity containing 10 mg of isosorbide dinitrate with 10 ml of *ether* and filter. Evaporate the filtrate to dryness at a temperature not exceeding 35° and dissolve the residue in 0.15 ml of *sulphuric acid (50 per cent)* containing a trace of *diphenylamine*; an intense blue colour is produced.

## Tests

**Inorganic nitrates**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H*.

*Mobile phase*. A mixture of 60 volumes of *toluene*, 30 volumes of *acetone* and 15 volumes of *glacial acetic acid*.

*Test solution*. A solution of the substance under examination in *ethanol (95 per cent)* containing the equivalent of 2.0 per cent w/v of isosorbide dinitrate.

*Reference solution*. Prepare freshly a 0.01 per cent w/v solution of *potassium nitrate* in *ethanol (90 per cent)*.

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of air until the acetic acid is completely removed. Spray copiously with freshly prepared *potassium iodide* and *starch solution*. Expose the plate to ultraviolet light at 254 nm for 15 minutes. Examine in daylight. Any spot corresponding to potassium nitrate in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Related substances**. Determine by liquid chromatography (2.4.14), as described under Assay, changing the detector setting to 210 to 215 nm and using injection volume of 10 µl.

Under the stated conditions, the retention times are: isosorbide dinitrate, about 5 minutes; isosorbide 2-nitrate, about 8 minutes; isosorbide 5-nitrate, about 11 minutes.

Inject reference solution (c). Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (c) is not less than 20 per cent of the full scale of the recorder.

Inject reference solution (e). The test is not valid unless in the chromatogram obtained with reference solution (e), the resolution between the peaks corresponding to isosorbide dinitrate and isosorbide 2-nitrate is at least 6.0.

Inject test solution (a), reference solution (c) and reference solution (d). In the chromatogram obtained with test solution (a) the area of any peak corresponding to isosorbide 2-nitrate is not greater than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent); the area of any peak corresponding to isosorbide 5-nitrate is not greater than the area of the principal peak in the chromatogram obtained with reference solution (d) (0.5 per cent).

**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 16 hours.



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**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution (a)*. Mix an accurately weighed quantity of the substance under examination containing about 25 mg of isosorbide dinitrate with 20 ml of the mobile phase with the aid of ultrasound for 15 minutes and dilute to 25.0 ml with the mobile phase. Filter the solution through a suitable membrane filter.

*Test solution (b)*. Dilute 1.0 ml of test solution (a) to 10.0 ml with the mobile phase.

*Reference solution (a)*. Mix a quantity of *isosorbide dinitrate RS* containing 25.0 mg of isosorbide dinitrate with 20 ml of the mobile phase with the aid of ultrasound for 15 minutes and dilute to 25.0 ml with the mobile phase. Filter the solution through a suitable membrane filter.

*Reference solution (b)*. Dilute 1.0 ml of reference solution (a) to 10.0 ml with the mobile phase.

*Reference solution (c)*. Dissolve 10.0 mg of *isosorbide 2-nitrate RS* in the mobile phase and dilute to 10.0 ml with the mobile phase. Dilute 0.1 ml of this solution to 20.0 ml with the mobile phase.

*Reference solution (d)*. Dissolve 20.0 mg of *isosorbide mononitrate RS* in the mobile phase and dilute to 10.0 ml with the mobile phase. Dilute 0.1 ml of this solution to 20.0 ml with the mobile phase.

*Reference solution (e)*. Dissolve 5 mg of *isosorbide 2-nitrate RS* in the mobile phase and dilute to 10 ml with the mobile phase. To 1 ml of this solution add 0.5 ml of reference solution (a) and dilute to 10 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with aminopropylmethylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 15 volumes of *ethanol* and 85 volumes of *trimethylpentane*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 20 µl.

Inject reference solution (b). Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained is not less than 50 per cent of the full scale of the recorder. If the areas of the peaks from two successive injections do not agree to within 1.0 per cent, then inject a further four times and calculate, for the six injections, the relative standard deviation. The assay is not valid unless the relative standard deviation for the six injections is at most 2.0 per cent.

Inject alternately test solution (b) and reference solution (b).

Calculate the content of isosorbide dinitrate as a percentage of the declared content.

**Storage**. Store protected from light, at a temperature not exceeding 15°.

**Labelling**. The label states the percentage content of isosorbide dinitrate.

# Isosorbide Dinitrate Tablets

Sorbide Dinitrate Tablets; Sorbide Nitrate Tablets

Isosorbide Dinitrate Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of isosorbide dinitrate, $C_6H_8N_2O_8$.

**Usual strengths**. The equivalent of 5 mg and 10 mg of isosorbide dinitrate.

## Identification

*CAUTION — Undiluted isosorbide dinitrate is a powerful explosive and can be exploded with percussion or excessive heat. Proper precautions must be taken in handling it and only exceedingly small amounts should be isolated.*

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase. Toluene.*

*Test solution*. Extract a quantity of the powdered tablets containing 2 mg of isosorbide dinitrate with 1 ml of *ether* and centrifuge.

*Reference solution*. Dissolve 2 mg of *isosorbide dinitrate RS* in 1 ml of *ether*.

Apply to the plate 20 µl of each solution. After development, dry the plate in a current of air, spray with a 1 per cent w/v solution of *diphenylamine* in *methanol* and expose for 15 minutes to ultraviolet light at 254 nm and 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution

B. Extract a quantity of the powdered tablets containing10mgoisosorbide dinitrate with 10 ml of *ether* and filter. Evaporate the filtrate to dryness at a temperature not exceeding 35° and dissolve the residue in 0.15 ml of *sulphuric acid (50 per cent)* containing a trace of *diphenyl-amine*; an intense blue colour is produced.

## Tests

**Inorganic nitrates**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H*.

*Mobile phase*. A mixture of 60 volumes of *toluene*, 30 volumes of *acetone* and 15 volumes of *glacial acetic acid*.



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*Test solution*. Shake a quantity of the powdered tablets containing 0.1 g of isosorbide nitrate with 5 ml of *ethanol (95 per cent)* and filter.

*Reference solution*. Prepare freshly a 0.01 per cent w/v solution of *potassium nitrate* in *ethanol (90 per cent)*.

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of air until the acetic acid is completely removed. Spray copiously with freshly prepared *potassium iodide* and *starch solution*. Expose the plate to ultraviolet light at 254 nm for 15 minutes. Examine in daylight. Any spot corresponding to potassium nitrate in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Add 20 ml of the mobile phase to a quantity of the powdered tablets containing 25 mg of isosorbide dinitrate, mix with the aid of ultrasound for 15 minutes and dilute to 25 ml with the same solvent. Filter through a glass-fibre filter (such as Whatman GF/C) and use the filtrate.

*Reference solution (a)*. A solution containing 0.0005 per cent w/v of *isosorbide 2-nitrate RS* in the mobile phase.

*Reference solution (b)*. A solution containing 0.0005 per cent w/v of *isosorbide mononitrate RS* in the mobile phase.

*Reference solution (c)*. A solution containing 0.005 per cent w/v each of *isosorbide dinitrate RS* and *isosorbide 2-nitrate RS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm packed with aminopropylmethylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 15 volumes of *ethanol* and 85 volumes of *2,2,4-trimethylpentane*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume. 20 µl.

Inject reference solution (c). The test is not valid unless, in the chromatogram obtained, the resolution between the peaks corresponding to isosorbide dinitrate and isosorbide 2-nitrate is at least 6.0.

In the chromatogram obtained with the test solution the area of any peak corresponding to isosorbide 2-nitrate is not greater than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent) and the area of any peak corresponding to isosorbide 5-nitrate is not greater than the area of the principal peak obtained with reference solution (b) (0.5 per cent).

**Dissolution** (2.5.2). (for tablets intended to be swallowed whole).

Apparatus No. 1,
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 50 rpm and 30 minutes.

Withdraw 10 ml of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first 1 ml of the filtrate.

Determine by liquid chromatography (2.4.14).

*Test solution*. The filtrate from the dissolution medium.

*Reference solution*. A solution of *isosorbide dinitrate RS* in the dissolution medium containing the same concentration of isosorbide dinitrate as that expected in the dissolution medium in the vessel.

Chromatographic system
- a stainless steel column 20 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil ODS),
- mobile phase: a mixture of equal volumes of *methanol* and *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 222 nm.
- injection volume. 100 µl.

Calculate the content of $C_6H_8N_2O_8$.

D. Not less than 80 per cent of the stated amount of $C_6H_8N_2O_8$.

**Uniformity of content**. Comply with the test stated under Tablets.

Crush one tablet, add 5 ml of *glacial acetic acid*, shake for 1 hour and centrifuge. To a suitable volume of the supernatant liquid containing 1.0 mg of isosorbide dinitrate add sufficient *glacial acetic acid*, if necessary, to produce 1.0 ml, add 2 ml of *phenoldisulphonic acid solution*, allow to stand for 15 minutes, add 25 ml of *water*, make alkaline with *strong ammonia solution*, cool and add sufficient *water* to produce 50.0 ml. Measure the absorbance of the resulting solution at about 405 nm (2.4.7), using as the blank 1.0 ml of *glacial acetic acid* treated in a similar manner beginning at the words "add 2 ml of *phenoldisulphonic acid solution*,....". Dissolve 0.2 g of *potassium nitrate*, previously dried at 105°, in 5 ml of *water* and add sufficient *glacial acetic acid* to produce 25.0 ml. To 5.0 ml add sufficient *glacial acetic acid* to produce 50.0 ml. Using 1.0 ml of this solution repeat the procedure beginning at the words "add 2 ml of *phenoldisulphonic acid solution*,.....". Calculate the content of $C_6H_8N_2O_8$ from the values of the absorbances so obtained.

1 ml of the potassium nitrate solution is equivalent to 0.000934 g of $C_6H_8N_2O_8$.

Calculate the content of $C_6H_8N_2O_8$ in the tablet.

**Other tests**. Comply with the tests stated under Tablets.



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**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh and powder 20 tablets. Add 20 ml of the mobile phase to an accurately weighed quantity of the powdered tablets containing about 25 mg of isosorbide dinitrate, mix with the aid of ultrasound for 15 minutes and dilute to 25.0 ml with the same solvent. Filter through a glass-fibre filter (such as Whatman GF/C) and dilute 1 volume to 10 volumes with the mobile phase.

*Reference solution (a)*. Add 20 ml of the mobile phase to a quantity of *isosorbide dinitrate RS* containing 25 mg of isosorbide dinitrate, mix with the aid of ultrasound for 15 minutes and dilute to 25 ml with the mobile phase. Filter through a glass-fibre filter (such as Whatman GF/C) and dilute 1 volume to 10 volumes with the mobile phase.

*Reference solution (b)*. A solution containing 0.005 per cent w/v each of *isosorbide dinitrate RS* and *isosorbide 2-nitrate RS* in the mobile phase.

Use chromatographic system as described under Related substances, using a detection wavelength of 230 nm.

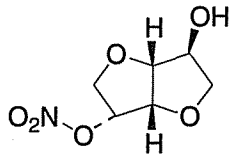
The test is not valid unless, in the chromatogram obtained with reference solution (b), the resolution between the peaks corresponding to isosorbide dinitrate and isosorbide 2-nitrate is at least 6.0.

Calculate the content of $C_6H_8N_2O_8$ in the tablets.

**Storage** Store at a temperature not exceeding 30°.

**Labelling**. The label states whether the tablets are to be swallowed whole, chewed before swallowing or allowed to dissolve in the mouth.

# Diluted Isosorbide Mononitrate




$C_6H_9NO_6$ Mol. Wt. 191.1

Diluted Isosorbide Mononitrate is a dry mixture of isosorbide mononitrate and lactose monohydrate or mannitol.

Diluted Isosorbide Mononitrate is 1,4:3,6-dianhydro-D-glucitol 5-nitrate.

Diluted Isosorbide Mononitrate contains not less than 95.0 per cent and not more than 105.0 per cent of $C_6H_9NO_6$.

**Description**. Undiluted isosorbide mononitrate is a white, crystalline powder.

## Identification

A. Shake a quantity containing 25 mg of isosorbide mononitrate with 15 ml of *acetone* for 2 minutes. Filter, evaporate the filtrate to dryness at a temperature not exceeding 35° and dry the residue over *phosphorus pentoxide* at a pressure of 0.7 kPa for 16 hours. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *isosorbide mononitrate RS* treated in the same manner.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 5 volumes of *methanol* and 95 volumes of *dichloromethane*.

*Test solution*. Shake a quantity of the substance under examination containing 10 mg of isosorbide mononitrate with 10 ml of *ethanol (95 per cent)* and filter.

*Reference solution*. A 0.1 per cent w/v solution of *isosorbide mononitrate RS* in *ethanol (95 per cent)*.

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of air, spray with a freshly prepared *potassium iodide* and *starch solution* and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with the reference solution.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 10 volumes of *water*, 15 volumes of *methanol*, 25 volumes of *anhydrous acetic acid* and 50 volumes of *dichloroethane*.

*Test solution*. Shake a quantity of the substance under examination containing 0.1 g of *lactose* or *mannitol* with 10 ml of *water*.

*Reference solution (a)*. Dissolve 0.1 g of *lactose* in 10 ml of *water*.

*Reference solution (b)*. Dissolve 0.1 g of *mannitol* in 10 ml of *water*.

*Reference solution (c)*. Mix equal volumes of reference solutions (a) and (b).

Apply to the plate 1 µl of each solution. After development, dry the plate in a current of warm air. Repeat immediately the development after renewing the mobile phase. Dry the plate in a current of warm air. Spray with *4-aminobenzoic acid solution*. Dry the plate in a cold air until the *acetone* is removed. Heat



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the plate at 100° for 15 minutes. Allow to cool and spray with a 0.2 per cent w/v solution of *sodium periodate*. Dry the plate in a current of cold air. Heat the plate at 100° for 15 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with reference solution (a) for lactose or to the principal spot in the chromatogram obtained with reference solution (b) for mannitol. The identification is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

### Tests

**Inorganic nitrates**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H*.

*Mobile phase*. A mixture of 15 volumes of *glacial acetic acid*, 30 volumes of *acetone* and 60 volumes of *toluene*.

*Test solution*. Shake a quantity of the substance under examination containing 0.1 g of isosorbide mononitrate with 5 ml of *ethanol (95 per cent)* and filter.

*Reference solution*. Dissolve 10 mg of *potassium nitrate* in 1 ml of *water* and dilute to 100 ml with *ethanol (95 per cent)*.

Apply separately to the plate 10 µl of each solution. After development, dry the plate in a current of air until the acetic acid is completely removed. Spray with a freshly prepared *potassium iodide* and *starch solution* and examine in ultraviolet light at 254 nm. Any spot corresponding to nitrate ion in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent, calculated as potassium nitrate).

**Isosorbide dinitrate and isosorbide 2-nitrate**. Determine by liquid chromatography (2.4.14) as described under Assay, changing the detection to 210 nm to 215 nm and using injection volume of 10 µl.

Under the stated conditions, the retention times are: isosorbide dinitrate about 5 minutes, isosorbide-2-nitrate about 8 minutes and isosorbide 5-nitrate about 11 minutes.

Inject reference solution (b). Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (b) is at least 20 per cent of the full scale of the recorder.

Inject reference solution (d). The test is not valid unless in the chromatogram obtained, the resolution between the peaks corresponding to isosorbide 2-nitrate and isosorbide 5-nitrate is at least 4.0.

Inject test solution (a), reference solution (b) and reference solution (c). In the chromatogram obtained with test solution (a), the area of any peak corresponding to isosorbide 2-nitrate is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent), the area of any peak corresponding to isosorbide dinitrate is not greater than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent).

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution (a)*. Weigh accurately a quantity of the substance under examination containing 25 mg of isosorbide mononitrate, dissolve in 25.0 ml of the mobile phase and filter.

*Test solution (b)*. Dilute 1.0 ml of test solution (a) to 10.0 ml with the mobile phase.

*Reference solution (a)*. A 0.01 per cent w/v solution of *isosorbide mononitrate RS* in the mobile phase.

*Reference solution (b)*. A 0.0005 per cent w/v solution of *isosorbide-2-nitrate RS* in the mobile phase.

*Reference solution (c)*. A 0.0005 per cent w/v solution of *isosorbide dinitrate RS* in the mobile phase.

*Reference solution (d)*. A solution containing 0.005 per cent w/v each of *isosorbide mononitrate RS* and *isosorbide-2-nitrate RS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with aminopropylmethylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 15 volumes of *ethanol* and 85 volumes of *trimethylpentane*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 20 µl.

Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject test solution (b) and reference solution (a).

Calculate the content of $C_6H_9NO_6$.

**Storage**. Store protected from light.

**Labelling**. The label states the percentage content of isosorbide mononitrate.

## Isosorbide Mononitrate Tablets

Sorbide Mononitrate Tablets

Isosorbide Mononitrate Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of $C_6H_9NO_6$.

### Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.



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*Mobile phase*. A mixture of 5 volumes of *methanol* and 95 volumes of *dichloromethane*.

*Test solution*. Extract a quantity of the powdered tablets containing 10 mg of isosorbide mononitrate with 10 ml of *ethanol (95 per cent)* and centrifuge.

*Reference solution*. Dissolve 10 mg of *isosorbide mononitrate RS* in 10 ml of *ethanol (95 per cent)*.

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of air, spray with a 1 per cent w/v solution of *diphenylamine* in *methanol* and expose for 15 minutes to ultraviolet at 254 nm and 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution (a).

## Tests

**Inorganic Nitrates**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H*.

*Mobile phase*. A mixture of 60 volumes of *toluene*, 30 volumes of *acetone* and 15 volumes of *glacial acetic acid*.

*Test solution*. Shake a quantity of the powdered tablets containing 0.1 g of isosorbide mononitrate with 5 ml of *ethanol (95 per cent)* and filter.

*Reference solution*. Prepare freshly a 0.01 per cent w/v solution of *potassium nitrate* in *ethanol (95 per cent)*.

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of air until the acetic acid is completely removed. Spray copiously with freshly prepared *potassium iodide and starch solution*. Expose the plate to ultraviolet light at 254 nm for 15 minutes and examine in daylight. Any spot corresponding to *potassium nitrate* in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm and 30 minutes.

Withdraw 10 ml of the medium and filter promptly, rejecting the first few ml of the filtrate.

Determine by liquid chromatography (2.4.14).

*Test solution*. The filtrate obtained from the dissolution medium.

*Reference solution*. A solution of *isosorbide mononitrate RS* in the dissolution medium containing the same concentration of isosorbide mononitrate as that expected in the dissolution medium in the vessel.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with endcapped octyldecylsilane bonded to porous silica (5 µm) (such as Spherisorb ODS 2),
- mobile phase: a mixture of equal volumes of *methanol* and *water*,
- flow rate 1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume. 100 µl.

Calculate the content of $C_6H_9NO_6$.

D. Not less than 80 per cent of the stated amount of $C_6H_9NO_6$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Shake a quantity of the powdered tablets containing 50 mg of isosorbide mononitrate with 15 ml of the mobile phase with the aid of ultrasound for 15 minutes and filter through a membrane filter with a nominal pore size not exceeding 0.45 µm.

*Reference solution (a)*. A solution containing 0.001 per cent w/v of *isosorbide 2-nitrate RS* in the mobile phase.

*Reference solution (b)*. A solution containing 0.001 per cent w/v of *isosorbide dinitrate RS* in the mobile phase.

*Reference solution (c)*. A solution containing 0.001 per cent each of *isosorbide 2-nitrate RS* and *isosorbide mononitrate RS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with endcapped octyldecylsilane bonded to porous silica (5 µm) (such as Spherisorb ODS 2),
- mobile phase: 30 volumes of *methanol* and 70 volumes of *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 20 µl.

Inject reference solution (c). The test is not valid unless, in the chromatogram obtained, the resolution between isosorbide mononitrate and isosorbide 2-nitrate is at least 2.4.

In the chromatogram obtained with the test solution the area of any peak corresponding to isosorbide 2-nitrate is not greater than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent) and the area of any peak corresponding to isosorbide dinitrate is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent).

**Uniformity of content**. Comply with the test stated under Tablets.



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Crush one tablet, add 5 ml of *glacial acetic acid*, shake for 1 hour and centrifuge. To a suitable volume of the supernatant liquid containing 1.0 mg of isosorbide mononitrate add sufficient *glacial acetic acid*, if necessary, to produce 1.0 ml, add 2 ml of *phenoldisulphonic acid solution*, allow to stand for 15 minutes, add 25 ml of *water*, make alkaline with *strong ammonia solution*, cool and add sufficient *water* to produce 50.0 ml. Measure the absorbance of the resulting solution at about 405 nm (2.4.7), using as the blank 2.0 ml of *glacial acetic acid* treated in a similar manner beginning at the words 'add 2 ml of *phenoldisulphonic acid solution*....'. Dissolve 0.2 g of *potassium nitrate*, previously dried at 105°, in 5 ml of *water* and add sufficient *glacial acetic acid* to produce 50.0 ml. To 5 ml, add sufficient *glacial acetic acid* to produce 50 ml. Using 1.0 ml of this solution repeat the procedure beginning at the words 'add 2 ml of *phenoldisulphonic acid solution*....'. Calculate the content of $C_6H_9NO_6$ from the values of the absorbances.

1 ml of the potassium nitrate solution is equivalent to 0.0007568 g of $C_6H_9NO_6$.

Calculate the content of $C_6H_9NO_6$ in the tablet.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 50 mg of isosorbide mononitrate, add 70 ml of the mobile phase, mix with the aid of ultrasound for 15 minutes and dilute to 100.0 ml with the mobile phase. Centrifuge. Mix 1 volume of the supernatant liquid with 5 volumes of the mobile phase and filter through a membrane filter with a nominal pore size not greater than 0.45 µm.

*Reference solution (a)*. Add 20 ml of the mobile phase to an accurately weighed quantity of *isosorbide mononitrate RS* containing 25 mg of isosorbide mononitrate, mix with the aid of ultrasound for 15 minutes, dilute to 25.0 ml with the mobile phase, mix and centrifuge. Dilute 1 volume of the clear supernatant liquid to 10 volumes with the mobile phase and filter through a membrane filter with a nominal pore size not greater than 0.45 µm.

*Reference solution (b)*. A solution containing 0.001 per cent w/v each of *isosorbide mononitrate RS* and *isosorbide 2-nitrate RS* in the mobile phase.

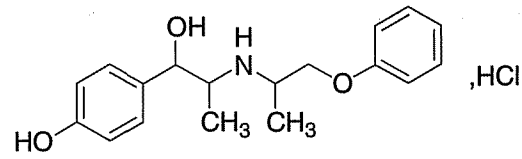
Use the chromatographic system described in the test for Related substances.

The test is not valid unless in the chromatogram obtained with reference solution (b), the resolution between the peaks corresponding to isosorbide mononitrate and isosorbide 2-nitrate is at least 2.4.

Calculate the content of $C_6H_9NO_6$ in the tablets.

**Storage**. Store at a temperature not exceeding 30°.

# Isoxsuprine Hydrochloride




$C_{18}H_{23}NO_3,HCl$ Mol. Wt. 337.9

Isoxsuprine Hydrochloride is (1*RS*,2*SR*)-1-(4-hydroxyphenyl)-2-[(1*RS*)-1-methyl-2-phenoxyethylamino]propan-1-ol hydrochloride.

Isoxsuprine Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{18}H_{23}NO_3,HCl$, calculated on the dried basis.

**Category**. Vasodilator; uterine relaxant.

**Dose**. As vasodilator, orally, 20 mg 4 times daily; by intramuscular injection, 5 to 10 mg upto 4 times daily; by intravenous infusion, initially 100 µg per minute, increased to 500 µg per minute if required. As uterine relaxant, by intravenous infusion, initially 200 µg to 300 µg per minute gradually increased to 500 µg per minute until labour is arrested; subsequently by intramuscular injection, 10 mg every 3 hours for 24 hours; then every 4 to 6 hours for 48 hours; then orally, 20 mg 4 times daily.

**Description**. A white or almost white, crystalline powder; odourless or almost odourless.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *isoxsuprine hydrochloride RS* or with the reference spectrum of isoxsuprine hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.01 per cent w/v solution in *0.1 M hydrochloric acid* shows absorption maxima at about 269 nm and 274 nm; absorbance at about 269 nm, about 0.73 and at about 274 nm, about 0.72.

C. Dissolve 10 mg in 1 ml of *water* and add 0.05 ml of *copper sulphate solution* and 1 ml of *5 M sodium hydroxide*; a blue colour is produced. Add 1 ml of *ether* and shake; the ether layer remains colourless.

D. Gives the reactions of chlorides (2.3.1).

## Tests

**pH** (2.4.24). 4.5 to 6.0, determined in a 1.0 per cent w/v solution, prepared with gentle warming if necessary.

**Appearence of solution**. A 1.0 per cent w/v solution is clear (2.4.1) and colourless (2.4.1).



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**Specific optical rotation** (2.4.22). – 0.05° to + 0.05°, determined in a 1.0 per cent w/v solution.

**Related substances.** Determine by gas chromatography (2.4.13).

*NOTE— Prepare the solutions immediately before use.*

*Internal standard solution (a).* Dissolve 0.1 g of *hexacosane* in 20.0 ml of *trimethylpentane.*

*Internal standard solution (b).* Dilute 1.0 ml of internal standard solution (a) to 50.0 ml with *trimethylpentane.*

*Test solution.* Add 0.5 ml of *N-trimethylsilylimidazole* to 10 mg of the substance under examination. Heat to 65° for 10 minutes, cool. Add 2.0 ml of internal standard solution (b) and 2.0 ml of *water*, shake. Use the upper layer.

*Reference solution (a).* Add 0.5 ml of *N-trimethylsilylimidazole* to 10 mg of the substance under examination. Heat to 65° for 10 minutes, cool. Add 2.0 ml of internal standard solution (a) and 2.0 ml of *water*, shake. Dilute 1.0 ml of the upper layer to 50.0 ml with *trimethylpentane.*

*Reference solution (b).* Add 0.5 ml of *N-trimethylsilylimidazole* to 10 mg of the substance under examination. Heat to 65° for 10.0 min, cool. Add 2.0 ml of *trimethylpentane* and 2.0 ml of *water*, shake. Use the upper layer.

Chromatographic system

- a glass column 1.5 m x 4 mm, packed with diatomaceous support (125 to 135 µm) coated with 3 per cent w/w of poly(dimethyl)siloxane,
- temperature:

| Column | time (min) | temperature (°) |
|---|---|---|
| | 0-25 | 195 |
| | 25-29 | 195-215 |
| | 29-39 | 215 |

- inlet and detector port 225°,
- flame ionization detector,
- flow rate. 30 ml per minute, nitrogen as the carrier gas.

Inject 1 µl of the test solution and reference solution (a). The test is not valid unless the resolution between the peaks due to isoxsurpine and hexacosane is not less than 5.0.

Inject the test solution and reference solution (a). In the chromatogram obtained with the test solution, calculate the ratio of the area of the peak due to the trimethylsilyl derivative of isoxsuprine to the area of the peak due to hexacosane from the chromatogram obtained with reference solution (a). The ratio of the sum of the areas of peaks, other than the principal peak and the peak due to hexacosane to the area of the peak due to the hexacosane is not more than 2.0 per cent.

**Phenones.** Absorbance of a 0.01 per cent w/v solution at about 310 nm, not more than 0.20 (2.4.7).

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay.** Weigh accurately about 0.6 g, dissolve in 150 ml of *anhydrous glacial acetic acid*, heating on a water-bath to effect dissolution. Cool and add 15 ml of *mercuric acetate solution.* Titrate with *0.1 M perchloric acid*, using *naphtholbenzein* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03378 g of $C_{18}H_{23}NO_3,HCl$.

**Storage.** Store protected from light.

# Isoxsuprine Injection

Isoxsuprine Hydrochloride Injection

Isoxsuprine Injection is a sterile solution of Isoxsuprine Hydrochloride in Water for Injections.

Isoxsuprine Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of isoxsuprine hydrochloride, $C_{18}H_{23}NO_3,HCl$.

**Usual strength.** 5 mg per ml.

## Identification

A. To a volume containing 50 mg of Isoxsuprine Hydrochloride add 20 ml of *water* and 10 ml of *ammonia buffer pH 10.0* and extract with three quantities, each of 15 ml, of *dichloromethane.* Shake the combined extracts with 5 g of *anhydrous sodium sulphate*, filter, evaporate the filtrate to dryness, dissolve the residue in 5 ml of *0.1 M methanolic hydrochloric acid* and evaporate to dryness. Dissolve the residue in 5 ml of *methanol,* evaporate to dryness, redissolve the residue in 2 ml of *methanol*, add 15 ml of *dichloromethane*, again evaporate to dryness and dry the residue at 60° at a pressure of 2 kPa for 1 hour. On the residue determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *isoxsuprine hydrochloride RS* treated in the same manner or with the reference spectrum of isoxsuprine hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), the final solution obtained in the Assay shows absorption maxima at about 269 nm and 274 nm.

## Tests

**pH** (2.4.24). 4.9 to 6.0.



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**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. To an accurately measured volume containing about 50 mg of Isoxsuprine Hydrochloride add sufficient *0.1 M hydrochloric acid* to produce 100.0 ml. Dilute 10.0 ml to 100.0 ml with the same solvent and measure the absorbance of the resulting solution at the maximum at about 274 nm (2.4.7). Calculate the content of $C_{18}H_{23}NO_3$,HCl taking 73 as the specific absorbance at 274 nm.

# Isoxsuprine Tablets

Isoxsuprine Hydrochloride Tablets

Isoxsuprine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of isoxsuprine hydrochloride, $C_{18}H_{23}NO_3$,HCl.

**Usual strength**. 20 mg.

## Identification

A. To a quantity of the powdered tablets containing 50 mg of Isoxsuprine Hydrochloride add 50 ml of *0.1 M hydrochloric acid* and heat on a water-bath for 30 minutes. Cool, filter, add 10 ml of *ammonia buffer pH 10.0* and extract with three quantities, each of 15 ml, of *dichloromethane*. Shake the combined extracts with 5 g of *anhydrous sodium sulphate*, filter, evaporate the filtrate to dryness, dissolve the residue in 5 ml of *0.1 M methanolic hydrochloric acid* and evaporate to dryness. Dissolve the residue in 5 ml of *methanol,* evaporate to dryness, redissolve the residue in 2 ml of *methanol*, add 15 ml of *dichloromethane*, again evaporate to dryness and dry the residue at 60° at a pressure of 2 kPa for 1 hour.

On the residue determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *isoxsuprine hydrochloride RS* treated in the same manner or with the reference spectrum of isoxsuprine hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), the final solution obtained in the Assay shows absorption maxima at about 269 nm and 274 nm.

## Tests

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 20 mg of Isoxsuprine Hydrochloride, add 50 ml of *0.1 M hydrochloric acid* and boil on a water-bath for 30 minutes. Cool, add sufficient *0.1 M hydrochloric acid* to produce 100 0 ml, mix and filter. Dilute 25.0 ml of the filtrate to 100.0 ml with *0.1 M hydrochloric acid* and measure the absorbance of the resulting solution at the maximum at about 274 nm (2.4.7). Calculate the content of $C_{18}H_{23}NO_3$,HCl taking 73 as the specific absorbance at 274 nm.



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| | | |
|---|---|---|
| Kanamycin Sulphate | .... | 1533 |
| Kanamycin Acid Sulphate | .... | 1534 |
| Kanamycin Injection | .... | 1535 |
| Heavy Kaolin | .... | 1537 |
| Light Kaolin | .... | 1537 |
| Ketamine Hydrochloride | .... | 1538 |
| Ketamine Injection | .... | 1539 |
| Ketoconazole | .... | 1540 |
| Ketoconazole Tablets | .... | 1541 |
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| Ketorolac Tromethamine Injection | .... | 1544 |
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| Ketotifen Fumarate | .... | 1546 |




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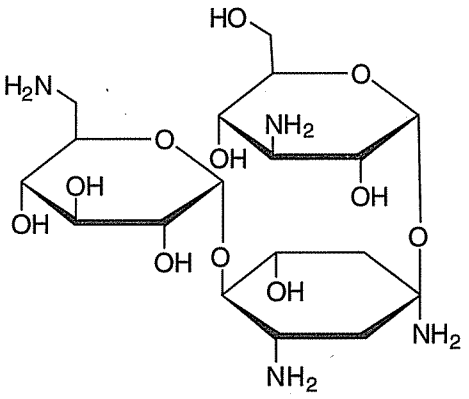
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# Kanamycin Sulphate




, $H_2SO_4$, $H_2O$

$C_{18}H_{36}N_4O_{11},H_2SO_4,H_2O$ Mol. Wt. 600.6

Kanamycin Sulphate is 6-*O*-(3-amino-3-deoxy-α-D-glucopyranosyl)-4-*O*-(6-amino-6-deoxy-α-D-glucopyranosyl)-2-deoxy-D-streptamine sulphate monohydrate, an antimicrobial substance produced by the growth of certain strains of *Streptomyces kanamyceticus*.

Kanamycin Sulphate has a potency of not less than 750 Units per mg, calculated on the dried basis.

**Category**. Antibacterial.

**Dose**. By intramuscular injection, the equivalent of 500 mg to 1 g (500,000 to 1,000,000 Units) of kanamycin daily, in divided doses. By slow intravenous infusion, 15 to 30 mg per kg body weight daily, in divided doses, every 8 to 12 hours.

**Description**. A white or almost white, crystalline powder; odourless or almost odourless.

## Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with a 0.75-mm layer of the following mixture. Mix 0.3 g of *carbomer* (Such as Carbopol 934) with 240 ml of *water* and allow to stand, with moderate shaking, for 1 hour; adjust to pH 7.0 by the gradual addition, with continuous shaking, of *2 M sodium hydroxide* and add 30 g of *silica gel H*. Heat the plate at 110° for 1 hour, allow to cool and use immediately.

*Mobile phase*. A 7 per cent w/v solution of *potassium dihydrogen phosphate*.

*Test solution*. A 0.1 per cent w/v solution of the substance under examination in *water*.

*Reference solution (a)*. A 0.1 per cent w/v solution of *kanamycin sulphate RS* in *water*.

*Reference solution (b)*. A solution containing 0.1 per cent w/v each of *kanamycin sulphate RS*, *neomycin sulphate RS* and *streptomycin sulphate RS* in *water*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, spray with a mixture of equal volumes of a 0.2 per cent w/v solution of *1,3-napthalenediol* in *ethanol (95 per cent)* and a 45 per cent w/v solution of *sulphuric acid* and heat at 150° for 5 to 10 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows three clearly separated spots.

B. Dissolve 0.5 g in 10 ml of *water* and add 10 ml of a 1 per cent w/v solution of *picric acid*. If necessary initiate crystallisation by scratching the walls of the container with a glass rod, allow to stand and filter. The crystals, after washing with 20 ml of *water* and drying at 105°, melt at about 235°, with decomposition (2.4.21).

C. Dissolve 50 mg in 2 ml of *water*, add 1 ml of a 1 per cent w/v solution of *ninhydrin* and heat for a few minutes on a water-bath; a violet colour is produced.

D. Gives the reactions of sulphates (2.3.1).

## Tests

**pH** (2.4.24). 6.5 to 8.5, determined in a 1.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). +112° to +123°, determined at 20° in a 1.0 per cent w/v solution.

**Kanamycin B**. Determine by thin-layer chromatography (2.4.17), coating the plate with a 0.75-mm layer of the following mixture. Mix 0.3 g of *carbomer* (Such as Carbopol 934) with 240 ml of *water* and allow to stand, with moderate shaking, for 1 hour; adjust to pH 7.0 by the gradual addition, with continuous shaking, of *2 M sodium hydroxide* and add 30 g of *silica gel H*. Heat the plate at 110° for 1 hour, allow to cool and use immediately.

*Mobile phase*. A 7 per cent w/v solution of *potassium dihydrogen phosphate*.

*Test solution*. A 0.5 per cent w/v solution of the substance under examination in *water*.

*Reference solution*. A 0.02 per cent w/v solution of *kanamycin B sulphate RS* in *water*.

Apply to the plate 4 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, spray with *ninhydrin and stannous chloride reagent* and heat at 110° for 15 minutes. Any spot corresponding to kanamycin B in the chromatogram obtained with the test



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solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Sulphates**. 15.0 to 17.0 per cent of $SO_4$, calculated on the dried basis and determined by the following method. Dissolve 0.25 g in 100 ml of *water* and adjust the pH to 11 using *strong ammonia solution*. Add 10.0 ml of *0.1 M barium chloride* and 0.5 mg of *metalphthalein*. Titrate with *0.1 M disodium edetate*; when the colour of the solution begins to change, add 50 ml of *ethanol (95 per cent)* and continue the titration until the blue colour disappears.

1 ml of *0.1 M barium chloride* is equivalent to 0.009606 g of $SO_4$.

**Sulphated ash** (2.3.18). Not more than 0.5 per cent.

**Loss on drying** (2.4.19). Not more than 1.5 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa for 3 hours.

**Assay**. Determine by the microbiological assay of antibiotics, Method A or B (2.2.10).

*Kanamycin Sulphate intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.*

**Bacterial endotoxins** (2.2.3). Not more than 0.67 Endotoxin Unit per mg of kanamycin.

*Kanamycin Sulphate intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.*

**Sterility** (2.2.11). Complies with the test for sterility.

**Storage**. Store protected from light and moisture. If the contents are intended for use in the manufacture of parenteral preparations, the container should be sterile and sealed so as to exclude micro-organisms.

**Labelling**. The label states (1) the number of Units per mg; (2) whether or not the material is intended for use in the manufacture of parenteral preparations.

# Kanamycin Acid Sulphate

Kanamycin Acid Sulphate is a form of kanamycin sulphate prepared by adding Sulphuric Acid to a solution of Kanamycin Sulphate and subsequent drying.

Kanamycin Acid Sulphate has a potency of not less than 650 Units per mg, calculated on the dried basis.

**Usual strength**. 50 mg per ml.

**Description**. A white or almost white powder; odourless or almost odourless; hygroscopic.

## Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with a 0.75-mm layer of the following mixture. Mix 0.3 g of *carbomer* (Such as Carbopol 934) with 240 ml of *water* and allow to stand, with moderate shaking, for 1 hour; adjust to pH 7.0 by the gradual addition, with continuous shaking, of *2 M sodium hydroxide* and add 30 g of *silica gel H*. Heat the plate at 110° for 1 hour, allow to cool and use immediately.

*Mobile phase*. A 7 per cent w/v solution of *potassium dihydrogen phosphate*.

*Test solution*. A 0.1 per cent w/v solution of the substance under examination in *water*.

*Reference solution (a)*. A 0.1 per cent w/v solution of *kanamycin sulphate* RS in *water*.

*Reference solution (b)*. A solution containing 0.1 per cent w/v each of *kanamycin sulphate RS*, *neomycin sulphate RS* and *streptomycin sulphate RS* in *water*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, spray with a mixture of equal volumes of a 0.2 per cent w/v solution of *1,3-napthalenediol* in *ethanol (95 per cent)* and a 45 per cent w/v of solution of *sulphuric acid* and heat at 150° for 5 to 10 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows three clearly separated spots.

B. Dissolve 0.5 g in 10 ml of *water* and add 10 ml of a 1 per cent w/v solution of *picric acid*. If necessary initiate crystallisation by scratching the walls of the container with a glass rod, allow to stand and filter. The melting point of the crystals, after washing with 20 ml of *water* and drying at 105°, is about 235°, with decomposition (2.4.21).

C. Dissolve 50 mg in 2 ml of *water*, add 1 ml of a 1 per cent w/v solution of *ninhydrin* and heat for a few minutes on a water-bath; a violet colour is produced.

D. Gives the reactions of sulphates (2.3.1).

## Tests

**pH** (2.4.24). 5.5 to 7.5, determined in a 1.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). +103° to +115°, determined at 20° in a 1.0 per cent w/v solution.

**Kanamycin B**. Determine by thin-layer chromatography (2.4.17), coating the plate with a 0.75-mm layer of the following mixture. Mix 0.3 g of *carbomer* (Such as Carbopol 934) with 240 ml of *water* and allow to stand, with moderate shaking, for 1 hour; adjust to pH 7.0 by the gradual addition, with continuous shaking, of *2 M sodium hydroxide* and add 30 g



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of *silica gel H*. Heat the plate at 110° for 1 hour, allow to cool and use immediately.

*Mobile phase*. A 7 per cent w/v solution of *potassium dihydrogen phosphate*.

*Test solution*. A 0.5 per cent w/v solution of the substance under examination in *water*.

*Reference solution*. A 0.02 per cent w/v solution of *kanamycin B sulphate RS* in *water*.

Apply to the plate 4 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, spray with *ninhydrin* and *stannous chloride reagent* and heat at 110° for 15 minutes. Any spot corresponding to kanamycin B in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Sulphates**. 23.0 to 26.0 per cent of $SO_4$, calculated on the dried basis and determined by the following method. Dissolve 0.25 g in 100 ml of *water* and adjust the pH to 11 using *strong ammonia solution*. Add 10.0 ml of *0.1 M barium chloride* and 0.5 mg of *metalphthalein*. Titrate with *0.1 M disodium edetate*; when the colour of the solution begins to change, add 50 ml of *ethanol (95 per cent)* and continue the titration until the blue colour disappears.

1 ml of *0.1 M barium chloride* is equivalent to 0.009606 g of $SO_4$.

**Sulphated ash** (2.3.18). Not more than 0.5 per cent.

**Loss on drying** (2.4.19). Not more than 5.0 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa for 3 hours.

**Assay**. Determine by the microbiological assay of antibiotics, Method A or B (2.2.10).

*Kanamycin Acid Sulphate intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.*

**Bacterial endotoxins** (2.2.3). Not more than 0.67 Endotoxin Unit per mg of kanamycin.

*Kanamycin Sulphate intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.*

**Sterility** (2.2.11). Complies with the test for sterility.

**Storage**. Store protected from light and moisture. If the material is intended for use in the manufacture of parenteral preparations, the container should be sterile and sealed so as to exclude micro-organisms.

**Labelling**. The label states (1) the number of Units per mg; (2) whether or not the material is intended for use in the manufacture of parenteral preparations.

# Kanamycin Injection

Kanamycin Injection is either a sterile solution of Kanamycin Sulphate in Water for Injections containing Sulphuric Acid and suitable buffering and stabilising agents or, is a sterile material consisting of Kanamycin Acid Sulphate with buffering agents and other excipients. It is filled in a sealed container.

### A. *Kanamycin Injection (Solution)*

Kanamycin Injection contains not less than 97.0 per cent and not more than 110.0 per cent of the stated number of Units of kanamycin.

**Usual strengths**. The equivalent of 250 mg (250,000 Units) of kanamycin per ml or per sealed container.

**Description**. A colourless to pale yellow solution.

## Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with a 0.75-mm layer of the following mixture. Mix 0.3 g of *carbomer* (Such as Carbopol 934) with 240 ml of *water* and allow to stand, with moderate shaking, for 1 hour; adjust the pH to 7.0 by the gradual addition, with continuous shaking, of *2 M sodium hydroxide* and add 30 g of *silica gel H*. Heat the plate at 110° for 1 hour, allow to cool and use immediately.

*Mobile phase*. A 7 per cent w/v solution of *potassium dihydrogen phosphate*.

*Test solution*. A suitable volume diluted with *water* to contain 800 Units per ml.

*Reference solution (a)*. A 0.1 per cent w/v solution of *kanamycin sulphate RS* in *water*.

*Reference solution (b)*. A solution containing 0.1 per cent w/v each of *kanamycin sulphate RS*, *neomycin sulphate RS* and *streptomycin sulphate RS* in *water*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, spray with a mixture of equal volumes of a 0.2 per cent w/v solution of *1,3-napthalenediol* in *ethanol (95 per cent)* and a 45 per cent w/v of solution of *sulphuric acid* and heat at 150° for 5 to 10 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows three clearly separated spots.



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## Tests

**pH** (2.4.24). 4.0 to 6.0.

**Kanamycin B**. Determine by thin-layer chromatography (2.4.17), coating the plate with a 0.75-mm layer of the following mixture. Mix 0.3 g of *carbomer* (Such asCarbopol 934) with 240 ml of *water* and allow to stand, with moderate shaking, for 1 hour; adjust to pH 7.0 by the gradual addition, with continuous shaking, of *2 M sodium hydroxide* and add 130 g of *silica gel H*. Heat the plate at 110° for 1 hour, allow to cool and use immediately.

*Mobile phase*. A 7 per cent w/v solution of *potassium dihydrogen phosphate*.

*Test solution*. A suitable volume diluted with *water* to contain 3750 Units per ml.

*Reference solution*. A 0.02 per cent w/v solution of *kanamycin B sulphate RS* in *water*.

Apply to the plate 4 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, spray with *ninhydrin* and *stannous chloride reagent* and heat at 110° for 15 minutes. Any spot corresponding to kanamycin B in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Bacterial endotoxins** (2.2.3). Not more than 0.67 Endotoxin Unit per mg of kanamycin.

**Assay**. Determine by the microbiological assay of antibiotics, Method A or B (2.2.10).

The upper fiducial limit of error is not less than 97.0 per cent and the lower fiducial limit of error is not more than 110.0 per cent of the stated number of Units.

**B. *Kanamycin Injection (Powder)***

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

*The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).*

**Storage**. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Kanamycin Injection contains not less than 95.0 per cent and not more than 115.0 per cent of the stated number of Units of kanamycin

*The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.*

## Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with a 0.75-mm layer of the following mixture. Mix 0.3 g of *carbomer* (Such as Carbopol 934) with 240 ml of *water* and allow to stand, with moderate shaking, for 1 hour; adjust the pH to 7.0 by the gradual addition, with continuous shaking, of *2 M sodium hydroxide* and add 30 g of *silica gel H*. Heat the plate at 110° for 1 hour, allow to cool and use immediately.

*Mobile phase*. A 7 per cent w/v solution of *potassium dihydrogen phosphate*.

*Test solution*. A suitable volume diluted with *water* to contain 800 Units per ml.

*Reference solution (a)*. A 0.1 per cent w/v solution of *kanamycin sulphate RS* in *water*.

*Reference solution (b)*. A solution containing 0.1 per cent w/v each of *kanamycin sulphate RS*, *neomycin sulphate RS* and *streptomycin sulphate RS* in *water*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, spray with a mixture of equal volumes of a 0.2 per cent w/v solution of *1,3-napthalenediol* in *ethanol (95 per cent)* and a 45 per cent w/v of solution of *sulphuric acid* and heat at 150° for 5 to 10 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows three clearly separated spots.

## Tests

**pH** (2.4.24). 5.5 to 7.5, determined in a 1.0 per cent w/v solution.

**Kanamycin B**. Determine by thin-layer chromatography (2.4.17), coating the plate with a 0.75-mm layer of the following mixture. Mix 0.3 g of *carbomer* (Such as Carbopol 934) with 240 ml of *water* and allow to stand, with moderate shaking, for 1 hour; adjust to pH 7.0 by the gradual addition, with continuous shaking, of *2 M sodium hydroxide* and add 30 g of *silica gel H*. Heat the plate at 110° for 1 hour, allow to cool and use immediately.

*Mobile phase*. A 7 per cent w/v solution of *potassium dihydrogen phosphate*.

*Test solution*. A suitable volume diluted with *water* to contain 3750 Units per ml.

*Reference solution*. A 0.02 per cent w/v solution of *kanamycin B sulphate RS* in *water*.

Apply to the plate 4 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, spray with *ninhydrin* and *stannous chloride reagent* and heat



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at 110° for 15 minutes. Any spot corresponding to kanamycin B in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Bacterial endotoxins** (2.2.3). Not more than 0.67 Endotoxin Unit per mg of kanamycin.

**Assay**. Determine the weight of the contents of 10 containers. Using the mixed contents of the 10 containers determine by the microbiological assay of antibiotics, Method A or Method B (2.2.10).

For a container of average content weight, the upper fiducial limit of error is not less than 95.0 per cent and the lower fiducial limit of error is not more than 115.0 per cent of the stated number of Units.

**Storage**. Store in single dose containers protected from light and moisture.

**Labelling**. The label states (1) the strength in terms of the number of Units or the equivalent weight of kanamycin in a suitable dose-volume or in the sealed container; (2) the volume of Water for Injections for constituting the solution (for contents of a sealed container).

## Heavy Kaolin

Heavy Kaolin is a purified, natural, hydrated aluminium silicate of variable composition.

**Category**. Pharmaceutical aid.

**Description**. Fine, white or greyish white, soft powder; odourless.

### Identification

A. To 0.5 g in a metal crucible add 1 g of *potassium nitrate* and 3 g of *anhydrous sodium carbonate*, heat until the mixture has melted and allow to cool. To the residue add 20 ml of boiling *water*, mix, filter and wash the residue with 50 ml of *water*. To the residue add 1 ml of *hydrochloric acid* and 5 ml of *water* and filter. To the filtrate add 1 ml of *10 M sodium hydroxide* and filter. To the filtrate add 3 ml of *ammonium chloride solution*; a gelatinous, white precipitate is produced.

B. 0.25 g gives the reaction of silicates (2.3.1).

### Tests

**Acidity or alkalinity**. To 1.0 g add 20 ml of *carbon dioxide-free water*, shake for 2 minutes and filter. To 10 ml of the filtrate add 0.1 ml of *phenolphthalein solution*. The solution is colourless and not more than 0.25 ml of *0.01 M sodium hydroxide* is required to change the colour of the solution to pink.

**Arsenic** (2.3.10). Disperse 5.0 g in 50 ml of *water* and add 10 ml of *stannated hydrochloric acid*. The resulting solution complies with the limit test for arsenic (2 ppm).

**Heavy metals** (2.3.13). Boil 5.0 g with 7.5 ml of *2 M hydrochloric acid* and 27.5 ml of *water* for 5 minutes, filter, wash the residue with *water* and dilute the combined filtrate and washings to 50 ml with *water* (solution A). To 5 ml of *solution* A add 5 ml of *water*, 10 ml of *hydrochloric acid* and 25 ml of *4-methylpentan-2-one*, shake for 2 minutes, allow the layers to separate and evaporate the aqueous layer to dryness on a water-bath. Dissolve the residue in 1 ml of *5 M acetic acid*, dilute to 25 ml with *water* and filter. 12 ml of the resulting solution complies with the limit test for heavy metals, Method D (50 ppm). Use *lead standard solution (1 ppm Pb)* to prepare the standard.

**Chlorides** (2.3.12). Shake 4.0 g with a mixture of 34 ml of *distilled water* and 6 ml of *5 M acetic acid* for 1 minute and filter. 10 ml of the filtrate complies with the limit test for chlorides (250 ppm).

**Sulphates** (2.3.17). 2 ml of the filtrate obtained in the test for chlorides diluted to 15 ml complies with the limit test for sulphates (750 ppm).

**Substances soluble in mineral acids**. Not more than 1 per cent, determined by the following method. To 10 ml of solution A add 1.5 ml of *1 M sulphuric acid*, evaporate to dryness on a water-bath, ignite, cool and weigh.

**Organic impurities**. Heat 0.3 g to redness in a calcination tube. The residue is only slightly more coloured than the original substance.

**Adsorption power**. In a ground-glass-stoppered test-tube shake 1.0 g with 10 ml of a 0.37 per cent w/v solution of *methylene blue* for 2 minutes and allow to settle. Centrifuge and dilute 1 volume of the solution to 100 volumes with *water*. The solution is not more intensely coloured than a 0.003 per cent w/v solution of *methylene blue*.

**Swelling power**. Triturate 2 g with 2 ml of *water*; the mixture does not flow.

**Loss on ignition** (2.4.20). Not more than 15.0 per cent, determined on 1.0 g.

## Light Kaolin

Light Kaolin is a native hydrated aluminium silicate, freed from most of its impurities by elutriation and dried. It may contain a suitable dispersing agent.

**Category**. Antidiarrhoeal.

**Dose**. 15 to 75 g.

**Description**. Light, white powder free from gritty particles; odourless; almost tasteless; unctuous to the touch.



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## Identification

A. Fuse 1 g with 2 g of *anhydrous sodium carbonate*, warm the residue with 10 ml of *water*, filter, wash the filter with 5 ml of *water* and reserve the residue. To the combined filtrate and washings add 3 ml of *hydrochloric acid*; a gelatinous precipitate is produced.

B. Dissolve the residue reserved in test A in 10 ml of *2 M hydrochloric acid*; the solution gives reaction B of aluminium salts (2.3.1).

C. Triturate 2 g with 2 ml of *water*; the resulting mixture flows.

Coarse particles. Transfer 5 g to a stoppered cylinder (16 cm × 35 mm), add 60 ml of a 1 per cent w/v solution of *sodium pyrophosphate*, shake thoroughly and allow to stand for 5 minutes. Using a pipette, withdraw 50 ml from a point about 5 cm below the surface of the liquid. To the remaining liquid add 50 ml of *water*, shake, allow to stand for 5 minutes and withdraw 50 ml in the same manner as before. Repeat the operation until a total of 400 ml of suspension has been withdrawn under the prescribed conditions. Transfer the remainder to an evaporating dish and evaporate to dryness on a water-bath. The residue, after drying at 105°, weighs not more than 25 mg.

Fine particles. Disperse 5 g in 250 ml of *water* by shaking vigorously for 2 minutes in a stoppered flask, pour immediately into a glass cylinder, 5 cm in diameter, and transfer 20 ml to a glass dish using a pipette. Evaporate to dryness and dry to constant weight at 105°. Allow the remainder of the suspension to stand for 4 hours at 20° and withdraw a second 20-ml portion using a pipette with its tip exactly 5 cm below the surface and without disturbing the sediment. Transfer the second portion to a glass dish, evaporate to dryness and dry to constant weight at 105°. The weight of the residue from the second portion is not less than 70 per cent of the weight of the residue from the first portion.

## Tests

**Arsenic** (2.3.10). Disperse 5 g in 50 ml of *water* and add 10 ml of *stannated hydrochloric acid*; the resulting solution complies with the limit test for arsenic (2 ppm).

**Heavy metals**. Heat 6.0 g for 15 minutes under a reflux condenser on a water-bath with a mixture of 70 ml of *water* and 10 ml of *hydrochloric acid* and filter. To 40 ml of the filtrate add 0.5 ml of *nitric acid* and evaporate to a low bulk. Add 20 ml of *water*, 2 g of *ammonium chloride* and 2 g of *ammonium thiocyanate* and extract with two quantities, each of 10 ml, of a mixture of equal volumes of *amyl alcohol* and *ether*. To the aqueous layer add 2 g of *citric acid* and sufficient *water* to produce 60 ml. 12 ml of the solution complies with the limit test for heavy metals, Method D (2.3.13) (20 ppm).

**Chlorides** (2.3.12). Boil 1.0 g with 40 ml of *water* and 20 ml of *2 M nitric acid* under a reflux condenser for 5 minutes, cool and filter. 30 ml of the filtrate complies with the limit test for chlorides (330 ppm).

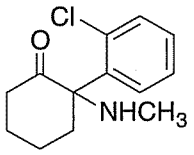
**Soluble matter**. Boil 2 g with 100 ml of *0.2 M hydrochloric acid* under a reflux condenser for 5 minutes, cool, filter and evaporate 50 ml to dryness. The residue, after ignition at about 600° for 30 minutes, weighs not more than 10 mg.

**Loss on drying** (2.4.19). Not more than 1.5 per cent, determined on 1 g by drying in an oven at 105°.

**Loss on ignition** (2.4.20). Not more than 15.0 per cent, determined on 1 g by igniting at 600°.

**Storage** . Store protected from moisture.

# Ketamine Hydrochloride



, HCl

$C_{13}H_{16}ClNO,HCl$ Mol. Wt. 274.2

Ketamine Hydrochloride is (*RS*)-2-(2-chlorophenyl)-2-methylaminocyclohexanone hydrochloride.

Ketamine Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{13}H_{16}ClNO,HCl$.

**Category**. General anaesthetic.

**Dose**. By slow intravenous injection, the equivalent of 1 to 2 mg of ketamine per kg body weight over 60 seconds, repeated according to patient's response; by deep intramuscular injection, the equivalent of 4 to 10 mg of ketamine per kg body weight, repeated according to patient's response.

(11.54 mg of ketamine hydrochloride is approximately equivalent to 10 mg of ketamine).

**Description**. A white, crystalline powder.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ketamine hydrochloride RS* or with the reference spectrum of ketamine hydrochloride.

B. A 10 per cent w/v solution gives the reactions of chlorides (2.3.1).



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C. Melting range (2.4.21). 258° to 261°.

### Tests

**Appearance of solution**. A 20.0 per cent w/v solution is clear (2.4.1), and colourless (2.4.1).

**pH** (2.4.24). 3.5 to 4.1, determined in a 10.0 per cent w/v solution.

**Optical rotation** (2.4.22). – 0.2° to + 0.2°, determined in 2.0 per cent w/v solution in *water*.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 50 mg of the substance under examination in 50 ml of the mobile phase.

*Reference solution*. Dilute 1.0 ml of the test solution to 10.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 20.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 12.5 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: dissolve 0.95 g of *sodium hexanesulphonate* in 1000 ml of a mixture of 25 volumes of *acetonitrile* and 75 volumes of *water*, add 4 ml of *acetic acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume. 20 µl.

Inject the test solution and the reference solution. Run the chromatogram 10 times the retention time of the principal peak. In the chromatogram obtained with the test solution the sum of areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with the reference solution, (0.5 per cent). Ignore any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent).

**Heavy metals** (2.3.13). 1.0 g complies with limit test for heavy metals, Method A (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Assay**. Weigh accurately about 0.5 g and dissolve in 1 ml of *anhydrous formic acid*. Add 50 ml of *anhydrous glacial acetic acid* and 10 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02742 g of $C_{13}H_{16}ClNO,HCl$.

**Storage**. Store protected from moisture.

## Ketamine Injection

Ketamine Hydrochloride Injection

Ketamine Injection is a sterile solution of Ketamine Hydrochloride in Water for Injections.

Ketamine Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of ketamine, $C_{13}H_{16}ClNO$.

**Usual strengths**. The equivalent of 10 mg per ml; 50 mg per ml; 100 mg of ketamine per ml (11.54 mg of ketamine hydrochloride is approximately equivalent to 10 mg of ketamine).

### Identification

A. Dilute a suitable volume with *0.1 M hydrochloric acid* to produce a solution containing 0.03 per cent w/v of ketamine. When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows absorption maxima at about 269 nm and 276 nm.

B. Dilute a suitable volume with a mixture of 49 volumes of *methanol* and 1 volume of *1 M sodium hydroxide* to produce a solution containing 0.07 per cent w/v of ketamine. When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum at about 301 nm.

### Tests

**pH** (2.4.24). 3.0 to 5.5.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Note—Prepare the solutions immediately before use.*

*Test solution*. Dilute a quantity of injection containing about 0.12 g of Ketamine in 100 ml of the mobile phase.

*Reference solution (a)*. Dilute 1.0 ml of the test solution to 200 ml with the mobile phase.

*Reference solution (b)*. Dilute 1.0 ml of reference solution (a) to 2 ml with the mobile phase.

Chromatographic system

- a stainless steel column 12.5 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm) ( Such as Lichrosorb RP18),
- mobile phase: a solution prepared by dissolving 0.95 g of *sodium hexanesulphonate* in 1000 ml of a mixture of 25 volumes of *acetonitrile* and 75 volumes of *water* and add 4 ml of *6 M acetic acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume. 20 µl.



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Inject the test solution, reference solution (a) and (b). Run the chromatogram 10 times the retention time of the principal peak. The retention time is about 3 to 4.5 minutes for ketamine. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent), and the area of one such peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) 0.25 per cent). Ignore any peak with an area less than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

**Bacterial endotoxins** (2.2.3). Not more than 0.40 Endotoxin Unit per mg of ketamine hydrochloride.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

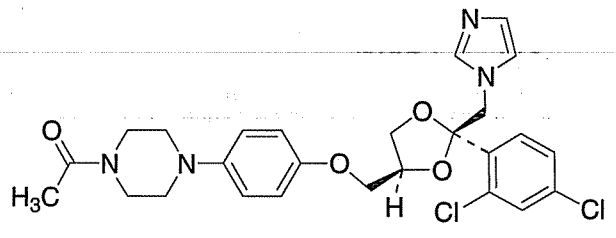
**Assay**. To an accurately measured volume containing about 0.5 g of ketamine add sufficient *water* to produce 200.0 ml and mix. To 20.0 ml of the resulting solution add 3 ml of *0.1 M sodium hydroxide* and extract with three quantities, each of 15 ml, of *chloroform*. Combine the *chloroform* extracts and shake successively with three quantities, each of 30 ml, of *0.05 M sulphuric acid*. Dilute the combined acid extracts to 200.0 ml with *0.05 M sulphuric acid* (saturated with *chloroform*), and measure the absorbance of the resulting solution at the maximum at about 269 nm (2.4.7).

Calculate the content of $C_{13}H_{16}ClNO$ from the absorbance obtained by repeating the determination using a standard solution of *ketamine hydrochloride RS* containing the equivalent of 250 µg per ml of ketamine base in the same medium in place of the substance under examination.

**Storage**. Store protected from light at a temperature not exceeding 30°.

**Labelling**. The label states the strength in terms of the equivalent amount of ketamine in a suitable dose-volume.

# Ketoconazole



$C_{26}H_{28}Cl_2N_4O_4$ Mol. Wt. 531.4

Ketoconazole is *cis*-1-acetyl-4-[[(2*RS*,4*RS*)-2-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxyl]phenylpiperazine.

Ketoconazole contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{26}H_{28}Cl_2N_4O_4$, calculated on the dried basis.

**Category**. Antifungal.

**Dose**. For an adult, 200 mg to 400 mg once daily with food for 14 days; for a child, 3 mg per kg body weight daily.

**Description**. A white to off-white, crystalline powder.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ketoconazole RS* or with the reference spectrum of ketoconazole.

B. In the test for Related substances, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

C. Melting range (2.4.21). 148° to 152°.

## Tests

**Specific optical rotation** (2.4.22). –1.0° to +1.0°, determined in a 4.0 per cent w/v solution in *methanol*.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 0.1 g of the substance to under examination in 10.0 ml of *methanol*.

*Reference solution (a)*. Dissolve 2.5 mg each of *ketoconazole RS* and *loperamide hydrochloride RS* in 50 ml of *methanol*.

*Reference solution (b)*. Dilute 5.0 ml of the test solution to 100.0 ml with *methanol*. Dilute 1.0 ml of this solution to 10.0 ml with *methanol*.

Chromatographic system

- stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- mobile phase: A. a mixture of 5 volumes of *acetonitrile* and 95 volumes of 0.34 per cent w/v solution of *tetrabutylammonium hydrogen sulphate*,
  B. a mixture of 50 volumes of *acetonitrile* and 50 volumes of 0.34 per cent w/v solution of *tetrabutylammonium hydrogen sulphate*,
- a linear gradient programme using the conditions given below,
- flow rate. 2 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 10 µl.



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| Time (min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0 - 10 | 100→0 | 0→100 |
| 10 – 15 | 0 | 100 |

Inject reference solution (b). Adjust the sensitivity of the system so that the height of the principal peak is at least 50 per cent of the full scale of the recorder.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to ketonazole and loperamide is not less than 15.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution the sum of the areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.1 times that of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

**Heavy metals** (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 80° at a pressure not exceeding 2.7 kPa for 4 hours.

**Assay**. Weigh accurately about 0.2 g, dissolve in 40 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25).Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02657 g of $C_{26}H_{28}Cl_2N_4O_4$.

**Storage**. Store protected from light.

# Ketoconazole Tablets

Ketoconazole Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ketoconazole, $C_{26}H_{28}Cl_2N_4O_4$.

**Usual strength**. 200 mg.

## Identification

Determine by thin- layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 42 volumes of *hexane*, 40 volumes of *ethyl acetate*, 15 volumes of *methanol*, 2 volumes of *water* and 1 volume of *glacial acetic acid*.

*Test solution*. Shake a quantity of powdered tablets containing 200 mg of Ketoconazole with 10 ml of *chloroform*, dilute to 20 ml with *chloroform* and filter.

*Reference solution*. A 1.0 per cent w/v solution of *ketoconazole RS* in *chloroform*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and expose to iodine vapour. The principal spot in the chromatogram obtained with the test solution corresponds to that obtained with the reference solution.

## Tests

**Dissolution** (2.5.2).

Apparatus No. 2,
Medium. 900 ml of *0.1M hydrochloric acid*,

Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted if necessary with the dissolution medium at 270 nm (2.4.7). Calculate the content of $C_{26}H_{28}Cl_2N_4O_4$, in the medium from the absorbances obtained from a solution of *ketoconazole RS*.

D. Not less than 80 per cent of the stated amount of $C_{26}H_{28}Cl_2N_4O_4$.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. Equal volumes of *methanol* and *dichloromethane*.

*Test solution*. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 200 mg of Ketoconazole, shake with 50.0 ml of the solvent mixture and centrifuge. To 5.0 ml of this solution, add 5.0 ml of a 0.5 per cent w/v solution of *terconazole RS* (internal standard) in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

*Reference solution*. Dissolve 20 mg of *ketoconazole RS* in 20 ml of the solvent mixture, add 5.0 ml of the internal standard solution and dilute to 50.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 7 volumes of 0.2 per cent w/v of *di-isopropylamine* in *methanol* and 3 volumes of a 0.5 per cent w/v solution of *ammonium acetate*,
- flow rate. 3 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume. 20 µl.

Inject the test solution and the reference solution. The relative retention times are about 0.6 for ketoconazole and 1.0 for terconazole.

Calculate the content of $C_{26}H_{28}Cl_2N_4O_4$ in the tablets.

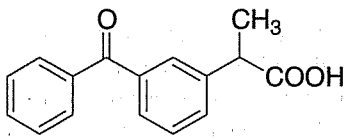


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# Ketoprofen




$C_{16}H_{14}O_3$ Mol. Wt. 254.3

Ketoprofen is (*RS*)-2-(3-benzoylphenyl)propionic acid.

Ketoprofen contains not less than 98.5 per cent and not more than 100.5 per cent of $C_{16}H_{14}O_3$, calculated on the dried basis.

**Category**. Anti-inflammatory; analgesic.

**Dose**. 100 to 200 mg daily, in 2 to 4 divided doses, with food.

**Description**. A white or almost white, crystalline powder; odourless or almost odourless.

## Identification

*Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ketoprofen RS* or with the reference spectrum of ketoprofen.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *methanol (75 per cent)* shows an absorption maximum only at about 258 nm; absorbance at about 258 nm, about 0.66.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 50 volumes of *acetone*, 49 volumes of *dichloromethane* and 1 volume of *glacial acetic acid*.

*Test solution*. Dissolve 0.1 g of the substance under examination in 100 ml of *acetone*.

*Reference solution (a)*. A 0.1 per cent w/v solution of *ketoprofen RS* in *acetone*.

*Reference solution (b)*. A mixture of equal volumes of 1.0 per cent w/v solution of *indomethacin RS* and refererence solution (a) in *acetone*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

## Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Note—Prepare the solutions immediately before use.*

*Test solution*. Dissolve 20 mg of the substance under examination in 20 ml of the mobile phase .

*Reference solution*. Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 2 volumes of freshly prepared *phosphate buffer pH 3.5* , 43 volumes of *acetonitrile* and 55 volumes of *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 233 nm,
- injection volume. 20 µl.

Inject the reference solution. Adjust the sensitivity of the system so that the height of the principal peak is at least 50 per cent of the full scale of the recorder.

Inject the test solution and the reference solution. Run the chromatogram 7 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (0.2 per cent). The sum of areas of all the secondary peaks is not more than twice the area of the peak due to impurity in the chromatogram obtained with the reference solution (0.4 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with the reference solution (0.02 per cent).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa.

**Assay**. Weigh accurately about 0.5 g, dissolve in 25 ml of *ethanol (95 per cent)* previously neutralised to *phenolphthalein solution*, add 25 ml of *water* Titrate with *0.1 M sodium hydroxide* using *phenolphthalein solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.02543 g of $C_{16}H_{14}O_3$.

**Storage**. Store protected from moisture.

# Ketoprofen Capsules

Ketoprofen Capsules contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of ketoprofen, $C_{16}H_{14}O_3$.



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**Usual strengths**. 50 mg; 100 mg.

## Identification

A.Shake a quantity of the contents of the capsules containing 0.5 g of Ketoprofen with 50 ml of *chloroform* for 5 minutes, filter, evaporate to dryness using a rotary evaporator, induce crystallisation by prolonged scratching of the inside wall of the container with a glass rod and separate the crystals by centrifugation or filtration.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ketoprofen RS* or with the reference spectrum of ketoprofen.

B. When examined in the range 230 nm to 360 nm (2.4.7), the final solution obtained in the Assay shows an absorption maximum only at about 258 nm.

## Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *phosphate buffer* prepared by dissolving 1.46 g of *potassium dihydrogen orthophosphate* and 20.06 g of *disodium hydrogen orthophosphate* in 1000 ml of *water,* adjusted to pH 7.5 with *orthophosphoric acid,*
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted if necessary with the dissolution medium to prepare a solution containing about 0.001 per cent w/v of ketoprofen at 260 nm (2.4.7). Calculate the content of $C_{16}H_{14}O_3$, in the medium from the absorbances obtained from a solution of *ketoprofen capsule RS* .

D. Not less than 80 per cent of the stated amount of $C_{16}H_{14}O_3$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Note—Prepare the solutions immediately before use.*

*Solvent mixture*. 40 volumes of *acetonitrile* and 60 volumes of *water.*

*Test solution.* Shake a quantity of the contents of capsules containing about 100 mg of Ketoprofen in 100 ml with the solvent mixture.

*Reference solution.* Dilute 1.0 ml of the test solution to 50 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) ,
- mobile phase: a mixture of 2 volumes of freshly prepared *phosphate buffer pH 3.5* , 43 volumes of *acetonitrile* and 55 volumes of *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 233 nm,
- injection volume. 20 µl.

Inject the test solution and the reference solution. The area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.2 per cent). The sum of the areas of all the secondary peaks is not more than 2.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with the reference solution (0.02 per cent).

**Other tests**. Comply with the tests stated under Capsules.

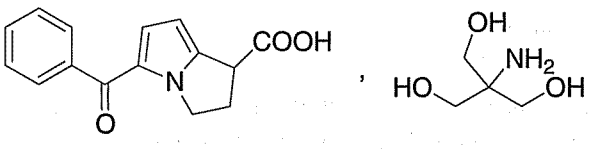
**Assay**. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 25 mg of Ketoprofen, shake for 10 minutes with 150 ml of *methanol (75 per cent)*, mix and dilute to 250.0 ml with *methanol (75 per cent)*. Allow to stand, dilute 10.0 ml of the supernatant liquid to 100.0 ml with *methanol (75 per cent)* and measure the absorbance of the resulting solution at the maximum at about 258 nm (2.4.7).

Calculate the content of $C_{16}H_{14}O_3$ taking 662 as the specific absorbance at 258 nm.

**Storage**. Store protected from moisture.

# Ketorolac Tromethamine

Ketorolac Trometamol




$C_{15}H_{13}NO_3,C_4H_{11}NO_3$ Mol. Wt. 376.4

Ketorolac Tromethamine is salt of (*RS*)-5-benzoyl-2,3-dihydro-1*H*-pyrrolizine-1-carboxylate with 2-amino-2-(hydroxymethyl) propane-1,3-diol.

Ketorolac Tromethamine contains not less than 98.5 per cent and not more than 101.5 per cent of $C_{15}H_{13}NO_3,C_4H_{11}NO_3$, calculated on the dried basis.

**Category**. Non-steroidal Antiinflamatory.

**Description**. A white or almost white, crystalline powder.



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## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ketorolac tromethamine RS* or with the reference spectrum of ketorolac tromethamine.

B. Determine by thin layer Chromatography ( 2.4.17), coating the plate with *silica gel G.*

*Mobile phase.* A mixture of 95 volumes of *dichloromethane,* 5 volumes of *acetone* and 2 volumes of *glacial acetic acid.*

*Test solution.* A 0.5 per cent w/v solution of the substance under examination in a mixture of 2 volumes of *dichloromethane* and 1 volume of *methanol.*

*Reference solution.* A 0.5 per cent w/v solution of *ketorolac tromethamine RS* in the same solvent.

Apply separately to the plate 40 µl of each solution. After development, dry the plate in air, spray with a freshly prepared alcoholic solution containing 30 mg of *ninhydrin* per ml, dry at 150° for 2 to 5 minutes and examine. The yellow spots with pink to purple borders obtained with the test solution corresponds to the spots obtained with the reference solution.

## Tests

**Appearance of solution.** A 3.0 per cent w/v solution in *carbon dioxide-free water* (solution A) is clear (2.4.1.).

**pH** (2.4.24). 5.7 to 6.7 determined on a solution prepared by diluting 5 ml of solution A to 15 ml with *carbon dioxide-free water.*

**Light absorption** (2.4.7). Absorbance of solution A at 430 nm, not more than 0.10.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. 30 volumes of *tetrahydrofuran* and 70 volumes of *water.*

*Test solution*. Dissolve 20.0 mg of the substance under examination in 50.0 ml of the solvent mixture.

*Reference solution (a)*. A 0.004 per cent w/v solution of *ketorolac tromethamine RS* in the solvent mixture. Dilute 1.0 ml of the solution to 100.0 ml with the solvent mixture.

*Reference solution (b)*. Prepare in situ 5-benzoyl-2,3-dihydro-1*H*-pyrrolizin-1-one (ketorolac impurity A) in the following manner. Place in a 250-ml separating funnel, 100 ml of *water*, 100 ml of *dichloromethane*, 30 mg of the substance under examination and 1 ml of a 10.3 per cent w/v solution of *hydrochloric acid.* Insert the stopper, shake, and allow the layers to separate. Transfer the lower *dichloromethane* layer to a stoppered borosilicate glass flask. Insert the stopper and expose to daylight for 10-15 minutes. Evaporate 1.0 ml of the solution to dryness in a current of air, or in a stream of nitrogen. Dissolve the residue in 1.0 ml of the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica ( 5 µm) ,
- column temperature. 40°,
- mobile phase: a mixture of 30 volumes of *tetrahydrofuran* and 70 volumes of a solution prepared by dissolving 5.75 g of *ammonium dihydrogen phosphate* in 900 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid* and diluting to 1000 ml with *water,*
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 313 nm,
- injection volume. 10 µl.

Inject reference solution (b). The resolution between the peaks due to ketorolac impurity A and ketorolac is not less than 1.5.

Inject the test solution and reference solution (a). Record the chromatogram of the test solution for three times the retention time of the principal peak. The area of any secondary peak obtained with the test solution is not more than the area of the peak in the chromatogram obtained with reference solution (a) (0.1 per cent ) and the sum of the areas of all the secondary peaks is not more than 10 times the area of the peak and the chromatogram obtained with reference solution (a) (1.0 per cent).

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in vacuum at 60° for 3 hours.

**Assay**. Dissolve 0.3 g in 60 ml of *anhydrous acetic acid.* Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03764 g of $C_{19}H_{24}N_2O_6$.

# Ketorolac Tromethamine Injection

Ketorolac Trometamol Injection

Ketorolac Tromethamine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ketorolac tromethamine $C_{15}H_{13}NO_3,C_4H_{11}NO_3$.

**Usual strengths**. 15 mg per ml; 30 mg per ml.



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### Identification

Prepare a mixture of equal volumes of the test solution and reference solution (a) and chromatograph the mixture as directed in the Assay. The chromatogram thus obtained exhibits two main peaks corresponding to ketorolac and naproxen.

### Tests

**pH** (2.4.24). 6.9 to 7.9.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Bacterial endotoxins** (2.2.3). Not more than 5.8 Endotoxin units per mg of ketorolac tromethamine.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. Equal volumes of *methanol* and *water*.

*Note — Protect the solutions from light.*

*Test solution*. Dilute an accurately measured volume containing 12 mg of Ketorolac Tromethamine to 50.0 ml with *methanol*. To 5.0 ml of this solution add 5.0 ml of reference solution (b) and dilute to 50.0 ml with the solvent mixture.

*Reference solution (a)*. A 0.024 per cent w/v solution of *ketorolac tromethamine RS* in *methanol*. To 5.0 ml of the solution, add 5.0 ml of reference solution (b) and dilute to 50.0 ml with the solvent mixture.

*Reference solution (b)*. A 0.03 per cent w/v solution of *naproxen RS* in *methanol*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 55 volumes of *methanol*, 44 volumes of *water* and 1 volume of *glacial acetic acid*,
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 100 µl.

Inject reference solution (a). The relative retention times are about 0.7 for ketorolac and 1.0 for naproxen. The resolution between the peaks due to ketorolac and naproxen is not less than 5.4, the column efficiency determined from the ketorolac peak is not less than 2700 theoretical plates, the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 1.5 per cent.

Inject the test solution and reference solution (a).

Calculate the content of $C_{15}H_{13}NO_3,C_4H_{11}NO_3$ in the injection.

**Storage**. Store protected from light, at a temperature not exceeding 30°.

## Ketorolac Tromethamine Tablets

Ketorolac Trometamol Tablets

Ketorolac Tromethamine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ketorolac tromethamine $C_{15}H_{13}NO_3,C_4H_{11}NO_3$.

**Usual strength**. 10 mg.

### Identification

Prepare a mixture of equal volumes of the test solution and reference solution (a) and determine by liquid chromatography (2.4.14) as described in the Assay, using the mixture. The chromatogram obtained exhibits two main peaks corresponding to ketorolac and naproxen.

### Tests

**Dissolution** (2.5.2).

Apparatus No.1,

Medium. 600 ml of *water*,

Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance (2.4.7) of the filtrate, suitably diluted with the medium if necessary, at the maximum at about 322 nm.

Calculate the content of $C_{15}H_{13}NO_3,C_4H_{11}NO_3$ in the medium from the absorbance obtained from a solution of known concentration of *ketorolac tromethamine RS* in the same medium.

D. Not less than 75 per cent of the stated amount of $C_{15}H_{13}NO_3,C_4H_{11}NO_3$.

**Uniformity of content.** Comply with the test stated under Tablets.

Powder 1 tablet and transfer to volumetric flask that will provide a final concentration of about 0.1 mg of ketorolac tromethamine per ml . Add a quantity of *water* equivalent to about 10 per cent of the volume of the flask and mix with the aid of ultrasound. Add a quantity of *methanol* equivalent to about 40 per cent of the volume of the flask and mix with the aid of ultrasound for about 10 minutes to dissolve the ketorolac tromethamine. Cool, dilute to volume with *methanol* and mix. Centrifuge. Dilute 5.0 ml of the clear supernatant to 50.0 ml with *methanol* and mix. Measure the absorbance of the resulting solution at the maximum at about 322 nm using *methanol* as blank. Calculate the content of $C_{15}H_{13}NO_3,C_4H_{11}NO_3$ from the absorbance obtained from a solution of known concentration of *ketorolac tromethamine RS*.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. Equal volumes of *methanol* and *water*.



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*NOTE— Protect the solutions from light.*

*Test solution.* Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 20 mg of Ketorolac Tromethamine, add 10 ml of *water* and shake well. Add 40 ml of *methanol* and mix with the aid of ultrasound for about 10 minutes to dissolve the ketorolac tromethamine. Cool, dilute to 100.0 ml with *methanol* and mix. Centrifuge To 5.0 ml of the clear supernatant liquid add 5.0 ml of reference solution (b), add sufficient solvent mixture to produce 50.0 ml and mix.

*Reference solution (a).* A 0.024 per cent w/v solution of *ketorolac tromethamine RS* in *methanol.* To 5.0 ml of the solution, add 5.0 ml of reference solution (b) and dilute to 50.0 ml with the solvent mixture.

*Reference solution (b).* A 0.03 per cent w/v solution of *naproxen RS* in *methanol.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm ),
- mobile phase : a mixture of 55 volumes of *methanol*, 44 volumes of *water* and 1 volume of *glacial acetic acid,*
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 100 µl.

Inject reference solution (a). The relative retention times are about 0.7 for ketorolac and 1.0 for naproxen. The resolution between the peaks due to ketorolac and naproxen is not less than 5.4, the column efficiency determined from the ketorolac peak is not less than 2700 theoretical plates, the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 1.5 per cent.

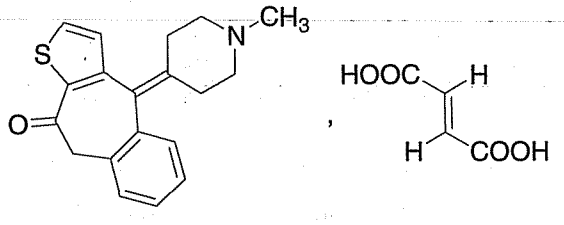
Inject the test solution and reference solution (a).

Calculate the content of $C_{15}H_{13}NO_3$,$C_4H_{11}NO_3$ in the tablets.

**Storage.** Store protected from light and moisture, at a temperature not exceeding 30°.

# Ketotifen Fumarate

Ketotifen Hydrogen Fumarate




$C_{19}H_{19}NOS$,$C_4H_4O_4$ Mol. Wt. 425.5

Ketotifen Fumarate is 4,9-dihydro-4-(1-methylpiperidin-4-ylidene)-10*H*-benzo[4,5]cyclohepta[1,2-*b*]thiophen-10-one hydrogen fumarate.

Ketotifen Fumarate contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{19}H_{19}NOS$,$C_4H_4O_4$, calculated on the dried basis.

**Category.** Antihistaminic.

**Description.** A white to brownish-yellow crystalline powder.

## Identification

A Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ketotifen hydrogen fumarate RS* or with the reference spectrum of ketotifen hydrogen fumarate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

*Mobile phase.* A mixture of 3 volumes of *water*, 7 volumes of *anhydrous formic acid* and 90 volumes of *di-isopropyl ether.*

*Test solution.* Dissolve 40 mg of the substance under examination in 10.0 ml of *methanol.*

*Reference solution.* A 0.11 per cent w/v solution of *fumaric acid RS* in *methanol.*

Apply separately to the plate 5 µl of each solution. Allow the mobile phase to rise 17 cm. Dry the plate in warm air and examine in ultraviolet light at 254 nm. Spray lightly with a 0.5 per cent w/v solution of *potassium permanganate* in 1.4 per cent v/v solution of *sulphuric acid.* In the chromatogram obtained with the test solution, the spot due to fumaric acid corresponds to the principal spot in the chromatogram obtained with the reference solution.

## Tests

**Appearance of solution.** A 2.0 per cent w/v solution in *methanol* is clear (2.4.1) and not more intensely coloured than reference solution YS4, BYS4 or BS4 (2.4.1) .

**Related substances.** Determine by liquid chromatography (2.4.14).

*NOTE— Protect the solutions from light.*

*Solvent mixture.* Equal volumes of *methanol* and *water.*

*Test solution.* Dissolve 30 mg of the substance under examination in 100.0 ml of a mixture of the solvent mixture.

*Reference solution (a).* Dilute 1.0 ml of the test solution to 50.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

*Reference solution (b).* Dissolve 3.0 mg of *4-(1-methylpiperidin-4-ylidene)-4H-benzo[4,5]cyclohepta[1,2-*



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*b]thiophen-9,10-dione RS* (*ketotifen impurity A RS*) in 10 ml of *methanol* and dilute to 20.0 ml with *water*.

*Reference solution (c)*. To 1.5 ml of reference solution (b), add 1.0 ml of the test solution and dilute to 10 ml with the solvent mixture.

*Reference solution (d)*. Dilute 0.5 ml of reference solution (b) to 50 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- column temperature 40°,
- mobile phase: A. a mixture of 175 µl of *triethylamine* and 500 ml of *water*,
  B. a mixture of 175 µl of *triethylamine* and 500 ml of *methanol*,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 297 nm,
- injection volume. 20 µl.

| Time (in min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0-12 | 40 | 60 |
| 12-20 | 40→10 | 60→90 |
| 20-25 | 10 | 90 |
| 25-26 | 10→40 | 90→60 |
| 26-31 | 40 | 60 |

Inject the test solution and reference solutions (a), (c) and (d). The relative retention time of ketotifen impurity A with reference to ketotifen is about 0.86. In the chromatogram obtained with reference solution (c), the resolution is not less than 1.5 between the peaks due to ketotifen and to ketotifen impurity A. In the chromatogram obtained with reference solution (d), the signal-to-noise ratio is not less than 70 for the principal peak.

For the calculation of contents, multiply the area of the corresponding peak by the following correction factor: 1.36 for impurity. In the chromatogram obtained with the test solution, the area of the peak due to ketotifen impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent), the area of the peak due to any other impurity is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent) and the sum of the areas of all the secondary peaks is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying at 105° for 4 hours.

**Assay**. Dissolve 0.35 g in a mixture of 30 ml of *anhydrous acetic acid* and 30 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04255 g of $C_{23}H_{23}NO_5S$.

**Storage**. Store protected from light.



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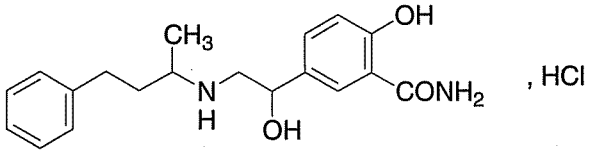


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# Labetalol Hydrochloride




$C_{19}H_{24}N_2O_3,HCl$ Mol. Wt. 364.9

Labetalol Hydrochloride is *all-rac*-2-hydroxy-5-[1-hydroxy-2-(1-methyl-3-phenylpropylamino)ethyl]benzamide hydrochloride.

Labetalol Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{19}H_{24}N_2O_3,HCl$, calculated on the dried basis.

**Category**. Antihypertensive.

**Dose**. Orally, initially, 100 mg twice daily with food, increased at fortnightly intervals to usual dose of 200 mg twice daily; upto 800 mg daily in 2 divided doses; maximum 2.4 g daily; by intravenous injection, 50 mg over 1 minute, repeated after 5 minutes, if necessary; maximum 200 mg.

**Description**. A white or almost white powder or granules.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *labetalol hydrochloride RS* or with the reference spectrum of labetalol hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.005 per cent w/v solution in *0.1 M hydrochloric acid* shows an absorption maximum only at 302 nm; absorbance at 302 nm, about 0.43.

C. Dissolve 10 mg in 5 ml of *water* and add 1 ml of *ferric chloride test solution*; a purple colour is produced.

D. A 1 per cent w/v solution gives the reactions of chlorides (2.3.1).

## Tests

**Appearance of solution**. A 1.0 per cent w/v solution is clear (2.4.1), and not more intensely coloured than reference solution YS6 (2.4.1).

**pH** (2.4.24). 4.0 to 5.0, determined in a 1.0 per cent w/v solution.

**Diasterioisomer ratio**. Determine by gas chromatography (2.4.13).

*Test solution*. Dissolve 2 mg of the substance under examination in 1 ml of a 1.2 per cent w/v solution of *1-butane-boronic acid* in *anhydrous pyridine* and allow to stand for 20 minutes.

Chromatographic system
- a stainless steel column 1.5 m × 4 mm, packed with acid-washed, silanised diatomaceous support (125 to 150 µm) coated with 3 per cent w/w of *polymethyl phenyl siloxane*,
- temperature:
  column. 270°,
  inlet port and detector at 300°,
- flow rate. 20 ml per minute of the carrier gas.

Two peaks due to the two diasterioisomers appear in the chromatogram. Adjust the sensitivity of the detector so that the chromatogram obtained, the height of the taller of the diasterioisomer peaks is about 80 per cent of the full-scale deflection. The area of each peak is not less than 45 per cent and not more than 55 per cent of the sum of the areas of the two peaks. The test is not valid unless the height of the trough separating the two diasterioisomers peaks is less than 5 per cent of the full-scale deflection.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 50 mg of the substance under examination in the mobile phase and dilute to 10 ml with the mobile phase.

*Reference solution*. Dilute 0.5 ml of the test solution to 100 ml with the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm),
- column temperature.30°,
- mobile phase: a degassed mixture of 150 ml of *tetrahydrofuran*, 300 ml of *methanol*, 550 ml of *water*, 0.82 g of *tetrabutylammonium hydrogen sulphate*, 1 g of *sodium octyl sulphate* and 10 ml of a 10 per cent w/v solution of *sulphuric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 229 nm,
- injection volume. 20 µl.

Equilibrate the column with the mobile phase for about 30 minutes.

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with the reference solution is at least 50 per cent of the full scale of the recorder. The retention time of the principal peak is 10 minutes to 15 minutes. If necessary, adjust the water content of the mobile phase ensuring that the 2:1 ratio of methanol to tetrahydrofuran is maintained.



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Inject each solution. Continue the chromatography for three times the retention time of the principal peak in the chromatogram obtained with the test solution; the area of any peak other than the principal peak is not greater than 0.6 times that of the principal peak in the chromatogram obtained with the reference solution (0.3 per cent); the sum of the areas of any such peaks is not greater than the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent). Ignore any peak due to the solvent and any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with the reference solution.

**Heavy metals** (2.3.13). Dissolve 2.0 g in a mixture of 20 ml of *water* and 5 ml of *dilute sodium hydroxide solution*. The solution complies with the limit test for heavy metals, Method C (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° at a pressure not exceeding 0.7 kPa.

**Assay**. Weigh accurately about 0.2 g, dissolve in 10 ml of *anhydrous formic acid* and 40 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03649 g of $C_{19}H_{24}N_2O_3$,HCl.

*NOTE — Mix thoroughly throughout the titration and stop the titration immediately after the end-point is reached.*

**Storage**. Store protected from moisture.

# Labetalol Tablets

Labetalol HydrochlorideTablets

Labetalol Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of labetalol hydrochloride, $C_{19}H_{24}N_2O_3$,HCl.

**Usual strengths**. 50 mg; 100 mg; 200 mg; 400 mg.

## Identification

A. To a quantity of the powdered tablets containing 50 mg of Labetalol Hydrochloride add 50 ml of *0.1 M hydrochloric acid* and heat on a water-bath for 30 minutes. Cool, filter, add 10 ml of *ammonia buffer pH 10.0* and extract with three quantities, each of 15 ml, of *dichloromethane*. Shake the combined extracts with 5 g of *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *labetalol RS* or with the reference spectrum of labetalol.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.004 per cent w/v solution of the residue obtained in test A in *0.1 M sodium hydroxide* shows an absorption maximum only at about 333 nm.

C. Disperse a quantity of the powered tablets containing 10 mg of Labetalol Hydrochloride in a mixture of 2 ml of *water* and 2 ml of *ferric chloride test solution*; a purple colour is produced.

## Tests

**Diasterioisomer ratio**. Determine by gas chromatography (2.4.13).

*Test solution*. Shake a quantity of the powdered tablets containing 0.5 g of Labetalol Hydrochloride with 10 ml of *methanol*, filter and evaporate the filtrate to dryness using a rotary evaporator.

Chromatographic system

- a stainless steel column 1.5 m x 4 mm, packed with acid-washed, silanised diatomaceous support (125 to 150 µm) coated with 3 per cent w/w of *polymethyl phenyl siloxane*,
- temperature:
  column. 270°,
  inlet port and detector at 300°,
- flow rate. 20 ml per minute of the carrier gas.

Two peaks due to the two diasterioisomers appear in the chromatogram. Adjust the sensitivity of the detector so that the chromatogram obtained, the height of the taller of the diasterioisomer peaks is about 80 per cent of the full-scale deflection. The area of each peak is not less than 45 per cent and not more than 55 per cent of the sum of the areas of the two peaks. The test is not valid unless the height of the trough separating the two diasterioisomers peaks is less than 5 per cent of the full-scale deflection.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 75 volumes of *dichloromethane*, 25 volumes of *methanol* and 5 volumes of *strong ammonia solution*.

*Test solution*. Shake a quantity of the powered tablets containing 0.5 g of Labetalol Hydrochloride with 10 ml of *methanol*, filter and use the filtrate.

*Reference solution (a)*. Dilute 1 volume of the test solution to 100 volumes with *methanol*.

*Reference solution (b)*. Dilute reference solution (a) with an equal volume of *methanol*.



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Apply to the plate 20 µl of each solution. After development, dry the plate in a current of warm air, heat at 105° for 30 minutes, cool and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

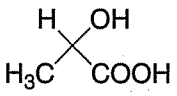
**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.5 g of Labetalol Hydrochloride, shake with 200 ml of *0.05 M sulphuric acid* for 30 minutes and dilute to 250.0 ml with *0.05 M sulphuric acid*, mix and filter. Dilute 10.0 ml of the filtrate to 250.0 ml with the same solvent and measure the absorbance of the resulting solution at the maximum at about 302 nm (2.4.7).

Calculate the content of $C_{19}H_{24}N_2O_3$,HCl taking 86 as the specific absorbance at 302 nm.

**Storage**. Store protected from moisture.

# Lactic Acid




$C_3H_6O_3$ Mol. Wt. 90.1

Lactic Acid consists of a mixture of 2-hydroxypropionic acid, its condensation products, such as lactoyl-lactic acid and other polylactic acids, and water. The equilibrium between lactic acid and polylactic acids depends on concentration and temperature.

It is usually in the form of the racemate [(*RS*)-lactic acid], but in some cases the (*S*)-isomer may predominate.

Lactic Acid contains the equivalent of not less than 88.0 per cent and not more than 92.0 per cent w/w of $C_3H_6O_3$.

**Category**. Pharmaceutical aid.

**Description**. A colourless or slightly yellow, viscous liquid; almost odourless; hygroscopic.

## Identification

A. Warm 1 g with 0.1 g of *potassium permanganate*; acetaldehyde is evolved.

B. Gives reaction A of lactates (2.3.1).

C. A 10 per cent w/v solution is strongly acidic.

## Tests

**Appearance of solution**. The substance under examination is not more intensely coloured than reference solution YS6 (2.4.1).

**Arsenic** (2.3.10). Mix 10.0 g with 50 ml of *water* and 10 ml of *stannated hydrochloric acid*. The resulting solution complies with the limit test for arsenic (1 ppm).

**Heavy metals** (2.3.13). Dissolve 5.0 g in 42 ml of *1 M sodium hydroxide* and dilute to 50 ml with *distilled water*. 12 ml of the resulting solution complies with the limit test for heavy metals, Method D (10 ppm).

**Citric, oxalic and phosphoric acids**. To 5 ml of the solution prepared in the test for Heavy metals add *6 M ammonia* until slightly alkaline. Add 1 ml of *calcium chloride solution* and heat on a water-bath for 5 minutes. Both before and after heating, any opalescence in the solution is not more intense than that in a mixture of 5 ml of the test solution and 1 ml of *water*.

**Ether-insoluble substances**. Dissolve 1.0 g in 25 ml of *ether*; the solution is not more opalescent than the solvent used for the test.

**Volatile fatty acids**. Cautiously heat 5 g in a glass-stoppered flask at 50° for 10 minutes; no unpleasant odour resembling that of the lower fatty acids is recognisable immediately after opening the flask.

**Methanol and methyl esters**. Place 2.0 g in a ground-glass-stoppered round-bottom flask and add 10 ml of *water*. Cool in ice, cautiously add 30 ml of a 30 per cent w/v solution of *potassium hydroxide* and cool in ice for a further 10 to 15 minutes. Steam distil the mixture into a 10-ml graduated cylinder containing 1 ml of *ethanol*, collecting a volume of at least 9.5 ml and dilute to 10.0 ml with *water*. To 1.0 ml of the distillate add 5 ml of *potassium permanganate and phosphoric acid solution* and mix. After 15 minutes add 2 ml of *oxalic acid and sulphuric acid solution*, stir with a glass rod until the solution is colourless and then add 5 ml of *decolorised magenta solution*. After 2 hours any colour in the solution is not more intense than that of 1 ml of a reference solution containing 100 µg of *methanol* and 0.1 ml of *ethanol* treated in the same manner beginning at the words "add 5 ml of *potassium permanganate* and *phosphoric acid solution*..".

**Reducing sugars**. Dilute 1 g with 10 ml of *water*, neutralise with *sodium hydroxide solution*, add 5 ml of *potassium cupri-tartrate solution*, and boil; no red or greenish precipitate is produced.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Assay**. Weigh accurately about 1.0 g in a ground-glass-stoppered flask and add 10 ml of *water*. Add 20.0 ml of *1 M sodium hydroxide*, stopper the flask and allow to stand



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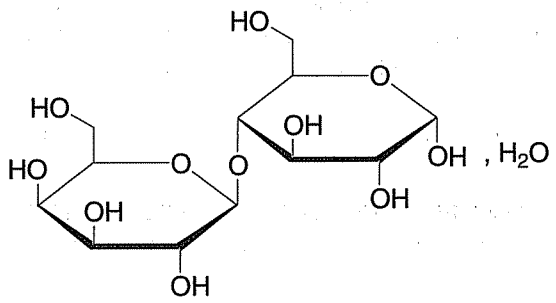
for 30 minutes. Titrate the excess of alkali with *1 M hydrochloric acid*, using *dilute phenolphthalein solution* as indicator until the pink colour is discharged.

1 ml of *1 M sodium hydroxide* is equivalent to 0.09008 g of $C_3H_6O_3$.

**Storage**. Store protected from light.

## Lactose

Lactose Monohydrate; Milk Sugar




$C_{12}H_{22}O_{11},H_2O$ Mol. Wt. 360.3

Lactose is *O*-β-D-galactopyranosyl-(1 → 4)-α-D-glucopyranose monohydrate.

**Category**. Pharmaceutical aid (excipient).

**Description**. A white or almost white, crystalline powder; odourless.

### Identification

*Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *lactose RS* or with the reference spectrum of lactose.

B. To 5 ml of a saturated solution add 5 ml of *1 M sodium hydroxide* and gently warm the mixture; the liquid becomes yellow and then brownish-red. Cool to room temperature and add 0.2 ml of *potassium cupri-tartrate solution*; a red precipitate is formed.

C. Heat 5 ml of a 5 per cent w/v solution with 5 ml of *10 M ammonia* in a water-bath at 80° for 10 minutes; a red colour develops.

### Tests

**Appearance of solution**. Dissolve 1.0 g in *water* by heating to 50°, dilute to 10 ml with *water* and allow to cool. The solution is clear (2.4.1), and not more intensely coloured than reference solution BYS7 (2.4.1).

**Acidity or alkalinity**. Dissolve 6 g in 25 ml of *carbon dioxide-free water* by boiling, cool and add 0.3 ml of *phenolphthalein solution*. The solution is colourless and not more than 0.4 ml of *0.1 M sodium hydroxide* is required to change the colour of the solution to pink.

**Specific optical rotation** (2.4.22). +54.4° to +55.9°, determined in a solution obtained by dissolving 10.0 g in 80 ml of *water* by heating to 50°, allowing to cool, adding 0.2 ml of *6 M ammonia*, allowing to stand for 30 minutes and diluting to 100.0 ml with *water*.

**Light absorption** (2.4.7). Dissolve 1.0 g in boiling *water* and dilute to 10 ml with the same solvent (solution A). Absorbance of solution A measured at the maximum at about 400 nm, not more than 0.04.

Dilute 1 ml of solution A to 10 ml with *water*.

When examined in the range 210 nm to 300 nm, absorbance is not more than 0.25 in the range 210 nm to 220 nm and not more than 0.07 in the range 270 nm to 300 nm.

**Arsenic** (2.3.10). Dissolve 10.0 g in 50 ml of *water* and add 10 ml of *stannated hydrochloric acid AsT*; the resulting solution complies with the limit test for arsenic (1 ppm).

**Heavy metals** (2.3.13). Dissolve 4.0 g in 20 ml of warm *water*, 1.0 ml of *0.1 M hydrochloric acid* and sufficient *water* to produce 25 ml. The solution complies with the limit test for heavy metals, Method A (5 ppm).

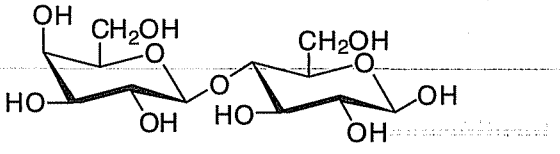
**Microbial contamination** (2.2.9). Total microbial count not more than 100 per g. It complies with the test for Escherichia Coli and Salmonella

**Sulphated ash**. Not more than 0.1 per cent, determined in the following manner. To 1.0 g add 1 ml of *sulphuric acid*, evaporate to dryness on a water-bath and ignite to constant weight.

**Water** (2.3.43). 4.5 to 5.5 per cent, determined on 0.5 g in a mixture of 1 ml of *formamide* and 2 ml of *methanol*.

**Storage**. Store protected from moisture.

## Anhydrous Lactose




$C_{12}H_{22}O_{11}$ Mol. Wt. 342.3

Anhydrous Lactose is 4-*O*-β-D-galactopyranosyl-D-glucose.

**Category**. Pharmaceutical aid (excipient).



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**Description.** A white or almost white, crystalline powder.

### Identification

*Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *anhydrous lactose RS* or with the reference spectrum of anhydrous lactose.

B. To 5 ml of a saturated solution add 5 ml of *1 M sodium hydroxide* and gently warm the mixture; the liquid becomes yellow and then brownish-red. Cool to room temperature and add 0.2 ml of *potassium cupri-tartrate solution*; a red precipitate is formed.

C. Heat 5 ml of a 5.0 per cent w/v solution with 5 ml of *10 M ammonia* in a water-bath at 80° for 10 minutes; a red colour develops.

### Tests

**Appearance of solution.** Dissolve 1.0 g in *water* by heating to 50°, dilute to 10 ml with *water* and allow to cool. The solution is clear (2.4.1), and not more intensely coloured than reference solution BYS7 (2.4.1).

**Acidity or alkalinity.** Dissolve 6 g of the substance under examination in 25 ml of *carbon dioxide-free water* by boiling, cool and add 0.3 ml of *phenolphthalein solution*. The solution is colourless and not more than 0.4 ml of *0.1 M sodium hydroxide* is required to change the colour of the solution to pink.

**Specific optical rotation** (2.4.22). +54.4° to +55.9°, determined in a solution prepared by dissolving 10 g in 80 ml of *water* by heating to 50°, allow to cool and add 0.2 ml of *6 M ammonia*. Allow to stand for 30 minutes and dilute to 100.0 ml with *water*.

**Light absorption** (2.4.7). A 10.0 per cent w/v solution in *water*, shows an absorption maxima at about 400 nm is not more than 0.04. Dilute 1.0 ml of the solution to 10.0 ml with *water*. When examined in the range 210 nm to 220 nm; absorbance is not more than 0.25 and in the range 270 nm to 300 nm, absorbance is not more than 0.07.

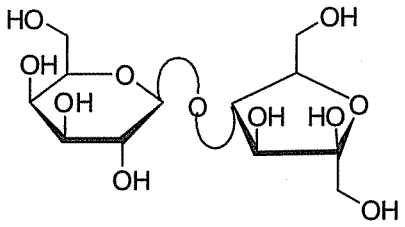
**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals, Method D (5 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 1.0 per cent, determined on 0.5 g in a mixture of 1 volume of *formamide* and 2 volumes of *methanol*.

**Microbial contamination** (2.2.9). Total microbial count not more than 100 CFU per g, 1 g is free from *Escherichia coli*.

## Lactulose




$C_{12}H_{22}O_{11}$ Mol. Wt. 342.3

Lactulose is 4-*O*-β-D-galactopyranosyl-D-fructose.

Lactulose contains not less than 95.0 per cent and not more than 102.0 per cent of lactulose, $C_{12}H_{22}O_{11}$, calculated on the anhydrous basis.

**Category.** Pharmaceutical aid.

**Description.** A white or almost white, crystalline powder.

### Identification

*Test A may be omitted if tests B, C and D are carried out and test B may be omitted if tests A, C and D are carried out.*

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase.* A mixture of 10 volumes of *glacial acetic acid*, 15 volumes of 5 per cent w/v solution of *boric acid*,, 20 volumes of *methanol* and 55 volumes of *ethyl acetate*.

*Test solution.* Dissolve 50 mg of the substance under examination in 10.0 ml of *water*.

*Reference solution.* A 0.5 per cent w/v solution of *lactulose RS* in *water*.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate at 105° for 5 minutes and allow to cool. Spray with a 0.1 per cent w/v solution of *1,3-dihydroxynaphthalene* in a mixture of 10 volumes of *sulphuric acid* and 90 volumes of *methanol*. Heat at 110° for 5 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with reference solution (b).

C. Dissolve 50 mg in 10 ml of *water*. Add 3 ml of *cupri-tartaric solution* and heat. A red precipitate is formed.

D. Dissolve 0.125 g in 5 ml of *water* and add 5 ml of *ammonia*. Heat on a water-bath at 80° for 10 minutes. A red colour develops.



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## Tests

*Solution A.* Dissolve 3.0 g in 50.0 ml of *carbon dioxide-free water.*

**Appearance of solution**. Solution A is clear (2.4.1) and not more intensely coloured than reference solution $BY_5$ (2.4.1).

**pH** (2.4.24). 3.0 to 7.0, determined in solution A.

**Specific optical rotation** (2.4.22). – 46.0° to – 50.0°, determined in a 5 per cent w/v solution in *water.*

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve 1 g of the substance under examination in 10 ml of *water*. Add 12.5 ml of *acetonitrile* with gentle heating and dilute to 25.0 ml with *water.*

*Reference solution (a).* To 3 ml of the test solution, add 47.5 ml of *acetonitrile* with gentle heating and dilute to 100.0 ml with *water*.

*Reference solution (b).* Dissolve 1.0 g of *lactulose RS* in 10 ml of *water,* add 12.5 ml of *acetonitrile* with gentle heating and dilute to 25.0 ml with *water*.

*Reference solution (c).* Dissolve the contents of a vial of *lactulose for system suitability RS* in 1 ml of a mixture of equal volumes of *acetonitrile* and *water*.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with aminopropylsilane bonded to porous silica (3 µm),
- column temperature. 38°,
- mobile phase: a mixture of 220 volumes of a solution prepared by dissolving 1.15 g of *sodium dihydrogen orthophosphate* in 1000 ml of *water* and 780 volumes of *acetonitrile,*
- flow rate. 1 ml per minute,
- refractive index detector,
- injection volume. 20 µl.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to lactulose and lactulose impurity A is not less than 1.3. The relative retention time with reference to lactulose for tagatose (lactulose impurity E) is about 0.38, for fructose (lactulose impurity D) is about 0.42, for galactose (lactulose impurity B) is about 0.57, for epilactose (lactulose impurity A) is about 0.9 and for lactose (lactulose impurity C) is about 1.17.

Inject reference solution (a) and the test solution. Run the chromatogram 2.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (3.0 per cent).

**Methanol**. Not more than 20 ppm.

Determine by gas chromatography (2.4.13).

*Test solution.* To 79 mg of the substance under examination in a 20 ml vial, add 1.0 ml of the internal standard solution and 5 µl of a 0.1 per cent v/v solution of *methanol.*

*Internal standard solution.* Mix 0.5 ml of *propanol* with 100.0 ml of *water.* Dilute 1.0 ml of this solution to 100.0 ml with *water*. Further dilute 5.0 ml of the solution to 50.0 ml with *water*.

*Reference solution.* To 1.0 ml of the internal standard solution in a 20 ml vial, add 5 µl of a 0.1 per cent v/v solution of *methanol.*

Chromatographic system
- a stainless steel column 2 m x 2 mm, packed with ethylvinylbenzene-divinylbenzene copolymer (180 µm),
- temperature:
  column 148°,
  injector port at 200° and detector at 220°,
- flame ionization detector,
- flow rate. 30 ml per minute using nitrogen as the carrier gas.

Inject 1 ml of the gaseous phase of each solution.

Calculate the content of methanol.

The ratio of the area of the peak due to methanol to the area of the peak due to the internal standard in the chromatogram obtained with the test solution is not more than 50 ppm.

**Boron**. Not more than 9 ppm.

*NOTE—Avoid where possible the use of glassware.*

*Reference solution.* Dissolve 50 mg of *boric acid* in 100.0 ml of *water*. Dilute 5.0 ml of this solution to 100.0 ml with *water*. *Keep in a well-closed polyethylene container.*

In first polyethylene 25-ml flask, dissolve 0.5 g of the substance under examination in 2.0 ml of *water* (solution A), in second polyethylene 25-ml flask, dissolve 0.5 g of the substance under examination dissolved in 1.0 ml of the reference solution and 1.0 ml of *water* (solution B), in third polyethylene 25 ml flask, dilute 1.0 ml of the reference solution with 1.0 ml of *water* (solution C) and in fourth polyethylene 25 ml flask, take 2.0 ml of *water* (solution D).

To each flask, add 4.0 ml of *acetate-edetate buffer solution pH 5.5*. Mix and add 4.0 ml of freshly prepared *azomethine solution*. Mix and allow to stand for 1 hour. Measure the absorbance of solutions A, B and C at 420 nm (2.4.7), using solution D as the compensation liquid. The test is not valid unless the absorbance of solution C is not less than 0.25. The absorbance of solution B is not less than twice that of solution A.

**Lead** (2.3.15). Not more than 0.5 ppm.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.



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**Water** (2.3.43). Not more than 2.5 per cent, determined on 0.5 g.

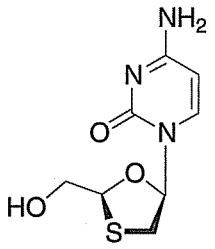
**Microbial contamination** (2.2.9). Total viable aerobic count not more than $10^2$ micro-organisms per gram, determined by plate count. It complies with the test for *Escherichia coli.*

**Assay**. Determine by liquid chromatography (2.4.14), as described in the test for Related substances with the following modification.

Inject reference solution (b) and the test solution.

Calculate the content of $C_{12}H_{22}O_{11}$.

# Lamivudine




$C_8H_{11}N_3O_3S$ Mol. Wt. 229.3

Lamivudine is (2*R*,5*S*)-4-amino-1-[2-(hydroxymethyl)-1,3-oxathiolan-5yl]-2(1*H*)-pyrimidinone.

Lamivudine contains not less than 98.0 per cent and not more than 102.0 per cent of $C_8H_{11}N_3O_3S$, calculated on the anhydrous basis.

**Category**. Antiretroviral.

**Dose**. 150 mg twice daily.

**Description**. A white or almost white powder.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *lamivudine RS* or with the reference spectrum of lamivudine.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to lamivudine in the chromatogram obtained with the reference solution.

C. Melting range (2.4.21). 172° to 178°.

## Tests

**Specific optical rotation** (2.4.22). –136° to –144°, determined in a 1.0 per cent w/v solution in *methanol.*

**Related substances**. Determine by liquid chromatography (2.4.14), as described in the Assay but using the following solutions.

*Test solution.* Dissolve 0.1 g of the substance under examination in 100 ml mobile phase.

*Reference solution.* A solution containing 0.001 per cent w/v each of *lamivudine RS* and *salicylic acid* in the mobile phase.

Inject the reference solution and record the chromatogram for at least twice the retention time of lamivudine. The order of elution is lamivudine and then, salicylic acid. The test is not valid unless the resolution between the peaks due to lamivudine and salicylic acid is not less than 10, the column efficiency determined from lamivudine peak is not less than 5000 theoretical plates and the tailing factor is not more than 1.5.

Separately inject the test solution and the reference solution and measure the peak responses for each impurity. In the chromatogram obtained with the test solution, the area of any peak other than the principal peak is not greater than half of the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of the areas of all such peaks is not greater than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent).

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Water** (2.3.43). Not more than 0.5 per cent determined on 2.0 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve 25 mg of the substance under examination in 100 ml of mobile phase.

*Reference solution.* A 0.025 per cent w/v solution of *lamivudine RS* in mobile phase.

Chromatographic system

- a stainless steel column 15 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 35°,
- mobile phase: a degassed mixture of 5 volumes of *methanol* and 95 volumes of a buffer prepared by dissolving 1.9 g of *ammonium acetate* in 1000 ml of *water* and adjusting the pH to 3.8 ± 0.2 with *glacial acetic acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 270 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency determined from the lamivudine peak is not



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less than 5000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternatively the test solution and the reference solution.

Calculate the content of $C_8H_{11}N_3O_3S$.

**Storage**. Store protected from light and moisture.

## Lamivudine Oral Solution

Lamivudine Oral Solution is a solution of Lamivudine in a suitable flavoured vehicle.

Lamivudine Oral Solution contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of lamivudine, $C_8H_{11}N_3O_3S$.

**Usual strengths**. 25 mg in 5 ml; 50 mg in 5 ml.

### Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 40 volumes of *1-butanol*, 30 volumes of *heptane*, 30 volumes of *acetone* and 10 volumes of *strong ammonia solution*.

*Test solution*. Dilute the preparation under examination with *methanol* to obtain a solution containing 2 mg of lamivudine per ml.

*Reference solution*. A 0.2 per cent w/v solution of *lamivudine RS* in a mixture of 75 volumes *methanol* and 25 volumes of *water*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to lamivudine in the chromatogram obtained with the reference solution.

### Tests

**pH** (2.4.24). 5.0 to 7.0.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Transfer an accurately measured volume of the preparation under examination containing 50 mg of lamivudine to a 50-ml volumetric flask, add about 30 ml of a solution prepared by mixing 10 volumes of *acetonitrile* and 90 volumes of *0.1 M potassium dihydrogen phosphate*, the pH of which is adjusted to 3.0 with *dilute phosphoric acid* (solution A), mix with the aid of ultrasound for 5 minutes, dilute to volume with solution A and filter. Dilute 5 ml of the filtrate to 50 ml with solution A.

*Reference solution*. Weigh accurately about 25 mg of *lamivudine RS* and transfer to a 50-ml volumetric flask, dissolve and dilute to volume with solution A. Further dilute 5 ml of this solution to 250 ml with solution A.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm),
- mobile phase: degassed gradient mixtures of *acetonitrile* and *0.05 M potassium dihydrogen phosphate*, adjusting the pH of the solution to 3.0 with *dilute phosphoric acid*,
- flow rate. 0.8 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 270 nm,
- injection volume. 20 µl.

| *Time* (in min.) | *Phosphate buffer (pH 3.0)* (per cent v/v) | *Acetonitrile* ( per cent v/v) |
|---|---|---|
| 0 | 98 | 02 |
| 10 | 85 | 15 |
| 25 | 30 | 70 |
| 35 | 30 | 70 |
| 40 | 98 | 02 |
| 50 | 98 | 02 |

Inject the reference solution. The test is not valid unless the column efficiency determined from the lamivudine peak is not less than 15,000 theoretical plates and the tailing factor is not more than 1.5.

Inject solution A and the test solution. Examine the chromatogram obtained with solution A for any extraneous peaks and ignore the corresponding peaks observed in the chromatogram obtained with the test solution. Ignore any peaks due to preservatives also.

Any secondary peak observed in the chromatogram obtained with the test solution should not be more than 1.0 per cent and the sum of the areas of all the secondary peaks should not be more than 2.0 per cent when calculated by percentage area normalisation.

**Other tests**. Comply with the tests stated under Oral Liquids.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh accurately a quantity of the preparation under examination containing about 100 mg of lamivudine in a 100-ml volumetric flask, add about 50 ml of *water*, mix with the



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aid of ultrasound for 10 minutes, dilute to volume with *water*, mix and filter. Dilute 5.0 ml of the filtrate to 50.0 ml with *water*.

*Reference solution*. Weigh accurately about 100 mg of *lamivudine RS* in a 100-ml volumetric flask, dissolve and dilute to volume with *water*. Dilute 5.0 ml of this solution to 50.0 ml with *water*.

Chromatographic system
- a stainless steel column 15 cm × 4.6 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm),
- mobile phase: 80 volumes of *water* and 20 volumes of *methanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 270 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency determined from the lamivudine peak is not less than 3000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for the replicate injections is not more than 2.0 per cent.

Inject separately the test solution and the reference solution and measure the responses for the major peak.

Determine the weight per ml (2.4.29) of the oral solution and calculate the content of $C_8H_{11}N_3O_3S$ weight in volume.

# Lamivudine Tablets

Lamivudine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of lamivudine, $C_8H_{11}N_3O_3S$.

**Usual strengths**. 100 mg; 150 mg; 300 mg.

## Identification

A. When examined in the range 200 nm to 400 nm (2.4.7), a 0.001 per cent w/v solution in a mixture of 50 volumes of *water* and 50 volumes of *acetonitrile*, shows an absorption maximum at about 270 nm.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to lamivudine in the chromatogram obtained with the reference solution.

## Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Shake a quantity of the powdered tablets with a suitable quantity of *water*, and disperse with the aid of ultrasound. Add a quantity of *acetonitrile* containing half of the final volume to get a final concentration of 0.6 per cent w/v of lamivudine. Mix with the aid of ultrasound for 10 minutes and make up the volume with *water*. Filter through a membrane filter disc with an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate.

*Reference solution*. A 0.024 per cent w/v solution of *lamivudine RS* in a mixture of 80 volumes of *water* and 20 volumes of *acetonitrile*.

Chromatographic system as described under Assay

Inject the reference solution. The test is not valid unless the column efficiency determined from the lamivudine peak is not less than 3000 theoretical plates and the tailing factor is not more than 2.0.

Separately inject the test solution. Calculate the amount of related substances by the area normalisation method. The content of any individual impurity is not more than 1.0 per cent and the sum of all impurities is not more than 2.0 per cent.

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.01 M hydrochloric acid*,
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first 1 ml of the filtrate. Measure the absorbance of the filtrate, suitably diluted if necessary, at the maximum at about 270 nm (2.4.7). Calculate the content of $C_8H_{11}N_3O_3S$, in the medium from the absorbance obtained from a solution of known concentration of *lamivudine RS* in *0.01 M hydrochloric acid*.

D. Not less than 70 per cent of the stated amount of $C_8H_{11}N_3O_3S$.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh and powder 20 tablets. Shake a quantity of the powder containing about 100 mg of Lamivudine with a mixture of 50 volumes of *water* and 50 volumes of *acetonitrile* to have a final concentration of 0.024 per cent of Lamivudine. Disperse with the aid of ultrasound and filter through a membrane filter disc with an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate.

*Reference solution*. A 0.024 per cent w/v solution of *lamivudine RS* in the same solvent mixture.

Chromatographic system
- a stainless steel column 15 cm × 4.6 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm),
- mobile phase: a degassed mixture of 5 volumes of *methanol* and 95 volumes of a buffer prepared by



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dissolving 1.0 g of *ammonium acetate* and 1.0 ml of *glacial acetic acid* in sufficient *water* to make 1000 ml and adjusting the pH to 3.8 with *glacial acetic acid*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 270 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency determined from the lamivudine peak is not less than 3000 theoretical plates and the tailing factor is not more than 2.0.

Separately inject the test solution and the reference solution and measure the peak responses of the major peak.

Calculate the content of $C_8H_{11}N_3O_3S$ in the tablets.

**Storage**. Store protected from moisture.

## Lamivudine and Tenofovir Tablets

Lamivudine and Tenofovir Disoproxil Fumarate Tablets

Lamivudine and Tenofovir Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of lamivudine, $C_8H_{11}N_3O_3S$ and tenofovir disoproxil fumarate, $C_{19}H_{30}N_5O_{10}P,C_4H_4O_4$.

**Usual strengths**. Lamivudine 300 mg and Tenofovir 600 mg .

### Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

### Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14)

*Test solution*. The filtrate obtained as given above. Dilute the filtrate if necessary, with the dissolution medium.

*Reference solution*. 5.0 ml of each of a 0.16 per cent w/v solution of *lamivudine RS* and of *tenofovir disoproxil fumarate RS* in *methanol*, diluted to 25 ml with the dissolution medium.

Use the chromatographic system given in the Assay.

Inject the reference solution. The resolution between the peaks due to lamivudine and tenofovir disoproxil is not less than 2.0.

Inject the test solution and the reference solution.

Calculate the contents of $C_8H_{11}N_3O_3S$ and $C_{19}H_{30}N_5O_{10}P,C_4H_4O_4$.

D. Not less than 75 per cent of the stated amounts of $C_8H_{11}N_3O_3S$ and $C_{19}H_{30}N_5O_{10}P,C_4H_4O_4$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*NOTE — Prepare the solutions immediately before use.*

*Test solution*. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 100 mg of Lamivudine, disperse in 100 ml of mobile phase A and filter.

*Reference solution (a)*. A solution containing 0.1 per cent w/v of *lamivudine RS* and *tenofovir disoproxil fumarate RS* equivalent to 0.1 per cent w/v of tenofovir disoproxil in mobile phase A.

*Reference solution (b)*. Dilute 1 ml of the solution to 100 ml with mobile phase A.

*Reference solution (c)*. A 0.02 per cent w/v solution of fumaric acid in mobile phase A.

Chromatographic system
- a stainless steel column 15 cm × 4.6 mm, packed octadecylsilane bonded to porous silica (5 µm),
- column temperature. 35°,
- mobile phase: A. a mixture of 95 volumes of a buffer solution prepared by dissolving 1.9 g of *ammonium acetate* in 1000 ml of *distilled water* and adjusting the pH to 3.8 with *glacial acetic acid* and 5 volumes of *methanol*,

  B. *methanol*,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 277 nm,
- injection volume. 10 µl.

| Time (in min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0 | 100 | 0 |
| 30 | 100 | 0 |
| 31 | 80 | 20 |
| 35 | 50 | 50 |
| 60 | 50 | 50 |
| 61 | 80 | 20 |
| 65 | 100 | 0 |
| 67 | 100 | 0 |



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Inject reference solution (a). The test is not valid unless the column efficiency determined from lamivudine and tenofovir disoproxil peaks is not less than 750 and 1500 theoretical plates respectively and the tailing factor is not more than 2.0.

Inject the test solution, reference solutions (b) and (c). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 3.5 times the area of any peak in the chromatogram obtained with the reference solution (b) (3.5 per cent) and the sum of areas of all the secondary peaks is not more than 6 times the area of any peak in the chromatogram obtained with the reference solution (b) (6.0 per cent). Ignore the peak corresponding to the peak in the chromatogram obtained with reference solution (c).

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*NOTE — Prepare the solutions immediately before use.*

*Test solution*. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 20 mg of Lamivudine, dissolve in 100 ml of the mobile phase and filter.

*Reference solution*. A 0.1 per cent w/v solution each of *lamivudine RS* and *tenofovir disoproxil fumarate RS* in the mobile phase. Dilute 20.0 ml of the solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica ( 5 µm),
- column temperature. 35°,
- mobile phase: a mixture of 50 volumes of a buffer solution prepared by dissolving 7.8 g of *sodium dihydrogen orthophosphate dihydrate* in 1000 ml of *distilled water*, adding 1 ml of *triethylamine* and adjusting the pH to 2.3 with *orthophosphoric acid*, and 50 volumes of acetonitrile,
- flow rate. 1 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency determined from the peaks due to lamivudine and tenofovir disoproxil is not less than 750 and 1500 theoretical plates respectively, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent for each component.

Inject the test solution and the reference solution.

Calculate the content of $C_8H_{11}N_3O_3S$ and $C_{19}H_{30}N_5O_{10}P,C_4H_4O_4$ in the tablets.

**Storage**. Store protected from moisture, at temperature not exceeding 30°.

# Lamivudine and Zidovudine Tablets

Lamivudine and Zidovudine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of lamivudine, $C_8H_{11}N_3O_3S$, and zidovudine, $C_{10}H_{13}N_5O_4$.

**Usual strength**. Lamivudine, 150 mg and zidovudine, 300 mg.

## Identification

In the Assay, the two principal peaks in the chromatogram obtained with the test solution correspond to the peaks due to lamivudine and zidovudine in the chromatogram obtained with the reference solution.

## Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first 1 ml of the filtrate.

Determine by liquid chromatography (2.4.14).

*Test solution*. Dilute 5.0 ml of the filtrate to 50.0 ml with the mobile phase.

*Reference solution*. Weigh accurately about 30 mg of *lamivudine RS* and 60 mg of *zidovudine RS*, transfer into a 100-ml volumetric flask, dissolve and dilute to volume with the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with *0.1 M hydrochloric acid*. Further dilute 5.0 ml of this solution to 20.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm),
- mobile phase: a degassed mixture of 60 volumes of a buffer solution containing *0.1 M ammonium acetate* in 0.1 per cent *acetic acid* and 40 volumes of *methanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 270 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections for each of the peaks corresponding to lamivudine and zidovudine is not more than 2.0 per cent.

Separately inject the test solution and the reference solution and measure the responses of the major peaks due to lamivudine and zidovudine. Calculate the contents of $C_8H_{11}N_3O_3S$ and $C_{10}H_{13}N_5O_4$.



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D. Not less than 75.0 per cent of the stated amounts of $C_8H_{11}N_3O_3S$ and $C_{10}H_{13}N_5O_4$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh accurately a quantity of the powdered tablets containing the average weight of one tablet, transfer to a 100-ml volumetric flask and add about 50 ml of *methanol*. Disperse with the aid of ultrasound for about 10 minutes with occasional shaking to obtain a uniform dispersion, cool to room temperature, dilute to volume with *methanol* and mix. Filter through a membrane filter disc with an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate.

*Reference solution (a)*. A 0.015 per cent w/v solution of *salicylic acid* in *methanol*.

*Reference solution (b)*. A solution containing 0.01 per cent w/v each of *thymine* and β-*thymidine* in *methanol*.

*Reference solution (c)*. Transfer 5 ml of reference solution (a) and 15 ml of reference solution (b) to a 100-ml volumetric flask and dilute to volume with *methanol*.

*Reference solution (d)*. Weigh accurately about 300 mg of *zidovudine RS* and about 150 mg of *lamivudine RS*, transfer to a 100-ml volumetric flask, add 30 ml of reference solution (b) and 20 ml of reference solution (a) and disperse with the aid of ultrasound for about 15 minutes to dissolve. Dilute to volume with *methanol* and filter through a membrane filter disc with an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm),
- column temperature. 35°,
- mobile phase: A. a buffer solution prepared by dissolving 1.945 g of *ammonium acetate* in 900 ml of *water*, adjusted to pH 3.8 with *glacial acetic acid* and diluting to 1000.0 ml with *water*,
  B. *methanol*,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 270 nm,
- injection volume. 10 µl.

| Time (in min.) | Mobile phase A ( per cent v/v) | Mobile phase B ( per cent v/v) |
|---|---|---|
| 0 | 90 | 10 |
| 10 | 90 | 10 |
| 25 | 80 | 20 |
| 40 | 90 | 10 |
| 45 | 90 | 10 |

Inject reference solution (d). The test is not valid unless the tailing factor of the lamivudine and zidovudine peaks is not more than 2.0 and the relative standard deviation for replicate injections for each of the peaks corresponding to lamivudine and zidovudine is not more than 2 per cent.

Separately inject the test solution and record the chromatograms for at least two times the retention time of the zidovudine peak. Separately inject reference solutions (a), (b) and (c).

Calculate the amounts of the related substances by the area normalisation method. The content of thymine is not greater than 2.0 per cent, of b-thymidine is not greater than 1.0 per cent, of salicylic acid is not greater than 0.2 per cent and of any unknown impurity not greater than 0.5 per cent. The sum of all the impurities is not greater than 3.0 per cent.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh and powder 20 tablets. Transfer an accurately weighed quantity of the powder equivalent to the average weight of one tablet into a 200-ml volumetric flask. Add 100 ml of the mobile phase and disperse with the aid of ultrasound for about 15 minutes with occasional shaking to obtain a uniform dispersion. Cool to room temperature and dilute to volume with the mobile phase. Filter the solution through a membrane filter disc with an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate. Dilute 5.0 ml of the filtrate to 25.0 ml with the mobile phase. Further dilute 5.0 ml to 50.0 ml with the mobile phase and mix.

*Reference* solution. Weigh accurately about 30 mg of *lamivudine RS* and 60 mg of *zidovudine RS*, transfer to a 100 ml volumetric flask, dissolve in the mobile phase and dilute to volume with the mobile phase. Further dilute 5.0 ml of this solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm),
- mobile phase: a degassed mixture of 60 volumes of a buffer solution containing *0.1 M ammonium acetate* in 0.1 per cent *acetic acid* and 40 volumes of *methanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 270 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections for each of the peaks corresponding to lamivudine and zidovudine is not more than 2.0 per cent.

Separately inject the test solution and the reference solution and measure the peak responses of the major peaks.



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Calculate the contents of $C_8H_{11}N_3O_3S$ and $C_{10}H_{13}N_5O_4$ in the tablets.

**Storage**. Store protected from moisture.

## Lamivudine, Nevirapine and Stavudine Dispersible Tablets

Lamivudine, Nevirapine and Stavudine Dispersible Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of lamivudine, $C_8H_{11}N_3O_3S$, nevirapine, $C_{15}H_{14}N_4O$ and stavudine, $C_{10}H_{12}N_2O_4$.

**Usual strengths**. Lamivudine 30 mg, Nevirapine 6 mg and Stavudine 50 mg; Lamivudine 60 mg, Nevirapine 12 mg and Stavudine 100 mg.

### Identification

In the Assay, the principal peaks in the chromatogram obtained with the test solution correspond to the peaks in the chromatogram obtained with the reference solution.

### Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

*Solvent mixture*. 50 volumes of *water* and 50 volumes of *methanol*.

*Test solution*. The filtrate obtained as given above, diluted suitably if necessary, with the dissolution medium.

*Reference solution*. A solution containing 0.02 per cent w/v of *stavudine RS*, 0.09 per cent w/v *lamivudine RS* and 0.15 per cent w/v of *nevirapine RS* dissolved in minimum quantity of *methanol* and makeup with solvent mixture. Dilute 5 ml of the solution to 100 ml with the dissolution medium.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 65 volumes of a buffer solution prepared by dissolving 1 g of *octane sulphonic acid sodium salts* and 0.68 g of *potassium dihydrogen phosphate* in 1000 ml of *water*, adding 1 ml of *triethylamine* and adjusted to pH 2.5 with *orthophosphoric acid*, and 35 volumes of *methanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 266 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5 for each component, the column efficiency in not less than 2000 theoretical plates for lamivudine and the relative standard deviation for replicate injections is not more than 2.0 per cent for each component.

Inject the test solution and the reference solution.

Calculate the content of $C_8H_{11}N_3O_3S$, $C_{15}H_{14}N_4O$ and $C_{10}H_{12}N_2O_4$.

D. Not less than 80 per cent of the stated amounts of $C_8H_{11}N_3O_3S$, $C_{15}H_{14}N_4O$ and $C_{10}H_{12}N_2O_4$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*NOTE — Prepare the solutions immediately before use.*

*Solvent mixture*. 70 volumes of 0.2 per cent v/v *orthophosphoric acid* and 30 volumes of *methanol*.

*Test solution*. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 75 mg of Lamivudine, dissolve in 100 ml of the solvent mixture and filter.

*Reference solution (a)*. Weigh accurately about 75 mg of *lamivudine RS*, 130 mg of *nevirapine RS* and 20 mg of *stavudine RS*, dissolve in 20 ml of *methanol* and dilute to 100 ml with the solvent mixture. Dilute 5 ml of the solution to 50 ml with the solvent mixture.

*Reference solution (b)*. Dissolve 0.38 mg of Thymine and 0.15 mg of Carboxylic acid in 50 ml of the solvent mixture, add 10 ml of reference solution (a), dilute to 100 ml with the solvent mixture and filter.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. *methanol*,
  B. a buffer solution prepared by dissolving 1.925 g of *ammonium acetate* in 1000 ml of *water* and adjusted to pH 3.0 with *trifluoroacetic acid*,
- flow rate. 1.2 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 266 nm,
- injection volume. 10 µl.

| Time (min.) | Mobile phase A ( per cent v/v) | Mobile phase B ( per cent v/v) |
|---|---|---|
| 0 | 5 | 95 |
| 15 | 15 | 85 |
| 30 | 45 | 55 |
| 40 | 60 | 40 |
| 48 | 60 | 40 |
| 51 | 5 | 95 |
| 60 | 5 | 95 |



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Inject reference solution (b). The relative retention times for carboxylic acid is 0.36, for thymine is 0.42, for lamivudine, 0.74, for stavudine, 1.0 and for nevirapine, 2.19. The test is not valid unless the tailing factor is not more than 1.5 and the resolution between carboxylic acid and thymine is not less than 2.0.

Inject the test solution. Any individual impurity each for lamivudine and nevirapine is not more than 1 per cent and for stavudine, is not more than 3 per cent and the sum of all impurities is not more than 5.0 per cent.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. 50 volumes of *water* and 50 volumes of *methanol*.

*Test solution*. Weigh accurately a quantity of the powdered tablets containing 50 mg of Lamivudine, dissolve in 100.0 ml of solvent mixture. Dilute 10.0 ml of the solution to 50.0 ml with the solvent mixture and filter.

*Reference solution*. A solution containing 0.10 per cent w/v of *lamivudine RS*, 0.175 per cent w/v *nevirapine RS* and 0.025 per cent w/v of *stavudine RS* dissolved in minimum quantity of *methanol* and makeup with solvent mixture. Dilute 5.0 ml of the solution to 50.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 65 volumes of a buffer solution prepared by dissolving 1 g of *octane sulphonic acid* and 0.68 g of *potassium dihydrogen phosphate* in 1000 ml of *water*, adding 1 ml of *triethylamine* and adjusting the pH to 2.5 with *orthophosphoric acid*, and 35 volumes of *methanol*.
- flow rate. 1 ml per minute.
- spectrophotometer set at 266 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5 for each component, the column efficiency in not less than 2000 theoretical plates for lamivudine and the relative standard deviation for replicate injections is not more than 2.0 per cent for each component.

Inject the test solution and the reference solution.

Calculate the content of $C_8H_{11}N_3O_3S$, $C_{15}H_{14}N_4O$ and $C_{10}H_{12}N_2O_4$ in the tablets.

**Storage**. Store protected from moisture, at a temperature not exceeding 25°.

# Lamivudine, Nevirapine and Stavudine Tablets

Lamivudine, Nevirapine and Stavudine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of lamivudine, $C_8H_{11}N_3O_3S$, nevirapine, $C_{15}H_{14}N_4O$, and stavudine, $C_{10}H_{12}N_2O_4$.

**Usual strengths**. Stavudine, 30 mg, Lamivudine, 150 mg and Nevirapine, 200 mg; Stavudine, 40 mg, Lamivudine, 150 mg and Nevirapine, 200 mg.

## Identification

In the Assay, the three principal peaks in the chromatogram obtained with the test solution have retention times similar to those of the peaks due to lamivudine, nevirapine and stavudine in the chromatogram obtained with the reference solution.

## Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.01 M hydrochloric acid,*
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate.

Determine by liquid chromatography (2.4.14).

*Test solution*. Use the filtrate as the test solution.

*Reference solution*. Weigh accurately about 150 mg of *lamivudine RS*, 200 mg of *nevirapine RS* and 30 mg of *stavudine RS* (if claim of stavudine per tablet is 30 mg) or 40 mg of *stavudine RS* (if claim of stavudine per tablet is 40 mg) and transfer to a 100-ml volumetric flask. Add about 20 ml of *methanol*, disperse with the aid of ultrasound to dissolve and dilute to volume with a solvent mixture of equal volumes of *methanol* and *water*. Dilute 5.0 ml of this solution to 50.0 ml with *0.01 M hydrochloric acid*.

Chromatographic system

- a stainless steel column 15 cm × 4.6 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm),
- mobile phase: a degassed mixture of 35 volumes of *methanol* and 65 volumes of a buffer prepared by dissolving 0.68 g of *potassium dihydrogen phosphate* and 1.0 g of *sodium octanesulphonate* in 1000.0 ml of *water* to which 1 ml of *triethylamine* is added and adjusted to pH 2.5 with *phosphoric acid*,



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- flow rate. 1 ml per minute,
- spectrophotometer set at 266 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency determined from the lamivudine peak is not less than 2000 theoretical plates, the tailing factor for the individual lamivudine, nevirapine and stavudine peaks is not more than 1.5 and the relative standard deviation for replicate injections of all the analyte peaks is not more than 1.0 per cent.

Separately inject the test solution and measure the peak responses of the major peaks due to lamivudine, nevirapine and stavudine. Calculate the contents of $C_8H_{11}N_3O_3S$, $C_{15}H_{14}N_4O$, and $C_{10}H_{12}N_2O_4$ respectively.

D. Not less than 70 per cent of the stated amounts of $C_8H_{11}N_3O_3S$, $C_{15}H_{14}N_4O$ and $C_{10}H_{12}N_2O_4$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh accurately a quantity of the powdered tablets (a minimum of 2 tablets should be powdered) containing 100 mg of nevirapine, transfer to a 200-ml volumetric flask and add about 150 ml of *water*. Disperse with the aid of ultrasound for 10 minutes with occasional shaking to obtain a uniform dispersion, cool to room temperature, dilute to volume with *water* and mix. Filter through a membrane filter disc with an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate.

*Reference solution (a)*. A solution containing 0.15 per cent w/v of *stavudine RS* and 0.015 per cent w/v of *thymine RS* in *water*.

*Reference solution (b)*. Weigh accurately about 75 mg of *lamivudine RS* and about 100 mg of *nevirapine RS*, transfer to a 200-ml volumetric flask, add 20 ml of *methanol* and mix with the aid of ultrasound to dissolve. Add 10 ml of the test solution to this solution and make up to volume with *water* and filter.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: A. *0.1 M ammonium acetate*,
  B. *acetonitrile*,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 270 nm,
- injection volume. 20 µl.

| Time (in min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0 | 95 | 05 |
| 05 | 95 | 05 |
| 25 | 20 | 80 |
| 30 | 20 | 80 |
| 31 | 95 | 05 |
| 35 | 95 | 05 |

Separately inject reference solutions (a) and (b). The test is not valid unless the column efficiency determined for the thymine, stavudine, lamivudine and nevirapine peaks is not less than 3000 theoretical plates and the tailing factor for the same peaks is not more than 2.0.

Separately inject the test solution and measure the peak responses of the major peaks due to lamivudine, nevirapine and stavudine. Calculate the amounts of related substances by the area normalisation method. The content of thymine is not greater than 3.0 per cent and that of any other impurity is not greater than 1.0 per cent. The sum of all the impurities is not greater than 3.5 per cent.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh and powder 20 tablets. Transfer an accurately weighed quantity of the powder containing about 150 mg of lamivudine to a 100-ml volumetric flask, add 20 ml of *methanol* and about 50 ml of a mixture of equal volumes of *water* and *methanol* and disperse with the aid of ultrasound for 5 minutes. Dilute suitably with the same solvent mixture to obtain a solution containing 0.15 mg of lamivudine per ml. Filter this solution through a membrane filter disc with an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate.

*Reference solution*. A solution containing 0.015 per cent w/v of *lamivudine RS* and 0.02 per cent w/v of *nevirapine RS* and a concentration of *stavudine RS* similar to that of the concentration of stavudine in the test solution.

The chromatographic procedure may be carried out using the conditions described under Dissolution.

Inject the reference solution. The test is not valid unless the column efficiency determined from the lamivudine peak is not less than 2000 theoretical plates, the tailing factor for the individual peaks due to lamivudine, nevirapine and stavudine is not more than 1.5 and the relative standard deviation for replicate injections of all the analyte peaks is not more than 1.0 per cent.

Inject test solution and the reference solution.

Calculate the contents of $C_8H_{11}N_3O_3S$, $C_{15}H_{14}N_4O$ and $C_{10}H_{12}N_2O_4$ in the tablets.

**Storage**. Store protected from moisture.

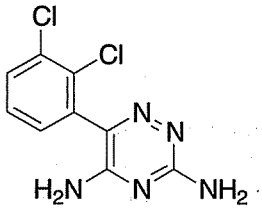


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# Lamotrigine



$C_9H_7Cl_2N_5$ Mol. Wt. 256.1

Lamotrigine is 6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5-diamine.

Lamotrigine contains not less than 98.5 per cent and not more than 101.5 per cent of $C_9H_7Cl_2N_5$, calculated on the dried basis.

**Category**. Anticonvulsant.

**Description**. A white to off - white powder.

## Identification

*Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *lamotrigine RS* or with the reference spectrum of lamotrigine.

B. When examined in the range 210 to 360 nm (2.4.7), a 0.0025 per cent w/v solution in *methanol* exhibits a maximum at about 309 nm.

C. In the Assay, the chromatogram obtained with test solution corresponds to the chromatogram obtained with reference solution.

## Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. 80 volumes of mobile phase A and 20 volumes of mobile phase B.

*Test solution*. Dissolve 100 mg of the substance under examination in 100 ml of solvent mixture.

*Reference solution (a)*. A 0.05 per cent w/v solution of *lamotrigine RS* in solvent mixture.

*Reference solution (b)*. Dilute 1 ml of reference solution (a) to 100 ml with solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. 0.174 per cent w/v solution of *dipotassium hydrogen phosphate* adjusted to pH 7.5 with *orthophosphoric acid* and filter,

B. *acetonitrile*,

- a linear gradient programme using the conditions given below,
- flow rate.1.5 ml per minute,
- spectrophotometer set at 250 nm,
- injection volume. 20 µl.

| Time (in mins.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0 | 80 | 20 |
| 5 | 80 | 20 |
| 15 | 25 | 75 |
| 30 | 25 | 75 |
| 35 | 80 | 20 |

Inject reference solution (b). Test is not valid unless the column effciency is not less than 5000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than 2 times the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent).

**Heavy metals** (2.3.13). 2.0 g complies with limit test for heavy metals, Method B (10 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°, under vacuum, for 3 hours.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 100 mg of the substance under examination in 100 ml of mobile phase. Dilute10.0 ml of the solution to 50.0 ml with mobile phase.

*Reference solution*. A 0.02 per cent w/v solution of *lamotrigine RS* in mobile phase.

Chromatographic system

- a stainless steel column 15 cm × 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 30 volumes of *acetonitrile* and 70 volumes of 0.408 per cent *potassium dihydrogen orthophosphate* adjusted to pH 7.0 with *dilute potassium hydroxide solution*.
- flow rate.1.2 ml per minute,
- spectrophotometer set at 308 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2500 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.



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Inject the test solution and the reference solution.

Calculate the content of $C_9H_7Cl_2N_5$.

**Storage**. Store protected from moisture, at a temperature not exceeding 30°.

## Lamotrigine Dispersible Tablets

Lamotrigine Dispersible Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of lamotrigine, $C_9H_7Cl_2N_5$.

**Usual strengths**. 25 mg; 50 mg; 100 mg.

### Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

### Tests

**Dissolution** (2.5.2).

Apparatus No. 1,

Medium. 900 ml of *0.1 M hydrochloric acid*,

Speed and time. 50 rpm for 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

*Test solution*. The filtrate obtained as given above.

*Reference solution*. Dissolve an accurately weighed quantity of *lamotrigine RS* in the dissolution medium and dilute with the dissolution medium to obtain a solution having a known concentration similar to the expected concentration of the test solution.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm packed with octadecylsilane bonded to porous ilica (5 µm),
- mobile phase: a mixture of 70 volumes of a 0.408 per cent w/v solution of *potassium dihydrogen phosphate* previously adjusted to pH 7.0 with *potassium hydroxide*, 30 volumes of *acetonitrile*
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 308 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

D. Not less than 75 per cent of the stated amount of $C_9H_7Cl_2N_5$.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Accurately transfer 10 intact tablets in a suitable volumetric flask, add mobile phase, sonicate to dissolve and dilute with mobile phase to obtain a concentration of 0.02 per cent w/v of Lamotrigine.

*Reference solution*. A 0.02 per cent w/v solution of *lamotrigine RS* in mobile phase.

Chromatographic system

- a stainless steel column 15 cm × 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 70 volumes of a 0.408 per cent w/v solution of *potassium dihydrogen phosphate* previously adjusted to pH 7.0 with *potassium hydroxide*, 30 volumes of *acetonitrile*,
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 308 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2500 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_9H_7Cl_2N_5$.

**Storage**. Store protected from light and moisture.

**Labelling**. The label states that the tablets should be dispersed in water immediately before use.

## Lamotrigine Sustained-release Tablets

Lamotrigine Sustained-release Tablets contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of lamotrigine, $C_9H_7Cl_2N_5$.

### Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

### Tests

**Dissolution** (2.5.2).

Apparatus No. 1,

Medium. 1000 ml of a buffer solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, adjusted to pH 6.8 with *0.2 M sodium hydroxide*,

Speed and time. 100 rpm and 1 hour, 2 hours and 12 hours.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 305 nm



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(2.4.7). Calculate the content of $C_9H_7Cl_2N_5$ in the medium from the absorbance obtained from a solution of known concentration of *lamotrigine RS* in the same medium.

D. Not less than 35 per cent and not more than 70 per cent in 1 hour, not less than 50 per cent and not more than 80 per cent in 2 hours and not less than 85 per cent in 12 hours of the stated amount of $C_9H_7Cl_2N_5$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. 60 volumes of *methanol* and 40 volumes of *water*.

*Test solution*. Weigh and transfer 5 tablets in to suitable volumetric flask, add *methanol* to 10 per cent of the volume of the flask and sonicate to disperse. Further add solvent mixture to 75 per cent of the volume of the flask and sonicate for 60 minutes in cool *water* with ocassional shaking. Make up the volume with the solvent mixture. Centrifuge at 3500 rpm for 15 minutes. Dilute it with the solvent mixture to prepare 0.1mg per ml solution.

*Reference solution*. Dissolve 50 mg of *lamotrigine RS* in 10.0 ml of *methanol*, sonicate to dissolve and dilute to 100.0 ml with the solvent mixture. Further dilute the solution with the solvent mixture to prepare 0.1mg per ml.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of *water*, 40 volumes of *methanol* and 0.01 volumes of *triethylamine*, adjusted to pH 7.0 with *10 per cent v/v* solution of *orthophosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 309 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the theoretical plates is not less than 3000 and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with the reference solution (1.5 per cent.)

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. 60 volumes of *methanol* and 40 volumes of *water*.

*Test solution*. Weigh and transfer 5 tablets in to suitable volumetric flask, add *methanol* to 10 per cent of the volume of the flask and sonicate to disperse. Further add solvent mixture to 75 per cent of the volume of the flask and sonicate for 60 minutes in cool *water* with ocassional shaking. Make up the volume with the solvent mixture. Centrifuge at 3500 rpm for 15 minutes. Dilute to obtain a solution of 0.01 per cent with the solvent mixture.

*Reference solution*. Dissolve 50 mg of *lamotrigine RS* in 10.0 ml of *methanol*, sonicate to dissolve and dilute to 100.0 ml with the solvent mixture. Further dilute the solution with the solvent mixture to make concentration of 0.1 mg per ml.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of *water*, 40 volumes of *methanol* and 0.01 volumes of *triethylamine*, adjusted to pH 7.0 with 10 per cent v/v solution of *orthophosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 309 nm,
- injection volume. 20 µl.

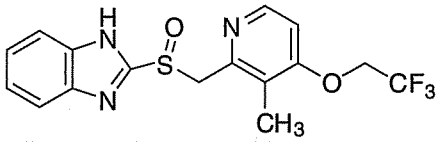
Inject the reference solution. The test is not valid unless the theoretical plates is not less than 3000 and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution.

Calculate the content of $C_{38}H_{72}N_2O_{12}$ in the tablets.

**Storage**. Store protected from light.

## Lansoprazole



$C_{16}H_{14}F_3N_3O_2S$ Mol. Wt. 369.4

Lansoprazole is (*RS*)-2-[[[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridinyl]methyl]sulphinyl]-1*H*-benzimidazole.

Lansoprazole contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{16}H_{14}F_3N_3O_2S$, calculated on the anhydrous basis.

**Category**. Antiulcer.

**Description**. A white to brownish-white powder.

### Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *lansoprazole RS* or with the reference spectrum of lansoprazole.



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B. When examined in the range of 200 nm to 400 nm (2.4.7), a 0.001 per cent w/v solution in *methanol* shows maxima at the same wavelength obtained with the solution having same concentration of the reference solution.

## Tests

**Related substances.** Determine by liquid chromatography (2.4.14).

*NOTE—Use the solutions at or below 5°.*

*Solvent mixture.* 75 volumes of *0.1 M sodium hydroxide solution* and 25 volumes of *methanol.*

*Test solution.* Dissolve about 125 mg of the substance under examination in 50 ml of *methanol.* Dilute 1.0 ml of this solution to 10.0 ml with solvent mixture.

*Reference solution (a).* A solution containing 5 mg each of *lansoprazole RS* and *lansoprazole impurity A RS* in 200 ml of *methanol.* Dilute 1 ml of this solution to 10 ml with solvent mixture.

*Reference solution (b).* A 0.00025 per cent w/v solution of *lansoprazole RS* in the *methanol.*

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. *water,*
  B. a mixture of 80 volumes of *acetonitrile,* 20 volumes of *water* and 0.5 volume of *triethylamine,* adjusted to pH 7.0 with *orthophosphoric acid,*
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 285 nm,
- injection volume. 40 µl.

| Time (in min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0-40 | 90 → 20 | 10 → 80 |
| 40-50 | 20 | 80 |
| 50-53 | 20 → 90 | 80 → 10 |
| 53-60 | 90 | 10 |

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to lansoprazole and lansoprazole impurity A is not less than 6 and the relative standard deviation for replicate injections is not more than 3.0 per cent. The relative retention time with reference to lansoprazole for lansoprazole sulfone is about 1.1, for lansoprazole *N*-oxide is about 0.8 and for lansoprazole sulphide is about 1.2

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of the peak due to lansoprazole sulphone is not more than 0.4 per cent the area of the principal peak in the chromatogram obtained with reference solution (b), the area of each peak due to lansoprazole *N*-oxide and lansoprazole sulphide is not more than 0.1 per cent the area of the principal peak in the chromatogram obtained with reference solution (b). The area of any other secondary peak is not more than 0.1 per cent the area of the principal peak in the chromatogram obtained with reference solution (b). Ignore any peak with an area less than 0.05 per cent the area of the principal peak in the chromatogram obtained with reference solution (b).

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 0.1 per cent, determined on a 1.0 g in a mixture of 90 volumes of *pyridine* and 10 volumes of *ethylene glycol.*

**Assay.** Determine by liquid chromatography (2.4.14).

*Solvent mixture.* 60 volumes of *water*, 40 volumes of *acetonitrile* and 1 volume of *triethylamine,* adjusted to pH 10.0 with *orthophosphoric acid.*

*Internal standard solution.* A 0.25 per cent w/v solution of *4-ethoxyacetophenone* in solvent mixture.

*Test solution.*Dissolve about 50 mg of the substance under examination in 10.0 ml of internal standard solution. Dilute 1.0 ml of this solution to 50 ml with solvent mixture.

*Reference solution (a).* A solution containing 0.01 per cent w/v each of *lansoprazole RS* and *lansoprazole impurity A RS* in the solvent mixture.

*Reference solution (b).* A 0.5 per cent w/v solution of *lansoprazole RS* in internal standard solution. Dilute 1.0 ml of this solution to 50 ml with solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of *water*, 40 volumes of *acetonitrile* and 1 volume of *triethylamine,* adjusted to pH 7.0 with *orthophosphoric acid,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 285 nm,
- injection volume. 10 µl.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due lansoprazole and lansoprazole impurity A is not less than 5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (b) and the test solution.

Calculate the content of $C_{16}H_{14}F_3N_3O_2S$.

**Storage.** Store protected from light and moisture.



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# Lansoprazole Sustained-release Capsules

Lansoprazole Sustained-release Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of lansoprazole, $C_{16}H_{14}F_3N_3O_2S$.

**Usual strength**. 500 mg.

## Identification

A. Shake the contents of Capsules containing about 5 mg of Lansoprazole in 5 ml of *methanol* and centrifuge. To 0.1 ml of the supernatant, add 10 ml of *methanol* and examine in the range 200 to 400 nm (2.4.7). The spectra obtained with the test solution exhibits the maxima at the same wavelength with that of the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

## Tests

**Dissolution** (2.5.2).

A. Apparatus No. 1,
Medium. 500 ml of *0.1 M hydrochloric acid*,
Speed and time. 75 rpm and 60 minutes.

Withdraw 25 ml of the medium and proceed as directed for test solution in the test B, leaving the remaining 475 ml in the vessel for use in the test B and measure the absorbance of the filtrate, suitably diluted if necessary with dissolution medium at 306 nm (2.4.7). Calculate the content of lansoprazole, $C_{16}H_{14}F_3N_3O_2S$ in the medium from the absorbance obtained from a solution of *lansoprazole RS* having a known concentration of about 8.0 per cent of the stated amount of lansoprazole dissolved per 500 ml of test A medium.

D. Not more than 10 per cent of the stated amount of $C_{16}H_{14}F_3N_3O_2S$.

B. Apparatus No. 1,
Medium. 500 ml of buffer solution prepared by dissolving 16.35 g of *monobasic sodium phosphate*, 7.05 g of *sodium hydroxide* and 3 g of *sodium dodecyl sulphate* in 1000 ml of *water*,
Speed and time. 75 rpm and 60 minutes.

Add 425 ml of buffer solution to the remaining 475 ml of solution in each vessel from the test A. Adjust the pH to 6.8 with either *orthophosphoric acid* or *sodium hydroxide*, filter and measure the absorbances at 286 nm and 650 nm. Calculate the content of lansoprazole, $C_{16}H_{14}F_3N_3O_2S$ in the medium from the difference between the absorbances at 286 nm and 650 nm obtained from a solution of *lansoprazole RS* having a known concentration of about 70 per cent of the stated amount of lansoprazole dissolved in 900 ml of buffer stage medium.

D. Not less than 80 per cent of the stated amount of $C_{16}H_{14}F_3N_3O_2S$.

**Uniformity of content**. Shake the contents of 1 Capsule with 30 ml of *0.1 M sodium hydroxide* in a 100-ml volumetric flask and sonicate to disintegrate. Dilute to volume with *acetonitrile*, centrifuge and filter. Dilute a volume of the filtrate with a mixture of 7 volumes of *acetonitrile* and 3 volumes of *0.1 M sodium hydroxide* to obtain a solution containing about 0.012 mg of lansoprazole per ml and measure the absorbance at about 294 nm.

Calculate the content of lansoprazole, $C_{16}H_{14}F_3N_3O_2S$ by using a 0.0012 per cent w/v solution of *lansoprazole RS* in a mixture of 7 volumes of *acetonitrile* and 3 volumes of *0.1 M sodium hydroxide*.

**Loss on drying** (2.4.19). Not more than 5.0 per cent, determined on 1 g of the contents of capsules by drying in an oven at 60° in vacuum over *phosphorus pentoxide*, at a pressure not exceeding 5 mm of Hg.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. 60 volumes of *water*, 40 volumes of *acetonitrile* and 1 volume of *triethylamine*, adjusted to pH 10.0 with *orthophosphoric acid*.

*Internal standard solution*. A 0.75 per cent w/v solution of *4'-ethoxyacetophenone* in *acetonitrile*.

*Test solution*. Shake the contents of 10 Capsules containing about 300 mg of Lansoprazole with 60 ml of *0.1 M sodium hydroxide* in a 300-ml conical flask and sonicate until completely disintegrated. Add 20.0 ml of *acetonitrile* and 20 ml of internal standard solution, shake well and centrifuge. Dilute a volume of supernatant with solvent mixture to obtain a concentration of 0.1 mg of lansoprazole per ml and filter.

*Reference solution (a)*. A solution containing 0.01 per cent w/v each of *lansoprazole RS* and *2-[[[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridyl]methyl]sulfonyl]benzimidazole RS (lansoprazole impurity A RS)* in the solvent mixture.

*Reference solution (b)*. A 0.3 per cent w/v solution of *lansoprazole RS* in a mixture of 3 volumes of *0.1 M sodium hydroxide* and 2 volumes of *acetonitrile*. To 25.0 ml of this solution, add 5.0 ml of internal standard solution and dilute to 50 ml with the solvent mixture to obtain a concentration of 0.1 mg of lansoprazole per ml.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of *water*, 40 volumes of *acetonitrile* and 1 volume of *triethylamine*, adjusted to pH 7.0 with *orthophosphoric acid*,



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- flow rate. 1 ml per minute,
- spectrophotometer set at 285 nm,
- injection volume. 10 µl.

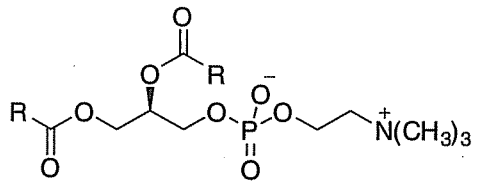
Inject reference solution (a). The test is not valid unless the resolution between the peaks due to lansoprazole and lansoprazole impurity A is not less than 5.0. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (b) and the test solution.

Calculate the content of $C_{16}H_{14}F_3N_3O_2S$ in the Capsules.

**Storage**. Store protected from moisture, at a temperature not exceeding 30°.

# Lecithin



R = Fatty acids

Lecithin is a complex mixture of acetone-insoluble phosphatides, which consist chiefly of phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine, and phosphatidyl inositol, combined with various amounts of other substances such as triglycerides, fatty acids, and carbohydrates, as separated from the crude vegetable oil source.

Lecithin contains not less than 50.0 per cent of stated amount of acetone-insoluble matter.

**Category**. Pharmaceutical aid (excipient).

## Identification

A. Transfer 1.0 g of Lecithin to a Kjeldahl flask, add 5.0 g of *potassium sulphate*, 0.5 g of *cupric sulpahte* and 20 ml of *sulphuric acid*. Incline the flask to a 45 degree angle, heat gently until the effervescence almost ceases, and raise the temperature to boiling. After the contents become a blue, transparent solution, heat for 1 to 2 hours, cool, and add an equal volume of *water*. To 5 ml of this solution, add 10 ml of 20 per cent w/v solution of *ammonium molybdate* and heat; a yellow precipitate is produced.

B. Determine by paper chromatography (2.4.15), coating the plate with cellulose.

*Mobile phase*. A mixture of 40 volumes of *n-butanol*, 20 volumes of *water* and 10 volumes of *acetic acid*.

*Test solution*. Dissolve 0.5 g of substance under examination in 5.0 ml of 50 per cent w/v solution of *hydrochloric acid*, heat in a water-bath for 2 hours, filter.

*Reference solution*. A 0.5 per cent w/v solution of *choline chloride RS*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in a current of air, spray with *dragendorff's reagent* and examine in day light. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with the reference solution.

## Tests

**Acid value**. Not more than 36 mg of potassium hydroxide, determined by the following method (2.3.23). Weigh accurately about 2.0 g of substance under examination in a 250-ml conical flask and dissolve it in 50 ml of *petroleum ether (40° to 60°)*, add 50 ml of *ethanol (95 per cent)*, previously neutralized to *phenolphthalein solution* with *0.1 M sodium hydroxide*, mix. Add *phenolphthalein solution* and titrate with *0.1 M sodium hydroxide* to a pink end point that persists for 5 seconds. Calculate the number of mg of *potassium hydroxide* required to neutralize the free acids in 1.0 g of lecithin.

**Peroxide value**. Not more than 10.

Weigh accurately about 5.0 g of substance under examination into a 250 ml Erlenmeyer flask with a ground-glass stopper; add 35 ml of a mixture of 2 volumes of *chloroform* and 1 volume of *acetic acid*, mix. Completely dissolve the substance while shaking gently. The solution becomes transparent. Completely replace the air in the flask with nitrogen. While purging with nitrogen, add 1 ml of 16.5 per cent w/v solution of *potassium iodide*, then stop the flow of the nitrogen, and immediately place a stopper in the flask. Shake for 1 minute, and allow to stand in a dark place for 5 minutes, add 75 ml of *water*, replace the stopper again, shake vigorously. Titrate with *0.01 M sodium thiosulphate*, adding starch as the endpoint is approached, and continue the titration until the blue colour of starch has just disappeared. Carry out a blank titration.

Calculate the content of peroxide value.

**Water** (2.3.43). Not more than 1.5 per cent, determined on 1 g.

**Hexane-insoluble matter**. Not more than 0.3 per cent. Weigh accurately about 10.0 g into a 250-ml conical flask, add 100 ml of *hexane*, and shake until solution is apparently complete or until no more of any residue seems to be dissolving. Pass through a coarse-porosity filtering funnel that previously has been heated at 105° for 1 hour, cooled, and weighed, wash the flask with two 25 ml portions of *hexane* and pour both washings through the funnel. Dry the funnel at 105° for 1 hour. Cool to room temperature, and determine the gain in weight: not more than 0.3 per cent is found.



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**Lead** (2.3.15). Not more than 0.001 per cent.

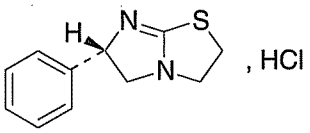
**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm). After completion of method proceed as directed in procedure under Method D.

**Acetone-insoluble matter**. Weigh accurately about 2.0 g of substance under examination to a 40 ml centrifuge tube that previously has been tared along with a stirring rod, cool, Add 15.0 ml of *acetone,* warm carefully in a water-bath to melt the substance without evaporating the *acetone*, but with stirring to aid complete dissolution, and place in an ice-water-bath for 5 minutes. Add *acetone* that previously has been chilled to 0° to 5° to the 40 ml mark on the tube, stirring during the addition. Cool in an ice-water-bath for 15 minutes, stir, remove the rod, clarify by centrifuging at about 2000 rpm for 5 minutes, and decant. Break up the residue with the stirring rod, and refill the centrifuge tube to the 40 ml mark with chilled *acetone,* while stirring. Cool in an ice-water-bath for 15 minutes, stir, remove the rod, centrifuge, and decant. Break up the residue with the stirring rod. Place the tube in a horizontal position until most of the acetone has evaporated, mix again, and heat the tube containing the acetone-insoluble residue and the stirring rod at 105° to constant weight. Determine the weight of the residue and calculate the percentage of acetone-insoluble matter.

**Storage**. Store protected from moisture at a temperature not exceeding 30°.

**Labelling**. The label states that it is flammable.

# Levamisole Hydrochloride




$C_{11}H_{12}N_2S,HCl$ Mol. Wt. 240.8

Levamisole Hydrochloride is (*S*)-2,3,5,6-tetrahydro-6-phenylimidazo[2,1-*b*]thiazole hydrochloride.

Levamisole Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{11}H_{12}N_2S,HCl$, calculated on the dried basis.

**Category**. Anthelmintic.

**Dose**. The equivalent of 150 mg of levamisole as a single dose.

**Description**. A white or almost white, crystalline powder.

## Identification

*Test A may be omitted if tests B, C, D and E are carried out. Tests B and D may be omitted if tests A, C and E are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *levamisole RS* or with the reference spectrum of levamisole.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) examined in ultraviolet light at 254 nm corresponds to that in the chromatogram obtained with reference solution (b).

C. Complies with the test for Specific optical rotation.

D. Dissolve 0.5 g in 20 ml of *water* and add 6 ml of *1 M sodium hydroxide*. Extract with 20 ml of *dichloromethane*, wash the lower layer with two quantities, each of 10 ml, of *water*, dry over *anhydrous sodium sulphate*, filter and evaporate the solvent at a temperature not exceeding 40° under reduced pressure. The residue melts at 58° to 61° (2.4.21).

E. Gives reaction A of chlorides (2.3.1).

## Tests

**Appearance of solution**. A 5.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and not more intensely coloured than reference solution YS7 (2.4.1).

**pH** (2.4.24). 3.0 to 4.5, determined in a 5.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). –121° to –128°, determined in a 5.0 per cent w/v solution.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254.*

*Mobile phase*. A mixture of 60 volumes of *toluene*, 40 volumes of *acetone* and 1 volume of *strong ammonia solution.*

*Test solution (a)*. Dissolve 0.5 g of the substance under examination in 10 ml of *methanol.*

*Test solution (b)*. Dissolve 0.5 g of the substance under examination in 100 ml of *methanol.*

*Reference solution (a)*. Dissolve 0.25 g of the substance under examination in 100 ml of *methanol.*

*Reference solution (b)*. A 0.5 per cent w/v solution of *levamisole hydrochloride RS* in *methanol.*

Apply to the plate 10 µl of each solution. After development, dry the plate at 105° for 15 minutes and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a). Expose the plate to iodine vapour in an airtight tank for 15 minutes. Any secondary spot in the chromatogram obtained with test solution (a), other than any spot with a very low $R_f$ value, is not more intense than the spot in the chromatogram obtained with reference solution (a).

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method A (20 ppm).



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**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 4 hours.

**Assay**. Weigh accurately about 0.2 g, dissolve in 30 ml of *ethanol (95 per cent)*, add 5 ml of *0.01 M hydrochloric acid*. Titrate with *0.1 M sodium hydroxide* determining the end-point potentiometrically (2.4.25). Record the volume added between the two inflections.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.02408 g of $C_{11}H_{12}N_2S$,HCl.

**Storage**. Store protected from light and moisture.

# Levamisole Tablets

Levamisole Hydrochloride Tablets

Levamisole Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of levamisole, $C_{11}H_{12}N_2S$.

**Usual strengths**. The equivalent of 50 mg, 150 mg of levamisole.

## Identification

In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

## Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.01 N hydrochloric acid,*
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 214 nm (2.4.7). Calculate the content of $C_{11}H_{12}N_2S$, in the medium from the absorbance obtained by repeating the determination using a solution of *levamisole RS* in the same medium.

D. Not less than 80 per cent of the stated amount of $C_{11}H_{12}N_2S$.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254*.

*Mobile phase*. A mixture of 60 volumes of *toluene*, 40 volumes of *acetone* and 1 volume of *strong ammonia solution*.

*Test solution (a)*. Shake a quantity of the powdered tablets containing 100 mg of levamisole with 5 ml of *methanol* for 2 minutes and filter.

*Test solution (b)*. Dilute 1 ml of test solution (a) to 10 ml with *methanol*.

*Reference solution* (a). A 0.24 per cent w/v solution of *levamisole hydrochloride RS* in *methanol*.

Referece solution (b). *Dilute 1 ml of reference solution (a)* to *20 ml with* methanol.

Apply to the plate 10 µl of each solution. After development, dry the plate at 105° for 15 minutes and examine in ultraviolet light at 254 nm as well as after exposure to iodine vapour. Any secondary spot the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b).

**Other tests**. Comply with the tests stated under Tablets.

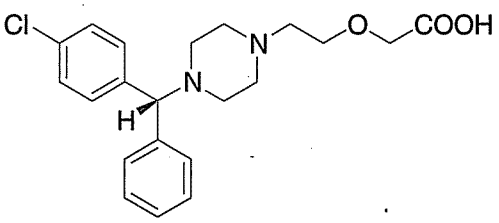
**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 300 mg of levamisole, add 30 ml of *water* and shake for 10 minutes. Filter, wash the filter with 20 ml of *water* and add the washings to the filtrate. To the combined filtrate add *dilute ammonia solution* to make it alkaline and extract with three quantities each of 25 ml, 15 ml and 15 ml, of *chloroform*, filter through cotton wool covered with a layer of *anhydrous sodium sulphate*. Combine the chloroform extracts and evaporate to dryness. Dissolve the residue in 50 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02043 g of $C_{11}H_{12}N_2S$.

**Storage**. Store protected from moisture.

**Labelling**. The label states the strength in terms of the equivalent amount of levamisole.

# Levocetirizine Hydrochloride




$C_{21}H_{25}N_2O_3Cl$,2HCl Mol. Wt. 461.8

Levocetirizine dihydrochloride is (*R*)-2-[2-[4-[(4-chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy]acetic acid dihydrochloride.

Levocetirizine Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{21}H_{25}N_2O_3Cl$,2HCl calculated on the dried basis.

**Category**. Antihistaminic.

**Description**. A white or almost white powder.



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### Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *levocetirizine dihydrochloride RS* or with the reference spectrum of levocetirizine dihydrochloride.

B. When examined in the range 200 nm and 350 nm (2.4.7), a 0.001 per cent w/v solution in *methanol* shows an absorption maximum at about 230 nm.

### Tests

**Specific optical rotation** (2.4.22). +10° to +14°, determined in a 1 per cent w/v solution in *carbon dioxide-free water* at 365 nm.

**Heavy metals** ( 2.3.13). Dissolve the residue obtained in the test for sulphated ash in 20 ml *water*. 12 ml of this solution complies with limit test for heavy metals, Method D (20 ppm).

**Enantiomeric purity**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 12.5 mg of the substance under examination in 1 ml of *ethanol ( 95 per cent)* and dilute to 25 with the mobile phase, filter.

*Reference solution*. Dissolve 12.5 mg of the *racemic cetirizine dihydrochloride RS* in 1 ml of *ethanol ( 95 per cent)* and dilute to 25 with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm packed with chiral Pak AD-H (5 µm),
- mobile phase: a mixture of 70 volumes of *n-hexane*, 15 volumes of *isopropyl alcohol,* 15 volumes of *ethanol (95 per cent)*, 0.2 volume of *tri-fluoro acetic acid* and 0.01 volume of *diethylamine,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 10 µl.

Inject the reference solution and the test solution.

The relative retention time of levocetirizine isomer is about 2 with respect to levocetirizine peak.

Calculate the chiral purity of levocetirizine dihydrochloride by area normalization method, the enantiomeric purity is not less than 98 per cent.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 20 mg of the substance under examination in 100 ml of mobile phase and filter.

*Reference solution (a)*. A 0.02 per cent w/v solution of *levocetrizine dihydrochloride RS* in mobile phase.

*Reference solution (b)*. Dilute 1 ml of reference solution (a) to 100 ml with mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with silica gel (5 µm),
- mobile phase: a mixture of 0.4 volume of 5.5 per cent v/v solution of *sulphuric acid,* 6.6 volumes of *water* and 93 volumes of *acetonitrile,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 20 µl.

Inject reference solution (b). Test is not valid unless the column effciency is not less than 5000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent).

**Sulphated ash** (2.3.18). Not more than 0.2 per cent, determined on 2 g.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1 g by drying in an oven at 100° at a pressure not exceeding 0.7 kPa.

**Assay**. Weigh accurately about 0.1 g, dissolve in 70 ml of a mixture of 30 ml of *water* and 70 ml of *acetone*. Titrate with *0.1 M sodium hydroxide* upto the second point of inflection. Determine the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.01539 g of $C_{21}H_{25}N_2O_3Cl,2HCl$.

**Storage**. Store protected from moisture.

## Levocetirizine Tablets

Levocetirizine Dihydrochloride Tablets

Levocetirizine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of levocetirizine hydrochloride, $C_{21}H_{25}N_2O_3Cl,2HCl$.

**Usual strength**. 5 mg.

### Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.



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## Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *phosphate buffer pH 6.8,*
Speed and time. 50 rpm for 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

*Test solution.* Use the filterate.

*Reference solution.* A 0.025 per cent w/v solution of *levocetirizine dihydrochloride RS* in mobile phase. Dilute 1 ml of the solution to 50 ml with dissolution medium.

Chromatographic system as described under Assay.

Calculate the content of $C_{21}H_{25}N_2O_3Cl,2HCl$.

D. Not less than 75 per cent of the stated amount of $C_{21}H_{25}N_2O_3Cl,2HCl$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh and powder 20 tablets. Weigh accurately a quantity of the powdered tablet containing 25 mg of levocetirizine dihydrochloride, dissolve in 25 ml of mobile phase.

*Reference solutionn (a).* A 0.1 per cent w/v solution of *levocetirizine hydrochloride RS* in mobile phase.

*Reference solution (b).* Dilute 1 ml of reference solution (a) to 100 ml with mobile phase.

Chromatographic system as described under Assay.

Inject reference solution (a). Test is not valid unless the column effciency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with the reference solution (b) (2.0 per cent).

**Uniformity of content**. Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay.

*Test solution.* Transfer 1 tablet in a suitable volumetric flask. Add about 20 ml of mobile phase to disperse with shaking. Further dilute with the mobile phase to obtain a final concentration of 0.0025 per cent w/v.

*Reference solution.* A 0.0025 per cent w/v solution of *levocetirizine dihydrochloride RS* in the mobile phase.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh and powder 20 tablets. Weigh accurately a quantity of the powdered tablet containing 25 mg of Levocetirizine Dihydrochloride, disperse in 100.0 ml of mobile phase and filter. Dilute 5.0 ml of the solution to 25.0 ml with mobile phase.

*Reference solution.* A 0.005 per cent w/v solution of *levocetrizine dihydrochloride RS* in mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane chemically bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of *0.05 M potassium dihydrogen phosphate* and 40 volumes of *acetonitrile*, adjust the pH to 6.0 with 10 per cent w/v of *sodium hydroxide,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0. The column efficiency in not less than 1500 theoretical plates. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

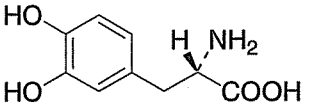
Calculate the content of $C_{21}H_{25}N_2O_3Cl,2HCl$.

**Storage**. Store protected from light and moisture.

**Labelling**. The label states the strength of Levocetirizine Dihydrochloride.

# Levodopa

L-Dopa




$C_9H_{11}NO_4$ Mol. Wt. 197.2

Levodopa is 3-(3,4-dihydroxyphenyl)-L-alanine.

Levodopa contains not less than 98.5 per cent and not more than 101.0 per cent of $C_9H_{11}NO_4$, calculated on the dried basis.

**Category**. Antiparkinsonian.

**Dose**. Initially, 125 to 500 mg daily, in divided doses after meals, increasing gradually in accordance with the needs of the



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patient; optimal dose, 1 to 8 g daily. Usually used in combination with Carbidopa.

**Description**. A white or slightly cream, crystalline powder; odourless.

### Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *levodopa RS* or with the reference spectrum of levodopa.

B. Dissolve about 2 mg in 2 ml of *water* and add 0.2 ml of *ferric chloride solution*; a green colour develops which changes to bluish violet on the addition of 0.1 g of *hexamine*.

C. Dissolve about 5 mg in a mixture of 5 ml of *1 M hydrochloric acid* and 5 ml of *water*. Add 0.1 ml of *sodium nitrite solution* containing 10 per cent w/v of *ammonium molybdate*; a yellow colour develops which changes to red on the addition of *10 M sodium hydroxide*.

D. Mix about 5 mg with 1 ml of *water*, 1 ml of *pyridine* and about 5 mg of *4-nitrobenzoyl chloride* and allow to stand for 3 minutes; a violet colour develops which changes to pale yellow on boiling. Add, while shaking, 0.2 ml of *sodium carbonate solution*; the violet colour reappears.

### Tests

**Appearance of solution**. A 4.0 per cent w/v solution in *1 M hydrochloric acid* is not more intensely coloured than reference solution BYS6 (2.4.1).

**pH** (2.4.24). 4.5 to 7.0, determined in a suspension prepared by shaking 0.1 g with 10 ml of *carbon dioxide-free water* for 15 minutes.

**Optical rotation** (2.4.22). –1.27° to –1.34°, determined at 20° in a solution prepared in the following manner. Dissolve a quantity containing 0.2 g of the substance on the dried basis and 5 g of *hexamine* in 10 ml of *1 M hydrochloric acid*, add sufficient *1 M hydrochloric acid* to produce 25 ml and allow to stand for 3 hours, protected from light.

**Light absorption**. Dissolve 30 mg in sufficient *0.1 M hydrochloric acid* to produce 100.0 ml and dilute 10.0 ml to 100.0 ml with *0.1 M hydrochloric acid*.

When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum only at about 280 nm; absorbance at 280 nm, 0.41 to 0.44.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *microcrystalline cellulose*.

*Mobile phase*. A mixture of 50 volumes of *1-butanol*, 25 volumes of *glacial acetic acid* and 25 volumes of *water*.

*Test solution*. Dissolve 0.1 g of the substance under examination in 5 ml of *anhydrous formic acid* and add sufficient *methanol* to produce 10 ml; prepare immediately before use.

*Reference solution (a)*. Dilute 1 volume of the test solution to 200 volumes with *methanol*.

*Reference solution (b)*. A mixture of equal volumes of the test solution and a solution prepared by dissolving 30 mg of *L-tyrosine* in 1 ml of *anhydrous formic acid* and diluting to 100 ml with *methanol*.

Apply to the plate 10 µl each of the test solution and reference solution (a) and 20 µl of reference solution (b) as bands. Allow the mobile phase to rise 20 cm. Dry the plate in a current of air. After development, dry the plate in a current of warm air, spray with a freshly prepared mixture of equal volumes of a 10 per cent w/v solution of *ferric chloride* and a 5 per cent w/v solution of *potassium ferricyanide* and examine the plate immediately. Any secondary band in the chromatogram obtained with the test solution is not more intense than the band in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows a distinct band, at a higher $R_f$ value than the principal band, which is more intense than the band in the chromatogram obtained with reference solution (a).

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.3 g, dissolve, heating if necessary, in 5 ml of *anhydrous formic acid* and add 25 ml of *anhydrous glacial acetic acid* and 25 ml of *dioxan*. Titrate with *0.1 M perchloric acid*, using 0.1 ml of *crystal violet solution* as indicator and titrating to a green end-point. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01972 g of $C_9H_{11}NO_4$.

**Storage**. Store protected from light and moisture.

## Levodopa and Carbidopa Tablets

Co-careldopa Tablets

Levodopa and Carbidopa Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of anhydrous carbidopa, $C_{10}H_{14}N_2O_4$, and not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of levodopa, $C_9H_{11}NO_4$.



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**Dose**. Expressed as levodopa, initially 100 to 125 mg thrice or four times daily after meals, adjusted according to response; usual maintenance dose, 0.75 to 1.5 g daily, in divided doses.

**Usual strengths**. Expressed in the form *x/y* where *x* and *y* are the strengths, in mg, of anhydrous carbidopa and levodopa respectively as 10/100; 25/100; 25/250.

### Identification

A. In the Assay, the chromatogram obtained with the test solution shows two principal peaks that correspond to those due to carbidopa and levodopa in the chromatogram obtained with the reference solution.

B. Shake a quantity of the powdered tablets containing 50 mg of Levodopa with 4 ml of *ethanol (95 per cent)* and 1 ml of *1 M sulphuric acid*. Add 2 ml of *cinnamaldehyde*, allow to stand for 20 minutes, add 50 ml of *0.1 M hydrochloric acid*, shake for 2 minutes and allow to stand. Filter the aqueous layer and to 5 ml of the filtrate add 0.1 ml of *ferric chloride test solution*. To half of the solution add an excess of *dilute ammonia solution*; a purple colour is produced. To the remainder add an excess of *sodium hydroxide solution*; a deep red colour is produced.

C. Shake a quantity of the powdered tablets containing 1 mg of anhydrous carbidopa with 5 ml of *0.05 M sulphuric acid* and filter. Add 5 ml of *dimethylaminobenzaldehyde reagent* to the filtrate; a yellow colour is produced.

### Tests

**Uniformity of Content**. *For tablets containing 10 mg or less of Carbidopa.*

Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14).

*Test solution*. Shake one tablet with 20 ml of *0.1 M phosphoric acid* for 30 minutes, add sufficient *water* to produce 200.0 ml, mix and filter.

*Reference solution*. Weigh accurately about 10 mg of *carbidopa RS*, dissolve in 20 ml of *0.1 M phosphoric acid* by gentle warming and add sufficient *water* to produce 200.0 ml.

Carry out the chromatographic procedure described under Assay.

Calculate the content of $C_{10}H_{14}N_2O_4$ in the tablet.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh and powder 20 tablets. Shake a quantity of the powder containing about 250 mg of Levodopa and 25 mg of Carbidopa, with 50 ml of *0.1 M phosphoric acid* for 30 minutes, add sufficient *water* to produce 500.0 ml, mix and filter.

*Reference solution*. Weigh accurately about 250 mg of *levodopa RS* and about 25 mg of *carbidopa RS*, dissolve in 2 ml of *0.1 M phosphoric acid* by gentle warming and add sufficient *water* to produce 500.0 ml.

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm, packed with octylsilane chemically bonded to porous silica (5 µm),
- mobile phase: mix 950 ml of *sodium dihydrogen phosphate solution (1.162 per cent w/v)* with 1.3 ml of *sodium 1-decanesulfonate solution (0.024 per cent w/v)*, adjust to a pH of about 2.8 with *phosphoric acid* and dilute with *water* to produce 1000 ml,
- flow rate. 2 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume. 20 µl.

Inject alternately the test solution and the reference solution. The retention times are about 4 minutes and 11 minutes for levodopa and carbidopa respectively.

Calculate the content of $C_{10}H_{14}N_2O_4$ and $C_9H_{11}NO_4$ in the tablets.

**Storage**. Store protected from light and moisture.

**Labelling**. The label states the quantity of Carbidopa in terms of the equivalent amount of anhydrous carbidopa, and the quantity of Levodopa in each tablet.

## Levodopa Capsules

L-Dopa Capsules

Levodopa Capsules contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of levodopa, $C_9H_{11}NO_4$.

**Usual strengths**. 125 mg; 250 mg; 500 mg.

### Identification

A. Dissolve as completely as possible a quantity of the contents of the capsules containing 0.5 g of Levodopa in 25 ml of *1 M hydrochloric acid* and filter. Adjust the pH to 3 with *5 M ammonia*, added dropwise with stirring, and allow to stand for several hours, protected from light. Filter, wash the precipitate and dry it at 105°.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *levodopa RS* or with the reference spectrum of levodopa.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *microcrystalline cellulose.*

*Mobile phase*. A mixture of 50 volumes of *1-butanol*, 25 volumes of *glacial acetic acid* and 25 volumes of *water*.



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*Test solution.* Shake a quantity of the contents of the capsules containing 0.1 g of Levodopa with 10 ml of *1 M hydrochloric acid* and filter.

*Reference solution.* A 1 per cent w/v solution of *levodopa RS* in *1 M hydrochloric acid.*

Apply to the plate 5 µl of each solution. After development, dry the plate in a current of warm air and spray with a freshly prepared mixture of equal volumes of a 10 per cent w/v solution of *ferric chloride* and a 5 per cent w/v solution of *potassium ferricyanide*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

### Tests

**Specific optical rotation** (2.4.22). –38.5° to –41.5°, determined in the following manner. Weigh accurately a quantity of the contents of the capsules containing 1.25 g of Levodopa, shake with 25.0 ml of *0.5 M hydrochloric acid* for 30 minutes, centrifuge and filter the supernatant liquid. To 10.0 ml of the filtrate add 10 ml of a 21.5 per cent w/v solution of *aluminium sulphate*, 20 ml of a 21.8 per cent w/v solution of *sodium acetate* and sufficient *water* to produce 50.0 ml and measure the optical rotation of the resulting solution at 20°. Separately dilute 5.0 ml of the filtrate to 200.0 ml with *0.1 M hydrochloric acid*, mix well and dilute 10.0 ml to 200.0 ml with *0.1 M hydrochloric acid.* Measure the absorbance of the resulting solution at the maximum at about 280 nm (2.4.7). Calculate the content of $C_9H_{11}NO_4$, in the filtrate taking 142 as the specific absorbance at 280 nm and from the result so obtained calculate the specific optical rotation.

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with *microcrystalline cellulose.*

*Mobile phase.* A mixture of 50 volumes of *1-butanol*, 25 volumes of *glacial acetic acid* and 25 volumes of *water.*

*Test solution.* A solution prepared immediately before use by shaking a quantity of the contents of the capsules containing 0.1 g of Levodopa with 10 ml of a mixture of equal volumes of *anhydrous formic acid* and *methanol.*

*Reference solution (a).* Dilute 1 volume of the test solution to 200 volumes with *methanol.*

*Reference solution (b).* A mixture of equal volumes of the test solution and a solution prepared by dissolving 30 mg of *L-tyrosine* in 1 ml of *anhydrous formic acid* and diluting to 100 ml with *methanol.*

Apply to the plate 10 µl of each of the test solution and reference solution (a) and 20 µl of reference solution (b) as bands. Allow the mobile phase to rise 20 cm. After development, dry the plate in a current of warm air, spray with a freshly prepared mixture of equal volumes of a 10 per cent w/v solution of *ferric chloride* and a 5 per cent w/v solution of *potassium ferricyanide* and examine the plate immediately. Any secondary band in the chromatogram obtained with the test solution is not more intense than the band in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows a distinct band, at a higher $R_f$ value than the principal band, which is more intense than the band in the chromatogram obtained with reference solution (a).

**Dissolution** (2.5.2).

Apparatus No. 2,
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance *of* the resulting solution at the maximum at about 280 nm (2.4.7). Calculate the content of $C_9H_{11}NO_4$ taking 141 as the specific absorbance at 280 nm.

D. Not less than 75 per cent of the stated amount of $C_9H_{11}NO_4$.

**Other tests.** Comply with the tests stated under Capsules.

**Assay.** Weigh accurately a quantity of the mixed contents of 20 capsules containing about 0.4 g of Levodopa, dissolve as completely as possible in 10 ml of *anhydrous formic acid*, add 80 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, using *oracet blue B solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01972 g of $C_9H_{11}NO_4$.

**Storage.** Store protected from light and moisture.

## Levodopa Tablets

L-Dopa Tablets

Levodopa Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of levodopa, $C_9H_{11}NO_4$.

**Usual strengths.** 250 mg; 500 mg.

### Identification

A. Dissolve as completely as possible a quantity of the tablet containing 0.5 g of Levodopa in 25 ml of *1 M hydrochloric acid* and filter. Adjust the pH to 3 with *5 M ammonia*, added dropwise with stirring, and allow to stand for several hours, protected from light. Filter, wash the precipitate and dry it at 105°.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that



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obtained with *levodopa RS* or with the reference spectrum of levodopa.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *microcrystalline cellulose.*

*Mobile phase*. A mixture of 50 volumes of *1-butanol*, 25 volumes of *glacial acetic acid* and 25 volumes of *water*.

*Test solution*. Shake a quantity of the powdered tablets containing 0.1 g of Levodopa with 10 ml of *1 M hydrochloric acid* and filter.

*Reference solution*. A 1 per cent w/v solution of *levodopa RS* in *1 M hydrochloric acid.*

Apply to the plate 5 µl of each solution. After development, dry the plate in a current of warm air and spray with a freshly prepared mixture of equal volumes of a 10 per cent w/v solution of *ferric chloride* and a 5 per cent w/v solution of *potassium ferricyanide*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

## Tests

**Specific optical rotation** (2.4.22). –38.5° to –41.5°, determined in the following manner. Weigh accurately a quantity of the powdered tablets containing 1.25 g of Levodopa, shake with 25.0 ml of *0.5 M hydrochloric acid* for 30 minutes, centrifuge and filter the supernatant liquid. To 10.0 ml of the filtrate add 10 ml of a 21.5 per cent w/v solution of *aluminium sulphate*, 20 ml of a 21.8 per cent w/v solution of *sodium acetate* and sufficient *water* to produce 50.0 ml and measure the optical rotation at 20°. Separately dilute 5.0 ml of the filtrate to 200.0 ml with *0.1 M hydrochloric acid*, mix well and dilute 10.0 ml to 200.0 ml with *0.1 M hydrochloric acid.* Measure the absorbance of the resulting solution at the maximum at about 280 nm (2.4.7). Calculate the content of $C_9H_{11}NO_4$, in the filtrate taking 142 as the specific absorbance at 280 nm and from the result so obtained calculate the specific optical rotation.

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with *microcrystalline cellulose.*

*Mobile phase*. A mixture of 50 volumes of *1-butanol*, 25 volumes of *glacial acetic acid* and 25 volumes of *water*.

*Test solution*. Shake a quantity of the powdered tablets containing 0.1 g of Levodopa with 10 ml of a mixture of equal volumes of *anhydrous formic acid* and *methanol.*

*Reference solution (a)*. Dilute 1 volume of the test solution to 200 volumes with *methanol.*

*Reference solution (b)*. A mixture of equal volumes of the test solution and a solution prepared by dissolving 30 mg of *L-tyrosine* in 1 ml of *anhydrous formic acid* and diluting to 100 ml with *methanol.*

Apply to the plate 10 µl each of the test solution and reference solution (a) and 20 µl of reference solution (b) as bands. Allow the mobile phase to rise 20 cm. Dry the plate in a current of warm air, spray with a freshly prepared mixture of equal volumes of a 10 per cent w/v solution of *ferric chloride* and a 5 per cent w/v solution of *potassium ferricyanide* and examine the plate immediately. Any secondary band in the chromatogram obtained with the test solution is not more intense than the band in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows a distinct band, at a higher $R_f$ value than the principal band, which is more intense than the band in the chromatogram obtained with reference solution (a).

**Dissolution** (2.5.2).

Apparatus No. 2,
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the resulting solution at the maximum at about 280 nm (2.4.7). Calculate the content of $C_9H_{11}NO_4$ taking 141 as the specific absorbance at 280 nm.

D. Not less than 75 per cent of the stated amount of $C_9H_{11}NO_4$.

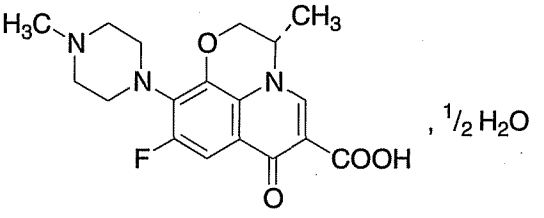
**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.4 g of Levodopa, dissolve as completely as possible in 10 ml of *anhydrous formic acid*, add 80 ml of anhydrous *glacial acetic acid.* Titrate with *0.1 M perchloric acid,* using *oracet blue B solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01972 g of $C_9H_{11}NO_4$.

**Storage**. Store protected from light and moisture.

# Levofloxacin Hemihydrate




$C_{18}H_{20}FN_3O_4, ½H_2O$ Mol. Wt. 370.4

Levofloxacin Hemihydrate is (*S*)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid hemihydrate.



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Levofloxacin Hemihydrate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{18}H_{20}FN_3O_4$, calculated on the anhydrous basis.

**Category**. Antibacterial.

**Description**. A yellowish white to yellow powder.

### Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *levofloxacin hemihydrate RS*.

### Tests

**D-Ofloxacin**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 10 mg of the substance under examination in 50 ml of mobile phase.

Chromatographic system
- a stainless steel column 25 cm × 4 mm packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 40°,
- mobile phase: dissolve 1.25 g of *copper(II)sulphate pentahydrate* and 1.31 g of *L-isolucine* in 113 ml of *methanol* and make 1000 ml with *water,*
- flow rate. 1.8 ml per minute,
- spectrophotometer set at 330 nm,
- injection volume. 10 µl.

Inject the test solution. The relative retention times are about 1.0 for levofloxacin and 1.2 for D-Ofloxacin. The area of peak corresponds to D-Ofloxacin in the chromatogram obtained with the test solution is not more than 2.0 per cent of the area of the principal peak.

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B ( 20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.2 per cent, determined on 1.0 g.

**Water** (2.3.43). Not more than 3.0 per cent, determined on 0.1 g.

**Assay**. Weigh accurately about 0.18 g, dissolve in 50 ml of *anhydrous acetic acid*. Titrate with *0.1 M perchloric acid,* determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml *0.1 M perchloric acid* is equivalent of 0.03614 g of $C_{18}H_{20}FN_3O_4$.

**Storage**. Store protected from light.

## Levofloxacin Infusion

Levofloxacin Infusion is a sterile solution of levofloxacin in water for injection.

Levofloxacin Infusion contain not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of levofloxacin, $C_{18}H_{20}FN_3O_4$.

**Usual strength**. 500 mg.

### Identification

In the Assay, the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution.

### Tests

**pH** (2.4.24). 3.8 to 5.8.

**Other tests**. Complies with the tests stated under Parenteral Preparation (Infusions).

**Assay**. Determine by liquid chromatography (2.4.14).

*NOTE — Use freshly prepared solutions and carry out the test protected from light.*

*Test solution*. Measure accurately a volume containing 50 mg of levofloxacin, dilute to 50.0 ml with *0.1 M hydrochloric acid*. Dilute 5.0 ml of the solution to 25.0 ml with *water*.

*Reference solution*. A 0.1 per cent w/v solution of *levofloxacin hemihydrate RS* in *0.1 M hydrochloric acid*. Dilute 5.0 ml of the solution to 25.0 ml with a *water*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 85 volumes of buffer solution prepared by mixing 84 volumes of *0.05 M citric acid monohydrate* and 1 volume of *1 M ammonium acetate* and 15 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 293 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and column efficiency is not less than 3000 theoretical plates and the relative standard deviation is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{18}H_{20}FN_3O_4$ in the infusion.

**Storage**. Store protected from light and moisture.

## Levofloxacin Tablets

Levofloxacin Tablets contain Levofloxacin hemihydrate.

Levofloxacin Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of levofloxacin, $C_{18}H_{20}FN_3O_4$.



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**Usual strengths**. 250 mg; 500 mg.

## Identification

In the Assay, the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution.

## Tests

**Dissolution** (2.5.2).

Apparatus No. 2,
Medium. 900 ml of *0.01 M hydrochloric acid,*
Speed and time. 100 rpm and 45 minutes.
Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 293 nm (2.4.7). Calculate the content of $C_{18}H_{20}FN_3O_4$ in the medium from the absorbance obtained from a solution of known concentration of *levofloxacin hemihydrate RS* in the same medium.

D. Not less than 70.0 per cent of the stated amount of $C_{18}H_{20}FN_3O_4$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*NOTE — Use freshly prepared solutions and carry out the test protected from light.*

*Test solution.* Weigh and powder 20 tablets. Weigh accurately a quantity of powdered tablet containing 100 mg of Levofloxacin, disperse in 100 ml of *0.1 M hydrochloric acid* and filter. Dilute 5 ml of the solution to 10 ml with *water.*

*Reference solution (a).* A 0.1 per cent w/v solution of *levofloxacin hemihydrate RS* in *0.1 M hydrochloric acid.* Dilute 5 ml of the solution to 10 ml with a *water.*

*Reference solution (b).* Dilute 1 ml of reference solution (a) to 100 ml with *water.*

Chromatographic system as described under Assay.

Inject reference solution (a). Test is not valid unless the column effciency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent).

**Other tests**. Comply with the tests stated under the Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*NOTE — Use freshly prepared solutions and carry out the test protected from light.*

*Test solution.* Weigh and powder 20 tablets. Weigh accurately a quantity of powdered tablet containing 100 mg of levofloxacin, disperse in 100.0 ml of *0.1 M hydrochloric acid* and filter. Dilute 5.0 ml of the solution to 50.0 ml with *water.*

*Reference solutionn.* A 0.1 per cent w/v solution of *levofloxacin hemihydrate RS* in *0.1 M hydrochloric acid.* Dilute 5 ml of the solution to 50 ml with *water.*

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Cosmosil C18 MS II),
- mobile phase: a mixture of 85 volume of buffer solution prepared by dissolving 84 volumes of *0.05 M citric acid monohydrate* and 1 volume of *1 M ammonium acetate*, filter and 15 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 293 nm,
- injection volume. 10 µl.

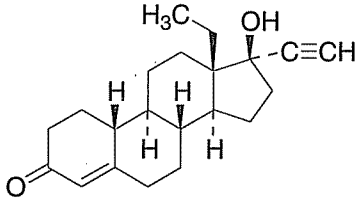
Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, column efficiency is not less than 3000 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{18}H_{20}FN_3O_4$ in the tablets.

**Storage**. Store protected from light and moisture.

# Levonorgestrel




$C_{21}H_{28}O_2$ Mol. Wt. 312.5

Levonorgestrel is 13β-ethyl-17β-hydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one.

Levonorgestrel contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{21}H_{28}O_2$, calculated on the dried basis.

**Category**. Progestogen.

**Dose**. As a contraceptive, 150 to 250 µg in combination with 20 to 50 µg of ethinyloestradiol daily.



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**Description**. A white or almost white, crystalline powder; odourless or almost odourless.

### Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *levonorgestrel RS* or with the reference spectrum of levonorgestrel.

B. When examined in the range 220 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *methanol* shows an absorption maximum only at about 240 nm.

C. Melts at about 237° (2.4.21).

### Tests

**Specific optical rotation** (2.4.22). –30.0° to –35.0°, determined in a 2.0 per cent w/v solution in *chloroform*.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 80 volumes of *dichloromethane* and 20 volumes of *ethyl acetate*.

*Test solution*. Dissolve 0.2 g of the substance under examination in 10 ml of *chloroform*.

*Reference solution (a)*. A 0.01 per cent w/v solution of the substance under examination in *chloroform*.

*Reference solution (b)*. A 0.004 per cent w/v solution of the substance under examination in *chloroform*

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with *phosphomolybdic acid solution*. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than two such spots are more intense than the spot in the chromatogram obtained with reference solution (b).

**Sulphated ash** (2.3.18). Not more than 0.3 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.1 g, dissolve in sufficient *ethanol (95 per cent)* to produce 100.0 ml, dilute stepwise with *ethanol (95 per cent)* to obtain a solution containing 0.001 per cent w/v of Levonorgestrel and measure the absorbance of the resulting solution at the maximum at about 241 nm (2.4.7).

Calculate the content of $C_{21}H_{28}O_2$ from the absorbance obtained with a 0.001 per cent w/v solution of *levonorgestrel RS* in *ethanol (95 per cent)*.

**Storage**. Store protected from light and moisture, at a temperature not exceeding 15°.

## Levonorgestrel and Ethinyloestradiol Tablets

Levonorgestrel and Ethinyloestradiol Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of levonorgestrel, $C_{21}H_{28}O_2$, and ethinyloestradiol, $C_{20}H_{24}O_2$.

**Category**. Oral contraceptive.

**Dose**. One tablet daily for 21 days starting from the fifth day of the menstrual cycle.

**Usual strengths**. Levonorgestrel, 150 µg and Ethinyloestradiol, 30 µg; Levonorgestrel, 250 µg and Ethinyloestradiol, 30 µg; Levonorgestrel, 250 µg and Ethinyloestradiol, 50 µg.

### Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 96 volumes of *dichloromethane* and 4 volumes of *ethanol (95 per cent)*.

*Test solution*. Powder 20 tablets finely, triturate with 20 ml of *dichloromethane*, allow the solids to sediment and use the clear supernatant liquid.

*Reference solution*. A solution containing 0.06 per cent w/v of *levonorgestrel RS* and 0.006 per cent w/v of *ethinyloestradiol RS* in *water*.

Apply to the plate 40 µl of each solution. After development, dry the plate in air, spray with *ethanolic sulphuric acid (80 per cent v/v)*, heat at 110° for 10 minutes and examine in ultraviolet light at 365 nm. The principal spots in the chromatogram obtained with the test solution correspond to the spots for levonorgestrel (red fluorescence) and ethinyloestradiol (orange-yellow fluorescence) in the chromatogram obtained with the reference solution.

### Tests

**Uniformity of content**. Comply with the test stated under Tablets.

Carry out the procedure described under Assay but using the following solutions.

*Test solution*. Add 2.0 ml of *methanol (70 per cent)* and 2.0 ml of a 0.00002 per cent w/v solution of *diphenyl* in *methanol (70 per cent)* (internal standard) to one tablet, shake for 20 minutes, centrifuge, filter the supernatant liquid through a membrane filter with a pore size of not more than 0.2 µm and use the filtrate.

*Reference solution (a)*. Weigh accurately a quantity of *norgestrel RS* containing 100 times the stated amount of



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Levonorgestrel per tablet, dissolve in sufficient *methanol (70 per cent)* to produce 200.0 ml. Take 2.0 ml of this solution, add 2.0 ml of the internal standard solution and use the resulting solution.

*Reference solution (b)*. Weigh accurately a quantity of *ethinyloestradiol RS* containing 100 times the stated amount of Ethinyloestradiol per tablet, dissolve in sufficient *methanol (70 per cent)* to produce 200.0 ml. Take 2.0 ml of the solution, add 2.0 ml of the internal standard solution and use the resulting solution.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh and finely powder 20 tablets. To a quantity of the powdered tablets containing about 3 mg of Levonorgestrel in a 200-ml volumetric flask, add the mobile phase and 10 ml of a 0.0025 per cent w/v solution of *diphenyl* (internal standard) in the mobile phase. Shake by mechanical means for 20 minutes and dilute to volume with the mobile phase. Centrifuge and use the clear supernatant liquid.

*Reference solution*. Transfer 15.0 ml of a solution of *levonorgestrel RS* in the mobile phase and 3.0 ml of a solution of *ethinyloestradiol RS* in the mobile phase, each solution having a concentration of about 0.1 mg per ml, into a 100 ml volumetric flask. Add 10 ml of a 0.0025 per cent w/v solution of *diphenyl* (internal standard) in the mobile phase. Dilute to volume with the mobile phase and mix. Each ml of this reference solution has a known concentration of about 15 µg and 3 µg of levonorgestrel and ethinyl oestradiol per ml respectively.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 35 volumes of *acetonitrile*, 15 volumes of *methanol* and 45 volumes of *water*,
- flow rate. 1 to 1.5 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume. 50 µl.

Inject the reference solution and record the peak response. The test is not valid unless the resolution between the two major peaks is not less than 2.5, and the relative standard deviation for replicate injections is not more than 2.0.

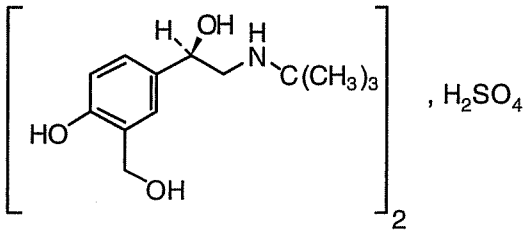
Inject separately the test solution and the reference solution and measure the responses for the major peaks. The relative retention times are about 0.7 for ethinyloestradiol and 1.0 for levonorgestrel.

Calculate the contents of $C_{21}H_{28}O_2$ and $C_{20}H_{24}O_2$ in the tablets.

**Storage**. Store protected from moisture.

# Levosalbutamol Sulphate

(*R*)-Albuterol Sulphate



$(C_{13}H_{21}NO_3)_2.H_2SO_4$ Mol. Wt. 576.7

Levosalbutamol Sulphate is (*R*)-2-*tert*-butylamino-1-(4-hydroxy-3-hydroxymethylphenyl)ethanol sulphate.

Levosalbutamol Sulphate contains not less than 98.0 per cent and not more than 102.0 per cent of $(C_{13}H_{21}NO_3)_2.H_2SO4$, calculated on the dried basis.

**Description**. A white to off-white powder.

## Identification

A. Determine by infrared absorption spectrphotometry (2.4.6). Compare the spectrum with that obtained with *levosalbutamol sulphate RS* or with the reference spectrum of levosalbutamol sulphate.

B. When examined in the range 200 to 350 nm (2.4.7), a 0.002 per cent w/v solution in *methanol* shows absorption maxima at about 227 nm and 278 nm.

## Tests

**Specific optical rotation** (2.4.22). - 30° to - 40°, determined on 1.0 per cent w/v solution.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve about 100 mg of the substance under examination in 50 ml of the mobile phase.

*Reference solution*. A 0.1 per cent w/v solution of *levosalbutamol sulphate RS* in the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 3.9 mm, packed with octylsilane bonded to porous silica (5 µm) (such as Waters symmetry C8),
- mobile phase. a mixture of 22 volumes of *acetonitrile* and 78 volumes of a solution containing 0.29 per cent w/v of *sodium heptanesulphonate* and 0.25 per cent w/v of *potassium dihydrogen phosphate dihydrate*, adjusted to pH 3.7 with *orthophosphoric acid*,



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- flow rate. 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent).

**Enantiomeric purity**. Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve about 30 mg of the substance under examination in 4 ml of *methanol* and dilute to 10.0 ml with the mobile phase.

*Reference solution.* Dissolve 30 mg of *salbutamol sulphate RS* in 4 ml of *methanol* and dilute to 10.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, bonded to porous silica (5 µm), (Such as Chirex 3022),
- mobile phase. a mixture of 70 volumes of *n-hexane*, 23 volumes of *dichloroethane*, 7 volumes of *methanol* and 0.1 volume of *trifluoroacetic acid*,
- flow rate. 0.8 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume. 15 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to levosalbutamol and dextrosalbutamol is not less than 1.0. The first peak is due to levosalbutamol and the second peak is due to dextrosalbutamol.

Inject the test solution and the reference solution. Run the chromatogram for 60 minutes. The content of the peak due to (S)-salbutamol sulphate is not more than 1.0 per cent.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 2.0 per cent, determined on 1.0 g at 105°.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve about 50 mg of the substance under examination in 100.0 ml of the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

*Reference solution.* A 0.005 per cent w/v solution of *levosalbutamol sulphate RS* in the mobile phase.

Chromatographic system as described under Related substances.

Inject the reference solution. The test is not valid unless the theoretical plates for the principal peak is not less than 2000, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

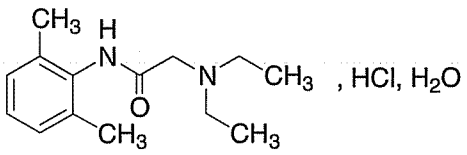
Inject the reference solution and the test solution.

Calculate the content of $(C_{13}H_{21}NO_3)_2.H_2SO_4$.

**Storage**. Store at a temperature not exceeding 30°.

# Lignocaine Hydrochloride

Lidocaine Hydrochloride




$C_{14}H_{22}N_2O,HCl,H_2O$ Mol. Wt. 288.8

Lignocaine Hydrochloride is 2-diethylaminoaceto-2′,6′-xylidide hydrochloride monohydrate.

Lignocaine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{14}H_{22}N_2O,HCl$, calculated on the anhydrous basis.

**Category**. Local anaesthetic; antiarrhythmic.

**Dose**. As local anaesthetic, upto 200 mg as a single dose, when given with adrenaline; as antiarrhythmic, by intravenous infusion, 50 to 100 mg at the rate of 1 to 2 mg per minute.

**Description**. A white, crystalline powder; odourless or practically odourless.

## Identification

*Test A may be omitted if tests B, C, D and E are carried out. Tests B, C, D and E may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *lignocaine hydrochloride RS* or with the reference spectrum of lignocaine hydrochloride.

B. To 10 ml of a 2.5 per cent w/v solution add *sodium hydroxide solution* till alkaline and filter. Wash the residue with *water*, dissolve half of the residue in 1 ml of *ethanol (95 per cent)* and add 0.5 ml of a 10 per cent w/v solution of *cobalt chloride*; a bluish-green precipitate is produced.



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C. To 5 mg add 0.5 ml of *fuming nitric acid*, evaporate to dryness on a water-bath, cool, dissolve the residue in 5 ml of *acetone* and add 1 ml of *0.1 M ethanolic potassium hydroxide*; a green colour is produced.

D. Dissolve 0.2 g in 10 ml of *water* and add 10 ml of *picric acid solution*. The precipitate, after washing with *water* and drying, melts at about 229° (2.4.21).

E. Gives reaction A of chlorides (2.3.1).

### Tests

**Appearance of solution**. A 5.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and colourless (2.4.1).

**pH** (2.4.24). 4.0 to 5.5, determined in a 0.5 per cent w/v solution.

**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals, Method A (10 ppm).

**Sulphates**. Dissolve 0.2 g in 20 ml of *water*, add 2 ml of *3 M hydrochloric acid*, mix and divide into two parts. To one part add 1 ml of *barium chloride solution*; no more opalescence is produced than in the remaining portion of the solution to which nothing has been added.

**2,6-Dimethylaniline**. To 2 ml of a 2.5 per cent w/v solution in *methanol* (solution A), add 1 ml of a 1 per cent w/v solution of *4-dimethylaminobenzaldehyde* in *methanol* and 2 ml of *glacial acetic acid* and allow to stand for 10 minutes at room temperature. Any yellow colour produced is more intense than that obtained by repeating the test using 2 ml of *methanol* in place of solution A and less intense than the colour produced using a mixture of 1 ml of a solution of *2,6-dimethylaniline* in *methanol* containing 5 µg per ml and 1 ml of *methanol* in place of solution A (100 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). 5.0 to 7.5 per cent, determined on 0.25 g.

**Assay**. Weigh accurately about 0.5 g, dissolve in 30 ml of *anhydrous glacial acetic acid*, add 6 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02708 g of $C_{14}H_{22}N_2O,HCl$.

**Storage**. Store protected from moisture.

## Lignocaine and Adrenaline Injection

Lidocaine Hydrochloride and Adrenaline Bitartrate Injection; Lidocaine and Adrenaline Injection

Lignocaine and Adrenaline Injection is a sterile solution of Lignocaine Hydrochloride and Adrenaline Bitartrate in Water for Injections.

Lignocaine and Adrenaline Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of lignocaine hydrochloride, $C_{14}H_{22}N_2O,HCl,H_2O$ and not less than 87.5 per cent and not more than 112.5 per cent of the stated amount of adrenaline, $C_9H_{13}NO_3$.

**Usual strength**. Lignocaine Hydrochloride, 20 mg per ml and adrenaline, 0.01 mg per ml.

**Description**. A clear colourless solution.

### Identification

A. To 5 ml add 1 ml of *hydrochloric acid*, cool to 0°, add 5 ml of a 1 per cent w/v solution of *sodium nitrite* and pour the mixture into 2 ml of *2-naphthol solution* containing 1 g of *sodium acetate*; no red colour is produced.

B. To 10 ml add 4 ml of *disodium hydrogen phosphate solution* and sufficient *0.1 M iodine* to produce a distinct brown colour. Add *0.01 M sodium thiosulphate* to remove the excess of iodine; a pink colour is produced.

C. To 3 ml add 3 ml of *water* and 6 ml of *picric acid solution*, shake gently and allow to stand until the precipitate becomes crystalline; the precipitate, after washing with *water* and drying at 105°, melts at about 229° (2.4.21).

### Tests

**pH** (2.4.24). 3.0 to 4.5.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. *For lignocaine hydrochloride* — Make an accurately measured volume containing 0.1 g of Lignocaine Hydrochloride alkaline with *2 M sodium hydroxide* and extract with three quantities, each of 20 ml, of *chloroform*, washing each extract with the same 10 ml of *water*. Filter the washed extracts through a filter paper moistened with *chloroform*, wash the filter with 10 ml of *chloroform*, combine the washings with the filtrate. Titrate with *0.02 M perchloric acid*, using *crystal violet solution* as indicator.

1 ml of *0.02 M perchloric acid* is equivalent to 0.005776 g of $C_{14}H_{22}N_2O,HCl,H_2O$.

*For adrenaline* — To an accurately measured volume containing 0.1 mg of adrenaline add 20 mg of *sodium metabisulphite*, 0.1 ml of *ferrous sulphate-citrate solution*, 1 ml of *glycine buffer solution* and mix. Allow to stand for 10 minutes, extract with 10 ml of ether, allow to separate, reject the *ether* and measure the absorbance of a 4-cm layer of the solution at about 540 nm (2.4.7). Calculate the content of adrenaline, $C_9H_{13}NO_3$, from a reference curve prepared by treating suitable aliquots of a solution of *adrenaline bitartrate RS* in the same manner.



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1 mg of adrenaline bitartrate is equivalent to 0.0005497 g of $C_9H_{13}NO_3$.

**Storage**. Store protected from light.

**Labelling**. The label states the strength of Adrenaline Bitartrate in terms of the equivalent amount of adrenaline.

## Lignocaine and Dextrose Injection

Lignocaine Hydrochloride and Dextrose Injection; Lidocaine Hydrochloride and Dextrose Injection; Lidocaine and Dextrose Injection

Lignocaine and Dextrose Injection is a sterile solution of Lignocaine Hydrochloride and Dextrose in Water for Injections.

Lignocaine and Dextrose Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amounts of lignocaine hydrochloride, $C_{14}H_{22}N_2O,HCl,H_2O$, and dextrose, $C_6H_{12}O_6,H_2O$.

**Usual strength**. Lignocaine Hydrochloride 50 mg per ml and Dextrose 75 mg per ml.

**Description**. A clear colourless or slightly yellow solution.

### Identification

A. To a volume containing about 0.5 g of Lignocaine Hydrochloride in a separator add 2 ml of *2 M sodium hydroxide* and extract with four quantities, each of 15 ml, of *chloroform*. Combine the chloroform extracts and evaporate the solution to dryness with the aid of a current of air. Dissolve the residue in 2 ml of *hexane*, evaporate with the aid of warm air and dry the residue over *silica gel* for 24 hours at a pressure not exceeding 0.7 kPa.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *lignocaine hydrochloride RS* treated in the same manner.

B. To a volume containing 0.1 g of Dextrose add 10 ml of *water* and 3 ml of *potassium cupri-tartrate solution* and heat; a red precipitate is produced.

### Tests

**pH** (2.4.24). 3.0 to 7.0.

**Other tests**. Complies with the tests described under Parenteral Preparations (Injections).

**Assay**. *For lignocaine hydrochloride* - Make an accurately measured volume containing about 0.1 g of Lignocaine Hydrochloride alkaline with *2 M sodium hydroxide* and extract with three quantities, each of 20 ml, of *chloroform*, washing each extract with the same 10 ml of *water*. Filter the washed extracts through a filter paper moistened with *chloroform*, wash the filter with 10 ml of *chloroform*, combine the washings with the filtrate. Titrate with *0.02 M perchloric acid,* using *crystal violet solution* as indicator.

1 ml of *0.02 M perchloric acid* is equivalent to 0.005776 g of $C_{14}H_{22}N_2O,HCl,H_2O$.

*For dextrose* — To an accurately measured volume containing 2 g to 5 g of Dextrose add sufficient *water* to produce 100.0 ml. Mix well, allow to stand for 30 minutes and determine the optical rotation in a 2-dm tube (2.4.22). The observed rotation in degrees multiplied by 1.0425 represents the weight, in g, of *dextrose*, $C_6H_{12}O_6,H_2O$ in the volume taken for assay.

**Storage**. Store in single dose containers in a cool place.

## Lignocaine Gel

Lignocaine Hydrochloride Gel; Lidocaine Hydrochloride Gel

Lignocaine Gel is a sterile solution of Lignocaine Hydrochloride in a suitable water-miscible base. It may contain suitable antioxidants, stabilisers and antimicrobial preservatives.

Lignocaine Gel contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of anhydrous lignocaine hydrochloride, $C_{14}H_{22}N_2O,HCl$.

**Usual strengths**. The equivalent of 1 per cent and 2 per cent w/v of anhydrous lignocaine hydrochloride.

### Identification

To a quantity of the gel containing 80 mg of anhydrous lignocaine hydrochloride add 4 ml of *hydrochloric acid* and heat on a water-bath for 10 minutes. Allow to cool, transfer to a separating funnel with the aid of 20 ml of *water*, add *5 M sodium hydroxide* until precipitation is complete and extract with two quantities, each of 20 ml, of *chloroform*. Filter the chloroform extracts through *anhydrous sodium sulphate* and evaporate the filtrate to dryness on a water-bath using a stream of nitrogen. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *lignocaine hydrochloride RS* or with the reference spectrum of lignocaine hydrochloride.

B. Dissolve 20 mg in 1 ml of *ethanol (95 per cent)*, add 0.5 ml of a 10 per cent w/v solution of *cobalt chloride* and 0.5 ml of *5 M sodium hydroxide* and shake for 2 minutes; a bluish green precipitate is produced.



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C. Dissolve 40 mg in 5 ml of 1 per cent w/v solution of *cetrimide*, add 1 ml of *5 M sodium hydroxide* and 1 ml of *bromine water*; a yellow colour is produced.

### Tests

**pH** (2.4.24). 6.0 to 7.0.

**2,6-Dimethylaniline**. Mix a quantity of the gel containing 15 mg of anhydrous lignocaine hydrochloride with sufficient *water* to produce 3 ml, using a rotary mixer. To 2 ml of the resulting solution, add 1 ml of a freshly prepared 1 per cent w/v solution of *4-dimethylaminobenzaldehyde* in *methanol*. Mix thoroughly using a rotary mixer. Add 2 ml of *glacial acetic acid* and allow to stand for 10 minutes. Any yellow colour produced is not more intense than that obtained by using a mixture of 2 ml of a solution of *2,6-dimethylaniline* in *methanol* containing 2 µg per ml in place of the solution of the gel (20 ppm).

**Sterility** (2.2.11). Complies with the test for sterility.

**Other tests**. Complies with the tests stated under Gels.

**Assay**. Weigh accurately a quantity containing about 10 mg of anhydrous lignocaine hydrochloride and disperse in 20 ml of *water*. Add 5 ml of *acetate buffer pH 2.8*, 120 ml of *chloroform* and 5 ml of *dimethyl yellow-oracet blue B solution* and titrate with *0.005 M dioctyl sodium sulphosuccinate* swirling vigorously, until near the end-point, then add the titrant dropwise and, after each addition, swirl vigorously, allow to separate and swirl gently for 5 seconds. The end-point is indicated when the colour of the chloroform layer changes from green to pinkish-grey. Carry out a blank titration.

1 ml of *0.005 M dioctyl sodium sulphosuccinate* is equivalent to 0.001354 g of $C_{14}H_{22}N_2O,HCl$.

Determine the weight per ml of the gel (2.4.29), and calculate the percentage of $C_{14}H_{22}N_2O,HCl$, weight in volume.

**Storage**. Store in suitable tamper-proof containers holding sufficient of the gel for use on one occasion, and at a temperature not exceeding 30°. The gel should not be frozen.

**Labelling**. The label states (1) that the contents are sterile; (2) the strength in terms of the equivalent amount of anhydrous lignocaine hydrochloride; (3) that any of the gel not used in a single application should be discarded.

## Lignocaine Injection

Lignocaine Hydrochloride Injection; Lidocaine Hydrochloride Injection; Lidocaine Injection

Lignocaine Injection is a sterile solution of Lignocaine Hydrochloride in Water for Injections.

Lignocaine Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of lignocaine hydrochloride, $C_{14}H_{22}N_2O,HCl,H_2O$.

**Usual strengths**. 0.2 per cent w/v; 1.0 per cent w/v; 5.0 per cent w/v.

**Description**. A clear colourless solution.

### Identification

A. To a volume containing 0.1 g of Lignocaine Hydrochloride add sufficient *sodium hydroxide solution* to make alkaline, filter, wash the residue with *water*, dissolve in 1 ml of *ethanol (95 per cent)*, add 0.5 ml of a 10 per cent w/v solution of *cobalt chloride* and shake for 2 minutes; a bluish-green precipitate is formed.

B. To a volume containing 0.1 g of Lignocaine Hydrochloride add 10 ml of *picric acid solution*; the precipitate, after washing with *water* and drying at 105°, melts at about 229° (2.4.21).

C. Gives the reactions of chlorides (2.3.1).

### Tests

**pH** (2.4.24). 5.0 to 7.0.

**2,6-Dimethylaniline**. To a volume containing 25 mg of Lignocaine Hydrochloride add *water* if necessary to produce 10 ml, add *2 M sodium hydroxide* until the solution is just alkaline and extract with three quantities, each of 5 ml, of *chloroform*. Dry the combined chloroform extracts over *anhydrous sodium sulphate*, filter, wash with a further 5 ml of *chloroform* and evaporate the filtrate to dryness at a pressure of 2 kPa. Dissolve the residue in 2 ml of *methanol*, add 1 ml of a 1 per cent w/v solution of *4-dimethylamino-benzaldehyde* in *methanol* and 2 ml of *glacial acetic acid* and allow to stand at room temperature for 10 minutes. Any yellow colour produced is not more intense than the colour produced by repeating the operation using 10 ml of a solution in *water* containing 1 µg per ml of *2,6-dimethylaniline* in place of the preparation under examination (400 ppm).

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. Make an accurately measured volume containing about 0.1 g of Lignocaine Hydrochloride alkaline with *2 M sodium hydroxide* and extract with three quantities, each of 20 ml, of *chloroform*, washing each extract with the same 10 ml of *water*. Filter the washed extracts through a filter paper moistened with *chloroform*, wash the filter with 10 ml of *chloroform*, combine the washings with the filtrate. Titrate with *0.02 M perchloric acid*, using *crystal violet solution* as indicator.

1 ml of *0.02 M perchloric acid* is equivalent to 0.005776 g of $C_{14}H_{22}N_2O,HCl,H_2O$.

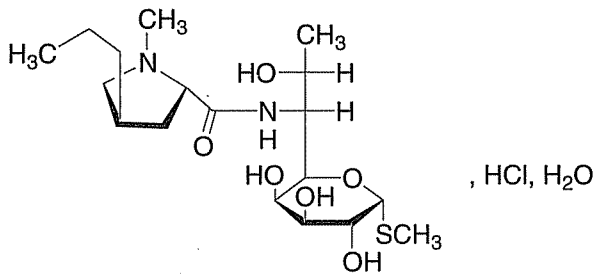


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# Lincomycin Hydrochloride




$C_{18}H_{34}N_2O_6S,HCl,H_2O$ Mol. Wt. 461.0

Lincomycin Hydrochloride consists mainly of methyl 6-amino-6,8-dideoxy-*N*-[(2*S*,4*R*)-1-methyl-4-propylprolyl]-1-thio-D-*erythro*-α-D-*galacto*-octopyranoside hydrochloride monohydrate, an antimicrobial substance produced by *Streptomyces lincolnensis* var. *lincolnensis* or by any other means.

Lincomycin Hydrochloride contains not less than 82.5 per cent and not more than 93.0 per cent of $C_{18}H_{34}N_2O_6S$, calculated on the anhydrous basis.

**Category**. Antibacterial.

**Dose**. Orally, the equivalent of 500 mg of lincomycin every 6 to 8 hours, 30 minutes before food; by intramuscular injection, the equivalent of 600 mg of lincomycin every 12 to 24 hours; by slow intravenous infusion, the equivalent of 600 mg of lincomycin every 8 to 12 hours.

**Description**. A white or almost white, crystalline powder.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *lincomycin hydrochloride RS* or with the reference spectrum of lincomycin hydrochloride.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. The upper layer obtained by shaking a mixture of 45 volumes of *ethyl acetate*, 40 volumes of a 15 per cent w/v solution of *ammonium acetate* previously adjusted to pH 9.6 with *10 M ammonia* and 20 volumes of *2-propanol*.

*Test solution*. Dissolve 0.1 g of the substance under examination in 100 ml of *methanol*.

*Reference solution (a)*. A 0.1 per cent w/v solution of *lincomycin hydrochloride RS* in *methanol*.

*Reference solution (b)*. A solution containing 0.1 per cent w/v each of *lincomycin hydrochloride RS* and *clindamycin hydrochloride RS* in *methanol*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and spray with a 0.1 per cent w/v solution of *potassium permanganate*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

C. Dissolve 10 mg in 2 ml of *2 M hydrochloric acid* and heat in a water-bath for 3 minutes. Add 3 ml of a 10.5 per cent w/v solution of *anhydrous sodium carbonate* and 1 ml of a 2 per cent w/v solution of *sodium nitroprusside*; a violet-red colour is produced.

D. A 1 per cent w/v solution gives reaction A of chlorides (2.3.1).

## Tests

**Appearance of solution**. A 10.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and not more intensely coloured than reference solution YS6 (2.4.1).

**pH** (2.4.24). 3.5 to 5.5, determined in a 10.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). +135° to +150°, determined in a 4.0 per cent w/v solution.

**Lincomycin B**. In the Assay, the chromatogram obtained with reference solution (a) shows a peak derived from lincomycin B which is eluted just before lincomycin. The area of the peak derived from lincomycin B is not more than 5 per cent of the area of the peak derived from lincomycin.

**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

**Sulphated ash** (2.3.18). Not more than 0.5 per cent.

**Water** (2.3.43). 3.0 to 4.6 per cent, determined on 0.5 g.

**Assay**. Determine by gas chromatography (2.4.13).

*Test solution*. Add 10.0 ml of a 0.8 per cent w/w solution of *dotriacontane* (internal standard) in *chloroform* to 0.1 g of substance under examination, dilute to 100.0 ml with a 2 per cent w/v solution of *imidazole* in *chloroform* and shake to dissolve. Place 4.0 ml of the resulting solution in a 15 ml glass-stoppered centrifuge tube, add 1.0 ml of a mixture of 99 volumes of N,O-*bis (trimethylsilyl)-acetamide* and 1 volume of *trimethylchlorosilane* and mix gently. Loosen the glass stopper and heat at 65° for 30 minutes.

*Reference solution (a)*. Prepare in the same manner as the test solution but omitting the internal standard.



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*Reference solution (b).* Prepare in the same manner as the test solution but using 0.1 g of *lincomycin hydrochloride RS.*

Chromatographic system
- a glass column 1.5 m × 3 mm, packed with acid-washed, silanised diatomaceous support impregnated with 3 per cent w/w of phenyl methyl silicone fluid (50 per cent phenyl),
- temperature:
  column 260°,
  inlet port and detector at 260° to 290°,
- flow rate. 45 ml per minute using helium as carrier gas.

Inject 1 µl of the test solution, reference solution (a) and reference solution (b).

Calculate the content of $C_{18}H_{34}N_2O_6S$.

*Lincomycin Hydrochloride intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.*

**Bacterial endotoxins** (2.2.3). Not more than 0.5 Endotoxin Unit per mg.

*Lincomycin Hydrochloride intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.*

**Sterility** (2.2.11). Complies with the test for sterility.

**Storage**. Store protected from moisture at a temperature not exceeding 30°. If the contents are sterile, the container should be sterile, tamper-evident and sealed so as to exclude micro-organisms.

**Labelling**. The label states whether or not the material is intended for use in the manufacture of parenteral preparations.

# Lincomycin Capsules

## Lincomycin Hydrochloride Capsules

Lincomycin Capsules contain Lincomycin Hydrochloride equivalent to not less than 90.0 per cent and not more than 110 per cent of the stated amount of lincomycin, $C_{18}H_{34}N_2O_6S$.

**Usual strength**. 500 mg.

### Identification

In the Assay, the retention time of the principal peak derived from lincomycin hydrochloride relative to that of the internal standard in reference solution (b) is the same as the retention time of the principal peak derived from *lincomycin hydrochloride RS* relative to that of the internal standard in the test solution.

### Tests

**Lincomycin B**. Examine reference solution (b) as described under the Assay but increase the sensitivity by 8 to 10 times while recording the peak derived from lincomycin B, which is eluted immediately before that derived from lincomycin. The area of the peak derived from lincomycin B, when corrected for the sensitivity factor, is not more than 5 per cent of the area of the peak derived from lincomycin.

**Water** (2.3.43). Not more than 7.0 per cent, determined on 0.3 g of the contents of the capsules.

**Other tests**. Comply with the tests stated under Capsules.

**Assay**. Determine by gas chromatography (2.4.13).

*Test solution.* Add 10.0 ml of a 0.8 per cent w/w solution of *dotriacontane* (internal standard) in *chloroform* to 0.1 g of *lincomycin hydrochloride RS*, dilute to 100.0 ml with a 2 per cent w/v solution of *imidazole* in *chloroform* and shake to dissolve. Place 4.0 ml of the resulting solution in a 15 ml glass-stoppered centrifuge tube, add 1.0 ml of a mixture of 99 volumes of N,O-*bis(trimethylsilyl)-acetamide* and 1 volume of *trimethylchlorosilane* and mix gently. Loosen the glass stopper and heat at 65° for 30 minutes.

*Reference solution (a).* Prepare in the same manner as the test solution but omitting the internal standard and using a quantity of the mixed contents of 20 capsules containing about 90 mg of lincomycin in place of *lincomycin hydrochloride RS.*

*Reference solution (b).* Prepare in the same manner as the test solution but using a quantity of the mixed contents of 20 capsules containing about 90 mg of lincomycin in place of *lincomycin hydrochloride RS.*

Chromatographic system
- a glass column 1.5 m × 3 mm, packed with acid-washed, silanised diatomaceous support impregnated with 3 per cent w/w of phenyl methyl silicone fluid (50 per cent phenyl),
- temperature:
  column 260°,
  inlet port and detector at 260° to 290°,
- flow rate. 45 ml per minute using helium as carrier gas.

Inject 1 µl of the test solution, reference solution (a) and reference solution (b).

Calculate the content of $C_{18}H_{34}N_2O_6S$ in the capsules.

**Storage**. Store protected from moisture.

**Labelling**. The label states the strength in terms of the equivalent amount of lincomycin.

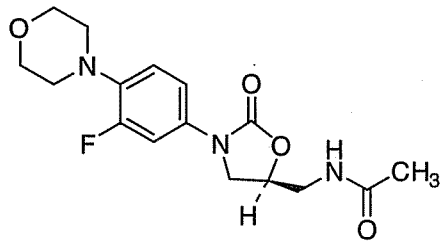


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# Linezolid



$C_{16}H_{20}FN_3O_4$ Mol. Wt. 337.4

Linezolid is *N*-[[(5*S*)-3-[3-fluoro-4-(4-morpholinyl)phenyl]-2-oxo-5-oxazolidinyl]methyl]acetamide.

Linezolid contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{16}H_{20}FN_3O_4$, calculated on the dried basis.

**Category.** Antibacterial.

**Description.** A white to off-white, crystalline powder.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *linezolid RS* or with the reference spectrum of linezolid.

B. When examined in the range of 200 nm to 400 nm (2.4.7), a 0.0001 per cent w/v solution in *methanol* shows maxima at the same wavelength as that of the reference solution.

## Tests

**Specific optical rotation** (2.4.22). – 9.0° to – 14.0°, determined on 0.9 per cent w/v solution in *chloroform.*

**Related substances.** Determine by liquid chromatography (2.4.14).

*Buffer solution.* A solution prepared by diluting 1.0 ml of *triethylamine* in 1000 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid.*

*Solvent mixture.* 60 volumes of buffer solution and 40 volumes of *methanol.*

*Test solution.* Dissolve 50 mg of the substance under examination in 50 ml of the solvent mixture.

*Reference solution (a).* A 0.1 per cent w/v solution of *linezolid RS* in the solvent mixture.

*Reference solution (b).* Dilute 2 ml of reference solution (a) to 100 ml with the solvent mixture. Further dilute 5 ml of this solution to 20 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Hypersil ODS),
- column temperature. 40°,
- mobile phase: A. a mixture 90 volumes of buffer solution and 10 volumes of *methanol*,
  B. a mixture 10 volumes of buffer solution and 90 volumes of *methanol*,
- a linear gradient programme using the conditions given below,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 250 nm,
- injection volume. 10 µl.

| Time (in min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0 | 90 | 10 |
| 40 | 70 | 30 |
| 50 | 20 | 80 |
| 57 | 20 | 80 |
| 60 | 90 | 10 |
| 65 | 90 | 10 |

Inject reference solution (b). The test is not valid unless the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent).

**Heavy Metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in oven at 105° for 3 hours.

**Assay.** Determine by liquid chromatography (2.4.14).

*Buffer solution.* A solution prepared by diluting 1.0 ml of *triethylamine* in 1000 ml of water, adjusted to pH 3.0 with *orthophosphoric acid.*

*Solvent mixture.* 60 volumes of buffer solution and 40 volumes of *methanol.*

*Test solution.* Dissolve about 100 mg of the substance under examination in 100.0 ml of the solvent mixture. Dilute 10.0 ml of this solution to 50.0 ml with the solvent mixture.



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*Reference solution (a).* A 0.1 per cent w/v solution of *linezolid RS* in the solvent mixture.

*Reference solution (b).* Dilute 10.0 ml of reference solution (a) to 50.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Hypersil ODS),
- column temperature. 40°,
- mobile phase: a mixture of 78 volumes of a solution containing 90 volumes of buffer solution and 10 volumes of *methanol*, and 22 volumes of a solution containing 10 volumes of buffer solution and 90 volumes of *methanol*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 250 nm,
- injection volume. 10 µl.

Inject reference solution (b). The test is not valid unless the theoretical plates is not less than 2000, tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (b) and the test solution.

Calculate content of $C_{16}H_{20}FN_3O_4$.

**Storage**. Store protected from light and moisture, at a temperature not exceeeding 30°.

# Linezolid Tablets

Linezolid Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of linezolid, $C_{16}H_{20}FN_3O_4$.

## Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

## Tests

**Dissolution** (2.5.2).

Apparatus No. 1,

Medium. 900 ml of buffer solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* and 0.9 g of *sodium hydroxide* in 1000 ml of *water*, adjusted to pH 6.8 with *sodium hydroxide solution*,

Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 250 nm (2.4.7). Calculate the content of $C_{16}H_{20}FN_3O_4$ in the medium from the absorbance obtained from a solution of known concentration of *linezolid RS* in the same medium.

D. Not less than 70 per cent of the stated amount of $C_{16}H_{20}FN_3O_4$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture.* A mixture of 75 volumes of buffer solution prepared by diluting 1.0 ml of *triethylamine* to 1000.0 ml with *water*, adjusted to pH 3.0 with *orthophosphoric acid* and 25 volumes of *methanol*.

*Test solution.* Weigh and powder 20 tablets. Disperse a quantity of powder containing about 40 mg of linezolid with 35 ml of the solvent mixture, sonicate for 20 minutes and dilute to 50.0 ml with the solvent mixture, filter.

*Reference solution.* A 0.0008 per cent w/v solution of *linezolid RS* in the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) ( Such as Hypersil ODS),
- column temperature. 40°,
- mobile phase: A. a mixture of 90 volumes of buffer solution prepared by mixing 1.0 ml of *triethylamine* in 1000 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid* and 10 volumes of *methanol*,
  B. a mixture of 90 volumes of buffer solution prepared by mixing 1.0 ml of *triethylamine* in 1000 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid* and 90 volumes of *methanol*,
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 250 nm,
- injection volume. 10 µl.

| Time (in min.) | Mobile Phase A (per cent v/v) | Mobile Phase B (per cent v/v) |
|---|---|---|
| 0 | 90 | 10 |
| 35 | 65 | 35 |
| 45 | 20 | 80 |
| 47 | 20 | 80 |
| 48 | 90 | 10 |
| 55 | 90 | 10 |

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the reference solution and test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with the reference solution (1.0 per cent) and the sum of areas of all the secondary peaks is not



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more than two times the area of the peak in the chromatogram obtained with the reference solution (2.0 per cent).

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. A mixture of 75 volumes of buffer solution prepared by diluting 1.0 ml of *triethylamine* to 1000 ml with *water*, adjusted pH 3.0 with *orthophosphoric acid* and 25 volumes of *methanol*.

*Test solution*. Weigh and powder 20 tablets. Disperse a quantity of powder containing about 40 mg of Linezolid with 35 ml of solvent mixture, sonicate for 20 minutes and dilute to 50 ml with the solvent mixture, filter. Dilute 5 ml of this solution to 50 ml with the solvent mixture.

*Reference solution*. A 0.08 per cent w/v solution of *linezolid RS* in the solvent mixture. Dilute 5.0 ml of this solution to 50.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil ODS),
- column temperature. 40°,
- mobile phase: a mixture of 70 volumes of a buffer solution prepared by diluting 1.0 ml of *triethylamine* to 1000 ml with *water*, adjusted to pH 3.0 with *orthophosphoric acid* and 30 volumes of *methanol,*
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 250 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the theoretical plates is not less than 2000, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent

Inject the reference solution and the test solution.

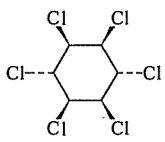
Calculate the content of $C_{16}H_{20}FN_3O_4$ in the tablets.

**Storage**. Store protected from light and moisture.

**Labelling**. The label states the strength in terms of the equivalent amount of Linezolid.

# Lindane

Gamma Benzene Hexachloride




$C_6H_6Cl_6$ Mol.Wt.290.8

Lindane is 1α,2α,3β,4α,5α,6β-hexachlorocyclohexane.

Lindane contains not less 99.0 per cent and not more than 100.5 per cent of $C_6H_6Cl_6$.

**Category**. Topical parasiticide.

**Description**. A white or almost white crystalline powder.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *lindane RS*.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

C. Dissolve about 5 mg in 4 ml of *ethanol (95 per cent)*. Add 1 ml of *0.5 M ethanolic potassium hydroxide* and allow to stand for 10 minutes. The solution gives reaction A of chlorides (2.3.1).

D. Melts at 112° to 115° (2.4.21).

## Tests

**Appearance of solution**. A 5.0 per cent w/v solution in *acetone* is clear (2.4.1), and not more intensely coloured than reference solution YS7 (2.4.1).

**Related substances**. Determine by thin-layer chromatography (2.4.17) coating the plate with *silica gel* G.

*Mobile phase*. A mixture of 90 volumes of *cyclohexane* and 10 volumes of *chloroform*.

*Test solution (a)*. Dissolve 1 g of the substance under examination in 10 ml of *chloroform*.

*Test solution (b)*. Dilute 1 ml of test solution (a) to 10 ml with *chloroform*.

*Reference solution (a)*. A 1 per cent w/v solution of *lindane RS* in *chloroform*.

*Reference solution (b)*. Dilute 1 ml of test solution (b) to 10 ml with *chloroform*.

*Reference solution (c)*. Dissolve 10 mg of *α-hexachlorocyclohexane RS* in sufficient of the test solution (a) to produce 5 ml.

Apply separately to the plate 1 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of dry air and irradiate with ultraviolet light at 254 nm for 15 minutes. Spray with a 0.6 per cent w/v solution of *dicarboxidine hydrochloride* in *ethanol (90 per cent)* and examine the spots in daylight. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.



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**Chlorides** (2.3.12). To 0.75 g, finely powdered, add 15 ml of *water* and boil for 1 minute. Allow to cool, shaking frequently, and filter. To 10 ml of the filtrate add 3 ml of *water* and 2 ml of *ethanol (95 per cent)*. The solution complies with the limit test for chlorides (l00 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Assay**. Weigh accurately about 0.2 g, add 10 ml of *ethanol (95 per cent)* and warm on a water-bath until dissolved. Cool, add 20 ml of *0.5 M ethanolic potassium hydroxide* and allow to stand for 10 minutes, swirling frequently. Add 50 ml of *water,* 20 ml of *2 M nitric acid,* 25 ml of *0.1 M silver nitrate* and 5 ml of *ferric ammonium sulphate solution.* Titrate with *0.1 M ammonium thiocyanate* until a reddish-yellow colour is obtained. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of silver nitrate required.

1 ml of *0.1 M silver nitrate* is equivalent to 0 009694 g of $C_6H_6Cl_6$.

**Storage**. Store protected from light.

## Absorbent Lint

Lint; Cotton Lint; Unmedicated Lint

Absorbent Lint is a cotton cloth of plain weave, on one side of which a nep has been raised from either warp or weft yarns. It absorbs water readily but its absorbency may be considerably reduced by medication, the absorbency of the product depending upon the medicament incorporated.

**Category**. Surgical dressing.

**Description.** Cotton cloth of plain weave, reasonably free from weaving defects, readily tearable in both directions and bleached to a good white having on one side a nep raised from either the warp or weft yarns and reasonably free from neps; it is clean and reasonably free from leaf, shell and other foreign substances. It is made of yarn that is reasonably free from slubs, snarls and other defects.

Absorbent Lint has not less than 98.0 per cent of the dimensions stated on the label.

### Tests

**Threads per cm**. Warp not less than 16 and weft not less than 10.

**Weight per unit area**. 25 g has a superficial area of 1350 to 1370 sq. cm.

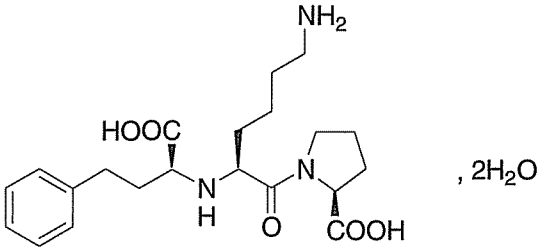
**Absorbency**. A piece 10 cm square, placed lightly by means of forceps, unraised side downwards, on the surface of water at 20°, becomes saturated within 10 seconds.

**Fluorescence**. Not more than a few points of fluorescence are visible under screened ultraviolet light.

**Storage**. Store protected from moisture in well-closed packages in a dry place, free from dust.

**Labelling**. The label states the dimensions viz. the length and width in cm.

## Lisinopril




$C_{21}H_{31}N_3O_5, 2H_2O$ Mol. Wt. 441.5

Lisinopril is (*S*)-1-[$N^2$-(1-carboxy-3-phenylpropyl)-L-lysyl]-L-proline dihydrate.

Lisinopril contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{21}H_{31}N_3O_5$, calculated on the anhydrous basis.

**Category**. Antihypertensive.

**Description**. A white crystalline powder.

### Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *lisinopril RS* or with the reference spectrum of lisinopril.

B. In the Assay, the chromatogram obtained with the test solution corresponds to the chromatogram obtained with the reference solution.

### Tests

**Specific optical rotation** (2.4.22). -115.0° to -123.0°, determined on 1.0 per cent w/v solution in *0.25 M zinc acetate* at 405 nm.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 20 mg of the substance under examination in 10 ml of mobile phase A

*Reference solution (a)*. A 0.2 per cent w/v solution of *lisinopril RS* in mobile phase A.

*Reference solution (b)*. Dilute 1 ml of reference solution (a) to 100 ml with mobile phase A.



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Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octylsilane bonded to porous silica (5 µm) (such as Symmetry C-8),
- column temperature 50°,
- mobile phase: A. a mixture of 30 volumes of *acetonitrile* and 970 volumes of *0.02 M sodium dihydrogen phosphate*, adjust the pH to 5.0 with a 5 per cent w/v solution of *sodium hydroxide* and filter.
  B. a mixture of 200 volumes of *acetonitrile* and 800 volumes of *0.02 M sodium dihydrogen phosphate,* adjust the pH to 5.0 with 5 per cent w/v solution of *sodium hydroxide* and filter.
- a linear gradient programme using the conditions given below,
- flow rate. 1.8 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

| Time (min.) | Mobile phase A ( per cent v/v) | Mobile phase B ( per cent v/v) |
|---|---|---|
| 0 | 100 | 0 |
| 35 | 70 | 30 |
| 45 | 70 | 30 |
| 55 | 0 | 100 |
| 65 | 100 | 0 |
| 75 | 100 | 0 |

Inject reference solution (b). Test is not valid unless the tailing factor is not more than 3.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent).

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B ( 20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). 8.0 to 9.5, determined on 0.1 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 30 mg of the substance under examination in 100 ml of *water* and filter.

*Reference solution*. A 0.03 per cent w/v solution of *lisinopril RS* in *water.*

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with dimethyloctylsilane (C8 alkyl chain) fully endcapped stationary phase (5 µm) (such as Hypersil MOS),
- column temperature 50°,
- mobile phase: a mixture of 96 volumes of buffer pH 5.0 prepared by dissolving 2.76 g of *monobasic sodium phosphate* in 1000 ml of *water.* adjust the pH to 5.0 with *1 M sodium hydroxide* and 4 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 180 theoretical plates. The tailing factor is not more than 2.0. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{21}H_{31}N_3O_5$.

**Storage**. Store protected from moisture, at a temperature not exceeding 25°.

## Lisinopril Tablets

Lisinopril Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of lisinopril, $C_{21}H_{31}N_3O_5$.

**Usual strengths**. 2.5 mg; 5 mg; 10 mg.

### Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

### Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 50 rpm for 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

*Test solution*. The filtrate obtained as given above.

*Reference solution*. A 0.001 per cent w/v solution of *lisinopril RS* in dissolution medium.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm packed with octylsilane bonded to porous silica (5 µm) (such as Symmetry C8),
- column temperature 50°,
- mobile phase: dissolve 1.0 g of *hexane sulphonic acid sodium salt* in 800 volumes of *phosphate solution* prepared by dissolving 4.1 g of *monobasic potassium phosphate* in 900 ml *water*, adjust pH to 2.0 with



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*orthophosphoric acid,* dilute to 1000 ml with *water* and 200 volumes of *acetonitrile*, mix, filter and degas.
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume. 50 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 850 theoretical plates, the tailing factor is not more than 2.0. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

D. Not less than 75 per cent of the stated amount of $C_{21}H_{31}N_3O_5$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh accurately a powdered tablet containing 100 mg of Lisinopril to a 50-ml volumetric flask. Add about 25 ml of mobile phase A and sonicate for 10 minutes with intermittent shaking. Make up the volume with the mobile phase A and filter.

*Reference solution (a)*. A 0.2 per cent w/v solution of *lisinopril RS* in the mobile phase A.

*Reference solution (b)*. Dilute 1 ml of reference solution (a) to 100 ml with mobile phase A.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with packed with dimethyloctylsilane (C8 alkyl chain) fully endcapped stationary phase (5 µm) (such as Hypersil MOS/ Symmetry C8),
- column temperature 50°,
- mbile phase: A. a mixture of 3 volumes of *acetonitrile* and 97 volumes of *0.02 M sodium dihydrogen phosphate*, adjust the pH to 5.0 with 5 per cent w/v solution of *sodium hydroxide* and filter,
  B. a mixture of 20 volumes of *acetonitrile* and 80 volumes of *0.02 M sodium dihydrogen phosphate*, adjust the pH to 5.0 with 5 per cent w/v solution of *sodium hydroxide* and filter,
- a linear gradient programme using the conditions given below,
- flow rate. 1.8 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

| Time (in min.) | Mobile phase A ( per cent, v/v) | Mobile phase B ( per cent, v/v) |
|---|---|---|
| 0 | 100 | 0 |
| 35 | 70 | 30 |
| 45 | 70 | 30 |
| 50 | 100 | 0 |
| 60 | 100 | 0 |

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with the reference solution (b) (2.0 per cent).

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. A mixture of 4 volumes of *water* and 1 volume of *methanol*.

*Test solution*. Transfer to a suitable size volumertric flask 10 tablets, add the solvent mixture to fill about half of the volumetric flask, shake the flask by mechanical means for 20 minutes and dilute with solvent mixture which will yield a solution having a concentration of about 0.02 per cent w/v and filter.

*Reference solution*. A 0.02 per cent w/v solution of *lisinopril RS* in solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octylsilane bonded to porous silica (5 µm), (such as Symmetry C-8),
- column temperature. 50°,
- mobile phase: dissolve 1.0 g of *hexane sulphonic acid sodium salt* in 800 volumes of *phosphate solution* prepared by dissolving 4.1 g of *monobasic potassium phosphate* in 900 ml *water,* adjust the pH to 2.0 with *orthophosphoric acid,* dilute to 1000 ml with *water* and 200 volumes of *acetonitrile*, mix, filter and degas.
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 850 theoretical plates, the tailing factor is not more than 2.0. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{21}H_{31}N_3O_5$

**Storage**. Store protect from moisture, at a temperature between 20° to 25°.

## Lithium Carbonate

$Li_2CO_3$ Mol.Wt.73.9

Lithium Carbonate contains not less than 98.5 per cent and not more than 100.5 per cent of $Li_2CO_3$.

**Category**. Antidepressant.



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**Dose**. 250 mg to 2 g daily, in divided doses.

**Description**. A white, crystalline powder; odourless.

### Identification

A. When moistened with *hydrochloric acid* and introduced on a platinum wire, it imparts a red colour to a non-luminous flame.

B. Dissolve 0.2 g in 1 ml of *hydrochloric acid* and evaporate to dryness on a water-bath; the residue is soluble in 3 ml of *ethanol (95 per cent)*.

C. Gives reaction A of carbonates (2.3.1).

### Tests

**Appearance of solution**. Suspend 10.0 g in 30 ml of *distilled water* and dissolve by adding 22 ml of *nitric acid*. Neutralise with *2 M sodium hydroxide* and dilute to 100.0 ml with *distilled water* (solution A). The solution is clear (2.4.1), and colourless (2.4.1).

**Arsenic** (2.3.10). Dissolve 5.0 g in 15 ml of *brominated hydrochloric acid*, add 45 ml of *water* and remove the excess of bromine with a few drops of *stannous chloride solution AsT*. The resulting solution complies with the limit test for arsenic (2 ppm).

**Calcium and magnesium**. Dissolve 1.0 g in 30 ml of *1 M hydrochloric acid* and neutralise with *dilute ammonia solution*, filter, if necessary, and divide into two portions; to one portion add 1 ml of *ammonium oxalate solution*; no turbidity or precipitate is produced on standing for 5 minutes. To the other portion add 1 ml of *disodium hydrogen phosphate solution*; no turbidity or precipitate is produced on standing for 5 minutes.

**Heavy metals** (2.3.13). Mix 1.0 g with 5 ml of *water* and 15 ml of *dilute hydrochloric acid*, heat to boiling and maintain that temperature for 1 minute. Add 1 drop of *phenolphthalein solution* and *sufficient ammonia solution* to give the solution a faint pink colour. Cool and dilute to 25 ml with *water*. The resulting solution complies with the limit test for heavy metals, Method A ( 20 ppm).

**Iron** (2.3.14). 20 ml of solution A complies with the limit test for iron (20 ppm).

**Potassium**. Dissolve 1.0 g in 10 ml of *7 M hydrochloric acid*, add sufficient *water* to produce 50 ml and determine by flame photometry (2.4.4), measuring at 766.5 nm, using *potassium solution FP*, suitably diluted with *water*, to prepare the standard solutions (500 ppm).

**Sodium**. Dissolve 1.0 g in 10 ml of *7 M hydrochloric acid*, add sufficient *water* to produce 50 ml and determine by flame photometry (2.4.4), measuring at 589 nm, using *sodium solution FP*, suitably diluted with *water*, to prepare the standard solutions (500 ppm).

**Chlorides** (2.3.12). 10 ml of solution A diluted to 15 ml with *water* complies with the limit test for chlorides (250 ppm).

**Sulphates** (2.3.17). Disperse 0.75 g in 5 ml of *distilled water* and dissolve by adding 5 ml of *7 M hydrochloric acid*. Boil for 2 minutes, cool, neutralise with *2 M sodium hydroxide* and dilute to 25 ml with *distilled water*. The resulting solution complies with the limit test for sulphates (200 ppm).

**Assay**. Weigh accurately about 0.5 g, dissolve in 25.0 ml of *1 M hydrochloric acid* and titrate with *1 M sodium hydroxide* using *methyl orange solution* as indicator.

1 ml of *1 M hydrochloric acid* is equivalent to 0.03695 g of $Li_2CO_3$.

**Storage**. Store protected from moisture.

## Lithium Carbonate Tablets

Lithium Carbonate Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of lithium carbonate, $Li_2CO_3$.

**Usual strength**. 300 mg.

### Identification

A small quantity of the powdered tablets, when moistened with *hydrochloric acid* and introduced on a platinum wire, imparts a red colour to a non-luminous flame.

### Tests

**Dissolution** (2.5.2).

Apparatus. No 1

Medium. 900 ml of *water*

Speed and time. 100 rpm and 30 minutes.

Withdraw 90.0 ml of the medium, add a drop of *hydrochloric acid* and dilute to 100.0 ml with *water*. Determine by flame photometry Method A (2.4.4), or by atomic absorption spectrophotometry, Method A (2.4.2), measuring at 671 nm and using *lithium solution FP*, or *lithium solution AAS*, as appropriate, suitably diluted with *water*, for the standard solution.

D. Not less than 60 per cent of the stated amount of $Li_2CO_3$.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 1 g of Lithium Carbonate, add 100 ml of *water* and 50.0 ml of *1 M hydrochloric acid* and boil for 1 minute to remove carbon dioxide. Cool and titrate with *1 M sodium hydroxide* using *methyl orange solution* as indicator.



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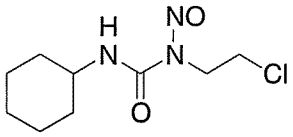
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1 ml of *1 M hydrochloric acid* is equivalent to 0.03695 g of $Li_2CO_3$.

**Storage**. Store protected from moisture.

# Lomustine



$C_9H_{16}ClN_3O_2$ Mol. Wt. 233.7

Lomustine is 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea.

Lomustine contains not less than 98.5 per cent and not more than 100.5 per cent of $C_9H_{16}ClN_3O_2$, calculated on the dried basis.

**Category**. Anticancer.

**Dose**. 120 to 130 mg per sq. m. body-surface every 6 to 8 weeks.

**Description**. A yellow, crystalline powder.

*Carry out the tests protected from light and prepare the solutions immediately before use.*

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *lomustine RS* or with the reference spectrum of lomustine.

B. When examined in the range 220 nm to 360 nm (2.4.7), a 0.002 per cent w/v solution in *ethanol (95 per cent)* shows an absorption maximum at about 230 nm; absorbance at about 230 nm, about 0.52.

C. In the test A for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (c).

D. Dissolve about 25 mg in 1 ml of *methanol*, add 0.1 ml of *2 M sodium hydroxide* and 2 ml of *water* and acidify by adding, dropwise, *1 M nitric acid*. The resulting solution gives the reactions of chlorides (2.3.1).

## Tests

**Related substances**. A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 80 volumes of *toluene* and 20 volumes of *glacial acetic acid*.

*Test solution (a)*. Dissolve 0.25 g of the substance under examination in 10 ml of *methanol*.

*Test solution (b)*. Dissolve 0.1 g of the substance under examination in 100 ml of *methanol*.

*Reference solution (a)*. A 0.01 per cent w/v solution of the substance under examination in *methanol*.

*Reference solution (b)*. A 0.005 per cent w/v solution of the substance under examination in *methanol*.

*Reference solution (c)*. A 0.1 per cent w/v solution of *lomustine RS* in *methanol*.

*Reference solution (d)*. A solution containing 0.1 per cent w/v each of *lomustine RS* and *1,3-dicyclohexylurea* in *methanol*.

Apply to the plate 5 µl of each solution. After development, dry the plate, heat it at 110° for 1 hour, exposing the hot plate in a closed tank containing chlorine, produced by adding *dilute hydrochloric acid* to a 5 per cent w/v solution of *potassium permanganate* contained in a beaker placed at the bottom of tank. Allow the plate to stand in contact with the chlorine vapours for 5 minutes. Remove the plate from the tank and dry it in a current of cold air until the excess of chlorine is removed and an area of the plate below the line of application produces at most a very faint blue colour with 0.05 ml of *potassium iodide and starch solution*; avoid prolonged exposure to cold air. Spray the plate with *potassium iodide and starch solution*. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b). The test is not valid unless the chromatogram obtained with reference solution (d) shows two clearly separated principal spots.

B. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 0.25 g of the substance under examination in 10 ml of *methanol*.

*Reference solution*. A 0.025 per cent w/v solution of the substance under examination in *methanol*.

Chromatographic system

- a stainless steel column 20 cm × 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: equal volumes of *methanol* and *water*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 20 µl.



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Inject the reference solution. The retention time of lomustine is about 25 minutes. When using a recorder, adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with the reference solution is not less than 50 per cent of the full scale of the recorder.

Inject separately each solution. In the chromatogram obtained with the test solution the sum of areas of any secondary peaks is not greater than the area of the peak in the chromatogram obtained with the reference solution. Ignore any peak with an area less than one-twentieth of that of the principal peak in the chromatogram obtained with the reference solution.

**Chlorides** (2.3.12). Dissolve 0.24 g in 4 ml of *methanol*, add 20 ml of *water*, allow to stand for 20 minutes and filter. To 10 ml of the filtrate add 5 ml of *methanol*. The resulting solution complies with the limit test for chlorides, replacing the 5 ml of *water* in the standard solution with 5 ml of *methanol (0.25 per cent)*.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 24 hours.

**Assay**. Weigh accurately about 0.2 g, add 20 ml of a 20 per cent w/v solution of *potassium hydroxide* and boil under a reflux condenser for 2 hours. Add 75 ml of *water* and 4 ml of *nitric acid*, cool. Titrate with *0.05 M silver nitrate*, determining the end-point potentiometrically (2.4.25). Repeat the operation without the substance under examination. The difference between the titrations represents the amount of *silver nitrate* required.

1 ml of *0.05 M silver nitrate* is equivalent to 0.01168 g of $C_9H_{16}ClN_3O_2$.

**Storage**. Store protected from light and moisture.

# Lomustine Capsules

Lomustine Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of lomustine, $C_9H_{16}ClN_3O_2$.

**Usual strengths**. 10 mg; 40 mg.

*Carry out the tests protected from light and prepare the solutions immediately before use.*

## Identification

Shake a quantity of the contents of the capsules containing 0.2 g of Lomustine with 10 ml of *methanol*, filter and evaporate the filtrate to dryness using a rotary evaporator on a water-bath maintained at not more than 60°. The residue, after drying at a pressure not exceeding 0.7 kPa at 60° for 30 minutes, complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *lomustine RS* or with the reference spectrum of lomustine.

B. Melting range. 89° to 91° (2.4.21).

## Tests

**Related substances**. A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 80 volumes of *toluene* and 20 volumes of *glacial acetic acid*.

*Test solution (a)*. Shake a quantity of the contents of the capsules containing 0.25 g of Lomustine with 10 ml of *methanol* and filter.

*Test solution (b)*. Dilute 1 volume of test solution (a) to 250 volumes with *methanol*.

*Reference solution (a)*. Dilute 1 volume of test solution (b) to 2 volumes with *methanol*.

*Reference solution (b)*. A 0.005 per cent w/v solution of the substance under examination in *methanol*.

*Reference solution (c)*. A 0.1 per cent w/v solution of *lomustine RS* in *methanol*.

*Reference solution (d)*. A solution containing 0.1 per cent w/v each of *lomustine RS* and *1,3-dicyclohexylurea* in *methanol*.

Apply to the plate 5 µl of each solution. After development, dry the plate, heat it at 110° for 1 hour, exposing the hot plate in a closed tank containing chlorine, produced by adding *dilute hydrochloric acid* to a 5 per cent w/v solution of *potassium permanganate* contained in a beaker placed at the bottom of tank. Allow the plate to stand in contact with the chlorine vapours for 5 minutes. Remove the plate and dry it in a current of cold air until the excess of chlorine is removed and an area of the plate below the line of application produces at most a very faint blue colour with 0.05 ml of *potassium iodide and starch solution*; avoid prolonged exposure to cold air. Spray the plate with *potassium iodide and starch solution*. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b). The test is not valid unless the chromatogram obtained with reference solution (d) shows two clearly separated principal spots.



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B. Determine by liquid chromatography (2.4.14).

*Test solution*. Shake a quantity of the contents of the capsules containing 0.25 g of Lomustine with 10 ml of *methanol* and filter.

*Reference solution*. Dilute 1 volume of the test solution to 50 volumes with *methanol*.

Chromatographic system
- a stainless steel column 20 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 mm),
- mobile phase: equal volumes of *methanol* and *water*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 20 µl.

Inject the reference solution. The retention time of lomustine is about 25 minutes. When using a recorder, adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with the reference solution is not less than 50 per cent of the full scale of the recorder.

Inject separately each solution. In the chromatogram obtained with the test solution the sum of areas of any secondary peaks is not greater than the area of the peak in the chromatogram obtained with the reference solution. Ignore any peak with an area less than one-twentieth of that of the principal peak in the chromatogram obtained with the reference solution.

**Uniformity of content**. *For capsules containing 10 mg or less*.

Comply with the test stated under Capsules.

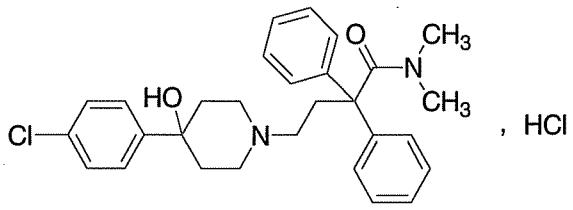
Transfer the contents of a capsule quantitatively to a 100 ml volumetric flask with the aid of 20 ml of *ethanol (95 per cent)*, shake well, make up to the volume with *ethanol (95 per cent)* and filter. Dilute suitably with the same solvent and measure the absorbance *of* the resulting solution at the maximum at about 230 nm (2.4.7). Calculate the content of $C_9H_{16}ClN_3O_2$ in the capsule taking 260 as the specific absorbance at 230 nm.

**Other tests**. Comply with the tests stated under Capsules.

**Assay**. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 40 mg of Lomustine and shake with 70 ml of *ethanol (95 per cent)* for 20 minutes, dilute to 100.0 ml with *ethanol (95 per cent)* and filter. Dilute 5.0 ml of the filtrate to 100.0 ml with *ethanol (95 per cent)* and measure the absorbance of the resulting solution at the maximum at about 230 nm (2.4.7). Calculate the content of $C_9H_{16}ClN_3O_2$ taking 260 as the specific absorbance at 230 nm.

**Storage**. Store protected from light and moisture.

# Loperamide Hydrochloride




$C_{29}H_{33}ClN_2O_2,HCl$ Mol. Wt. 513.5

Loperamide Hydrochloride is 4-(4-chlorophenyl)-4-hydroxy-piperidino]-*N*,*N*-dimethyl-2,2-diphenylbutyramide hydrochloride.

Loperamide Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{29}H_{33}ClN_2O_2,HCl$, calculated on the dried basis.

**Category**. Antidiarrhoeal.

**Dose**. Maximum daily dose, 0.48 mg per kg of body weight.

**Description**. A white or almost white powder.

## Identification

*Test B may be omitted if tests A and C are carried out. Test A may be omitted if tests B and C are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *loperamide hydrochloride RS*.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *octadecylsilyl silica gel*.

*Mobile phase*. A mixture of 40 volumes of *dioxan*, 40 volumes of *methanol* and 20 volumes of *ammonium acetate*.

*Test solution*. Dissolve 0.6 g of the substance under examination in 100 ml of the mobile phase.

*Reference solution (a)*. A 0.6 per cent w/v solution of *loperamide hydrochloride RS* in the mobile phase.

*Reference solution (b)*. A solution containing 0.6 per cent w/v each of *loperamide hydrochloride RS* and *ketaconazole RS* in the mobile phase.

Apply to the plate 5 µl of each solution. After development, dry the plate in air for 15 minutes and expose it to iodine vapours until the spots appear. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

C. Dissolve 50 mg in a mixture of 0.4 ml of *strong ammonia solution* and 2 ml of *water*. Mix, allow to stand for 5 minutes



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and filter. Acidify the filtrate with *2 M nitric acid*. It gives reaction A of chlorides (2.3.1).

### Tests

**Appearance of solution**. A 10.0 per cent w/v solution in *methanol* is clear (2.4.1), and not more intensely coloured than reference solution BYS7 (2.4.1).

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 0.1 g of the substance under examination in 10 ml of *methanol*.

*Reference solution (a)*. A solution containing 0.0025 per cent w/v each of *loperamide hydrochloride RS* and *haloperidol RS* in *methanol*.

*Reference solution (b)*. Dilute 1 ml of the test solution to 100 ml with *methanol*. Dilute 5 ml of this solution to 20 ml with *methanol*.

Chromatographic system

- a stainless steel column 10 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a 1.7 per cent w/v solution of *tetrabutylammoniumhydrogen sulphate*,
  B. *acetonitrile*,
- flow rate. 2 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 220 nm,
- injection volume. 10 µl.

| Time (in min) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0 | 90 | 10 |
| 10 | 30 | 70 |
| 15 | 30 | 70 |
| 20 | 90 | 10 |

Equilibrate the column for at least 30 minutes with *acetonitrile* and then equilibrate at the initial eluent composition for at least 5 minutes.

Adjust the sensitivity of the detector so that the height of the principal peak in the chromatogram obtained with reference solution (b) is 70 per cent to 90 per cent of the full scale of the recorder.

Inject reference solution (a) and record the peak responses. The retention times are: haloperidol, about 3 minutes and loperamide hydrochloride, about 4.5 minutes. The test is not valid unless the resolution between the peaks corresponding to haloperidol and loperamide hydrochloride is at least 8.0.

Inject separately *methanol* as a blank, the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peaks is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent) and the sum of the areas of any secondary peaks is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.4 g, dissolve in 25 ml of anhydrous *glacial acetic acid*, add 10 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, using *alpha-naphtholbenzein solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.05135 g of $C_{29}H_{33}ClN_2O_2,HCl$.

**Storage**. Store protected from light and moisture.

## Loperamide Capsules

Loperamide Hydrochloride Capsules

Loperamide Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the labeled amount of loperamide hydrochloride $C_{29}H_{33}ClN_2O_2,HCl$.

### Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 85 volumes of *chloroform*, 10 volumes of *methanol*, and 5 volumes of *formic acid*.

*Test solution*. To a quantity of the contents of the capsules, containing about 10 mg of Loperamide Hydrochloride, add 10 ml of *methanol*, shake for 5 minutes, and filter.

*Reference solution*. A 0.1 per cent w/v solution of *loperamide hydrochloride RS* in *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and expose to iodine vapour. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.



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### Tests

**Dissolution** (2.5.2)

Apparatus No. 2,
Medium. 500 ml of pH 4.7 acetate buffer, prepared by mixing 200 ml of *1 M acetic acid* with 600 ml of *water,* adjusting with *1 M sodium hydroxide* to a pH of 4.70 ± 0.05, diluting with *water* to 1000 ml and mixing,
Speed and time.100 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

*Test solution.* The filtrate from the dissolution medium.

*Reference solution.* A solution of *loperamide hydrochloride RS* in the dissolution medium containing the same concentration of loperamide hydrochloride as that expected in the dissolution medium in the vessel.

Determine by liquid chromatography (2.4.14) using the chromatographic system described under Assay.

Calculate the content of $C_{29}H_{33}ClN_2O_2$,HCl.

D. Not less than 80 per cent of the stated amount of $C_{29}H_{33}ClN_2O_2$, HCl.

**Uniformity of content**. Comply with the test stated under Tablets.

Transfer the contents of one capsule to a 200-ml volumetric flask. Add 35 ml of *0.5 M hydrochloric acid* and mix with the aid of ultrasound for 15 minutes. Add 35 ml of *acetonitrile* and mix with the aid of ultrasound for another 15 minutes. Dilute to volume with a mixture of equal volumes of *0.5 M hydrochloric acid* and *acetonitrile* mix and filter.

Determine by liquid chromatography (2.4.14) using the chromatographic system and the reference solution described under Assay.

Calculate the content of $C_{29}H_{33}ClN_2O_2$, HCl in the capsule.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Transfer an accurately weighed portion of the mixed contents of 20 capsules containing about 20 mg of Loperamide Hydrochloride, to a 100 ml volumetric flask. Add about 35 ml of *0.5 M hydrochloric acid* and mix with the aid of ultrasound for 15 minutes. Add 35 ml of *acetonitrile* and mix with the aid of ultrasound for an additional 15 minutes. Dilute with a mixture of equal volumes of *acetonitrile* and *0.5 M hydrochloric acid*, mix, and filter. Transfer 5.0 ml of this solution to a 100 ml volumetric flask, dilute to volume with the same solvent mixture and mix.

*Reference solution.* A 0.001 per cent w/v solution of *loperamide hydrochloride RS* in a mixture of equal volumes of *acetonitrile* and *water.*

Chromatographic system
- a stainless steel column 25 cm × 4 mm, packed with nitrile groups chemically bonded to porous silica particles (10 µm),
- mobile phase: dilute 500 ml of *acetonitrile* to 1000.0 ml with *water*, add 20 drops of *phosphoric acid,* mix, and filter,
- flow rate. 2 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 50 µl.

Inject the reference solution. The column efficiency, determined from the analyte peak is not less than 1900 theoretical plates, the capacity factor, is not less than 3.5, and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_{29}H_{33}ClN_2O_2$,HCl in the capsules.

## Loperamide Tablets

Loperamide Hydrochloride Tablets

Loperamide Hydrochloride Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the labeled amount of loperamide hydrochloride $C_{29}H_{33}ClN_2O_2$,HCl.

**Usual strength**. 2 mg.

### Identification

A. Transfer a quantity of finely powdered tablets containing about 10 mg of Loperamide Hydrochloride, to a test-tube, add 20.0 ml of *isopropyl alcohol.* shake by mechanical means for one minute, and allow to settle. Dilute 9.0 ml of the supernatant to 10 ml with *0.1 M hydrochloric acid.* The solution so obtained shows absorption maxima and minima at the same wavelengths as that of a similar preparation of *loperamide hydrochloride RS.*

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

### Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 of *0.01 M hydrochloric acid,*
Speed and time. 50 rpm and 30 minutes.

Withdraw 10 ml of the medium from each vessel, mix and filter.

*Test solution.* The mixed filtrate from the dissolution medium.



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*Reference solution.* A solution of *loperamide hydrochloride RS* in the dissolution medium containing the same concentration of loperamide hydrochloride as that expected in the dissolution medium in the vessel.

Determine by liquid chromatography (2.4.14) using the chromatographic system described under Assay.

Calculate the content of $C_{29}H_{33}ClN_2O_2$, HCl in the medium.

D. Not less than 80 per cent of the stated amount of $C_{29}H_{33}ClN_2O_2$, HCl.

**Uniformity of content**. Comply with the test stated under Tablets.

*Test solution.* Crush 1 tablet and transfer to a 200-ml volumetric flask. Add 4 ml of a 5 per cent w/v solution of *phosphoric acid* and 20 ml of *methanol* shake and dilute to volume with *water.*

*Reference solution.* Dissolve an accurately weighed quantity of *loperamide hydrochloride RS* in *methanol* to obtain a solution having a known concentration of about 2 mg per ml. Quantitatively dilute this solution with *water* to obtain a solution having a known concentration of about *0.2* mg per ml. To 10.0 ml of this solution add 5.0 ml of 5 per cent *phosphoric acid solution* and 25 ml of *methanol,* dilute with *water* to 200.0 ml and mix.

Determine by liquid chromatography (2.4.14) using the chromatographic system described under Assay.

Calculate the content of $C_{29}H_{33}ClN_2O_2$, HCl in the tablet.

**Assay**. Determine by liquid chromatography (2.4.14).

*Buffer solution.* To 3.0 g of *triethylamine hydrochloride* and 1.0 ml of *phosphoric acid* add 550 ml of *water* and mix.

*Test solution.* Weigh and finely powder 20 Tablets. Transfer an accurately weighed quantity of the powder containing about 16 mg of Loperamide Hydrochloride, to a 200-ml volumetric flask. Add 4 ml of a 5 per cent w/v solution of *phosphoric acid* and 20 ml of *methanol,* dilute with *water* to volume, and mix. To 10.0 ml of this solution, add 2.0 ml of 5 per cent w/v solution of *orthophosphoric acid* and 2 ml of *methanol* and dilute to 100.0 ml with *water*.

*Reference solution.* Dissolve an accurately weighed quantity of *loperamide hydrochloride RS* in *methanol* to obtain a solution having a known concentration of about 2 mg per ml. Quantitatively dilute this solution with *water* to obtain a solution having a known concentration of about 0.2 mg per ml. Transfer 10.0 ml of this solution to a 250-ml volumetric flask, add 5.0 ml of 5 per cent *phosphoric acid solution* and 25 ml of *methanol,* dilute with *water* to volume, and mix.

Chromatographic system

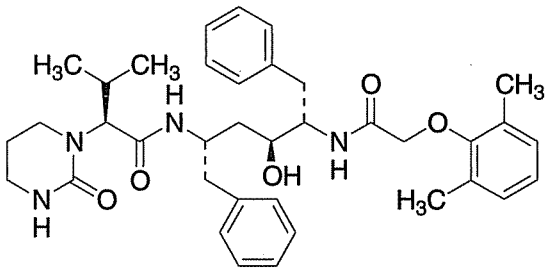
- a stainless steel column 8 cm × 4 mm, packed with octylsilane chemically bonded to totally porous silica particles (5 µm),
- mobile phase: a mixture of 45 volumes of *acetonitrile* and 55 volumes of buffer solution,
- flow rate. 2 ml per minute,
- spectrophotometer set at 214 nm,
- injection volume. 20 µl.

Inject the reference solution. The tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_{29}H_{33}ClN_2O_2$,HCl in the tablets.

# Lopinavir



$C_{37}H_{48}N_4O_5$ Mol. Wt. 628.8

Lopinavir is (α*S*)-*N*-[(1*S*,3*S*,4*S*)-4-[[(2,6-dimethylphenoxy)acetyl]amino-3-hydroxy-5-phenyl-1-(phenylmethyl)pentyl]tetrahydro-α-(1-methylethyl)-2-oxo-1-(2*H*)-pyrimidineacetamide.

Lopinavir contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{37}H_{48}N_4O_5$, calculated on the anhydrous basis.

**Category**. Antiretroviral.

**Description**. A white or almost white powder.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *lopinavir RS* or with the reference spectrum of lopinavir.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to lopinavir in the chromatogram obtained with the reference solution.

## Tests

**Specific optical rotation** (2.4.22). –22.0° to –26.0°, determined in a 0.4 per cent w/v solution in *methanol* and calculated on the anhydrous basis.



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**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. 70 volumes of a buffer and 30 volumes of *acetonitrile*, the buffer being prepared by dissolving 2.72 g of *potassium dihydrogen phosphate* in 900 ml of *water*, pH of which is adjusted to 2.5 with *phosphoric acid*, and diluting to 1000 ml with *water*.

*Test solution*. Dissolve 0.1 g of the substance under examination in 100 ml of solvent mixture.

*Reference solution*. A 0.01 per cent w/v solution of *lopinavir RS* in solvent mixture.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: A. buffer solution pH 2.5,
  B.*acetonitrile,*
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

| Time (in min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0 | 70 | 30 |
| 30 | 30 | 70 |
| 40 | 20 | 80 |
| 50 | 20 | 80 |

Inject the reference solution. The test is not valid unless the column efficiency determined from the lopinavir peak is not less than 5000 theoretical plates and the tailing factor is not more than 1.5.

Inject separately the test solution and the reference solution. Any secondary peak should not be more than 0.3 per cent and the sum of the areas of all the secondary peaks should not be more than 1.0 per cent when calculated by percentage area normalisation.

**Heavy metals** (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Water** (2.3.43). Not more than 4.0 per cent, determined on 0.1 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. 70 volumes of a buffer and 30 volumes of *acetonitrile*, the buffer being prepared by dissolving 2.72 g of *potassium dihydrogen orthophosphate* in 900 ml of *water*, pH of which is adjusted to 2.5 with *orthophosphoric acid*, and diluting to 1000 ml with *water*.

*Test solution*. Dissolve about 50 mg, accurately weighed, of the substance under examination in a suitable quantity of solution A in a 50-ml volumetric flask and dilute to volume with solvent mixture. Dilute 10.0 ml of this solution to 50.0 with solvent mixture.

*Reference solution*. Dissolve about 50 mg, accurately weighed, of *lopinavir RS* in a suitable quantity of solution A in a 50 ml volumetric flask and dilute to volume with solvent mixture. Dilute 10.0 ml of this solution to 50.0 ml with solvent mixture.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: 55 volumes of solution B prepared by mixing 80 volumes of *acetonitrile* and 20 volumes of *methanol* and 45 volumes of *0.05 M potassium dihydrogen phosphate*, the pH of which is adjusted to 3.0 with *dilute phosphoric acid*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency determined from the lopinavir peak is not less than 1500 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject separately the test solution and the reference solution and measure the responses for the principal peak.

Calculate the content of $C_{37}H_{48}N_4O_5$.

**Storage**. Store protected from light and moisture.

# Lopinavir and Ritonavir Capsules

Lopinavir and Ritonavir Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of lopinavir, $C_{37}H_{48}N_4O_5$ and ritonavir, $C_{37}H_{48}N_6O_5S_2$.

**Usual strength**. Lopinavir 200 mg and Ritonavir 50 mg.

## Identification

In the Assay, the principal peaks in the chromatogram obtained with the test solution correspond to the peaks in the chromatogram obtained with the reference solution.

## Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of a solution prepared by dissolving 15.7 g of *polyoxyethylene 10-lauryl ether* in 1000 ml of a 0.85 per cent v/v solution of *hydrochloric acid,*
Speed and time. 75 rpm and 120 minutes



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Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14)

*Test solution.* The filtrate obtained as given above. Dilute the filtrate if necessary, with the dissolution medium.

*Reference solution.* A solution containing 0.15 per cent w/v of *lopinavir RS* and 0.04 per cent w/v of *ritonavir RS* in *methanol.* Dilute 5 ml of the solution to 50 ml with the dissolution medium.

Use the chromatographic system given in the Assay.

Inject the test solution and the reference solution.

Calculate the contents of $C_{37}H_{48}N_4O_5$ and $C_{37}H_{48}N_6O_5S_2$.

D. Not less than 75 per cent of the stated amounts of $C_{37}H_{48}N_4O_5$ and $C_{37}H_{48}N_6O_5S_2$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*For Lopinavir*

*Solvent mixture.* 40 volumes of *buffer solution* and 60 volumes of *acetonitrile.*

*Test solution.* Weigh accurately a quantity of the contents of the capsules containing 100 mg of Lopinavir, disperse in 100 ml of the solvent mixture and filter.

*Reference solution (a).* A 0.1 per cent w/v solution of *lopinavir RS* in the solvent mixture.

*Reference solution (b).* Dilute 1 ml of reference solution (a) to 100 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm),
- mobile phase: A. a mixture of 45 volumes of *acetonitrile* and 55 volumes of a buffer solution prepared by dissolving 1.36 g of *potassium dihydrogen orthophosphate dihydrate* in 1000 ml of *water* and adjusting the pH to 4.0 with *orthophosphoric acid,*

  B. a mixture of 80 volumes of *acetonitrile* and 20 volumes of the buffer solution,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

| Time (in min) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0 | 100 | 0 |
| 80 | 100 | 0 |
| 90 | 0 | 100 |
| 120 | 0 | 100 |
| 130 | 100 | 0 |
| 140 | 100 | 0 |

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with the reference solution (b) (2.0 per cent).

*For Ritonavir*

*Solvent mixture.* 40 volumes of the buffer solution and 60 volumes of *acetonitrile.*

*Test solution.* Weigh accurately a quantity of the contents of the capsules containing 50 mg of Ritonavir, disperse in 100 ml of the solvent mixture and filter.

*Reference solution (a).* A 0.05 per cent w/v solution of *ritonavir RS* in the solvent mixture.

*Reference solution (b).* Dilute 1 ml of reference solution (a) to 100 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm × 4.6 mm, packed with silica gel consisting of porous spherical particles with chemically bonded butyl group ( 3 µm) (such as YMC C4),
- column temperature. 60°,
- mobile phase: A. a mixture of 69 volumes of buffer solution prepared by dissolving 4.1 g of *monobasic potassium phosphate* in 1000 ml of *distilled water* and filtering, and 18 volumes of *acetonitrile*, 8 volumes of *tetrahydrofuran* and 5 volumes of *n-Butanol.*

  B. a mixture of 40 volumes of the buffer solution, 47 volumes of *acetonitrile*, 8 volumes of *tetrahydrofuran* and 5 volumes of *n-Butanol,*
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 240 nm,
- injection volume. 50 µl.

| Time (in min) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0 | 100 | 0 |
| 60 | 100 | 0 |
| 120 | 0 | 100 |
| 130 | 100 | 0 |
| 155 | 100 | 0 |

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 5000 theoretical plates and the tailing factor is not more than 2.0.



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Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 2.5 times the area of the peak in the chromatogram obtained with the reference solution (b) (2.5 per cent) and the sum of areas of all the secondary peaks is not more than 5 times the area of the peak in the chromatogram obtained with the reference solution (b) (5.0 per cent).

**Other tests**. Comply with the tests stated under Capsules.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Mix the contents of 20 capsules. Weigh accurately a quantity of the mixed contents containing 70 mg of Lopinavir and disperse in 100.0 ml of *methanol* and filter. Dilute 5.0 ml of the filtrate to 50.0 ml with the mobile phase.

*Reference solution*. A solution containing 0.070 per cent w/v of *lopinavir RS* and 0.0175 per cent w/v of *ritonavir RS* in *methanol*. Dilute 10.0 ml of the solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 5 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica ( 5 µm),
- mobile phase: a mixture of 45 volumes of *buffer solution* prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate dihydrate* in 1000 ml with *water* and adjusting the pH to 3.0 with *orthophosphoric acid,* 42.5 volumes of *acetonitrile* and 12.5 volumes of *methanol,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the resolution between lopinavir and ritonavir peak is not less than 2.5, the column efficiency for each component is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the contents of $C_{37}H_{48}N_4O_5$ and $C_{37}H_{48}N_6O_5S_2$ in the capsules.

**Storage**. Store protected from moisture in a refrigerator (2° to 8°).

# Lopinavir and Ritonavir Tablets

Lopinavir and Ritonavir Tablets contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of lopinavir, $C_{37}H_{48}N_4O_5$ and ritonavir, $C_{37}H_{48}N_6O_5S_2$.

**Usual strength**. Lopinavir 200 mg and Ritonavir 50 mg.

## Identification

In the Assay, the principal peaks in the chromatogram obtained with test solution correspond to the principal peaks in the chromatogram obtained with the reference solution.

## Tests

**Dissolution** (2.5.2).

Apparatus. No 1

Medium. 900 ml of a solution prepared by dissolving 15.7 g of *polyoxyethylene 10 lauryl ether* in 1000 ml of a 0.85 per cent v/v solution of *hydrochloric acid.*

Speed and time. 75 rpm and 120 minutes

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14)

*Test solution*. The filtrate obtained as given above. Dilute the filtrate if necessary, with the dissolution medium.

*Reference solution*. A solution containing 0.22 per cent w/v of *lopinavir RS* and 0.055 per cent w/v of *ritonavir RS* in *methanol*. Dilute 5 ml of the solution to 50 ml with the dissolution medium.

Use the chromatographic system described under Assay.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

D. Not less than 70 per cent of the stated amounts of $C_{37}H_{48}N_4O_5$ and $C_{37}H_{48}N_6O_5S_2$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*For Lopinavir*

*Test solution*. Disperse accurately a quantity of the powdered tablets containing 100 mg of Lopinavir in 100 ml of the mobile phase.

*Reference solution (a)*. A 0.1 per cent w/v solution of *lopinavir RS* in the mobile phase.

*Reference solution (b)*. Dilute 1 ml of reference solution (a) to 100 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5mm),
- mobile phase: a mixture of 55 volumes of a buffer solution prepared by dissolving 1.36 g of *potassium dihydrogen orthophosphate in* 1000 ml of *water* and adjusting the pH to 4.0 with *orthophosphoric acid*, and 45 volumes of *acetonitrile,*
- flow rate.1.5 ml per minute,



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– spectrophotometer set at 210 nm,
– injection volume. 20 µl.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 3000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with the reference solution (b) (2.0 per cent).

*For Ritonavir*

*Solvent mixture.* A mixture of 40 volumes of a buffer solution prepared by dissolving 4.1 g of *potassium dihydrogen phosphate* in 1000 ml of *water*, and 60 volumes of *acetonitrile*,

*Test solution.* Disperse an accurately weighed quantity of the powdered tablets containing 100 mg of Ritonavir in 100 ml of the solvent mixture.

*Reference solution (a).* A 0.1 per cent w/v solution of *ritonavir RS* in the solvent mixture.

*Reference solution (b).* Dilute 1 ml of reference solution (a) to 100 ml with the solvent mixture.

Chromatographic system

– a stainless steel column 15 cm × 4.6 mm, packed with silica gel consisting of porous spherical particles with chemically bonded with butyl group (3µm) (such as YMC C4),
– column temperature 60°,
– mobile phase: A. a mixture of 69 volumes of a buffer solution prepared by dissolving 4.1 g of *potassium dihydrogen phosphate* in 1000 ml of *water,* 18 volumes of *acetonitrile*, 8 volumes of *tetrahydrofuran* and 5 volumes of *n- butanol*,

B. a mixture of 40 volumes of *buffer solution*, 47 volumes of *acetonitrile*, 8 volumes of *tetrahydrofuran* and 5 volumes of *n-butanol*,

– flow rate.1 ml per minute,
– a linear gradient programme using the conditions given below,
– spectrophotometer set at 240 nm,
– injection volume. 50 µl.

| Time (in min.) | Mobile phase A (per cent w/v) | Mobile phase B (per cent w/v) |
|---|---|---|
| 0 | 100 | 0 |
| 60 | 100 | 0 |
| 120 | 0 | 100 |
| 121 | 100 | 0 |
| 135 | 100 | 0 |

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 3000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 2.5 times the area of the peak in the chromatogram obtained with the reference solution (b) (2.5 per cent) and the sum of areas of all the secondary peaks is not more than 5 times the area of the peak in the chromatogram obtained with the reference solution (b) (5.0 per cent).

**Other tests**. Comply with the tests stated under Tablets.

**Water** (2.3.43). Not more than 6.0 per cent, determined on 0.5 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh and powder 20 tablets. Dissolve an accurately weighed quantity of the powder containing 200 mg of Lopinovir in 250.0 ml of *methanol.* Dilute 5.0 ml of the resulting solution to 50.0 ml with the mobile phase.

*Reference solution.* 5.0 ml of each of a 0.08 per cent w/v solution of *lopinavir RS* and a 0.02 per cent w/v solution of *ritonavir RS* in *methanol,* diluted to 50.0 ml with the mobile phase.

Chromatographic system

– a stainless steel column 15 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
– mobile phase: A. a mixture of 80 volumes of *acetonitrile* and 20 volumes of *methanol,*

B. a mixture of 45 volumes of a buffer solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate anhydrous* in 1000 ml of *water* and adjusting the pH to 3.0 with *orthophosphoric acid*, and 55 volumes of mobile phase A.

– flow rate. 1.5 ml per minute,
– spectrophotometer set at 210 nm,
– injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the resolution between the peak due to lopinavir (retention time, about 6 minutes) and the peak due to ritonavir (retention time, about 5 minutes) is less than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the contents of $C_{37}H_{48}N_4O_5$ and $C_{37}H_{48}N_6O_5S_2$ in the tablets.

**Storage**. Store protected from moisture, at a temperature not exceeding 30°.

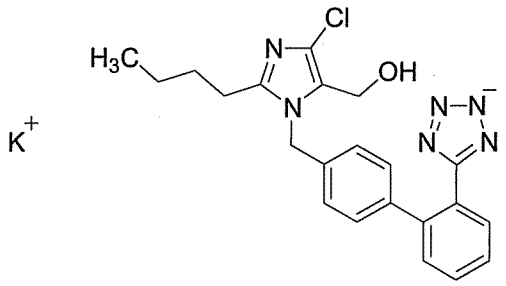


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# Losartan Potassium



$C_{22}H_{22}ClKN_6O$ Mol. Wt. 461.0

Losartan Potassium is monopotassium salt of 2-butyl-4-chloro-1-[[2′-(1*H*-tetrazol-5-yl)[1,1′-biphenyl]-4-yl]methyl]-1*H*-imidazole-5-methanol.

Losartan Potassium contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{22}H_{22}ClKN_6O$, calculated on the anhydrous basis.

**Category**. Antihypertensive.

**Description**. A white to off-white crystalline powder.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *losartan potassium RS* or with the reference spectrum of losartan potassium.

B. When examined in the range 200 to 400 nm (2.4.7), a 0.001 per cent w/v solution of *methanol* and compares with the absorbance obtained with a solution of *losartan potassium RS* prepared in a similar manner.

C. Gives reaction A of potassium (2.3.1).

## Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 30 mg of the substance under examination in 100 ml of *methanol*.

*Reference solution (a)*. A 0.03 per cent w/v solution of *losartan potassium RS* and 0.0002 per cent of *triphenyl-methanol* in *methanol*.

*Reference solution (b)*. A 0.0003 per cent w/v solution of *losartan potassium RS* in *methanol*.

Chromatographic system

- a stainless steel column 25 cm × 4.0 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. 0.1 per cent solution of *ortho-phosphoric acid* in *water* and filter,
  B. *acetonitrile*,
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 10 µl.

| Time (in min.) | Mobile phase A ( per cent v/v) | Mobile phase B ( per cent v/v) |
|---|---|---|
| 0 | 75 | 25 |
| 35 | 10 | 90 |
| 50 | 75 | 25 |

Inject reference solution (a). The relative retention times are about 1.0 for losartan and 1.9 for triphenylmethanol and the tailing factor for losartan is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent).

**Heavy metals** (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

**Water** (2.3.43). Not more than 0.5 per cent, determined on 1.0 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 25 mg of the substance under examination in 100.0 ml of *methanol*.

*Reference solution*. A 0.025 per cent w/v solution of *losartan potassium RS* in *methanol*.

Chromatographic system

- a stainless steel column 25 cm × 4.0 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. 0.1 per cent w/v solution of *ortho-phosphoric acid* in *water* and filter,
  B. *acetonitrile*,
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

| Time (in min.) | Mobile phase A ( per cent v/v) | Mobile phase B ( per cent v/v) |
|---|---|---|
| 0 | 75 | 25 |
| 35 | 10 | 90 |
| 50 | 75 | 25 |



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Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5, the column efficiency in not less than 5000 theoretical plates. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{22}H_{22}ClKN_6O$.

**Storage**. Store protected from moisture.

# Losartan Tablets

Losartan Potassium Tablets

Losartan Tablets contain Losartan Potassium.

Losartan Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of losartan potassium, $C_{22}H_{22}ClKN_6O$.

**Usual strength**. 50 mg.

## Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

## Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *water,*
Speed and time. 50 rpm for 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 250 nm (2.4.7). Calculate the content of $C_{22}H_{22}ClKN_6O$ in the medium from the absorbance obtained from a solution of known concentration of *losartan potassium RS* in the same medium.

D. Not less than 75 per cent of the stated amount of $C_{22}H_{22}ClKN_6O$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh and powder 20 tablets. Weigh accurately a quantity of the powdered tablet containing 100 mg of Losartan Potassium, disperse in 100 ml of *water* and filter.

*Reference solution (a)*. A 0.1 per cent w/v solution of *losartan potassium RS* in *water.*

*Reference solution (b)*. Dilute 1 ml of reference solution (a) to 100 ml with *water*.

Chromatographic system
- a stainless steel column 25 cm × 4.0 mm packed with octylsilane bonded to porous silica (5 µm), (such as Lichrosphere RP8e),
- mobile phase: a mixture of 75 volumes of buffer solution prepared by mixing 770 mg of *ammonium acetate* in 1000 ml of *water,* add 2.0 ml of *triethylamine,* adjust pH to 6.5 with *glacial acetic acid* and 25 volumes of *acetonitrile*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume. 10 µl.

Inject reference solution (b). The test is not valid unless the tailing factor is not more than 3.0 and the column efficiency in not less than 1000 theoretical plates.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than 2 times the area of the peak in the chromatogram obtained with the reference solution (b) (2.0 per cent).

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Transfer intact tablets in a suitable volumetric flask, dissolve in mobile phase and disperse completely. Dilute with mobile phase to obtain a final concentration of 0.0125 per cent w/v.

*Reference solution*. A 0.125 per cent w/v solution of *losartan potassium RS* in mobile phase. Dilute 10.0 ml of the solution to 100.0 ml with mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.0 mm packed with octylsilane bonded to porous silica (5 mm), (such as Lichrosphere RP8e)
- mobile phase: a mixture of 65 volumes of *0.005 M ammonium acetate*, 30 volumes of *acetonitrile,* 5 volumes of *methanol* and 0.2 volumes of *triethylamine*, adjust the pH to 6.6 with *glacial acetic acid* and filter,
- flow rate. 1 ml per minute,
- spectrophotometer set at 237 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, the column efficiency is not less than 5000 theoretical plates and the relative standard deviation for replicate injections is not more than 2 per cent.

Inject the test solution and the reference solution.



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Calculate the content of $C_{22}H_{22}ClKN_6O$.

**Storage**. Store protected from light and moisture.

**Labelling**. The label states the strength of Losartan Potassium.

## Losartan Potassium and Amlodipine Tablets

Amlodipine and Losartan Potassium Tablets; Losartan Potassium and Amlodipine Besylate Tablets

Losartan Potassium and Amlodipine Tablets contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of losartan potassium, $C_{22}H_{22}ClKN_6O$, and amlodipine besylate equivalent to amlodipine, $C_{20}H_{25}ClN_2O_5$.

### Identification

In the Assay, the two principle peaks in the chromatogram obtained with the test solution corresponds to the peaks due to losartan potassium and amlodipine in the chromatogram obtained with the reference solution.

### Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.01 M sodium acetate solution*, adjusted to pH 4.5 with *glacial acetic acid,*
Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14), using the chromatographic system as described under Assay, except using injection volume. 50 µl.

*Test solution*. Dilute the filtrate, if necessary, with the dissolution medium.

*Reference solution*. Dissolve an accurately weighed quantity of *amlodipine besylate RS* and *losartan potassium RS* in the dissolution medium and dilute with the dissolution medium to obtain a solution having a known concentration similar to the expected concentration of the test solution.

Calculate the contents of $C_{20}H_{25}ClN_2O_5$ and $C_{22}H_{22}ClKN_6O$.

D. Not less than 70.0 per cent of the stated amounts of $C_{22}H_{22}ClKN_6O$ and $C_{20}H_{25}ClN_2O_5$.

**Uniformity of content** *(for amlodipine)*. Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), using the chromatographic system as described under Assay.

*Test solution.* Transfer 1 tablet to a 100 ml volumetric flask. Add 5 m of *water* and sonicate for 10 minutes. Add 70 ml of the mobile phase, sonicate for 10 minutes and dilute to the volume with the mobile phase, filter.

*Reference solution.* A 0.007 per cent w/v solution of *amlodipine besylate RS* in the mobile phase.

Inject the test solution and the reference solution.

Calculate the content of $C_{20}H_{25}ClN_2O_5$.

1 mg of Amlodipine Besylate is equivalent to 0.000721 g of Amlodipine.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh 10 intact tablets and transfer to 250-ml volumetric flask. Add 20 ml of *water* and sonicate for 10 minutes. Add about 170 ml of the mobile phase and sonicate for 10 minutes. Make up the volume with the mobile phase, mix and centrifuge. Dilute 5 ml of the supernatant to 25 ml with the mobile phase and filter.

*Reference solution.* A 0.0055 per cent w/v solution of *amlodipine besylate RS* and 0.04 per cent w/v solution of *losartan potassium RS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 55 volumes of *phosphate buffer pH 5.0*, prepared by dissolving 0.68 g of *potassium dihydrogen orthophosphate* and 4.0 ml of *triethylamine* in 1000 ml of *water*, adjusted to pH 5.0 with *dilute orthophosphoric acid*, 22 volumes of *acetonitrile* and 18 volumes of *methanol*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 237 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections for each of the peaks corresponding to losartan potassium (first peak) and amlodipine (second peak) is not more than 2.0 per cent. The tailing factor for both the peaks due to amlodipine and losartan potassium is not more than 2.0.

Inject the reference solution and the test solution.

Calculate the content $C_{20}H_{25}ClN_2O_5$ and $C_{22}H_{22}ClKN_6O$ in the tablets.

1 mg of Amlodipine besylate is equivalent to 0.000721 g of Amlodipine.

**Storage**. Store protected from light and moisture.

**Labeling**. The quantity of Amlodipine besylate is mentioned in the equivalent terms of Amlodipine.



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# Losartan Potassium and Hydrochlorothiazide Tablets

Hydrochlorothiazide and Losartan Potassium Tablets

Losartan Potassium and Hydrochlorothiazide Tablets contain not less than 90.0 per cent and not more than110.0 per cent of the stated amount of losartan potassium, $C_{22}H_{22}ClKN_6O$ and hydrochlorothiazide,$C_7H_8ClN_3O_4S_2$.

## Identification

In the Assay, the principal peaks in the chromatogram obtained with the test solution corresponds to the peaks in the chromatogram obtained with reference solution (c) of losartan potassium & hydrochlorothiazide.

## Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *water,*
Speed and time. 100 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

*Test solution.* Use the filtrate obtained above.

*Reference solution (a).* A 0.05 per cent w/v solution of *losartan potassium RS* in dissolution medium.

*Reference solution (b).* Dissolve about 25 mg of *hydrochlorothiazide RS* in 10 ml of *acetonitrile* and dilute to 100.0 ml with dissolution medium.

*Reference solution (c).* Dilute 10.0 ml of reference solution (a) and 5.0 ml of reference solution (b) to 100.0 ml with dissolution medium.

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 60 volumes of buffer solution prepared by dissolving 0.78 g of *sodium dihydrogen orthophosphate* in 500 ml of *water*, adjusted to pH 2.5 with *orthophosphoric acid* and 40 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 20 µl.

Inject reference solution (c ).The test is not valid unless the tailing factor of both the principal peaks is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (c) and the test solution.

Calculate the content of $C_{22}H_{22}ClK_6O$ and $C_7H_8ClN_3O_4S_2$ in the dissolution medium.

D. Not less than 70 per cent of the stated amount of $C_{22}H_{22}ClKN_6O$ and $C_7H_8ClN_3O_4S_2$.

**Uniformity of content**. Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), using the chromatographic system as described under Dissolution.

*For Hydrochlorothiazide —*

*Test solution.* Disperse 1 whole tablet in 100 ml of the mobile phase. Dilute 5 ml of this solution to 50 ml with the mobile phase.

*Reference solution.* A 0.025 per cent w/v solution of *hydrochlorothiazide RS* in the mobile phase. Dilute 5.0 ml of this solution to 100.0 ml with the mobile phase.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_7H_8ClN_3O_4S_2$.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh and powder 20 tablets. Disperse a quantity of powder containing about 50 mg of Losartan Potassium and 12.5 mg of Hydrochlorothiazide in 100.0 ml of the mobile phase, filter. Dilute 5 ml of the filtrate to 50 ml with the mobile phase.

*Reference solution (a).* A 0.05 per cent w/v solution of *losartan potassium RS* in the mobile phase.

*Reference solution (b).* A 0.025 per cent w/v solution of *hydrochlorothiazide RS* in the mobile phase.

*Reference solution (c).* Dilute 10 ml of reference solution (a) and 5 ml of reference solution (b) to 100 ml with the mobile phase.

Use chromatographic system as described under Dissolution.

Inject reference solution (c). The test is not valid unless the tailing factor is not more than 2.0 and relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (c) and the test solution.

Calculate the content of $C_{22}H_{22}ClKN_6O$ and $C_7H_8ClN_3O_4S_2$ in the tablets.

**Storage**. Store protected from light and moisture.



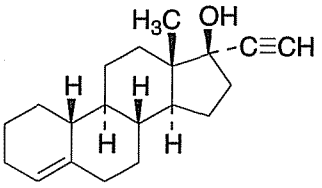
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# Lynoestrenol

Lynestrenol




$C_{20}H_{28}O$ Mol. Wt. 284.4

Lynoestrenol is 19-nor-17α-pregn-4-en-20-yn-17β-ol.

Lynoestrenol contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{20}H_{28}O$, calculated on the dried basis.

**Category**. Progestogen.

**Description**. A white or almost white, crystalline powder.

## Identification

*Test A may be omitted if tests B and C are carried out. Tests B and C may omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *lynoestrenol RS* .

B. In the test of Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

C. Melting range. 161° to 165° (2.4.21).

## Tests

**Appearance of solution**. A 2.0 per cent w/v solution in *ethanol (95 per cent)* is clear (2.4.1), and colourless (2.4.1).

**Specific optical rotation** (2.4.22). –9.5° to –11.0°, determined in a 3.6 w/v solution in *ethanol (95 per cent)*.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 80 volumes of *n-heptane* and 20 volumes of *acetone*.

*Test solution (a)*. Dissolve 0.5 g of the substance under examination in 100 ml of *chloroform*.

*Test solution (b)*. Dissolve 0.25 g of the substance under examination in 100 ml of *chloroform*.

*Reference solution (a)*. A 0.0025 per cent w/v solution of the substance under examination in *chloroform*.

*Reference solution (b)*. A 0.25 per cent w/v solution of *lynoestrenol RS* in *chloroform*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with *0.25 M ethanolic sulphuric acid*, heat at 105° for 10 minutes and examine in ultraviolet light at 365 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a).

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 0.5g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.15 g, dissolve in 40 ml of *tetrahydrofuran*, add 5 ml of a 10 per cent w/v solution of *silver nitrate*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.02844 g of $C_{20}H_{28}O$.

**Storage**. Store protected from light and moisture.



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# M


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# Magaldrate

$Al_5Mg_{10}(OH)_{31}(SO_4)_2,xH_2O$ Mol. Wt. 1097.4 (anhydrous)

Magaldrate is a chemical combination of aluminium and magnesium hydroxides and sulphates corresponding approximately to the formula $Al_5Mg_{10}(OH)_{31}(SO_4)_2,xH_2O$.

Magaldrate contains not less than 90.0 per cent and not more than 105.0 per cent of $Al_5Mg_{10}(OH)_{31}(SO_4)_2$, calculated on the dried basis.

**Category**. Antacid.

**Dose**. 800 mg to 1.6 g.

**Description**. A white or almost white, crystalline powder; odourless.

## Identification

A. Dissolve 0.8 g in 20 ml of *3 M hydrochloric acid*, dilute with *water* to 50 ml, add 3 drops of *methyl red solution* and heat to boiling. Add *dilute ammonia solution* until the colour changes to just yellow, continue boiling for 2 minutes and filter; the filtrate gives the reactions of magnesium salts (2.3.1).

B. Wash the precipitate obtained in test A with 50 ml of a hot 2 per cent w/v solution of *ammonium chloride*, then dissolve the precipitate in 15 ml of *3 M hydrochloric acid*; the solution gives the reactions of aluminium salts (2.3.1).

## Tests

**Arsenic** (2.3.10). To 1.0 g add 15 ml of *hydrochloric acid*, 0.1 ml of *stannous chloride solution AsT* and 5 ml of *potassium iodide solution* and allow to stand for 15 minutes. The resulting solution complies with the limit test for arsenic (10 ppm).

**Heavy metals** (2.3.13). Dissolve 0.33 g in 10 ml of *3 M hydrochloric acid*, filtering to get a clear solution and diluting to 25 ml with *water*. The solution complies with the limit test for heavy metals, Method A (60 ppm).

**Soluble chloride**. Boil 1.0 g, accurately weighed, with 50.0 ml of *water* for 5 minutes, cool, add *water* to the original volume, mix and filter. To 25.0 ml of the filtrate add 0.1 ml of *potassium chromate solution* and titrate with *0.1 M silver nitrate* until a persistent pink colour is obtained; Not more than 5.0 ml of *0.1 M silver nitrate* is required (3.5 per cent).

**Soluble sulphate**. Dilute 2.5 ml of the filtrate obtained in the test for Soluble chloride to 40 ml with *water* in a Nessler cylinder, add 1 ml of *3 M hydrochloric acid* and 3 ml of *barium chloride solution*, dilute to 50 ml, mix and allow to stand for 10 minutes; any turbidity produced is not greater than that produced by treating 1.0 ml of *0.01 M sulphuric acid* in the same manner (1.9 per cent).

**Sodium**. Transfer 2.0 g, accurately weighed, to a 100 ml volumetric flask, place in an ice-bath, add 5 ml of *nitric acid* and swirl to dissolve. Allow to warm to room temperature, dilute with *water* to volume and mix. Filter, if necessary, to obtain a clear solution. Dilute 10.0 ml of the filtrate with *water* to 100.0 ml. The emission intensity of this solution, determined by flame photometry (2.4.4), at about 589 nm and corrected for background transmission at about 580 nm, is not greater than that produced by treating similarly a standard solution containing 2.2 µg of Sodium per ml.

**Aluminium hydroxide**. 32.1 to 45.9 per cent of $Al(OH)_3$, calculated on the dried basis and determined by the following method. Dissolve about 100 mg, accurately weighed, in 3 ml of *dilute hydrochloric acid* and dilute to 30 ml with *water*. Add with stirring 25.0 ml of *0.05 M disodium edetate*, mix and allow to stand for 5 minutes. Add 20 ml of *acetic acid-ammonium acetate buffer*, 60 ml *ethanol (95 per cent)* and 2 ml of *dithizone solution* and titrate with *0.05 M zinc sulphate* to a bright rose-pink colour. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of *disodium edetate* required.

1 ml of *0.05 M disodium edetate* is equivalent to 0.0039 g of $Al(OH)_3$.

**Magnesium hydroxide**. 49.2 to 66.6 per cent of $Mg(OH)_2$, calculated on the dried basis and determined by the following method. Dissolve about 100 mg, accurately weighed, in 3 ml of *dilute hydrochloric acid* and dilute with *water* to about 200 ml. Add with stirring 1 g of *ammonium chloride*, 20 ml of *triethanolamine*, 10 ml of *ammonia-ammonium chloride buffer* and 0.1 ml of *eriochrome black T solution* and titrate with *0.05 M disodium edetate* to a blue colour. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of *disodium edetate* required.

1 ml of *0.05 M disodium edetate* is equivalent to 0.002916 g of $Mg(OH)_2$.

**Sulphates**. 16.0 to 21.0 per cent, calculated on the dried basis and determined by the following method. Weigh accurately about 0.875 g, transfer to a 25-ml volumetric flask, dissolve in 10 ml of water and 5 ml of *glacial acetic acid*, dilute to volume with *water* and mix. Transfer 5.0 ml of this solution to a glass chromatographic column, 1 cm in internal diameter, prepared by filling with 15 ml of a strongly acidic styrene-divinylbenzene cation exchange resin (50 to 100 mesh) (such as Dowex 50W-X8 or Amberlite 120) and washing the resin with 30 ml of *water*. Elute the column with 15 ml of *water* and collect the eluate in a 125-ml conical flask. To the eluate add 5 ml of a 5.38 per cent w/v solution of *magnesium acetate* and 32 ml of *methanol*. Titrate with *0.05 M barium chloride* using a 0.2 per cent w/v solution of *sodium alizarinsulphonate* as the indicator and adding about 5 ml of the titrant in the beginning



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and continuing the titration slowly thereafter until the yellow colour disappears and a pink tinge is visible.

1 ml of *0.05 M barium chloride* is equivalent to 0.004803 g of Sulphates.

**Microbial contamination** (2.2.9). 1 g is free from *Escherichia coli.*

**Loss on drying** (2.4.19). 10.0 to 20.0 per cent, determined on 0.5 g by drying in an oven at 200° for 4 hours.

**Assay**. Weigh accurately about 3.0 g and transfer to a 250 ml conical flask. Add 100.0 ml of *1 M hydrochloric acid* and stir well until a clear solution is obtained. Titrate the excess acid with *1 M sodium hydroxide* to a pH of 3.0, determined potentiometrically (2.4.25). Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of hydrochloric acid required.

1 ml of *1 M hydrochloric acid* is equivalent to 0.0354 g of $Al_5Mg_{10}(OH)_{31}(SO_4)_2$.

**Storage**. Store protected from moisture.

# Magaldrate Oral Suspension

Magaldrate Suspension

Magaldrate Oral Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of anhydrous magaldrate, $Al_5Mg_{10}(OH)_{31}(SO_4)_2$.

**Usual strength**. The equivalent of 800 mg of anhydrous magaldrate per 5 ml.

## Identification

A. Dissolve an amount of the suspension containing about 0.8 g of anhydrous magaldrate in 20 ml of *3 M hydrochloric acid*, dilute with *water* to 50 ml, add 3 drops of *methyl red solution* and heat to boiling. Add *dilute ammonia solution* until the colour changes to just yellow, continue boiling for 2 minutes and filter; the filtrate gives the reactions of magnesium salts (2.3.1).

B. Wash the precipitate obtained in test A with 50 ml of a hot 2 per cent w/v solution of *ammonium chloride*, then dissolve the precipitate in 15 ml of *3 M hydrochloric acid*; the solution gives the reactions of aluminium salts (2.3.1).

## Tests

**Aluminium hydroxide**. 32.1 to 45.9 per cent of the stated content of anhydrous magaldrate, determined by the following method. To an accurately measured quantity containing about 1.0 g of magaldrate add 30 ml of *dilute hydrochloric acid*, shake to dissolve, dilute to 100.0 ml with *water* and mix (solution A). To 10.0 ml of this solution add 20 ml of *water* and Add with stirring 25.0 ml of *0.05 M disodium edetate*, mix and allow to stand for 5 minutes. Add 20 ml of *acetic acid-ammonium acetate buffer*, 60 ml *ethanol (95 per cent)* and 2 ml of *dithizone solution* and titrate with *0.05 M zinc sulphate* to a bright rose-pink colour. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of disodium edetate required.

1 ml of *0.05 M disodium edetate* is equivalent to 0.0039 g of $Al(OH)_3$.

**Magnesium hydroxide**. 49.2 to 66.6 per cent of the stated content of anhydrous magaldrate, determined by the following method. Take 10.0 ml of solution A and dilute with *water* to about 200 ml. Add with stirring 1 g of *ammonium chloride*, 20 ml of *triethanolamine*, 10 ml of *ammonia-ammonium chloride buffer* and 0.1 ml of *eriochrome black T solution* and titrate with *0.05 M disodium edetate* to a blue colour. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of disodium edetate required.

1 ml of *0.05 M disodium edetate* is equivalent to 0.002916 g of $Mg(OH)_2$.

**Neutralising capacity**. To an accurately weighed quantity of the well-shaken suspension containing 0.8 g of anhydrous magaldrate in a 250-ml beaker add *water* to make a total volume of about 70 ml, heat to 37° and stir continuously, maintaining the temperature at 37°. Add 30.0 ml of *1 M hydrochloric acid* previously heated to 37° and maintain at 37° for 15 minutes, stirring continuously. Titrate the excess acid with *1 M sodium hydroxide* to a pH of 3.5. Not more than 12 ml of *1 M sodium hydroxide* is required.

**Microbial contamination** (2.2.9). Total microbial count, not more than 100 per ml; 0.01 ml is free from *Escherichia coli.*

**Other tests**. Complies with the tests stated under Oral Liquids.

**Assay**. To an accurately measured volume containing about 3.0 g of anhydrous magaldrate in a beaker add 100.0 ml of *1 M hydrochloric acid* and stir well until a solution is obtained. Titrate the excess acid with *1 M sodium hydroxide* to a pH of 3.0, determined potentiometrically (2.4.25). Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of hydrochloric acid required.

1 ml of *1 M hydrochloric acid* is equivalent to 0.0354 g of $Al_5Mg_{10}(OH)_{31}(SO_4)_2$.

**Storage**. Store protected from moisture.

**Labelling**. The label states the strength in terms of mg of anhydrous magaldrate per 5 ml.



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# Magaldrate Tablets

Magaldrate Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of anhydrous magaldrate, $Al_5Mg_{10}(OH)_{31}(SO_4)_2$.

**Usual strength**. The equivalent of 800 mg of anhydrous magaldrate.

## Identification

To a quantity of the powdered tablets containing about 2 g of anhydrous magaldrate, add about 60 ml of *water*, shake for 3 minutes, centrifuge and discard the supernatant solution. Repeat the washing with three more quantities, each of 60 ml, of *water*. Transfer the residue to a beaker and heat on a water-bath to dryness.

A. Dissolve 0.8 g of the residue in 20 ml of *3 M hydrochloric acid*, dilute with *water* to 50 ml, add 3 drops of *methyl red solution* and heat to boiling. Add *dilute ammonia solution* until the colour changes to just yellow, continue boiling for 2 minutes and filter; the filtrate gives the reactions of magnesium salts (2.3.1).

B. Wash the precipitate obtained in test A with 50 ml of a hot 2 per cent w/v solution of *ammonium chloride*, then dissolve the precipitate in 15 ml of *3 M hydrochloric acid*; the solution gives the reactions of aluminium salts (2.3.1).

## Tests

**Aluminium hydroxide**. 32.1 to 45.9 per cent of the stated content of anhydrous magaldrate, determined by the following method. Weigh and finely powder 20 tablets. To an accurately weighed quantity of the powder containing about 1.0 g of anhydrous magaldrate add 30 ml of *dilute hydrochloric acid*, shake well to dissolve, dilute to 100.0 ml with *water* and mix (solution A). To 10.0 ml of this solution add 20 ml of *water* and add with stirring 25.0 ml of *0.05 M disodium edetate*, mix and allow to stand for 5 minutes. Add 20 ml of *acetic acid-ammonium acetate buffer*, 60 ml *ethanol (95 per cent)* and 2 ml of *dithizone solution* and titrate with *0.05 M zinc sulphate* to a bright rose-pink colour. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of disodium edetate required.

1 ml of *0.05 M disodium edetate* is equivalent to 0.0039 g of $Al(OH)_3$.

**Magnesium hydroxide**. 49.2 to 66.6 per cent of the stated content of anhydrous magaldrate, determined by the following method. Take 10.0 ml of solution A and dilute with *water* to about 200 ml. Add with stirring 1 g of *ammonium chloride*, 20 ml of *triethanolamine*, 10 ml of *ammonia-ammonium chloride buffer* and 0.1 ml of *eriochrome black T solution* and titrate with *0.05 M disodium edetate* to a blue colour. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of disodium edetate required.

1 ml of *0.05 M disodium edetate* is equivalent to 0.002916 g of $Mg(OH)_2$.

**Neutralising capacity**. To an accurately weighed quantity of the powdered tablets containing 0.8 g of anhydrous magaldrate in a 250-ml beaker add about 75 ml of *water* heat to 37° and stir continuously, maintaining the temperature at 37°. Add 30.0 ml of *1 M hydrochloric acid* previously heated to 37° and maintain at 37° for 15 minutes, stirring continuously. Titrate the excess acid with *1 M sodium hydroxide* to a pH of 3.5 determined potentiometrically (2.4.25). Not more than 12 ml of *1 M sodium hydroxide* is required.

**Disintegration** (2.5.1). 2 minutes for tablets labelled to be swallowed.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and finely powder 20 tablets. To an accurately weighed quantity of the powder containing about 3.0 g of anhydrous magaldrate in a 200-ml volumetric flask add 100.0 ml of *1 M hydrochloric acid*, shake well for 30 minutes. Dilute to volume and filter. Transfer 100.0 ml to a conical flask. Titrate the excess acid with *1 M sodium hydroxide* to a pH of 3.0, determined potentiometrically (2.4.25). Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of hydrochloric acid required.

1 ml of *1 M hydrochloric acid* is equivalent to 0.03540 g of $Al_5Mg_{10}(OH)_{31}(SO_4)_2$.

**Storage**. Store protected from moisture.

**Labelling**. The label states (1) the strength in terms of the equivalent amount of anhydrous magaldrate; (2) whether the tablets are to be swallowed or chewed.

# Heavy Magnesium Carbonate

Heavy Magnesium Carbonate is a hydrated basic magnesium carbonate.

Heavy Magnesium Carbonate contains the equivalent of not less than 40.0 per cent and not more than 45.0 per cent of MgO.

**Category**. Antacid; osmotic laxative.

**Dose**. As antacid, 300 to 600 mg; as laxative, 2 to 4 g.

**Description**. A white powder; odourless. 15 g occupies a volume of about 30 ml.

## Identification

A. Gives reaction A of carbonates (2.3.1).



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B. Dissolve about 15 mg in 2 ml of *2 M nitric acid* and neutralise with *2 M sodium hydroxide*. The resulting solution gives reaction A of magnesium salts (2.3.1).

### Tests

**Appearance of solution**. Dissolve 5.0 g in a mixture of 70 ml of *5 M acetic acid* and 30 ml of *water*. Filter, if necessary, through a previously ignited and weighed porcelain or silica crucible of a suitable porosity to give a clear filtrate (solution A). Reserve any residue (residue R) for the test for Substances insoluble in *acetic acid*. Solution A is not more intensely coloured than reference solution BS3 (2.4.1).

**Arsenic** (2.3.10). Dissolve 5.0 g in 15 ml of *brominated hydrochloric acid* and 45 ml of *water* and remove the excess of bromine with a few drops of *stannous chloride solution AsT*. The resulting solution complies with the limit test for arsenic (2 ppm).

**Heavy metals** (2.3.13). To 20 ml of solution A add 15 ml of *7 M hydrochloric acid* and shake with 25 ml of *4-methylpentan-2-one* for 2 minutes. Separate the layers, evaporate the aqueous layer to dryness, dissolve the residue in 1 ml of *5 M acetic acid* and dilute to 20 ml with *water*. 12 ml of the resulting solution complies with the limit test for heavy metals, Method D (20 ppm). Use *lead standard solution (1 ppm Pb)* for standard preparation.

**Iron** (2.3.14). Dissolve 0.1 g in 3 ml of *2 M hydrochloric acid* and dilute to 10 ml with *water*. The resulting solution complies with the limit test for iron (400 ppm).

**Chlorides** (2.3.12). 5.0 ml of solution A complies with the limit test for chlorides (0.1 per cent).

**Sulphates** (2.3.17). 0.5 ml of solution A diluted to 15 ml with *distilled water* complies with the limit test for sulphates (0.6 per cent).

**Calcium**. To 0.2 ml of *ethanolic calcium standard solution (100 ppm Ca)* add 1 ml of *ammonium oxalate solution*. After 1 minute add a mixture of 1 ml of *2 M acetic acid* and 15 ml of a solution prepared by diluting 2.6 ml of solution A to 150 ml with *distilled water* and shake. After 15 minutes any opalescence produced is not more intense than that of a standard prepared in the same manner using a mixture of 10 ml of *calcium standard solution (10 ppm Ca)* and 5 ml of *distilled water* in place of solution A (0.75 per cent).

**Copper**. Dissolve 1 g in 5 ml of *hydrochloric acid* and 25 ml of *water*, boil to remove carbon dioxide, make alkaline with *dilute ammonia solution*; no blue colour is produced.

**Soluble substances**. Mix 2.0 g with 100 ml of *water*, boil for 5 minutes, filter whilst hot through a sintered-glass filter (porosity No. 3), allow to cool and dilute to 100.0 ml with *water*. Evaporate 50.0 ml of the filtrate to dryness and dry the residue at 105°; the residue weighs not more than 10 mg (1.0 per cent).

**Substances insoluble in acetic acid**. Residue R when washed, dried and ignited at 600°, weighs not more than 2.5 mg (0.05 per cent).

**Assay**. Weigh accurately about 0.15 g, dissolve in a mixture of 20 ml of *water* and 2 ml of *2 M hydrochloric acid* and add 50 ml of *water*, 10 ml of *strong ammonia-ammonium chloride solution* and titrate with *0.05 M disodium edetate*, using 0.1 g of *mordant black II mixture* as indicator, until a blue colour is obtained.

1 ml of *0.05 M disodium edetate* is equivalent to 0.002015 g of MgO.

**Storage**. Store protected from moisture.

## Light Magnesium Carbonate

Light Magnesium Carbonate is a hydrated basic magnesium carbonate.

Light Magnesium Carbonate contains the equivalent of not less than 40.0 per cent and not more than 45.0 per cent of MgO.

**Category**. Antacid; osmotic laxative.

**Dose**. As antacid, 300 to 600 mg; as laxative, 2 to 4 g.

**Description**. A very light, white powder. 15 g occupies a volume of about 125 ml.

### Identification

A. Gives reaction A of carbonates (2.3.1).

B. Dissolve about 15 mg in 2 ml of *2 M nitric acid* and neutralise with *2 M sodium hydroxide*. The resulting solution gives reaction A of magnesium salts (2.3.1).

### Tests

**Appearance of solution**. Dissolve 5.0 g in a mixture of 70 ml of *5 M acetic acid* and 30 ml of *water*. Filter, if necessary, through a previously ignited and weighed porcelain or silica crucible of a suitable porosity to give a clear filtrate (solution A). Reserve any residue (residue R) for the test for Substances insoluble in *acetic acid*. Solution A is not more intensely coloured than reference solution BS3 (2.4.1).

**Arsenic** (2.3.10). Dissolve 5.0 g in 15 ml of *brominated hydrochloric acid* and 45 ml of *water* and remove the excess of bromine with a few drops of *stannous chloride solution AsT*. The resulting solution complies with the limit test for arsenic (2 ppm).

**Heavy metals** (2.3.13). To 20 ml of solution A add 15 ml of *7 M hydrochloric acid* and shake with 25 ml of *4-methylpentan-2-one* for 2 minutes. Separate the layers, evaporate the aqueous



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layer to dryness, dissolve the residue in 1 ml of *5 M acetic acid* and dilute to 20 ml with *water*. 12 ml of the resulting solution complies with the limit test for heavy metals, Method D (20 ppm). Use *lead standard solution (1 ppm Pb)* to prepare the standard.

**Iron** (2.3.14). Dissolve 0.1 g in 3 ml of *2 M hydrochloric acid* and dilute to 10 ml with *water*. The resulting solution complies with the limit test for iron (400 ppm).

**Chlorides** (2.3.12). 5.0 ml of solution A complies with the limit test for chlorides (0.1 per cent).

**Calcium**. To 0.2 ml of *ethanolic calcium standard solution (100 ppm Ca)* add 1 ml of *ammonium oxalate solution*. After 1 minute add a mixture of 1 ml of *2 M acetic acid* and 15 ml of a solution prepared by diluting 2.6 ml of solution A to 150 ml with *distilled water* and shake. After 15 minutes any opalescence produced is not more intense than that of a standard prepared in the same manner using a mixture of 10 ml of *calcium standard solution (10 ppm Ca)* and 5 ml of *distilled water* in place of solution A (0.75 per cent).

**Copper**. Dissolve 1 g in 5 ml of *hydrochloric acid* and 25 ml of *water*, boil to remove carbon dioxide, make alkaline with *dilute ammonia solution*; no blue colour is produced.

**Soluble substances**. Mix 2.0 g with 100 ml of *water*, boil for 5 minutes, filter whilst hot through a sintered-glass filter (porosity No. 3), allow to cool and dilute to 100.0 ml with *water*. Evaporate 50.0 ml of the filtrate to dryness and dry the residue at 105°; the residue weighs not more than 10 mg (1.0 per cent).

**Substances insoluble in acetic acid**. Residue R when washed, dried and ignited at 600°, weighs not more than 2.5 mg (0.05 per cent).

**Sulphates** (2.3.17). 1 ml of solution A diluted to 15 ml with *distilled water* complies with the limit test for sulphates (0.3 per cent).

**Assay**. Weigh accurately about 0.15 g, dissolve in a mixture of 20 ml of *water* and 2 ml of *2 M hydrochloric acid* and add 50 ml of *water*, 10 ml of *strong ammonia-ammonium chloride solution* and titrate with *0.05 M disodium edetate*, using 0.1 g of *mordant black II mixture* as indicator, until a blue colour is obtained.

1 ml of *0.05 M disodium edetate* is equivalent to 0.002015 g of MgO.

**Storage**. Store protected from moisture

# Magnesium Chloride

$MgCl_2,6H_2O$ Mol. Wt. 203.3

Magnesium Chloride contains not less than 98.0 per cent and not more than 101.0 per cent of $MgCl_2,6H_2O$.

**Category**. Constituent of peritoneal dialysis solutions.

**Description**. Colourless crystals; hygroscopic.

## Identification

A. Gives reaction A of chlorides (2.3.1).

B. Gives reaction A of magnesium salts (2.3.1).

## Tests

**Appearance of solution**. Solution A is clear (2.4.1), and colourless (2.4.1).

**Acidity or alkalinity**. Dissolve 10.0 g in *carbon dioxide-free water* prepared from *distilled water* and dilute to 100 ml with the same solvent (solution A). To 5 ml of solution A add 0.05 ml of *phenol red solution*. Not more than 0.3 ml of either *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the solution.

**Arsenic** (2.3.10). Dissolve 5.0 g in 50 ml of *water* and add 10 ml of *stannated hydrochloric acid*. The resulting solution complies with the limit test for arsenic (2 ppm).

**Heavy metals** (2.3.13). Dissolve 1.0 g in 20 ml of *water*, adding 1 g of *ammonium chloride* and sufficient *water* to produce 25 ml. The resulting solution complies with the limit test for heavy metals, Method A (20 ppm).

**Iron** (2.3.14). 4.0 g complies with the limit test for iron (10 ppm).

**Calcium**. To 25 ml of solution A add sufficient *water* to produce 50 ml and determine by Method B for flame photometry (2.4.4), or by Method B for atomic absorption spectrophotometry (2.4.2), measuring at 423 nm and using *calcium solution FP*, or *calcium solution AAS*, as appropriate, diluted if necessary, with *0.1 M hydrochloric acid* for the standard solution (0.1 per cent).

**Sulphates** (2.3.17). 15 ml of solution A complies with the limit test for sulphates (100 ppm).

**Assay**. Weigh accurately about 0.3 g, dissolve in 30 ml of *water*, add 10 ml of *strong ammonia-ammonium chloride solution* and titrate with *0.05 M disodium edetate* using 0.1 g of *mordant black II mixture* as indicator.

1 ml of *0.05 M disodium edetate* is equivalent to 0.01017 g of $MgCl_2,6H_2O$.

*Magnesium Chloride intended for use in the preparation of dialysis solutions complies with the following additional requirement.*

**Aluminium**. Dissolve 4.0 g in 100 ml of *water* and add 10 ml of *acetate buffer pH 6.0*. Extract with successive quantities of 20, 20 and 10 ml of a 0.5 per cent w/v solution of *8-hydroxyquinoline* in *chloroform* and dilute the combined extracts to



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50 ml with *chloroform*. Use as the standard solution a mixture of 2 ml of *aluminium standard solution (2 ppm Al)*, 10 ml of *acetate buffer pH 6.0* and 98 ml of *water* treated in the same manner and as the blank solution a mixture of 10 ml of *acetate buffer pH 6.0* and 100 ml of *water* treated in the same manner. Measure the fluorescence of the test solution and the standard solution (2.4.5), using an excitation wavelength of about 392 nm and emission wavelength of about 518 nm, and setting the instrument to zero with the blank solution in each case. The fluorescence of the test solution is not greater than that of the standard solution (1 ppm).

**Storage**. Store protected from moisture.

## Magnesium Hydroxide

$Mg(OH)_2$ Mol. Wt. 58.3

Magnesium Hydroxide contains not less than 95.0 per cent and not more than 100.5 per cent of $Mg(OH)_2$.

**Category**. Antacid; osmotic laxative.

**Dose**. As antacid, 500 to 750 mg; as laxative, 2 to 4 g.

**Description**. A bulky white powder.

### Identification

Dissolve about 15 mg in 2 ml of *2 M nitric acid* and neutralise with *2 M sodium hydroxide*. The resulting solution gives reaction A of magnesium salts (2.3.1).

### Tests

**Appearance of solution**. Dissolve 5.0 g in a mixture 50 ml of *5 M acetic acid* and 50 ml of *distilled water*; not more than a slight effervescence is produced. Boil for 2 minutes, cool and dilute to 100 ml with *2 M acetic acid*. Filter, if necessary, through a previously ignited and weighed porcelain or silica crucible of a suitable porosity to give a clear filtrate (solution A). Reserve any residue (residue R) for the test for Substances insoluble in *acetic acid*. Solution A is not more intensely coloured than reference solution BS3 (2.4.1).

**Arsenic** (2.3.10). Dissolve 2.5 g in 18 ml of *brominated hydrochloric acid* and 42 ml of *water* and remove the excess of bromine with a few drops of *stannous chloride solution AsT*. The resulting solution complies with the limit test for arsenic (4 ppm).

**Heavy metals** (2.3.13). To 20 ml of solution A add 15 ml of *7 M hydrochloric acid* and shake with 25 ml of *4-methylpentan-2-one* for 2 minutes. Separate the layers, evaporate the aqueous layer to dryness, dissolve the residue in 1 ml of *5 M acetic acid* and dilute to 20 ml with *water*. 12 ml of the resulting solution complies with the limit test for heavy metals, Method D (30 ppm). Use *lead standard solution (2 ppm Pb)* to prepare the standard.

**Iron** (2.3.14). Dissolve 0.2 g in 7 ml of *2 M hydrochloric acid* and dilute to 20 ml with *water*. 5 ml of the resulting solution complies with the limit test for iron (0.08 per cent).

**Chlorides** (2.3.12). 5.0 ml of solution A diluted to 15 ml with *distilled water* complies with the limit test for chlorides (0.1 per cent).

**Sulphates** (2.3.17). 0.6 ml of solution A diluted to 15 ml with *distilled water* complies with the limit test for sulphates (0.5 per cent).

**Calcium**. To 0.2 ml of *ethanolic calcium standard solution (100 ppm Ca)* add 1 ml of *ammonium oxalate solution*. After 1 minute add a mixture of 1 ml of *2 M acetic acid* and 15 ml of a solution prepared by diluting 1.3 ml of solution A to 150 ml with *distilled water* and shake. After 15 minutes any opalescence produced is not more intense than that of a standard prepared in the same manner using a mixture of 10 ml of *calcium standard solution (10 ppm Ca)* and 5 ml of *distilled water* in place of solution A (1.5 per cent).

**Soluble substances**. Mix 2.0 g with 100 ml of *water*, boil for 5 minutes, filter whilst hot through a sintered-glass filter (porosity No. 3), allow to cool and dilute to 100.0 ml with *water*. Evaporate 50.0 ml of the filtrate to dryness and dry the residue at 105°; the residue weighs not more than 10 mg (1.0 per cent).

**Substances insoluble in acetic acid**. Residue R when washed, dried and ignited at 600°, weighs not more than 5.0 mg (0.1 per cent).

**Loss on ignition** (2.4.20). 30.0 to 32.5 per cent, determined on 0.5 g by igniting at 900° increasing the heat gradually.

**Assay**. Weigh accurately about 0.1 g, dissolve in 20 ml of *2 M hydrochloric acid* and dilute to 100.0 ml with *water*. To 50.0 ml of this solution add 5 ml of *strong ammonia-ammonium chloride solution* and titrate with *0.05 M disodium edetate*, using about 50 mg of *mordant black II mixture* as indicator.

1 ml of *0.05 M disodium edetate* is equivalent to 0.002916 g of $Mg(OH)_2$.

**Storage**. Store protected from moisture.

## Magnesium Hydroxide Oral Suspension

Magnesium Hydroxide Mixture; Milk of Magnesia; Cream of Magnesia

Magnesium Hydroxide Oral Suspension is an aqueous suspension of hydrated magnesium oxide. It may be prepared from a suitable grade of Light Magnesium Oxide.



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Magnesium Hydroxide Oral Suspension contains not less than 7.0 per cent and not more than 8.5 per cent w/w of hydrated magnesium oxide, calculated as $Mg(OH)_2$.

**Dose**. As antacid, 5 to 10 ml; as laxative, 15 to 30 ml.

**Description**. A white, uniform suspension, which does not separate readily on standing.

### Identification

A solution of 1 ml in 2 ml of *dilute hydrochloric acid* gives the reactions of magnesium salts (2.3.1).

### Tests

**Soluble alkalis**. Filter about 25 ml and discard the first 10 ml of the filtrate. Dilute 5 ml of the filtrate with 40 ml of *water*, add 0.05 ml of *methyl red solution* and titrate with *0.05 M sulphuric acid* to a persistent pink colour. Not more than 1.0 ml of *0.05 M sulphuric acid* is required.

**Soluble salts**. To 5 ml of the clear filtrate obtained in the test for Soluble alkalis add 0.15 ml of *sulphuric acid*, evaporate to dryness on a water-bath and then ignite gently to constant weight; the residue does not weigh more than 12 mg.

**Heavy metals** (2.3.13). Dissolve 12.5 g in 10 ml of *hydrochloric acid* and 20 ml of *water*, add 0.5 ml of *nitric acid*, boil to remove any carbon dioxide and filter. To the cooled filtrate add 2 g of *ammonium chloride* and 2 g of *ammonium thiocyanate* and extract with two successive quantities, each of 10 ml, of *ether*. To the aqueous layer add 2 g of *citric acid* and sufficient *water* to produce 50 ml. 12 ml of the solution complies with the limit test for heavy metals, Method D (4 ppm). Use *lead standard solution (1 ppm Pb)* to prepare the standard.

**Sulphates** (2.3.17). Dissolve 2.5 ml in 20 ml of *hydrochloric acid* and dilute to 500 ml with *water*. 15 ml of the resulting solution, filtered if necessary, complies with the limit test for sulphates (0.2 per cent).

**Microbial contamination** (2.2.9). Total microbial count, not more than 100 per ml; 1 ml is free from *Escherichia coli.*

**Other tests**. Complies with the tests stated under Oral Liquids.

**Assay**. Weigh accurately about 10.0 g, mix with 50 ml of *water*, add 50.0 ml of *0.5 M sulphuric acid* and titrate the excess of acid with *1 M sodium hydroxide* using *methyl orange solution* as indicator.

1 ml of *0.5 M sulphuric acid* is equivalent to 0.02916 g of hydrated magnesium oxide calculated as $Mg(OH)_2$.

**Storage**. Store protected from moisture. Do not keep in a refrigerator.

## Heavy Magnesium Oxide

Heavy Magnesia

MgO Mol. Wt. 40.3

Heavy Magnesium Oxide contains not less than 98.0 per cent and not more than 100.5 per cent of MgO, calculated on the ignited basis.

**Category**. Antacid; osmotic laxative.

**Dose**. As antacid, 300 to 600 mg; as laxative, 2 to 4 g.

**Description**. A fine, white powder. 15 g occupies a volume of about 30 ml.

### Identification

Dissolve about 15 mg in 2 ml of *2 M nitric acid* and neutralise with *2 M sodium hydroxide*. The resulting solution gives reaction A of magnesium salts (2.3.1).

### Tests

**Appearance of solution**. Dissolve 5.0 g in a mixture of 70 ml of *5 M acetic acid* and 30 ml of *water*. Filter, if necessary, through a previously ignited and weighed porcelain or silica crucible of a suitable porosity to give a clear filtrate (solution A). Reserve any residue (residue R). Solution A is not more intensely coloured than reference solution BS3 (2.4.1).

**Arsenic** (2.3.10). Dissolve 2.5 g in 15 ml of *brominated hydrochloric acid*, add 45 ml of *water* and remove the excess of bromine with a few drops of *stannous chloride solution AsT*. The resulting solution complies with the limit test for arsenic (4 ppm).

**Heavy metals** (2.3.13). 20 ml of solution A complies with the limit test for heavy metals, Method D (30 ppm).

**Iron** (2.3.14). Dissolve 0.2 g in 7 ml of *2 M hydrochloric acid* and dilute to 20 ml with *water*. 5 ml of the resulting solution complies with the limit test for iron (800 ppm).

**Chlorides** (2.3.12). 5.0 ml of solution A complies with the limit test for chlorides (0.1 per cent).

**Sulphates** (2.3.17). 0.3 ml of solution A complies with the limit test for sulphates (1.0 per cent).

**Calcium**. To 0.2 ml of *ethanolic calcium standard solution (100 ppm Ca)* add 1 ml of *ammonium oxalate solution*. After 1 minute add a mixture of 1 ml of *2 M acetic acid* and 15 ml of a solution prepared by diluting 1.3 ml of solution A to 150 ml with *distilled water* and shake. After 15 minutes any opalescence produced is not more intense than that of a standard prepared in the same manner using a mixture of 10 ml of *calcium standard solution (10 ppm Ca)* and 5 ml of distilled water in place of solution A (1.5 per cent).



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**Soluble substances**. Mix 2.0 g with 100 ml of *water*, boil for 5 minutes, filter whilst hot through a sintered-glass filter (porosity No. 3), allow to cool and dilute to 100.0 ml with *water*. Evaporate 50.0 ml of the filtrate to dryness and dry the residue at 105°. The residue weighs not more than 20 mg (2.0 per cent).

**Substances insoluble in acetic acid**. Residue R when washed with *water*, dried and ignited at 600°, weighs not more than 5 mg (0.1 per cent).

**Loss on ignition** (2.4.20). Not more than 8.0 per cent, determined on 0.5 g when ignited at 900°.

**Assay**. Weigh accurately about 0.35 g, dissolve in 10 ml of *2 M hydrochloric acid* and dilute with *water* to 100.0 ml. To 10.0 ml add 5 ml of *strong ammonia-ammonium chloride solution* and titrate with *0.05 M disodium edetate* using about 50 mg of *mordant black II mixture* as indicator.

1 ml of *0.05 M disodium edetate* is equivalent to 0.002015 g of MgO.

**Storage**. Store protected from moisture.

# Light Magnesium Oxide

Light Magnesia

MgO Mol. Wt. 40.3

Light Magnesium Oxide contains not less than 98.0 per cent and not more than 100.5 per cent of MgO, calculated on the ignited basis.

**Category**. Antacid; osmotic laxative.

**Dose**. As antacid, 300 to 600 mg; as laxative, 2 to 4 g.

**Description**. A very fine, light, white powder. 15 g occupies a volume of about 150 ml.

## Identification

Dissolve about 15 mg in 2 ml of *2 M nitric acid* and neutralise with *2 M sodium hydroxide*. The resulting solution gives reaction A of magnesium salts (2.3.1).

## Tests

**Appearance of solution**. Dissolve 5.0 g in a mixture of 70 ml of *5 M acetic acid* and 30 ml of *water*. Filter, if necessary, through a previously ignited and weighed porcelain or silica crucible of a suitable porosity to give a clear filtrate (solution A). Reserve any residue (residue R) for the test for Substances insoluble in acetic acid. Solution A is not more intensely coloured than reference solution BS2 (2.3.1).

**Arsenic** (2.3.10). Dissolve 2.5 g in 15 ml of *brominated hydrochloric acid*, add 45 ml of *water* and remove the excess of bromine with a few drops of *stannous chloride solution AsT*. The resulting solution complies with the limit test for arsenic (4 ppm).

**Heavy metals** (2.3.13). To 20 ml of solution A add 15 ml of *7 M hydrochloric acid* and shake with 25 ml of *4-methylpentan-2-one* for 2 minutes. Separate the layers, evaporate the aqueous layer to dryness, dissolve the residue in 1 ml of *5 M acetic acid* and dilute to 20 ml with *water*. 12 ml of the resulting solution complies with the limit test for heavy metals, Method D (30 ppm). Use 7.5 ml of *lead standard solution (2 ppm Pb)* for preparing the standard.

**Iron** (2.3.14). Dissolve 40 mg in 5 ml of *2 M hydrochloric acid* and dilute to 10 ml with *water*. The resulting solution complies with the limit test for iron (0.1 per cent).

**Chlorides** (2.3.12). 4.0 ml of solution A complies with the limit test for chlorides (0.125 per cent).

**Sulphates** (2.3.17). 0.3 ml of solution A complies with the limit test for sulphates (1.0 per cent).

**Calcium**. To 0.2 ml of *ethanolic calcium standard solution (100 ppm Ca)* add 1 ml of *ammonium oxalate solution*. After 1 minute add a mixture of 1 ml of *2 M acetic acid* and 15 ml of a solution prepared by diluting 1.3 ml of solution A to 150 ml with *distilled water* and shake. After 15 minutes any opalescence produced is not more intense than that of a standard prepared in the same manner using a mixture of 10 ml of *calcium standard solution (10 ppm Ca)* and 5 ml of *distilled water* in place of solution A (1.5 per cent).

**Soluble substances**. Mix 2.0 g with 100 ml of *water*, boil for 5 minutes, filter whilst hot through a sintered-glass filter (porosity No. 3), allow to cool and dilute to 100.0 ml with *water*. Evaporate 50.0 ml of the filtrate to dryness and dry the residue at 105°. The residue weighs not more than 20 mg (2.0 per cent).

**Substances insoluble in acetic acid**. Residue R when washed with *water*, dried and ignited at 600°, weighs not more than 5 mg (0.1 per cent).

**Loss on ignition** (2.4.20). Not more than 8.0 per cent, determined on 0.5 g when ignited at 900°.

**Assay**. Weigh accurately about 0.35 g, dissolve in 10 ml of *2 M hydrochloric acid* and dilute with *water* to 100.0 ml. To 10.0 ml add 5 ml of *strong ammonia-ammonium chloride solution* and titrate with *0.05 M disodium edetate* using about 50 mg of *mordant black II mixture* as indicator.

1 ml of *0.05 M disodium edetate* is equivalent to 0.002015 g of MgO.

**Storage**. Store protected from moisture.



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# Magnesium Stearate

Magnesium Stearate consists mainly of magnesium stearate $(C_{17}H_{35}CO_2)_2Mg$ with variable proportions of magnesium palmitate, $(C_{15}H_{31}CO_2)_2Mg$ and magnesium oleate, $(C_{17}H_{33}CO_2)_2Mg$.

Magnesium Stearate contains not less than 3.8 per cent and not more than 5.0 per cent of Mg, calculate on the dried basis. The fatty acid fraction contains not less than 40.0 per cent of stearic acid and the sum of stearic acid and palmitic acid is not less than 90.0 per cent.

**Category**. Pharmaceutical aid (lubricant).

**Description**. A very fine, light, white powder; odourless or with a very faint odour of stearic acid; unctuous and free from grittiness.

## Identification

To 5.0 g add 50 ml of *peroxide-free ether*, 20 ml of *dilute nitric acid* and 20 ml of *water* and heat under a reflux condenser until dissolution is complete. Allow to cool. In a separating funnel, separate the aqueous layer and shake the ether layer with 2 quantities, each of 4 ml, of *water*. Combine the aqueous layers, wash with 15 ml of *peroxide-free ether* and dilute to 50 ml with *water* (solution A). Evaporate the organic layer to dryness and dry the residue at 105°.

A. The residue obtained in the preparation of solution A has a freezing point (2.4.11) not less than 53°.

B. The acid value of the fatty acids is 195 to 210, determined on 0.2 g of the residue obtained in the preparation of solution A, dissolved in 25 ml of the prescribed mixture of solvents (2.3.23).

C. In the test for fatty acid composition, the principle peaks in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

D. 1 ml of solution A gives the reaction of magnesium (2.3.1).

## Tests

**Appearance of solution**. Solution A is not more intensely coloured than reference solution YS6 (2.4.1).

**Appearance of solution of the fatty acids**. Dissolve 0.5 g of the residue obtained in the preparation of solution A in 10 ml of *chloroform*. The solution is clear (2.4.1), and not more intensely coloured than reference solution YS5 (2.4.1).

**Acidity or alkalinity**. Mix 1.0 g with 20 ml of *carbon dioxide-free water*, boil for 1 minute, shaking continuously, cool and filter. To 10 ml of filtrate add 0.05 ml of *bromothymol blue solution*. Not more than 0.05 ml of *0.1 M hydrochloric acid* or *0.1 M sodium hydroxide* is required to change the colour of the solution.

**Acid value of the fatty acids**. 195 to 210, determined on 0.2 g of the residue obtained in the preparation of solution A, dissolved in 25 ml of the prescribed mixture of solvents (2.3.23).

**Free stearic acid**. Not more than 3 per cent, determined by the following method. Weigh accurately about 1.0 g into a stoppered flask, add 50 ml of *chloroform*, stopper the flask and shake well. Filter into a beaker through two thicknesses of filter paper taking care to avoid evaporation of the solvent. Wash the filter with 10 ml of *chloroform* and collect the washings in the beaker. Evaporate the chloroform on a water-bath in a current of air. Dissolve the residue in about 10 ml of *ethanol (95 per cent)* previously neutralised to *phenolphthalein solution* and titrate with *0.1 M sodium hydroxide* using *phenolphthalein solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.0284 g of stearic acid.

**Zinc stearate**. Heat 5.0 g with shaking in a mixture of 50 ml of *water* and 50 ml of *dilute sulphuric acid* until the fatty acids separate as an oily layer. Cool, filter the aqueous layer and wash the residue with two successive quantities, each of 5 ml, of hot *water*, combine the filtrate and the washings and dilute to 100 ml with *water*. To 5 ml of the resulting solution add 0.5 ml of *ammonium mercurithiocyanate solution* and 0.05 ml of *copper sulphate solution*. Scratch the walls of the container with a glass rod and allow to stand for 15 minutes; no violet precipitate is formed.

**Heavy metals** (2.3.13). Heat 5.0 g with 40 ml of *2 M acetic acid* and allow to cool. Filter, wash the residue with two quantities, each of 5 ml, of warm *water* and dilute to 100 ml with *water*. 12 ml of the resulting solution complies with the limit test for heavy metals, Method D (20 ppm). Use 1.0 ml of *lead standard solution (10 ppm Pb)* to prepare the standard.

**Chlorides** (2.3.12). 10.0 ml of solution A diluted to 15 ml complies with the limit test for chlorides (250 ppm).

**Sulphates** (2.3.17). Dilute 5.0 ml of solution A to 50.0 ml with *water*. 2.5 ml of this solution diluted to 15 ml with *water* complies with the limit test for sulphates (0.6 per cent)

**Loss on drying** (2.4.19). Not more than 6.0 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.75 g, add 50 ml of a mixture of equal volumes of *1-butanol* and *ethanol*, 5 ml of *strong ammonia solution*, 3 ml of *ammonia buffer pH 10.0*, 30.0 ml of *0.1 M disodium edetate* and 15 mg of *mordant black II mixture*, heat to 45° to 50° and titrate with *0.1 M zinc sulphate* until the colour changes from blue to violet. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of disodium edetate required.

1 ml of *0.1 M disodium edetate* is equivalent to 0.002431 g of Mg.



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**Fatty acid composition**. Determine by gas chromatography (2.4.13).

*Test solution.* Dissolve 100 mg of the substance under examination in 5 ml of *boron trifluoride-methanol* solution. Boil under a reflux condenser for 10 minutes, add 4.0 ml of *heptane* through the condenser for 10 minutes, and add 20.0 ml of a saturated *sodium chloride* solution. Shake and allow the layers to separate. Remove about 2.0 ml of the organic layer and dry 20 mg of *anhydrous sodium sulphate*. Dilute 1.0 ml of the solution to 10.0 ml with *heptane.*

*Reference solution.* Dissolve 50 mg each of *palmitic acid RS* and *stearic acid RS* in 5.0 ml of *boron trifluoride-methanol* solution. Boil under a reflux condenser for 10 minutes, add 4.0 ml of *heptane* through the condenser for 10 minutes, and add 20.0 ml of a saturated *sodium chloride* solution. Shake and allow the layers to separate. Remove about 2.0 ml of the organic layer and dry 20 mg of *anhydrous sodium sulphate*. Dilute 1.0 ml of this solution to 10.0 ml with *heptane*.

Chromatographic system

- a stainless steel column 30 m x 0.32 mm, packed with fused silica coated with macrogol 20000 (film thickness 0.5 µm);
- temperature:

| Column | time (min) | temperature (°) |
|---|---|---|
| | 0-2 | 70 |
| | 2-36 | 70-240 |
| | 36-41 | 240 |

- Inlet port at 220° and detector at 260°,
- flame ionization detector,
- flow rate. 2.4 ml per minute, nitrogen as the carrier gas.

Inject 1µl of the reference solution. The relative retention with reference to methyl stearate for methyl palmitate is about 0.8. The test is not valid unless the resolution between the peaks due to methyl stearate and methyl palmitate is not less than 5.0.

Inject 1µl of the test solution and the reference solution.

Calculate the percentage content of stearic acid and palmitic acid.

# Magnesium Sulphate

Epsom Salts

$MgSO_4,7H_2O$ Mol. Wt. 246.5

Magnesium Sulphate contains not less than 99.0 per cent and not more than 100.5 per cent of $MgSO_4$, calculated on the dried basis.

**Category**. Osmotic laxative; used in the treatment of electrolyte deficiency.

**Dose**. 2 to 16 g.

**Description**. Colourless crystals or a white, crystalline powder.

## Identification

A. Gives the reactions of sulphates (2.3.1).

B. Gives reaction A of magnesium salts (2.3.1).

## Tests

**Appearance of solution**. Dissolve 5.0 g in sufficient *carbon dioxide-free water* to produce 50 ml (solution A). Solution A is clear (2.4.1), and colourless (2.4.1).

**Acidity or alkalinity**. To 10 ml of solution A add 0.05 ml of *phenol red solution*. Not more than 0.2 ml of either *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the solution.

**Arsenic** (2.3.10). Dissolve 5.0 g in 50 ml of *water* and add 10 ml of *stannated hydrochloric acid*. The resulting solution complies with the limit test for arsenic (2 ppm).

**Heavy metals** (2.3.13). Dissolve 2.0 g in 10 ml of *water*, 2 ml of *dilute acetic acid* and sufficient *water* to produce 25 ml. The solution complies with the limit test for heavy metals, Method A (10 ppm).

**Iron** (2.3.14). 2.0 ml of solution A diluted to 20 ml with *water* complies with the limit test for iron (200 ppm).

**Chlorides** (2.3.12). 8.0 ml of solution A complies with the limit test for chlorides (300 ppm).

**Loss on drying** (2.4.19). 48.0 to 52.0 per cent, determined on 0.5 g by drying in an oven at 110° to 120° for 1 hour and then at 400°.

**Assay**. Weigh accurately about 0.3 g, dissolve in 50 ml of *water*, add 10 ml of *strong ammonia-ammonium chloride solution* and titrate with *0.05 M disodium edetate*, using 0.1 g of *mordant black II mixture* as indicator, until a blue colour is obtained.

1 ml of *0.05 M disodium edetate* is equivalent to 0.00602 g of $MgSO_4$.

**Storage**. Store protected from moisture.

# Magnesium Trisilicate

Magnesium Trisilicate is a hydrated magnesium silicate of the approximate composition $2MgO,3SiO_2,xH_2O$.

Magnesium Trisilicate contains not less than the equivalent of 29.0 per cent of MgO and not less than the equivalent of 65.0 per cent of $SiO_2$, both calculated on the ignited basis.



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**Category**. Antacid.

**Dose**. 500 mg to 2 g, repeated in accordance with the needs of the patient.

**Description**. A fine, white or nearly white powder, free from grittiness; slightly hygroscopic.

### Identification

A. To 2.0 g add a mixture of 4 ml of *nitric acid* and 4 ml of *distilled water* and heat to boiling, shaking frequently. Add 12 ml of *distilled water*, allow to cool, filter or centrifuge to obtain a clear solution and dilute the filtrate to 20 ml with *distilled water* (solution A). 1 ml of solution A, after neutralisation with *2 M sodium hydroxide*, gives reaction A of magnesium salts (2.3.1).

B. 0.25 g gives the reaction of silicates (2.3.1).

### Tests

**Alkalinity**. In a tared 200-ml conical flask on a water-bath heat 10 g with 100 g of *water* for 30 minutes, allow to cool and restore the initial weight with *water*. Allow to stand and filter or centrifuge until a clear liquid is obtained. To 10 ml of the clear liquid add 0.1 ml of *phenolphthalein solution*. Not more than 1.0 ml of *0.1 M hydrochloric acid* is required to change the colour of the solution.

**Arsenic** (2.3.10). Disperse 2.5 g in 50 ml of *water* and add 10 ml of *stannated hydrochloric acid AsT*. The resulting solution complies with the limit test for arsenic (4 ppm).

**Heavy metals** (2.3.13). Neutralise 7.5 ml of solution A with *dilute ammonia solution* using *metanil yellow solution* as external indicator, dilute to 15 ml with *water* and filter if necessary. 12 ml of the resulting solution complies with the limit test for heavy metals, Method D (40 ppm).

**Chlorides** (2.3.12). 5.0 ml of solution A diluted to 15 ml with *distilled water* complies with the limit test for chlorides (500 ppm).

**Sulphates** (2.3.17). 0.3 ml of solution A diluted to 15 ml with *distilled water* complies with the limit test for sulphates (0.5 per cent).

**Acid absorption**. Not less than 100 ml of *0.1 M hydrochloric acid* per g, determined by the following method. Suspend 0.25 g in 100.0 ml of *0.1 M hydrochloric acid*, allow to stand in a water-bath at 37° ± 0.5° for 2 hours, shaking frequently and allow to cool. Add 0.1 ml of *bromophenol blue solution* to 20.0 ml of the supernatant liquid and titrate with *0.1 M sodium hydroxide* until a blue colour is produced.

**Water-soluble salts**. In a tared platinum dish evaporate to dryness on a water-bath 20 ml of the clear liquid obtained in the test for Alkalinity and ignite the residue to constant weight at 900°. The residue weighs not more than 30 mg.

**Loss on ignition** (2.4.20). 17.0 to 34.0 per cent, determined on 0.5 g when ignited in a platinum crucible at 900°.

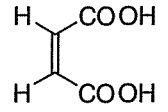
**Assay**. *For MgO* — Weigh accurately about 1.0 g, add 35 ml of *hydrochloric acid* and 50 ml of *water* and allow to stand for 15 minutes on a water-bath. Allow to cool, filter, wash the residue with *water* and dilute the combined filtrate and washings to 250.0 ml with *water*. Neutralise 50.0 ml with about 8 ml of *10 M sodium hydroxide*, add 10 ml of *ammonia buffer pH 10.0*, 50 mg of *mordant black II mixture*, heat to 40° and titrate with *0.05 M disodium edetate* until the colour changes to a deep blue.

1 ml of *0.05 M disodium edetate* is equivalent to 0.002015 g of MgO.

*For $SiO_2$* — Weigh accurately about 0.7 g, add 10 ml of *1 M sulphuric acid* and 10 ml of water and heat on a water-bath for 1.5 hours, shaking frequently and replacing the evaporated *water*. Allow to cool, decant onto an ashless filter paper (7 cm in diameter), wash the precipitate by decantation with three quantities, each of 5 ml, of hot *water*, transfer it to the filter paper and wash it with hot water until 1 ml of the filtrate remains clear on the addition of 2 ml of *barium chloride solution* and 0.05 ml of *2 M hydrochloric acid*. Ignite the filter paper and its contents in a tared platinum crucible at 900° to constant weight; the residue is $SiO_2$.

**Storage**. Store protected from moisture.

## Maleic Acid




$C_4H_4O_4$ Mol. Wt. 116.1

Maleic Acid is (*Z*)-butenedioic acid.

Maleic Acid contains not less than 99.0 per cent and not more than 101.0 per cent of $C_4H_4O_4$, calculated on the anhydrous basis.

**Category**. Pharmaceutical aid.

**Description**. A white or almost white, crystalline powder.

### Identification

A. In the test for fumaric acid, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).



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B. Dissolve 0.1 g of the substance under examination in 10 ml of *water*. To 0.3 ml of this solution add a solution of 10 mg of *resorcinol* in 3 ml of *sulphuric acid*. Heat on a water-bath for 15 minutes, no colour develops. Further to 3 ml of this solution, add 1 ml of *bromine water*. Heat on a water-bath for 15 minutes to remove the bromine, heat to boiling and cool. To 0.2 ml of this solution add a solution of 10 mg of *resorcinol* in 3 ml of *sulphuric acid*. Heat on a water-bath for 15 minutes. A violet-pink colour develops.

### Tests

**Appearance of solution**. A 0.01 per cent w/v solution is clear (2.4.1) and not more intensely coloured than reference solution YS7 (2.4.1).

**Fumaric acid**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 12 volumes of *anhydrous formic acid*, 16 volumes of *chloroform*, 32 volumes of *butanol* and 44 volumes of *heptane*.

*Test solution (a)*. Dissolve about 0.5 g of the substance under examination in 5.0 ml of *acetone*.

*Test solution (b)*. Dilute 1.0 ml of test solution (a) to 50.0 ml with *acetone*.

*Reference solution (a)*. A 0.2 per cent w/v solution of *maleic acid RS* in *acetone*.

*Reference solution (b)*. A 0.15 per cent w/v solution of *fumaric acid RS* in *acetone*.

*Reference solution (c)*. A mixture of 5 ml each of reference solution (a) and (b).

Apply to the plate 5 µl of the test solution (a), (b), reference solution (a) and (b) and 10 µl of reference solution (c). Allow the mobile phase to rise 10 cm. Dry the plate at 100° for 15 minutes and examine in ultraviolet light at 254 nm (2.4.7). Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b) (1.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

**Iron** (2.3.14). To 10 ml of a 0.01 per cent w/v solution in *water*, add 2 ml of *dilute hydrochloric acid* and 0.05 ml of *bromine water*. After 5 minutes, remove the excess of bromine by passing a current of air and add 3 ml of *potassium thiocyanate solution* and shake. Prepare reference solution at the same time and in the same manner, using a mixture of 5 ml of *iron standard solution (1 ppm Fe)*, 1 ml of *dilute hydrochloric acid*, 6 ml of *water* and 0.05 ml of *bromine water*. Allow both solutions to stand for 5 minutes. Any red colour in the test solution is not more intense than that in the reference solution (5 ppm).

**Heavy metals** (2.3.13). Weigh in a silica crucible 2 g of the substance under examination, mix with 0.5 g of *magnesium oxide*. Ignite to dull redness until a homogeneous white or greyish-white mass is obtained. After 30 minutes of ignition if mixture remains coloured, allow to cool, mix using a fine glass rod and repeat the ignition. If necessary repeat the operation. Heat at 800° for about 1 hour. Dissolve the residue in 5 ml of a mixture of equal volumes of *hydrochloric acid* and *water*. Add 0.1 ml of *phenolphthalein solution* and then *concentrated ammonia* until a pink colour is obtained. Cool, add *glacial acetic acid* until the solution is decolourised and add 0.5 ml in excess. Filter if necessary and wash the filter. Dilute to 20 ml with *water*, 12 ml of the resulting solution complies with the limit test for heavy metals, Method D (10 ppm). Use lead standard solution (10 ppm pb), diluting 1 ml standard solution to 10 ml with a mixture of equal volumes of *hydrochloric acid* and *water*.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

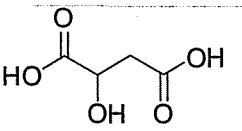
**Water** (2.3.43). Not more than 2.0 per cent, determined on 1.0 g.

**Assay**. Weigh accurately 0.5 g dissolve in 50 ml of *water*. Titrate with *1 M sodium hydroxide* using 0.5 ml of *phenolphthalein solution* as indicator.

1 ml of *1 M sodium hydroxide* is equivalent to 0.05804 g of $C_4H_4O_4$.

**Storage**. Store protected from light.

## Malic Acid




$C_4H_6O_5$ Mol. Wt. 134.1

Malic Acid is (*RS*)-hydroxybutanedioic acid.

Malic Acid contains not less than 99.0 per cent and not more than 101.0 per cent of $C_4H_6O_5$, calculated on the anhydrous basis.

**Category**. Pharmaceutical aid.

**Description**. A white or almost white, crystalline powder.

### Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *malic acid RS* or with the reference spectrum of malic acid.

### Tests

**Appearance of solution**. A 0.02 per cent w/v solution is clear (2.4.1) and colourless (2.4.1).



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**Specific optical rotation** (2.4.22). - 0.10° to + 0.10°, determined on 0.02 per cent w/v solution in *water*.

**Water insoluble substances**. Not more than 0.1 per cent w/w, determined on 0.025 per cent w/v solution of the substance under examination in *water*. Filter the solution through a tared sintered-glass filter, wash the filter with hot *water* and dry to constant weight at 105°. The residue weighs a maximum of 25 mg.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve about 100 mg of the substance under examination in 100.0 ml of the mobile phase.

*Reference solution (a)*. Dissolve 10 mg of *fumaric acid* and 4 mg of *maleic acid* in 50.0 ml of the mobile phase.

*Reference solution (b)*. Dilute 2.5 ml of reference solution (a) to 100.0 ml with the mobile phase.

*Reference solution (c)*. Dissolve 20 mg of the substance under examination in the mobile phase, add 1.0 ml of reference solution (a) and dilute to 20.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 30 cm x 7.8 mm, packed with ion-exclusion resin (9 µm),
- column temperature. 37°,
- mobile phase: *0.005 M sulphuric acid*,
- flow rate. 0.6 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to malic acid and malic acid impurity B is not less than 2.5. The relative retention time with reference to malic acid for fumaric acid (malic acid impurity A) is about 1.5 and for maleic acid (malic acid impurity B) is about 0.8.

Inject reference solution (b) and the test solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of the peak due to fumaric acid is not more than twice the area of the corresponding peak in the chromatogram obtained with reference solution (b) (1.0 per cent), the area of the peak due to maleic acid is not more than 0.25 times the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.05 per cent). The area of any other secondary peak is not more than 0.5 times the area of the peak due to maleic acid in the chromatogram obtained with reference solution (b) (0.1 per cent). The sum of the areas of all secondary peaks is not more than 2.5 times the area of the peak due to maleic acid in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.1 times the area of the peak due to maleic acid in the chromatogram obtained with reference solution (b) (0.02 per cent).

**Heavy metals** (2.3.13). Not more than 20 ppm, determined by the following method .Weigh 1 g of the substance under examination in a 100-ml long necked combination flask. Clamp the flask at an angle of 45°, add a sufficient volume of a mixture of 8 ml of *sulphuric acid* and 10 ml of *nitric acid* to moisten the substance, warm gently until the reaction starts, allow the reaction to subside and add additional portions of the same acid mixture, heating after each addition. Increase the amount of heat and boil gently until the solution darkens. Cool, add 2 ml of *nitric acid* and heat again until the solution darkens. Continue the heating, followed by the addition of *nitric acid* until no further darkening occur, then heat strongly until dense, white fumes are produced. Cool, add 5 ml of *water*, boil gently until dense, white fumes are produced and continue heating to reduce to 2-3 ml. Cool and add 5 ml of *water* and examine the colour of the solution. If the colour is yellow, cautiously add 1 ml of strong *hydrogen peroxide solution* and again evaporate until dense, white fumes are produced and reduce to a volume of 2 to 3 ml. If the solution is still yellow in colour, repeat the addition of 5 ml of *water* and 1 ml of *strong hydrogen peroxide solution* until the solution is colourless. Cool, and dilute to 25 ml with *water* into a 50-ml Nessler cylinder. Adjust the pH to 3.0 with concentrated *ammonia*, dilute with *water* to 40 ml and mix. Add 2 ml of *buffer solution pH 3.5*, mix and add 1.2 ml of *thioacetamide reagent* mix immediately and dilute to 50 ml with *water* and. Any colour produced is not more intense than that produced by treating 2 ml of *lead standard solution* (10 ppm).

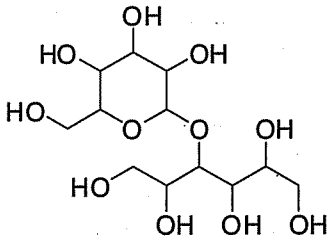
**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 2.0 per cent, determined on 1.0 g.

**Assay**. Weigh accurately 0.5 g, dissolved in 50 ml of *water*. Titrate with *1 M sodium hydroxide* determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *1 M sodium hydroxide* is equivalent to 0.06705 g of $C_4H_6O_5$.

# Maltitol




$C_{12}H_{24}O_{11}$ Mol. Wt. 344.3

Maltitol is α-D-glucopyranosyl-1,4-D-glucitol.



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Maltitol contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{12}H_{24}O_{11}$, calculated on the anhydrous basis.

**Category**. Pharmaceutical aid.

**Description**. A white or almost white crystalline powder.

## Identification

*Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *maltitol RS* or with the reference spectrum of maltitol.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 10 volumes of *water*, 20 volumes of *ethyl acetate* and 70 volumes of *propanol*.

*Test solution*. Dissolve 25 mg of the substance under examination in 10 ml of *water*.

*Reference solution (a)*. A 0.25 per cent w/v solution of *maltitol RS* in *water*.

*Reference solution (b)*. A solution containing 0.25 per cent w/v each of *maltitol RS* and *sorbitol RS* in *water*.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 17 cm. Dry the plate in air, spray with *4-aminobenzoic acid solution*. Remove the plate and place it in a current of cold air until the acetone is removed. Heat at 105° for 15 minutes and allow to cool, spray with a 0.2 per cent w/v solution of *sodium periodate*. Dry in a current of cold air. Heat at 100° for 15 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated principal spots.

C. Melting point (2.4.21). 148° to 151°.

## Tests

**Appearance of solution**. A 10.0 per cent w/v solution in *water* is clear (2.4.1) and colourless (2.4.1).

**Specific optical rotation** (2.4.22). + 105.5° to 108.5°, determined in a 5 per cent w/v solution in *water*, calculated on the anhydrous basis.

**Reducing sugars**. Not more than 0.2 per cent, expressed as glucose equivalent.

Dissolve 5.0 g in 6 ml of *water* with the aid of gentle heat. Cool and add 20 ml of *cupri-citric solution* and a few glass beads, boil for 10 minutes. Cool rapidly and add 100 ml of a 2.4 per cent v/v solution of *glacial acetic acid* and 20.0 ml of *0.025 M iodine*. With continuous shaking, add 25 ml of a mixture of 6 volumes of *hydrochloric acid* and 94 volumes of *water* and, when the precipitate has dissolved, titrate the excess of iodine with *0.05 M sodium thiosulphate* using 1 ml of *starch solution*, added towards the end of the titration as indicator. Not less than 12.8 ml of *0.05 M sodium* thiosulphate is required.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 5 g of the substance under examination in 100.0 ml of *water*.

*Reference solution (a)*. A 5.0 per cent w/v solution of *maltitol RS* in *water*.

*Reference solution (b)*. Dilute 1.0 ml of the test solution to 100.0 ml with *water*.

*Reference solution (c)*. Dilute 10.0 ml of reference solution (b) to 100.0 ml with *water*.

*Reference solution (d)*. A solution containing 5.0 per cent w/v each of *maltitol RS* and *sorbitol RS* in *water*.

Chromatographic system

- a stainless steel column 30 cm × 7.8 mm, packed with strong cation exchange resin (calcium form) (9 µm),
- column temperature. 85°,
- mobile phase: *water*,
- flow rate. 0.5 ml per minute,
- refractive index detector,
- injection volume. 20 µl.

Inject reference solution (d). The test is not valid unless the resolution between the peaks due to maltitol and maltitol impurity A is not less than 2.0. The relative retention time with reference to maltitol for sorbitol (maltitol impurity A) is about 1.8, for maltotriitol (maltitol impurity B) is about 0.8.

Inject reference solution (b), (c) and the test solution. Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). The sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent). Ignore any peak with the area less than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent).

**Lead** (2.3.15). Not more than 0.5 ppm.

**Nickel**. Dissolve 10.0 g in sufficient *water* to produce 20 ml, add 3 ml of *bromine water* and 2 ml of a 20 per cent w/v solution of *citric acid*, mix and 10 ml of 6 M *ammonia* and 1 ml of a 1 per cent w/v solution of *dimethylglyoxime* in *ethanol* (*95 per cent*). Mix, dilute of 50 ml with *water* and allow to stand for 5 minutes; any colour produced is not more intense



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than that produced by treating in the same manner and at the same time 1.0 ml of *nickel standard solution (10 ppm Ni)* diluted to 20 ml with *water* (1 ppm).

**Water** (2.3.43). Not more than 1.0 per cent, determined on 1 g

**Microbial contamination** (2.2.9). Total microbial count is not more than $10^2$ bacteria and $10^2$ fungi per gram, determined by plate count. It complies with the tests for *Escherichia coli* and *Salmonella.*

**Bacterial endotoxins** (2.2.3). Not more than 4 Endotoxin Unit per g for parenteral dosage forms having a concentration of less than 100 g per litre of maltitol and not more than 2.5 Endotoxin Unit per g for parenteral dosage forms having a concentration of less than 100 g per litre or more of maltitol.

**Assay**. Determine by liquid chromatography (2.4.14), as described under Related substances.

Inject the test solution and reference solution (a).

Calculate the content of $C_{12}H_{24}O_{11}$.

**Labelling**. The label states (a) where applicable, the maximum concentration of bacterial endotoxins; (b) where applicable, that the substance is suitable for use in the manufacture of parenteral dosage forms.

# Liquid Maltitol

Aqueous solution of a hydrogenated, partly hydrolysed starch, composed of a mixture of mainly 4-*O*-α-D-glucopyranosyl-D-glucitol (D-maltitol) with D-glucitol (D- sorbitol) and hydrogenated oligo- and polysaccharides.

Liquid Maltitol contains not less than 50.0 per cent of *D-maltitol*, $C_{12}H_{24}O_{11}$, calculated on the anhydrous basis and 95.0 per cent to 105.0 per cent of the content stated on the label; not more than 8.0 per cent of *D-sorbitol*, $C_6H_{14}O_6$, calculated on the anhydrous basis; not less than 68.0 per cent and not more than 85.0 per cent, calculated on the anhydrous basis.

**Category**. Pharmaceutical aid.

**Description**. A clear, colourless and syrupy liquid.

## Identification

*Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.*

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with reference solution (a).

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

*Mobile phase*. A mixture of 10 volumes of *water*, 20 volumes of *ethyl acetate* and 70 volumes of *propanol.*

*Test solution*. Disperse a volume of solution containing about 0.35 g of maltitol to 100.0 ml with *water.*

*Reference solution (a)*. A 0.2 per cent w/v solution of *maltitol RS* in *water.*

*Reference solution (b)*. A solution containing 0.2 per cent w/v each of *maltitol RS* and *sorbitol RS* in *water.*

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 17 cm. Dry the plate in air, spray with *4-aminobenzoic acid solution*. Remove the plate and place it in a current of cold air until the acetone is removed. Heat at 105° for 15 minutes and allow to cool, spray with a 0.2 per cent w/v solution of *sodium periodate*. Dry in a current of cold air. Heat at 100° for 15 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated principal spots.

C. To 3 ml of a freshly prepared 10 per cent w/v solution of *pyrocatechol*, add 6 ml of *sulphuric acid* while cooling in iced water. To 3 ml of the cooled mixture, add 0.3 ml of solution A. Heat gently over a naked-flame for about 30 seconds, a pink colour is produced.

## Tests

**Appearance of solution**. A 14.0 per cent w/v solution in *water* is clear (2.4.1) and colourless (2.4.1).

**Conductivity** (2.4.9). Not more than 10 µS $cm^{-1}$, measured on undiluted liquid maltitol while gently stirring with a magnetic stirrer.

**Reducing sugars**. Not more than 0.2 per cent, calculated as glucose equivalent.

To 5.0 g add 6 ml of *water*, 20 ml of *cupri-citric solution* and a few glass beads. Heat so that boiling begins after 4 minutes and maintain boiling for 3 minutes. Cool rapidly and add 100 ml of a 2.4 per cent v/v solution of *glacial acetic acid* and 20.0 ml of *0.025 M iodine.* With continuous shaking, add 25 ml of a mixture of 6 volumes of *hydrochloric acid* and 94 volumes of *water* and when the precipitate has dissolved, titrate the excess of iodine with *0.05 M sodium thiosulphate* using 1 ml of *starch solution*, added towards the end of the titration, as indicator. Not less than 12.8 ml of *0.05 M sodium thiosulphate* is required.

**Lead** (2.3.15). Not more than 0.5 ppm.

**Nickel**. Dissolve 10.0 g in sufficient *water* to produce 20 ml, add 3 ml of *bromine water* and 2 ml of a 20 per cent w/v solution of *citric acid*, mix and 10 ml of *6 M ammonia* and 1 ml of a 1 per cent w/v solution of *dimethylglyoxime* in *ethanol*



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(*95 per cent*). Mix, dilute of 50 ml with *water* and allow to stand for 5 minutes; any colour produced is not more intense than that produced by treating in the same manner and at the same time 1.0 ml of *nickel standard solution* (*10 ppm Ni*) diluted to 20 ml with *water* (1 ppm).

**Water** (2.3.43). 15.0 per cent to 32.0 per cent, determined on 0.1 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Disperse a volume of solution containing about 1.0 g of Maltitol in 50 ml of *water*.

*Reference solution (a)*. A 1.0 per cent w/v solution of *maltitol RS* in *water*.

*Reference solution (b)*. A 0.16 per cent w/v solution of *sorbitol RS* in *water*.

*Reference solution (c)*. A solution containing 1.0 per cent w/v each of *maltitol RS* and *sorbitol RS* in *water*.

Chromatographic system
- a stainless steel column 30 cm × 7.8 mm, packed with strong cation exchange resin (calcium form) (9 µm),
- column temperature. 85°,
- mobile phase: *water*,
- flow rate. 0.5 ml per minute,
- refractive index detector,
- injection volume. 20 µl.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to sorbitol and maltitol is not less than 2.0. The relative retention time with reference to maltitol for sorbitol is about 1.8.

Inject reference solution (c) and the test solution. Run the chromatograms 3 times the retention time of maltitol.

Calculate the content of *D-maltitol*, $C_{12}H_{24}O_{11}$ and *D-sorbitol*, $C_6H_{14}O_6$.

**Labelling**. The label states the content of D-maltitol.

# Maltodextrin

Maltodextrin is a mixture of glucose, disaccharides and polysaccharides, obtained by the partial hydrolysis of starch.

The degree of hydrolysis, expressed as dextrose equivalent (DE), is not more than 20 (nominal value).

**Category**. Pharmaceutical aid.

**Description**. A white or almost white, slightly hygroscopic powder or granules.

## Identification

A. Dissolve 0.1 g in 2.5 ml of *water* and heat with 2.5 ml of *cupri-tartaric solution*; a red precipitate is produced.

B. Dip for 1 second, a suitable stick with a reactive pad containing glucose-oxidase, peroxidase and a hydrogen-donating substance, such as tetramethylbenzidine, in a 10 per cent w/v solution of the substance under examination. Observe the colour of the reactive pad; within 60 seconds the colour changes from yellow to green or blue.

## Tests

*Solution A*. A 25 per cent w/v solution of the substance under examination in *carbon dioxide-free water*.

**pH** (2.4.24). 4.0 to 7.0, determined in mixture of 1 ml of a 22.36 per cent w/v solution of *potassium chloride* and 30 ml of solution A.

**Sulphur dioxide** (2.3.40). Not more than 20 ppm.

**Heavy metals** (2.3.13). Dilute 4 ml of solution A to 30 ml with *water*, complies with the limit test for heavy metals, (10 ppm).

Prepare the filtration apparatus by adapting the barrel of a 50 ml syringe without its piston to a support containing, on the plate, a membrane filter (pore size 3 µm) and above it a prefilter. Transfer the test solution into the syringe barrel, put the piston in place and then apply an even pressure on it until the whole of the liquid has been filtered. In opening the support and removing the prefilter, check that the membrane filter remains uncontaminated with impurities. If this is not the case replace it with another membrane filter and operation under the same conditions.

To the prefiltrate add 2 ml of *buffer solution pH 3.5* mix and add 1.2 ml of *thioacetamide reagent*. Mix immediately and allow to stand for 10 minutes and again filter as described above, but inverting the order of the filters, the liquid passing first through the membrane filter before passing through the prefilter. The filtration must be carried out slowly and uniformly by applying moderate and constant pressure to the piston of the syringe. After complete filtration, open the support, remove the membrane filter, and dry using filter paper.

Prepare the standard solution using 10 ml of *lead standard solution (1 ppm Pb)*, treat the standard solution in the same manner as the test solution. The colour of the spot obtained with the test solution is not more intense than that obtained with the standard solution.

**Sulphated ash** (2.3.18). Not more than 0.5 per cent.

**Loss on drying** (2.4.19). Not more than 6.0 per cent, determined on 1.0 g by drying in an oven at 105°.

**Dextrose equivalent (DE)**. Within 2 DE units of the nominal value.

Weigh an amount of the substance under examination equivalent to 2.85 to 3.15 g of reducing carbohydrates, calculated as dextrose equivalent, into a 500-ml volumetric



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flask. Dissolve in *water* and dilute to 500.0 ml with the same solvent. Transfer the solution to a 50 ml burette. Pipette 25.0 ml of *cupri-tartaric solution* into a 250 ml flask and add 18.5 ml of the test solution from the burette, mix and add a few glass beads. Place the flask on a hot plate, previously adjusted so that the solution begins to boil within 2 minutes ± 15 seconds. Allow to boil for exactly 120 seconds, add 1 ml of a 0.1 per cent w/v solution of *methylene blue* and titrate with the test solution (V1), until the blue colour disappears. Maintain the solution at boiling throughout the titration. Standardize the cupri-tartaric solution using a 0.6 per cent w/v solution of *glucose (V0).*

$$\frac{300 \times V_0 \times 100}{V_1 \times M \times D}$$

Where, $V_0$ = total volume of glucose standard solution, in millilitres,

$V_1$ = total volume of test solution, in millilitres,

$M$ = sample mass, in grams,

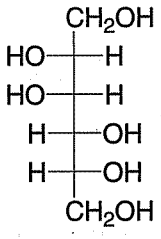
$D$ = percentage content of dry matter in the substance.

**Microbial contamination** (2.2.9). Total microbial count is not more than $10^3$ bacteria and $10^2$ fungi per gram, determined by plate count. It complies with the tests for *Escherichia coli* and *Salmonella.*

**Labelling**. The label states the dextrose equivalent (DE) (nominal value).

# Mannitol

D-Mannitol




$C_6H_{14}O_6$ Mol. Wt. 182.2

Mannitol is D-mannitol, a hexahydric alcohol related to mannose.

Mannitol contains not less than 98.0 per cent and not more than 102.0 per cent of $C_6H_{14}O_6$, calculated on the dried basis.

**Category**. Osmotic diuretic; diagnostic aid (for renal function).

**Dose**. As diuretic, by intravenous infusion, 50 to 200 g over 24 hours but not more than 50 g on one occasion preceded by a test dose of 200 mg per kg of body weight by slow intravenous injection; as diagnostic aid, by intravenous injection, 200 mg per kg of body weight in a 15 to 25 per cent w/v solution.

**Description**. A white, crystalline powder or free-flowing granules.

## Identification

A. Determine by infrared absorption spectrophotometery (2.4.6). Compare the spectrum with that obtained with *mannitol RS* or with the reference spectrum of mannitol.

B. To 1 ml of a saturated solution add 0.5 ml of *ferric chloride test solution* followed by 0.25 ml of *sodium hydroxide solution* and shake well; a clear solution is obtained which remains clear on the further addition of *sodium hydroxide solution.*

C. Dissolve 5 g in sufficient *carbon dioxide-free water* prepared from *distilled water* to produce 50 ml (solution A). Add 0.3 ml of solution A to 3 ml of a cooled mixture prepared by adding 6 ml of *sulphuric acid* to 3 ml of a freshly prepared 10 per cent w/v solution of *catechol* while cooling in ice. Heat gently over a naked flame for about 30 seconds; a pink colour is produced.

D. Melting range 165° to 170° (2.4.21).

## Tests

**Appearance of solution**. Solution A is clear (2.4.1), and colourless (2.4.1).

**Acidity or alkalinity**. To 5 ml of solution A add 5 ml of *carbon dioxide-free water* and 0.05 ml of *dilute phenolphthalein solution*; not more than 0.2 ml of *0.01 M sodium hydroxide* is required to change the colour of the solution to pink. To a further 5 ml of solution A add 5 ml of *carbon dioxide-free water* and 0.05 ml of *methyl red solution.* Not more than 0.3 ml of *0.01 M hydrochloric acid* is required to change the colour of the solution to red.

**Specific optical rotation** (2.4.22). +23.0° to +25.0°, determined in a solution prepared by dissolving 2.0 g of the substance under examination and 2.6 g of *sodium tetraborate* in 20 ml of *water* previously heated at 30° and shaking continuously for 15 to 30 minutes without further heating. Dilute the resulting clear solution to 25.0 ml with *water.*

**Arsenic** (2.3.10). Dissolve 5.0 g in 50 ml of *water* and add 10 ml of *stannated hydrochloric acid.* The resulting solution complies with the limit test for arsenic (2 ppm).

**Chlorides** (2.3.12). A solution of 5.0 g in 10 ml of *water* complies with the limit test for chlorides (50 ppm).

**Sulphates** (2.3.17). A solution of 1.5 g in 10 ml of *water* complies with the limit test for sulphates (100 ppm).



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**Reducing sugars**. Dissolve 5.0 g in 25 ml of *water* with the aid of gentle heat. Cool and add 20 ml of *cupri-citric solution* and a few glass beads. Heat so that boiling begins 4 minutes later and continue to boil for 3 minutes. Cool rapidly and add 100 ml of a 2.4 per cent v/v solution of *glacial acetic acid* and 20.0 ml of *0.025 M iodine*. With continuous shaking add 25 ml of a mixture of 6 volumes of *hydrochloric acid* and 94 volumes of *water* and, when the precipitate has dissolved, titrate the excess of iodine with *0.05 M sodium thiosulphate* using 1 ml of *starch solution*, added towards the end of titration, as indicator. Not less than 12.8 ml of *0.05 M sodium thiosulphate* is required.

**Sorbitol**. Determine by thin-layer chromatography (2.4.17), coating the plate with a uniform 0.75-mm layer of the following mixture. Mix 0.1 g of *carbomer* with 110 ml of *water* and allow to stand, with gentle stirring, for 1 hour. Adjust to pH 7 by the gradual addition, with continuous shaking, of *2 M sodium hydroxide* and add 30 g of *silica gel H*. Heat the plate at 110° for 1 hour, allow to cool and use immediately.

*Mobile phase*. A mixture of 85 volumes of *2-propanol* and 15 volumes of a *0.2 per cent w/v solution of boric acid*.

*Test solution*. Shake 0.5 g of the substance under examination, in fine powder, with 10 ml of *ethanol (95 per cent)* for 30 minutes and filter.

*Reference solution*. A 0.1 per cent w/v solution of *sorbitol RS* in *ethanol (95 per cent)*.

Apply to the plate 2 µl of each solution. After development, dry the plate at 100° to 105° for 15 minutes, allow to cool, spray with a 0.5 per cent w/v solution of *potassium permanganate* in *1 M sodium hydroxide* and heat at 100° for 2 minutes. Any spot corresponding to sorbitol in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent, determined on 2.0 g.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 5.0 g of the substance under examination in 25 ml of *water* and dilute to 100.0 ml with *water*.

*Reference solution*. Dissolve 0.5 g of *mannitol RS* in 2.5 ml of *water* and dilute to 10.0 ml with *water*.

Chromatographic system
- a stainless steel column 30 cm x 7.8 mm packed with strong cation-exchange resin (calcium form) (9 µm),
- column temperature. 85 ± 1°,
- mobile phase: degassed *water*,
- flow rate. 0.5 ml per minute,
- refractometer at constant temperature,
- injection volume. 20 µl.

Inject the test solution and the reference solution. Continue the chromatography for twice the retention time of mannitol.

Calculate the content of $C_6H_{14}O_6$.

*Mannitol intended for use in the manufacture of parenteral preparations without a further appropriate procedure for removal of bacterial endotoxins complies with the following additional requirement.*

**Bacterial endotoxins** (2.2.3). Not more than 4 Endotoxin Unit per g for parenteral preparation having a concentration of 100 g per litre or less of mannitol, and less than 2.5 Endotoxin per g for parenteral preparations having a concentration of more than 100 g per litre of mannitol.

**Labelling**. The label states where applicable, the maximum concentration of bacterial endotoxins; whether or not the substance is suitable for use in the manufacture of parenteral preparations.

**Storage**. Store protected from moisture.

# Mannitol Injection

Mannitol Intravenous Infusion

Mannitol Injection is a sterile solution of Mannitol in Water for Injections.

Mannitol Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of mannitol, $C_6H_{14}O_6$.

**Usual strengths**. 10, 15, 20 and 25 per cent w/v.

**Description**. A colourless or almost colourless clear solution.

## Identification

A. Evaporate to dryness on a water-bath a volume containing 0.2 g of Mannitol. The residue melts at 165° to 170° (2.4.21).

B. Determine by thin layer chromatography (2.4.17), coating the plate with the *silica gel G*.

*Mobile phase*. A mixture of 10 volumes of *water*, 70 volumes of *propan-1-ol* and 20 volumes of *ethyl acetate*.

*Test solution*. Dilute a volume of injection containing 0.25 g of Mannitol to 10 ml with *water*.

*Reference solution*. A 0.25 per cent w/v solution of *mannitol RS* in *water*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air and spray with the 0.2 per cent w/v solution



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of *sodium periodate*. Dry the plate in air for 15 minutes and spray with a 2.0 per cent w/v solution of *4,4'-methylenebis-N,N-dimethylanaline* in a mixture of 1 volume of *glacial acetic acid* and 4 volumes of *acetone*, heat at 105° for 30 minutes and examine in ultraviolet light at 365 nm. The principal spot in chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

C. Dissolve 0.5 g of the residue obtained in test A in sufficient *carbon dioxide-free water* prepared from *distilled water* to produce 5 ml (solution A). Add 0.3 ml of solution A to 3 ml of a cooled mixture prepared by adding 6 ml of *sulphuric acid* to 3 ml of a freshly prepared 10 per cent w/v solution of *catechol* while cooling in ice. Heat gently over a naked flame for about 30 seconds; a pink colour is produced.

### Tests

**pH** (2.4.24). 4.5 to 7.0, determined in a solution containing not more than 10.0 per cent w/v solution of Mannitol, diluted if necessary with *water* and to which 0.3 ml of a saturated solution of *potassium chloride* has been added for each 100 ml of solution.

**Particulate contamination** (2.5.9). When supplied in a container with a nominal content of 100 ml or more, complies with the limit test for particulate contamination.

**Bacterial endotoxins** (2.2.3). Not more than 0.5 Endotoxin Unit per ml of a solution containing not more than 10 per cent w/v of Mannitol. For solutions of higher strength, dilute the injection under examination with *water BET* so that the final solution contains 10 per cent w/v of Mannitol.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Intravenous Infusions).

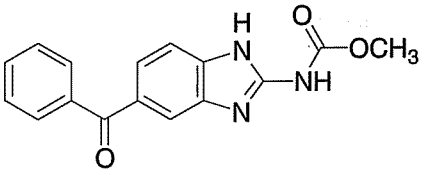
**Assay**. Dilute an accurately measured volume containing about 0.4 g of Mannitol to 100.0 ml with *water*, transfer 10.0 ml to a stoppered flask, add 20.0 ml of *0.1 M sodium periodate* and 2 ml of *1 M sulphuric acid* and heat on a water-bath for 15 minutes. Cool, add 3 g of *sodium bicarbonate*, in small quantities, and 25.0 ml of *0.1 M sodium arsenite*, mix, add 5 ml of a 20 per cent w/v solution of *potassium iodide* and allow to stand for 15 minutes. Titrate with *0.05 M iodine* until the first trace of yellow colour appears. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of iodine required.

1 ml of *0.05 M iodine* is equivalent to 0.001822 g of $C_6H_{14}O_6$.

**Storage**. Store at temperatures between 20° and 30°. Exposure to lower temperatures may cause the deposition of crystals, which should be dissolved by warming before use.

**Labelling**. The label states (1) the strength as a percentage w/v of Mannitol; (2) that the injection should not be used if it contains visible solid particles that do not dissolve on warming.

## Mebendazole




$C_{16}H_{13}N_3O_3$ Mol. Wt. 295.3

Mebendazole is methyl *N*-(5-benzoyl-1*H*-benzimidazol-2-yl)carbamate.

Mebendazole contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{16}H_{13}N_3O_3$, calculated on the dried basis.

**Category**. Anthelmintic.

**Dose**. For threadworm infestation, 100 mg as a single dose; for other infestations, 100 mg twice daily, for three days.

**Description**. A white to slightly yellow, amorphous powder; almost odourless.

### Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mebendazole RS* or with the reference spectrum of mebendazole.

B. In the test for Related substances, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with the reference solution.

C. To about 10 mg add 5 ml of *ethanol (95 per cent)*, 1 ml of *dinitrobenzene solution* and 1 ml of *sodium hydroxide solution*; an intense yellow colour is produced.

### Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 25 mg of the substance under examination in 25 ml of *dimethylformamide*.

*Reference solution*. Dilute 1.0 ml of the test solution to 100.0 ml with *dimethylformamide*. Dilute 5.0 ml of this solution to 20.0 ml with *dimethylformamide*.

Chromatographic system

– a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),



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- column temperature. 40°,
- mobile phase: A. a mixture of 0.75 per cent w/v solution of *ammonium acetate*,
  B. a*cetonitrile*,
- a linear gradient programme using the conditions given below,
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 250 nm,
- injection volume. 10 µl.

| Time (in min.) | Mobile phase A ( per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0-15 | 80-70 | 20-30 |
| 15-20 | 70-10 | 30-90 |
| 20-25 | 10 | 90 |
| 25-26 | 10-80 | 90-20 |
| 26-30 | 80 | 20 |

Inject the test solution and the reference solution. In the chromatogram obtained with the test solution the area of any secondary peak is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent), the sum of all the secondary peaks is not more than 4 times the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent) Ignore any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 4 hours.

**Assay**. Weigh accurately about 0.25 g, dissolve in 3 ml of *anhydrous formic acid* and 30 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02953 g of $C_{16}H_{13}N_3O_3$.

**Storage**. Store protected from light and moisture.

## Mebendazole Tablets

Mebendazole Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of mebendazole, $C_{16}H_{13}N_3O_3$.

**Usual strength**. 100 mg.

### Identification

A. Shake a quantity of the powdered tablets containing 50 mg of Mebendazole with 10 ml of a mixture of 10 volumes of *anhydrous formic acid* and 90 volumes of *chloroform* for 30 minutes, filter, evaporate the filtrate to dryness and dry the residue at a pressure not exceeding 0.7 kPa.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mebendazole RS* or with the reference spectrum of mebendazole.

B. In the test for Related substances, the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

### Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution.* Disperse a quantity of powdered tablets containing about 25 mg of Mebendazole in 25 ml of *dimethylformamide*.

*Reference solution.* Dilute 1.0 ml of the test solution to 100.0 ml with *dimethylformamide*. Dilute 5.0 ml of this solution to 20.0 ml with *dimethylformamide*.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3µm),
- column temperature 40°,
- mobile phase: A. a mixture of 0.75 per cent w/v solution of *ammonium acetate*,
  B. *acetonitrile*,
- a linear gradient programme using the conditions given below,
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 250 nm,
- injection volume. 10 µl.

| Time (in min.) | Mobile phase A ( per cent v/v) | Mobile phase B ( per cent v/v) |
|---|---|---|
| 0-15 | 80-70 | 20-30 |
| 15-20 | 70-10 | 30-90 |
| 20-25 | 10 | 90 |
| 25-26 | 10-80 | 90-20 |
| 26-30 | 80 | 20 |

Inject the test solution and the reference solution. In the chromatogram obtained with the test solution the area of any secondary peak is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent), the sum of all the secondary peaks is not more than 4 times the area of the principal peak in the chromatogram



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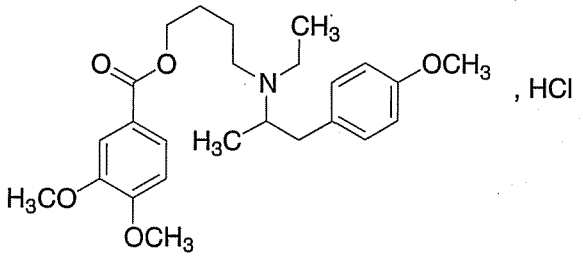
obtained with the reference solution (1.0 per cent) Ignore any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 50 mg of Mebendazole, add 50 ml of *0.5 M methanolic hydrochloric acid* shake for 30 minutes and dilute to 100.0 ml with *0.5 M methanolic hydrochloric acid*. Filter and discard the first 10 ml of the filtrate. Dilute 10.0 ml of the filtrate to 100.0 ml with *0.5 M methanolic hydrochloric* acid and mix. Further dilute 5.0 ml to 50.0 ml with the same solvent and mix. Measure the absorbance of the resulting solution at the maximum at about 234 nm (2.4.7). Calculate the content of $C_{16}H_{13}N_3O_3$ from the absorbance obtained by repeating the operation using *mebendazole RS* in place of the substance under examination.

**Storage**. Store protected from light and moisture.

# Mebeverine Hydrochloride




$C_{25}H_{35}NO_5$,HCl Mol. Wt. 466.0

Mebeverine Hydrochloride is (*RS*)-4-[ethyl(4-methoxy-α-methylphenethyl)amino]butyl veratrate hydrochloride.

Mebeverine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{25}H_{35}NO_5$,HCl, calculated on the dried basis.

**Category**. Antispasmodic.

**Dose**. 135 mg thrice daily, preferably 20 minutes before meals.

**Description**. A white or almost white, crystalline powder.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mebeverine hydrochloride RS* or with the reference spectrum of mebeverine hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.003 per cent w/v solution in *0.1 M hydrochloric acid* shows an absorption maximum at about 263 nm and a less well-defined maximum at about 292 nm; absorbance at about 263 nm, about 0.79 and at about 292 nm, about 0.41.

C. Dissolve 25 mg in 2 ml of *water*, acidify with *2 M nitric acid* and centrifuge. The supernatant liquid gives the reactions of chlorides (2.3.1).

## Tests

**pH** (2.4.24). 4.5 to 6,5, determined in a 2.0 per cent w/v solution.

**Ether-soluble extractive**. Dissolve 40 mg in 25 ml of *2 M hydrochloric acid* and shake with 50 ml of *ether* for 1 minute. Wash the *ether* layer with three quantities, each of 25 ml, of *water*, evaporate the ether to dryness using a rotary evaporator and dissolve the residue in sufficient *methanol* to produce 20 ml; absorbance of the resulting solution at about 260 nm, not more than 0.23 (2.4.7).

**Non-tertiary amine**. Dissolve 0.5 g in 5 ml of *pyridine*, add 5 ml of *copper chloride-pyridine reagent* and heat at 50° for 30 minutes. Cool, add sufficient *acetone* to produce 50 ml and measure the absorbance of the resulting solution at about 405 nm (2.4.7), using as the blank a solution obtained by treating 5 ml of *pyridine* in the same manner. The absorbance is not more than that obtained by repeating the test using 5 ml of a 0.006 per cent w/v solution of *di-n-butylamine* in *pyridine* and beginning at the words "add 5 ml of *copper chloride-pyridine reagent....*".

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel F254*.

*Mobile phase*. A mixture of 50 volumes of *ethanol*, 50 volumes of *chloroform* and 1 volume of *18 M ammonia*.

*Test solution*. Dissolve 0.2 g of the substance under examination in 10 ml of *acetone*.

*Reference solution (a)*. Dissolve 10 mg of the substance under examination in 100 ml of *acetone*.

*Reference solution (b)*. A 0.002 per cent w/v solution of *veratric acid* in *acetone*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Expose the plate to iodine vapour for 1 hour. When viewed in ultraviolet light, any spot corresponding to veratric acid in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b).Using both methods of visualisation any other secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 1 hour.



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**Assay**. Weigh accurately about 0.4 g, dissolve in 75 ml of *anhydrous glacial acetic acid* and add 7 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04660 g of $C_{25}H_{35}NO_5$,HCl.

**Storage**. Store protected from light and moisture at a temperature not exceeding 30°.

# Mebeverine Tablets

Mebeverine Hydrochloride Tablets

Mebeverine Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of mebeverine hydrochloride, $C_{25}H_{35}NO_5$,HCl. The tablets are coated.

**Usual strength**. 135 mg.

## Identification

A. Suspend a quantity of the powdered tablets containing 0.2 g of Mebeverine Hydrochloride in 20 ml of *water*, add 5 ml of *5 M sodium hydroxide* and extract with two quantities, each of 25 ml, of *chloroform*. Dry the combined extracts over *anhydrous sodium sulphate* and evaporate to dryness using a rotary evaporator.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mebeverine hydrochloride RS* or with the reference spectrum of mebeverine hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), the solution obtained in the Assay shows an absorption maximum at about 263 nm and a less well-defined maximum at about 292 nm.

## Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel F254*.

*Mobile phase*. A mixture of 50 volumes of *ethanol*, 50 volumes of *chloroform* and 1 volume of *18 M ammonia*.

*Test solution*. Shake a quantity of the powdered tablets containing 0.2 g of Mebeverine Hydrochloride with 10 ml of *acetone* and filter.

*Reference solution (a)*. Dilute 1 volume of the test solution to 200 volumes with *acetone*.

*Reference solution (b)*. A 0.01 per cent w/v solution of *veratric acid* in *acetone*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Expose the plate to iodine vapour for 1 hour. When viewed in ultraviolet light, any spot corresponding to veratric acid in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b). Using both methods of visualisation any other secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a).

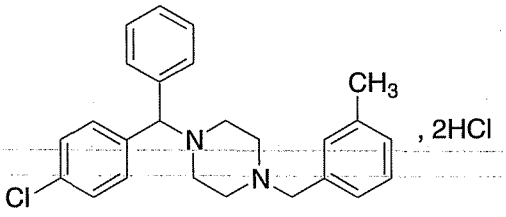
**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.5 g of Mebeverine Hydrochloride, dissolve in 100 ml of *0.1 M hydrochloric acid* and heat for 10 minutes on a water-bath, shaking occasionally. Cool, add sufficient *0.1 M hydrochloric acid to* produce 250.0 ml and filter. To 10.0 ml of filtrate add sufficient *0.1 M hydrochloric acid* to produce 100.0 ml and dilute 10.0 ml of this solution to 100.0 ml with the same solvent. Measure the absorbance of the resulting solution at the maximum at about 263 nm (2.4.7). Calculate the content of $C_{25}H_{35}NO_5$,HCl. taking 263 as the specific absorbance at 263 nm.

**Storage**. Store protected from light and moisture.

# Meclizine Hydrochloride

Meclozine Hydrochloride




$C_{25}H_{27}ClN_2$, 2HCl Mol. Wt. 463.9

Meclizine Hydrochloride is (*RS*)-1-(4-chlorobenzhydryl)-4-(3-methylbenzyl)piperazine dihydrochloride.

Meclizine Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{25}H_{27}ClN_2$, 2HCl, calculated on the anhydrous basis.

**Category**. Antiemetic.

**Dose**. 25 to 50 mg daily.

**Description**. A white or yellowish white, crystalline powder; odour, slight.



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### Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *meclizine hydrochloride RS* or with the reference spectrum of meclizine hydrochloride.

B. When examined in the range 220 nm to 360 nm (2.4.7), a 0.0015 per cent w/v solution in *0.1 M hydrochloric acid* shows an absorption maximum at about 232 nm and weak absorption without a defined maximum in the range 260 nm to 300 nm; absorbance at the maximum at about 232 nm, 0.51 to 0.57.

C. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

D. Dissolve about 15 mg in 2 ml of *ethanol (95 per cent)*; the solution gives reaction A of chlorides (2.3.1).

### Tests

**Appearance of solution**. A 2.0 per cent w/v solution in *ethanol (95 per cent)* is clear (2.4.1), and not more intensely coloured than reference solution YS6 (2.4.1).

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Solvent mixture*. Equal volumes of *dichloromethane* and *methanol*.

*Mobile phase*. A mixture of 60 volumes of *dichloromethane*, 30 volumes of *toluene*, 5 volumes of *methanol* and 0.5 volume *of strong ammonia solution*.

*Test solution (a)*. Dissolve 0.5 g of the substance under examination in 10 ml of the solvent mixture.

*Test solution (b)*. Dissolve 0.5 g of the substance under examination in 100 ml of the solvent mixture.

*Reference solution (a)*. Dissolve 25.0 mg of the substance under examination in 100 ml of the solvent mixture.

*Reference solution (b)*. A 0.5 per cent w/v solution of *meclizine hydrochloride RS* in the solvent mixture.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with *dilute potassium iodobismuthate solution*. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a). Ignore any yellowish white spot on the line of application.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 5.0 per cent, determined on 0.2 g.

**Assay**. Weigh accurately about 0.35 g, dissolve in 50 ml of *chloroform*, add 50 ml of *anhydrous glacial acetic acid*, 5 ml of *acetic anhydride* and 12 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, using a 0.1 per cent w/v solution of *quinaldine red* in *anhydrous glacial acetic acid* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02319 g of $C_{25}H_{27}ClN_2$, 2HCl.

**Storage**. Store protected from light and moisture.

## Meclizine Tablets

Meclizine HydrochlorideTablets; Meclozine Hydrochloride Tablets; Meclozine Tablets

Meclizine Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of meclizine hydrochloride, $C_{25}H_{27}ClN_2$, 2HCl.

**Usual strength**. 25 mg.

### Identification

Triturate a quantity of the powdered tablets containing 0.5 g of Meclizine Hydrochloride with three quantities, each of 15 ml, of *chloroform*. Filter the extracts and evaporate the clear filtrate to dryness on a water-bath.The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *meclizine hydrochloride RS* or with the reference spectrum of meclizine hydrochloride.

B. When examined in the range 220 nm to 360 nm (2.4.7), a 0.0015 per cent w/v solution in *0.1 M hydrochloric acid* shows an absorption maximum at about 232 nm and weak absorption without a defined maximum in the range 260 nm to 300 nm; absorbance at the maximum at about 232 nm, 0.51 to 0.57.

C. Dissolve about 15 mg in 2 ml of *ethanol (95 per cent)*; the solution gives reaction A of chlorides (2.3.1).

### Tests

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and reduce to a fine powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.35 g of Meclizine Hydrochloride and extract with three quantities, each of 50 ml, of *chloroform*, stirring the mixture each time for 30 minutes, then allowing the undissolved matter to settle and decanting the supernatant liquid on to a sintered-glass filter



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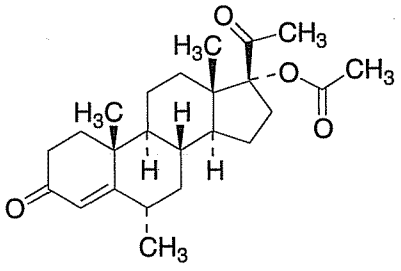


(porosity No. 4). Transfer the residue to the filter with the aid of *chloroform* and wash the vessel and filter with 20 ml of *chloroform*. Combine the extracts and washing and evaporate on a water-bath to 50 ml. Cool and add 50 ml of *anhydrous glacial acetic acid*, 5 ml of *acetic anhydride* and 12 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, using a 0.1 per cent w/v solution of *quinaldine red* in *anhydrous glacial acetic acid as* indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02319 g of $C_{25}H_{27}ClN_2, 2HCl$.

**Storage**. Store protected from light and moisture.

# Medroxyprogesterone Acetate



$C_{24}H_{34}O_4$ Mol. wt. 386.5

Medroxyprogesterone Acetate is 6α-methyl-3,20-dioxo-pregn-4-en-17α-yl acetate.

Medroxyprogesterone Acetate contains not less than 97.0 per cent and not more than 103.0 per cent of $C_{24}H_{34}O_4$, calculated on the dried basis.

**Category**. Progestogen.

**Description**. A white or almost white, crystalline powder.

## Identification

*Test A may be omitted if tests B and C are carried. Test B may be omitted if tests A and C are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *medroxyprogesterone acetate RS*.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Solvent mixture*. 90 volumes of *chloroform* and 10 volumes of *methanol*

*Mobile phase*. A mixture of 70 volumes of *toluene*, 40 volumes of *ethyl acetate* and 10 volumes of *light petroleum (50° to 70°)*.

*Test solution*. Dissolve 0.1 g of the substance under examination in 100 ml of the solvent mixture.

*Reference solution (a)*. A 0.1 per cent w/v solution of *medroxyprogesterone acetate RS* in the solvent mixture.

*Reference solution (b)*. A solution containing 0.05 per cent w/v each of *progesterone RS* and *medroxyprogesterone acetate RS* in the solvent mixture.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

Spray with *ethanolic sulphuric acid (20 per cent)*, heat at 120° for 10 minutes or until spots appear and allow to cool. Examine the plate in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

C. Melting range 205° to 209° (2.4.21).

## Tests

**Specific optical rotation** (2.4.22). +45.0° to +51.0°, determined in a 1.0 per cent w/v solution in *dioxan*.

**Related substances**. Determine by liquid chromatography (2.4.17).

*Test solution (a)*. Dissolve 5 mg of the substance under examination in 100 ml of the mobile phase.

*Test solution (b)*. Dissolve 0.25 g of the substance under examination in 100 ml of the mobile phase.

*Reference solution*. Evaporate 1 ml of a solution containing 0.1 per cent w/v each of *medroxyprogesteronge acetate RS* and *megestrol acetate RS* in *ethanol*, to dryness in a water bath at 45° and dissolve the residue in sufficient mobile phase to produce 25 ml.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 mm),
- mobile phase: a mixture of 60 volumes of *acetonitrile* and 40 volumes of *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Adjust the sensitivity so that the height of the principal peak in the chromatogram obtained with test solution (a) is 70 to 90 per cent of the full-scale deflection.



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Equilibrate the column with the mobile phase at a flow rate of 1 ml per minute for about 45 minutes. When the chromatograms are recorded under the conditions described above, the retention times are 12.5 minutes for megestrol acetate and 13.5 minutes for medroxyprogesterone acetate.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to megestrol acetate and medroxyprogesterone acetate is at least 2.0. If this resolution is not achieved, adjust the concentration of *acetonitrile* in the mobile phase. Verify the repeatability by making five separate injections of test solution (a). The system is not suitable unless the relative standard deviation for the area of the principal peak in the chromatogram obtained with test solution (a). The system is not suitable unless the relative standard deviation for the area of the principal peak in the chromatogram obtained with test solution (a) is less than 2.0 per cent.

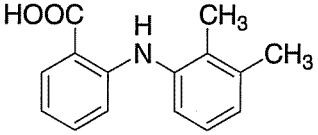
Inject separately test solutions (a) and (b) and record the chromatograms for 1.5 times the retention time of the principal peak. In the chromatogram obtained with test solution (b) the area of any secondary peak is not greater than half the area of the principal peak in the chromatogram obtained with test solution (a) and the sum of the areas of the secondary peaks is not greater than the area of the principal peak in the chromatogram obtained with test solution (a). Ignore any peak with an area less than 0.025 times the area of the principal peak in the chromatogram obtained with test solution (a).

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 0.5 g by drying in an oven at 105° for 3 hours.

**Assay**. Weigh accurately about 25 mg, dissolve in sufficient *ethanol (95 per cent)* to produce 100.0 ml, dilute 5.0 ml to 100.0 ml with *ethanol (95 per cent)* and measure the absorbance of the resulting solution at the maximum at about 241 nm (2.4.7). Calculate the content of $C_{24}H_{34}O_4$ taking 426 as the specific absorbance at 241 nm.

**Storage**. Store protected from light and moisture.

# Mefenamic Acid




$C_{15}H_{15}NO_2$ Mol. Wt. 241.3

Mefenamic Acid is *N*-(2,3-xylyl)anthranilic acid.

Mefenamic Acid contains not less than 99.0 per cent and not more than 100.5 per cent of $C_{15}H_{15}NO_2$, calculated on the dried basis.

**Category**. Antiinflammatory; analgesic.

**Dose**. 250 to 500 mg thrice daily, after food.

**Description**. A white to greyish-white, microcrystalline powder; odourless or almost odourless.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mefenamic acid RS* or with the reference spectrum of mefenamic acid.

B. Dissolve 25 mg in 15 ml of *chloroform* and examine in ultraviolet light at 365 nm; the solution exhibits a strong greenish-yellow fluorescence. Carefully add 0.5 ml of a saturated solution of *trichloroacetic acid* drop wise and examine again in ultraviolet light at 365 nm; the solution does not exhibit fluorescence.

C. Dissolve 5 mg in 2 ml of *sulphuric acid* and add 0.05 ml of *0.0167 M potassium dichromate*; an intense blue colour is produced immediately which fades rapidly to brownish-green.

## Tests

**Light absorption** (2.4.7). Absorbance of a 0.002 per cent w/v solution in a mixture of 99 volumes of *methanol* and 1 volume of *1 M hydrochloric acid* at the maximum at about 279 nm, 0.69 to 0.74 and at the maximum at about 350 nm, 0.56 to 0.60.

**Copper**. Moisten 1.0 g with *sulphuric acid* and ignite until all the carbon is removed. Add 10 ml of *1 M sulphuric acid* to the residue and allow to stand for 10 minutes. Transfer to a separating funnel using 20 ml of *water* and add 10 ml of a solution containing 20 per cent w/v *diammonium hydrogen citrate* and 5 per cent w/v solution of *disodium edetate*. Add 0.2 ml of *thymol blue solution* and neutralise with *5 M ammonia*. Add 10 ml of *sodium diethyldithiocarbamate solution* and 15 ml of *carbon tetrachloride*, shake and allow to separate. The yellow colour of the *carbon tetrachloride* layer is not more intense than that produced by treating 2 ml of *copper standard solution (10 ppm Cu)* in the same manner beginning at the words "Transfer to a separating funnel using....." (20 ppm).

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Solvent mixture*. 3 volumes of *chloroform* and 1 volume of *methanol*.

*Mobile phase*. A mixture of 90 volumes of *toluene*, 25 volumes of *dioxan* and 1 volume of *glacial acetic acid*.

*Test solution*. Dissolve 0.25 g of the substance under examination in 10 ml of the solvent mixture.

*Reference solution*. Dissolve 5.0 mg of the substance under examination in 100 ml of the solvent mixture.



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Apply to the plate 20 µl of each solution. After development, dry the plate in air, expose to iodine vapour for 5 minutes and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**2,3-Dimethylaniline**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Solvent mixture*. 3 volumes of *chloroform* and 1 volume of *methanol*.

*Mobile phase*. A mixture of 90 volumes of *toluene*, 25 volumes of *dioxan* and 1 volume of *18 M ammonia*.

*Test solution*. Dissolve 0.25 g of the substance under examination in 10 ml of the solvent mixture.

*Reference solution*. A 0.00025 per cent w/v solution of *2,3-dimethylaniline* in the solvent mixture.

Apply to the plate 40 µl of each solution. After development, dry the plate in a current of warm air. Spray the plate with *ethanolic sulphuric acid (20 per cent)*, heat at 105° for 30 minutes and immediately expose to nitrous fumes in a closed glass chamber for 15 minutes (the nitrous fumes may be generated by adding *dilute sulphuric acid* dropwise to a solution containing 10 per cent w/v of *sodium nitrite* and 3 per cent w/v of *potassium iodide*). Place the plate in a current of warm air for 15 minutes and spray with a 0.5 per cent w/v solution of *N-(1-naphthyl)ethylenediamine dihydrochloride* in *ethanol (95 per cent)*. If necessary, allow to dry and repeat the spraying. Any spot corresponding to 2,3-dimethylaniline in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.5 g and dissolve in 100 ml of warm *ethanol (95 per cent)* previously neutralised to *phenol red solution* and titrate with *0.1 M sodium hydroxide* using *phenol red solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.02413 g of $C_{15}H_{15}NO_2$.

**Storage**. Store protected from light and moisture.

# Mefenamic Acid Capsules

Mefenamic Acid Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of mefenamic acid, $C_{15}H_{15}NO_2$.

**Usual strength**. 250 mg.

## Identification

Extract a quantity of the contents of the capsules containing 0.25 g of Mefenamic Acid with two quantities, each of 30 ml, of *ether*. Wash the combined extracts with *water* and evaporate to dryness on a water-bath. The residue, after drying at 105°, complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mefenamic acid RS* or with the reference spectrum of mefenamic acid.

B. Dissolve 25 mg in 15 ml of *chloroform* and examine in ultraviolet light at 365 nm; the solution exhibits a strong greenish-yellow fluorescence. Carefully add 0.5 ml of a saturated solution of *trichloroacetic acid* drop wise and examine again in ultraviolet light at 365 nm; the solution does not exhibit fluorescence.

C. Dissolve 5 mg in 2 ml of *sulphuric acid* and add 0.05 ml of *0.0167 M potassium dichromate*; an intense blue colour is produced immediately which fades rapidly to brownish-green.

## Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 90 volumes of *toluene*, 25 volumes of *dioxan* and 1 volume of *glacial acetic acid*.

*Test solution*. The supernatant liquid obtained by shaking a quantity of the contents of the capsules containing 0.25 g of Mefenamic Acid with a mixture of 7.5 ml of *chloroform* and 2.5 ml of *methanol*.

*Reference solution*. Dissolve 5.0 mg of the substance under examination in 100 ml of a mixture of 3 volumes of *chloroform* and 1 volume of *methanol*.

Apply to the plate 20 µl of each solution. After development, dry the plate in air, expose to iodine vapour for 5 minutes and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**2,3-Dimethylaniline**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 90 volumes of *toluene*, 25 volumes of *dioxan* and 1 volume of *18 M ammonia*.

*Test solution*. The supernatant liquid obtained in the test for Related substances.

*Reference solution*. A 0.00025 per cent w/v solution of *2, 3-dimethylaniline* in a mixture of 3 volumes of *chloroform* and 1 volume of *methanol*.



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Apply to the plate 40 µl of each solution. After development, dry the plate in a current of warm air. Spray the plate with *ethanolic sulphuric acid (20 per cent)*, heat at 105° for 30 minutes and immediately expose to nitrous fumes in a closed glass chamber for 15 minutes (the nitrous fumes may be generated by adding *dilute sulphuric acid* dropwise to a solution containing 10 per cent w/v of *sodium nitrite* and 3 per cent w/v of *potassium iodide*). Place the plate in a current of warm air for 15 minutes and spray with a 0.5 per cent w/v solution of *N-(1-naphthyl)ethylenediamine dihydrochloride* in *ethanol (95 per cent)*. If necessary, allow to dry and repeat the spraying. Any spot corresponding to 2,3-dimethylaniline in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Dissolution** (2.5.2).

Apparatus No. 2,
Medium. 900 ml of *0.05 M tris buffer* prepared by dissolving 60.5 g *tris(hydroxymethyl) aminomethane* in 6000 ml *water*, diluting to 10,000 ml with *water* and adjusting with phosphoric acid to a pH 9.0 ± 0.05. 100 g of *sodium lauryl sulphate* is dissolved in 6000 ml of the above solution and further mixed with the remaining quantity of the solution,
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

*Test solution.* Use the filtrate as the test solution.

*Reference solution.* A 0.02 per cent w/v solution of *mefenamic acid RS* in the dissolution medium.

Use the chromatographic system described under Assay.

Calculate the content of $C_{15}H_{15}NO_2$ in the medium.

D. Not less than 75 per cent of the stated amount of $C_{15}H_{15}NO_2$.

**Other tests**. Comply with the tests stated under Capsules.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh accurately a quantity of the mixed contents of 20 capsules containing about 50 mg of Mefenamic acid in a 250.0-ml volumetric flask, add 5 ml of *tetrahydrofuran*, shake for 10 minutes with the aid of ultrasound, dilute to volume with the mobile phase and filter.

*Reference solution.* A solution containing 0.02 per cent w/v of *mefenamic acid RS* in the mobile phase.

*NOTE- Protect the solutions from light.*

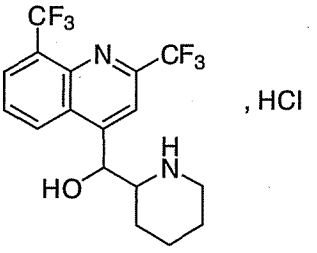
Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 23 volumes of *acetonitrile*, 20 volumes of *0.05 M of monobasic ammonium phosphate* adjusted to a pH of 5.0 with *3 M ammonia* and 7 volumes of *tetrahydrofuran*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency determined from the mefenamic acid peak is not less than 8200 theoretical plates, the tailing factor is not more than 1.6 and the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_{15}H_5NO_2$ in the capsules.

# Mefloquine Hydrochloride




$C_{17}H_{17}ClF_6N_2O$ Mol. Wt. 414.8

Mefloquine Hydrochloride is (*RS*)-[2,8-bis(trifluoromethyl)quinolin-4-yl][(2*SR*)-piperidin-2-yl]methanol hydrochloride.

Mefloquine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{17}H_{17}ClF_6N_2O$ calculated on the anhydrous basis.

**Category**. Antimalarial.

**Description**. A white or slightly yellow crystalline powder.

## Identification

*Tests A and E may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if tests A and E are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mefloquine hydrochloride RS* or with the reference spectrum of mefloquine hydrochloride.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.



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*Mobile phase.* A mixture of 10 volumes of *anhydrous acetic acid*, 10 volumes of *methanol*, and 80 volumes of *dichloromethane*.

*Test solution.* Dissolve 8.0 mg of the substance under examination in 5 ml of *methanol*.

*Reference solution (a).* Dissolve 8.0 mg of *mefloquine hydrochloride RS* in 5 ml of *methanol*.

*Reference solution (b).* Dilute 2.5 ml of the test solution to 100 ml with *methanol*.

*Reference solution (c).* To 1 ml of reference solution (b), add 1 ml of a 0.0016 per cent w/v solution of *quinidine sulphate* in *methanol*.

Apply to the plate 20 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in a current of warm air for 15 minutes and examine under ultraviolet light at 254 nm; lightly spray with a mixture, prepared immediately before use, of 1 volume of *sulphuric acid* and 40 volumes of *iodoplatinate reagent*; spray with *strong hydrogen peroxide solution*. In reference solution (c) the chromatogram shows two clearly separated spots. The principal spot in the chromatogram obtained with the test solution corresponds to the spot in the chromatogram obtained with reference solution (a).

C. Mix about 10 mg of the substance under examination with 45 mg of *heavy magnesium oxide* and ignite in a crucible until a practically white residue is obtained. Allow to cool, then add 2 ml of *water*, 0.05 ml of *phenolphthalein solution* and about 1 ml of *dilute hydrochloric acid* to make the solution colourless. Filter. To the filtrate add a freshly prepared mixture of 0.1 ml of *alizarin S solution* and 0.1 ml of *zirconyl nitrate solution*. Mix, allow to stand for 5 minutes and compare the colour of the solution with a blank prepared in the same manner. The test solution is yellow and the blank is red.

D. To about 20 mg of the substance under examination, add 0.2 ml of *sulphuric acid*. Blue fluorescence appears in ultraviolet light at 365 nm.

E. Gives reaction B of chlorides (2.3.1).

## Tests

**Appearance of solution.** A 5.0 per cent solution in *methanol* is clear (2.4.1) and not more intensely coloured than reference solution BYS7 (2.4.1).

**Optical rotation** (2.4.22). – 0.2° to + 0.2°, determined in a 5.0 per cent w/v solution in *methanol*.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve 100 mg of the substance under examination in 25.0 ml of the mobile phase.

*Reference solution (a).* Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 20.0 ml with the mobile phase.

*Reference solution (b).* A solution containing 0.016 per cent w/v each of *mefloquine hydrochloride RS* and *quinidine sulphate* in the mobile phase. Dilute 5.0 ml of this solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: dissolve 100 mg of *tetraheptylammonium bromide* in a mixture of 20 volumes of *methanol*, 40 volumes of a 0.15 per cent w/v solution of *sodium hydrogen sulphate* and 40 volumes of *acetonitrile*,
- flow rate. 0.8 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to quinidine and mefloquine is not less than 8.5.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of secondary peak with a relative retention time with reference to mefloquine is about 0.7 is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent). The sum of areas of secondary peaks other than the peak with a relative retention time with reference to mefloquine is about 0.7 is not more than five times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

**Heavy metals** (2.3.13). 1.0 g complies with limit test for heavy metals, Method C (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 3.0 per cent, determined on 1.0 g.

**Assay**. Dissolve 0.35 g in 15 ml of *anhydrous formic acid* and add 40 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04148 g of $C_{17}H_{17}ClF_6N_2O$.

**Storage**. Store protected from light.

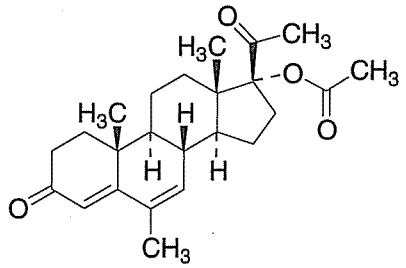


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# Megestrol Acetate



$C_{24}H_{32}O_4$ Mol.Wt. 384.5

Megestrol Acetate is 6-methyl-3,20-dioxopregna-4,6-dien-17-yl acetate.

Megestrol Acetate contains not less than 97.0 per cent and not more than 103.0 per cent of $C_{24}H_{32}O_4$, calculated on the dried basis.

**Category**. Progestogen.

**Dose**. 40 to 320 mg daily, in divided doses.

**Description**. A white to creamy-white, crystalline powder; odourless or almost odourless.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *megestrol acetate RS* or with the reference spectrum of megestrol acetate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Solvent mixture*. 90 volumes of *acetone* and *1,2-propanediol*.

*Mobile phase*. A mixture of 40 volumes of *cyclohexane* and 10 volumes of *toluene*.

*Test solution*. Dissolve 25 mg of the substance under examination in 10 ml of the solvent mixture.

*Reference solution (a)*. Dissolve 25 mg of *megestrol acetate RS* in 10 ml of the solvent mixture.

*Reference solution (b)*. Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 1 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v)*. Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

C. Gives the reaction of acetyl groups (2.3.1).

## Tests

**Specific optical rotation** (2.4.22). +9.0° to +12.0°, determined at 20° in a 5.0 per cent w/v solution in *chloroform*.

**Light absorption**. When examined in the range 230 nm to 360 nm (2.4.7), the solution obtained in the Assay shows an absorption maximum only at about 287 nm; ratio of the absorbance at about 240 nm to that at the maximum at about 287 nm, not more than 0.17.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. 145 volumes of *tetrahydrofuran* and 255 volumes of *acetonitrile*.

*Test solution*. Dissolve 25 mg of the substance under examination in 20 ml of the solvent mixture, dilute to 50 ml with *water*.

*Reference solution (a)*. Dissolve 25 mg of *medroxyprogesterone acetate RS (megestrol acetate impurity A RS)* in 20 ml of the solvent mixture, dilute to 50 ml with *water*.

*Reference solution (b)*. Dilute 1.0 ml of reference solution (a) to 200.0 ml with the mobile phase.

*Reference solution (c)*. To 3.0 ml of the test solution, add 1 ml of reference solution (a) and dilute to 50 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 14.5 volumes of *tetrahydrofuran*, 22.5 volumes of *acetonitrile* and 60 volumes of *water*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to megestrol acetate and megestrol acetate impurity A is not less than 4.0.

Inject the test solution and reference solution (b). Run the chromatogram 1.5 times the retention time of the principal



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peak. In the chromatogram obtained with the test solution, the area of the peak corresponding to megestrol acetate impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). The sum of areas of all the secondary peaks other than megestrol acetate impurity A is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principle peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

**Heavy metals** (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 20 mg, dissolve in sufficient *ethanol (95 per cent)* to produce 100.0 ml, dilute 5.0 ml to 100.0 ml with *ethanol (95 per cent)* and measure the absorbance of the resulting solution at the maximum at about 287 nm (2.4.7). Calculate the content of $C_{24}H_{32}O_4$ taking 630 as the specific absorbance at 287 nm.

**Storage**. Store protected from light and moisture.

## Megestrol Tablets

Megestrol AcetateTablets

Megestrol Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of megestrol acetate, $C_{24}H_{32}O_4$.

**Usual strengths**. 20 mg; 160 mg.

### Identification

Extract a quantity of the powdered tablets containing 40 mg of Megestrol Acetate with 10 ml of *chloroform*, filter and evaporate the filtrate to dryness in a current of air. Dry the residue at 60° at a pressure not exceeding 0.7 kPa for 1 hour.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *megestrol acetate RS* or with the reference spectrum of megestrol acetate.

### Tests

**Disintegration** (2.5.1). 30 minutes.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 50 mg of Megestrol Acetate and dissolve as completely as possible in sufficient *methanol* to produce 100.0 ml. Mix well and filter. Dilute 2.0 ml of the filtrate with *methanol* to 100.0 ml and mix. Measure the absorbance of the resulting solution at the maximum at about 287 nm (2.4.7). Calculate the content of $C_{24}H_{32}O_4$ taking 630 as the specific absorbance at 287 nm.

**Storage**. Store protected from light and moisture.

## Meloxicam Oral Suspension

Meloxicam Oral Suspension is a suspension of Meloxicam in a suitable vehicle.

Meloxicam Oral Suspension contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of meloxicam, $C_{14}H_{13}N_3O_4S_2$.

**Usual strengths**. 7.5 mg; 15 mg.

### Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel F254*.

*Mobile phase*. A mixture of 1 volume of *13.5 M ammonia*, 20 volumes of *methanol* and 80 volumes of *dichloromethane*.

*Test solution*. Dilute a quantity of the oral suspension containing 3 mg of Meloxicam to 10 ml with *acetone*, stir for 10 minutes, filter and use the filtrate.

*Reference solution*. Dissolve 3 mg of *meloxicam RS* in about 5 ml of *acetone*, add 0.5 ml of *water* and dilute to 10 ml with *acetone*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 and 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. Disperse a quantity of the substance under examination containing 1.5 mg of Meloxicam in 5 ml of *0.1M sodium hydroxide*, dilute to 100 ml with *methanol* and filter. The light absorption of the filtrate (2.4.7), in the range 340 to 450 nm exhibits a maximum at 362 nm.

### Tests

**Other tests**. Complies with the tests stated under Oral Liquids.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Shake a quantity of the oral suspension containing 15 mg of Meloxicam with sufficient of the mobile phase to produce 50 ml, stir for 30 minutes and filter.

*Reference solution*. A 0.03 per cent w/v solution of *meloxicam RS* in the mobile phase.



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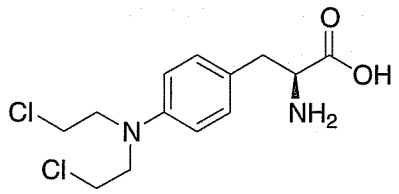
Chromatographic system
- a stainless steel column 10 cm × 4 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- column temperature. 40°,
- mobile phase: a mixture of 35 volumes of a solution containing 10 volumes of *propan-2-ol* and 65 volumes of *methanol* and 65 volumes of a 0.2 per cent w/v solution of *diammonium hydrogen orthophosphate* previously adjusted to pH 7.0 with *orthophosphoric acid*,
- flow rate. 0.8 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Determine the weight per ml of the suspension (2.4.29) and calculate the content of $C_{14}H_{13}N_3O_4S_2$.

# Melphalan




$C_{13}H_{18}Cl_2N_2O_2$ Mol. Wt. 305.2

Melphalan is 4-bis(2-chloroethyl)amino-L-phenylalanine.

Melphalan contains not less than 93.0 per cent and not more than 100.5 per cent of $C_{13}H_{18}Cl_2N_2O_2$, calculated on the dried basis.

**Category**. Anticancer.

**Dose**. Orally, 2 to 4 mg daily for 4 to 6 days; by injection, 50 mg.

**Description**. A white or almost white powder; odourless or almost odourless.

## Identification

A. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *methanol* shows an absorption maximum at about 260 nm and a less well-defined maximum at about 301 nm.

B. Dissolve 20 mg in 50 ml of *methanol* with the aid of gentle heat, add 1 ml of a 5 per cent w/v solution of *4-(4-nitrobenzyl)pyridine* in *acetone* and evaporate to dryness. Dissolve the residue in 1 ml of hot *methanol* and add 0.1 ml of *strong ammonia solution*; a red colour is produced.

C. Heat 0.1 g with 10 ml of *0.1 M sodium hydroxide* for 10 minutes on a water-bath. The resulting solution, after acidification with *2 M nitric acid*, gives reaction A of chlorides (2.3.1).

## Tests

**Specific optical rotation** (2.4.22). –30.0° to –36.0°, determined in a 0.7 per cent w/v solution in *methanol* prepared with the aid of gentle heat.

**Ionisable chlorine**. Dissolve 0.4 g in a mixture of 75 ml of *water* and 2 ml of *nitric acid*. Allow to stand for 2 minutes and titrate with *0.1 M silver nitrate*, determining the end-point potentiometrically (2.4.25); not more than 0.8 ml is required.

**Sulphated Ash** (2.3.18). Not more than 0.3 per cent.

**Loss on drying** (2.4.19). Not more than 7.0 per cent, determined on 0.5 g by drying in an oven at 105° at a pressure not exceeding 0.7 kPa for 2 hours.

**Assay**. Weigh accurately about 0.4 g, add 20 ml of a 20 per cent w/v solution of *potassium hydroxide*, heat on a water-bath for 2 hours, replacing the *water* lost by evaporation. Cool, add 75 ml of *water* and 4 ml of *nitric acid*, cool. Titrate with *0.1 M silver nitrate*, determining the end-point potentiometrically (2.4.25). Subtract the equivalent volume of *0.1 M silver nitrate* used in the test for Ionisable chlorine. The difference between the volumes represents the amount of *0.1 M silver nitrate* required by melphalan.

1 ml of *0.1 M silver nitrate* is equivalent to 0.01526 g of $C_{13}H_{18}Cl_2N_2O_2$.

**Storage**. Store protected from light and moisture.

# Melphalan Injection

Melphalan Injection is a sterile material consisting of Melphalan Hydrochloride with or without auxiliary substances. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

*The constituted solution complies with the requirement for Particulate matter stated under Parenteral Preparations (Injections) and with the following tests.*

**Appearance of solution**. Not more opalescent than opalescence standard OS2 (2.4.1).



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**pH** (2.4.24). 6.0 to 7.0.

**Storage**. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Melphelan Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of anhydrous melphalan, $C_{13}H_{18}Cl_2N_2O_2$.

**Usual strength**. 50 mg.

*The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.*

### Identification

A. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *methanol* shows an absorption maximum at about 260 nm and a less well-defined maximum at about 301 nm.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

C. Dissolve a quantity containing 20 mg of anhydrous melphalan in 50 ml of *methanol* with the aid of gentle heat, add 1 ml of a 5 per cent w/v solution of *4-(4-nitrobenzyl)pyridine* in *acetone* and evaporate to dryness. Dissolve the residue in 1 ml of hot *methanol* and add 0.1 ml of *strong ammonia solution*; a red colour is produced.

D. Heat a quantity of the powder containing 0.1 g of anhydrous melphalan with 10 ml of *0.1 M sodium hydroxide* for 10 minutes on a water-bath. The resulting solution, after acidification with *2 M nitric acid*, gives reaction A of chlorides (2.3.1).

### Tests

**Ionisable chlorine**. Dissolve a quantity containing 0.1 g of anhydrous melphalan in a mixture of 15 ml of *water* and 0.5 ml of *nitric acid*, allow to stand for 2 minutes and titrate with *0.02 M silver nitrate* determining the end-point potentiometrically (2.4.25); not more than 1.7 ml is required.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh accurately a quantity of the mixed contents of 10 containers containing about 50 mg of Melphalan, dissolve in a mixture of 4 volumes of *acetonitrile* and 1 volume of *0.1 M hydrochloric acid* and dilute with sufficient of the same solvent mixture to produce a final solution containing the equivalent of 0.01 per cent w/v of anhydrous melphalan.

*Reference solution*. A 0.01 per cent w/v solution of *melphalan RS* in the same solvent.

Chromatographic system
- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica (10 µm),
- mobile phase: 200 volumes of a 0.375 per cent w/v solution of *ammonium carbonate*, 180 volumes of *methanol* and 2.7 volumes of *glacial acetic acid*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject alternately the test solution and the reference solution.

Calculate the amount of $C_{13}H_{18}Cl_2N_2O_2$ in the injection.

**Storage**. Store protected from light and moisture at a temperature not exceeding 30°.

**Labelling**. The label on the sealed container states (1) the equivalent amount of anhydrous melphalan contained in it; (2) that it should be used immediately after preparation.

## Melphalan Tablets

Melphalan Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of melphalan, $C_{13}H_{18}Cl_2N_2O_2$. The tablets are coated.

**Usual strengths**. 2 mg; 5 mg.

### Identification

A. Shake a quantity of the powdered tablets containing about 5 mg of Melphalan with 100 ml of hot *methanol*. Filter and dilute 10 ml of the filtrate to 50 ml with *methanol*.

When examined in the range 230 nm to 360 nm (2.4.7), the filtrate shows an absorption maximum at about 260 nm and a less well-defined maximum at about 301 nm.

B. To the remainder of the filtrate obtained in test A add 1 ml of a 5 per cent w/v solution of *4-(4-nitrobenzyl)pyridine* in *acetone* and evaporate to dryness. Dissolve the residue in 1 ml of hot *methanol* and 0.1 ml of *strong ammonia solution*; a red colour is produced.

### Tests

**Uniformity of content**. Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14)

*Test solution*. Add 20 ml of a mixture of 4 volumes of *acetonitrile* and 1 volume of *0.1 M hydrochloric acid* to one tablet, mix with the aid of ultrasound for 10 minutes or until the tablet disintegrates, filter, discarding the first 5 ml of filtrate, and use the filtrate.



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*Reference solution*. A 0.01 per cent w/v solution of *melphalan RS* in the same solvent.

Chromatographic system
- a stainless steel column 20 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 200 volumes of a 0.375 per cent w/v solution of *ammonium carbonate*, 180 volumes of *methanol* and 2.7 volumes of *glacial acetic acid*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject the test solution and the reference solution.

Calculate the content of $C_{13}H_{18}Cl_2N_2O_2$ in the tablet.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh and powder 20 tablets and add about 150 ml of a mixture of 4 volumes of *acetonitrile* and 1 volume of *0.1 M hydrochloric acid* to an accurately weighed quantity of the powdered tablets containing about 25 mg of Melphalan, shake and mix with the aid of ultrasound for 5 minutes, dilute to 250 ml with the same solvent. Filter, discarding the first 20 ml of filtrate, and use the filtrate.

*Reference solution*. A 0.01 per cent w/v solution of *melphalan RS* in the same solvent.

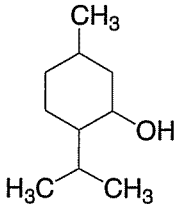
Use the chromatographic system described under Uniformity of content.

Calculate the content of $C_{13}H_{18}Cl_2N_2O_2$ in the tablets (for tablets containing more than 2 mg of Melphalan).

For tablets containing 2 mg or less of Melphalan, use the average of 10 individual results obtained in the test for Uniformity of content.

**Storage**. Store protected from light and moisture in a cool place.

# Menthol




$C_{10}H_{20}O$ Mol. Wt.156.3

Menthol is 2-isopropyl-5-methylcyclohexanol. It is obtained from the volatile oils of various species of *Mentha* or prepared synthetically. It may be levo-rotatory [(–)-menthol] or racemic [(±)-menthol].

**Category**. Topical antipruritic.

**Description**. Colourless, hexagonal or needle-like crystals, or infused masses or a crystalline powder; odour, pleasant and characteristic.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *menthol RS* or with the reference spectrum of menthol.

B. Dissolve 10 mg in 1 ml of *sulphuric acid* and add 1 ml of a 1 per cent w/v solution of *vanillin* in *sulphuric acid*; an orange-yellow colour is produced. Add 1 ml of *water*; the colour changes to violet (distinction from thymol).

C. When triturated with about an equal weight of *camphor* or *chloral hydrate* or *phenol*, the mixture liquefies.

## Tests

**Appearance of solution**. Dissolve 1.0 g in 10 ml of *ethanol (95 per cent)*. The solution is not more opalescent than opalescence standard OS4 (2.4.1), and not more intensely coloured than reference solution RS6 (2.4.1).

**Acidity**. To 1.0 g in a 100-ml glass-stoppered conical flask add 20 ml of *water*, boil until dissolution is complete, cool, stopper the flask and shake vigorously for 1 minute. Add a few crystals of the substance under examination to initiate crystallisation, shake vigorously for 1 minute and filter. To 5 ml of the filtrate add 0.05 ml of *methyl red solution* and 0.05 ml of *0.01M sodium hydroxide*; the solution is yellow.

**Specific optical rotation** (2.4.22). (for (–)-menthol) –49.0° to –51.0°; (for (±)-menthol) –2.0 ° to +2.0°, determined in a 10.0 per cent w/v solution in *ethanol (95 per cent)*.

**Congealing range** (2.4.10). (for (±)-menthol) 27.0° to 28.0°; on prolonged stirring, the temperature rises 30° to 32°.

**Related substances**. Determine by gas chromatography (2.4.13).

*Test solution*. Dissolve 0.1 g in sufficient *ethanol (95 per cent)* to produce 10 ml.

*Reference solution (a)*. Dilute 1 ml of the test solution to 100 ml with *ethanol (95 per cent)*.

*Reference solution (b)*. Dilute 1 ml of reference solution (a) to 20 ml with *ethanol (95 per cent)*.

Chromatographic system
- a glass or stainless steel column 4 m x 2 mm, packed with diatomaceous support (125 to 180 mesh) impregnated with 5 per cent carbowax 20 M (Polyethylene glycol compund 20 M),



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- temperature :
  column 80°,
  injection port at 250° and the detector at 240°,
- flow rate. 30 ml per minute of the carrier gas.

Inject separately of each solution. Run the chromatogram obtained with the test solution for 3 times the retention time of the principal peak.

In the chromatogram obtained with the test solution the sum of the areas of any secondary peaks is not greater than the area of the principal peak in the chromatogram obtained with reference solution (a). Ignore any peak with an area less than the principal peak in the chromatogram obtained with reference solution (b).

**Residue on evaporation**. Evaporate 2.0 g on a water-bath and heat at 105° for 1 hour. The residue weighs not more than 1.0 mg (0.05 per cent).

**Storage**. Store protected from light and moisture at a temperature not exceeding 30°.

**Labelling**. The label states whether the contents are levo-rotatory or racemic menthol.

## Menthol and Benzoin Inhalation

Menthol and Benzoin Inhalation is an inhalation vapour, solution of racementhol or levomenthol 20 g in sufficient benzoin inhalation to produce 1000 ml.

Menthol and Benzoin Inhalation contains not less than 2.8 per cent w/v of total balsamic acids, calculated as cinnamic acid, $C_9H_8O_2$.

### Tests

**Total solids** (2.6.5). 9.0 per cent to 12.0 per cent w/v, determined on 2 ml of the solution by drying at 105° for 4 hours.

**Other tests**. Complies with the tests stated under Inhalation Preparations.

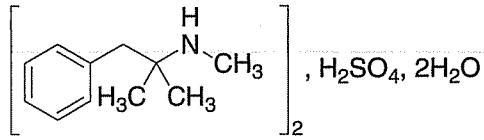
Follow the procedure described under Assay with suitable dilution of the reference solution wherever the amount of active substance is to be determined in any test.

**Assay**. Boil 10 ml with 25 ml of *ethanolic potassium hydroxide solution* under a reflux condenser for 1 hour. Evaporate the *ethanol (95 per cent)*, disperse the residue in 50 ml of hot *water*, cool, add 80 ml of *water* and 1.5 g of *magnesium sulphate* dissolved in 50 ml of *water*. Mix thoroughly and allow to stand for 10 minutes. Filter, wash the residue on the filter with 20 ml of *water*, acidify the combined filtrate and washings with *hydrochloric acid* and extract with four 40 ml quantities of *ether*. Discard the aqueous solution, combine the ether extracts and extract with successive quantities of 20, 20, 10, 10 and 10 ml of *sodium hydrogen carbonate solution*, washing each aqueous extract with the same 20 ml of *ether*. Discard the ether layers, carefully acidify the combined aqueous extracts with *hydrochloric acid* and extract with successive quantities of 30, 20, 20 and 10 ml of *chloroform*, filtering each extract through *anhydrous sodium sulphate* supported on absorbent cotton. Distil the *chloroform* from the combined filtrates until 10 ml remains and remove the remainder in a current of air. Dissolve the residue, with the aid of gentle heat, in 10 ml of *ethanol (95 per cent)*, previously neutralised to *phenol red solution*, cool and titrate with *0.1 M sodium hydroxide* using *phenol red solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.01482 g of total balsamic acids, calculated as cinnamic acid, $C_9H_8O_2$.

**Labelling**. The label states the amount of active ingredient delivered per inhalation.

## Mephentermine Sulphate




$(C_{11}H_{17}N)_2,H_2SO_4,2H_2O$ Mol. Wt.460.6

Mephentermine Sulphate is *N*,α,α-trimethylphenethylamine sulphate dihydrate.

Mephentermine Sulphate contains not less than 98.0 per cent and not more than 102.0 per cent of $(C_{11}H_{17}N)_2,H_2SO_4$, calculated on the dried basis.

**Category**. Sympathomimetic.

**Dose**. By intramuscular or intravenous injection, the equivalent of 30 to 45 mg of mephentermine. (21 mg of mephentermine sulphate is approximately equivalent to 15 mg of mephentermine).

**Description**. White crystals or a crystalline powder.

### Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mephentermine sulphate RS*.

B. A 0.2 per cent w/v solution yields a precipitate with *iodine solution* and with *potassium mercuri-iodide solution*.

C. Dissolve 0.1 g in 5 ml of *water*, add with stirring 10 ml of *picric acid solution*. Allow to stand for 30 minutes, filter and wash the precipitate with small quantities of cold *water* until



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the last washing is colourless; the precipitate, after drying at 105° melts at 154° to 158° (2.4.21).

D. Gives the reactions of sulphates (2.3.1).

### Tests

**pH** (2.4.24). 4.0 to 6.5, determined in a 2.0 per cent w/v solution in *carbon dioxide-free water*.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). 5.0 to 8.0 per cent, determined on 0.5 g by drying in an oven at 105° for 3 hours.

**Assay**. Weigh accurately about 0.4 g, dissolve in 250 ml of *water*, add 5 g of *sodium chloride*, shake well and add 5 ml of *5 M sodium hydroxide*. Extract with 30 ml and then with further quantities, each of 20 ml, of *ether* until the base is completely extracted. Combine the ether extracts, wash with two quantities, each of 10 ml, of *water* and extract the aqueous washings with 10 ml of *ether*, adding this ether to the main ether extract. Add to the ether solution 30.0 ml of *0.05 M sulphuric acid*, stir thoroughly and warm gently until the ether is evaporated. Cool and titrate with *0.1 M sodium hydroxide* using *methyl red solution* as indicator. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of sulphuric acid required.

1 ml of *0.05 M sulphuric acid* is equivalent to 0.02123 g of $(C_{11}H_{17}N)_2,H_2SO_4$.

**Storage**. Store protected from light and moisture.

## Mephentermine Injection

Mephentermine Sulphate Injection

Mephentermine Injection is a sterile solution of Mephentermine Sulphate in Water for Injections.

Mephentermine Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of mephentermine, $C_{11}H_{17}N$.

**Usual strength**. The equivalent of 15 mg of mephentermine per ml (21 mg of mephentermine sulphate is approximately equivalent to 15 mg of mephentermine).

### Identification

A. A 0.2 per cent w/v solution yields a precipitate with *iodine solution* and with *potassium mercuri-iodide solution*.

B. Dissolve 0.1 g in 5 ml of *water*, add with stirring 10 ml of *picric acid solution*. Allow to stand for 30 minutes, filter and wash the precipitate with small quantities of cold *water* until the last washing is colourless; the precipitate, after drying at 105° melts at 154° to 158° (2.4.21).

C. Gives the reactions of sulphates (2.3.1).

### Tests

**pH** (2.4.24). 4.0 to 6.5.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. Measure accurately a volume containing about 0.2 g of mephentermine, add *water* if necessary to produce 20 ml add 5 g of *sodium chloride*, shake well and add 5 ml of *5 M sodium hydroxide*. Extract with 30 ml and then with further quantities, each of 20 ml, of *ether* until the base is completely extracted. Combine the ether extracts, wash with two quantities, each of 10 ml, of *water* and extract the aqueous washings with 10 ml of *ether*, adding this ether to the main ether extract. Add to the ether solution 30.0 ml of *0.05 M sulphuric acid*, stir thoroughly and warm gently until the ether is evaporated. Cool and titrate with *0.1 M sodium hydroxide* using *methyl red solution* as indicator. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of sulphuric acid required.

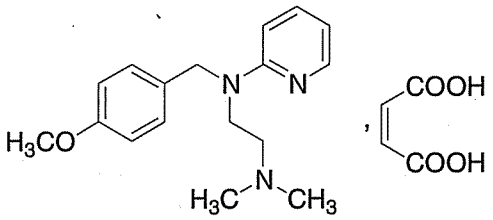
1 ml of *0.05 M sulphuric acid* is equivalent to 0.0163 g of $C_{11}H_{17}N$.

**Storage**. Store protected from light and moisture.

**Labelling**. The label states the strength in terms of the equivalent amount of mephentermine in a suitable dose-volume.

## Mepyramine Maleate

Pyrilamine Maleate




$C_{17}H_{23}N_3O,C_4H_4O_4$ Mol. Wt. 401.5

Mepyramine Maleate is 2-(*N*-4-methoxybenzyl-*N*-2-pyridylamino)ethyldimethylamine hydrogen maleate.

Mepyramine Maleate contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{17}H_{23}N_3O,C_4H_4O_4$, calculated on the dried basis.

**Category**. Histamine $H_1$-receptor antagonist.

**Dose**. Orally, 300 to 600 mg daily, in divided doses; by intramuscular or intravenous injection, 25 to 50 mg.



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**Description**. A white or slightly yellowish, crystalline powder; odourless or almost odourless.

### Identification

*Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mepyramine maleate RS* or with the reference spectrum of mepyramine maleate.

B. When examined in the range 220 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *0.01 M hydrochloric acid* shows absorption maxima, at about 239 nm and 316 nm; absorbance at about 239 nm, 0.43 to 0.477 and at about 316 nm, 0.2 to 0.22.

C. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

### Tests

**Appearance of solution**. A 4.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and not more intensely coloured than reference solution YS6 (2.4.1).

**pH** (2.4.24). 4.9 to 5.2, determined in a 2.0 per cent w/v solution.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 100 volumes of *ethyl acetate* and 2 volumes of *diethylamine*.

*NOTE—Prepare the following solutions immediately before use.*

*Test solution (a)*. Dissolve 0.4 g of the substance under examination in 10 ml of *chloroform*.

*Test solution (b)*. Dissolve 0.4 g of the substance under examination in 100 ml of *chloroform*.

*Reference solution (a)*. A 4.0 per cent w/v solution of *mepyramine maleate RS* in *chloroform*.

*Reference solution (b)*. A 0.4 per cent w/v solution of *mepyramine maleate RS* in *chloroform*.

*Reference solution (c)*. A 0.008 per cent w/v solution of *mepyramine maleate RS* in *chloroform*.

*Reference solution (d)*. A 0.004 per cent w/v solution of *mepyramine maleate RS* in *chloroform*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (c). The test is not valid unless the $R_f$ values of the principal spots in the chromatograms obtained with test solution (a) and reference solution (a) are at least 0.2 and unless the spot in the chromatogram obtained with reference solution (d) is clearly visible. Ignore the spot due to maleic acid on the line of application.

**Heavy metals** (2.3.13).1.0 g complies with the limit test for heavy metals, Method A (20 ppm).

**Chlorides** (2.3.12). 2.5 g dissolved in 15 ml of *water* complies with the limit test for chlorides (100 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.25 per cent, determined on 2.0 g by drying in an oven at 80°.

**Assay**. Weigh accurately about 0.15 g, dissolve in 40 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02007 g of $C_{17}H_{23}N_3O,C_4H_4O_4$.

**Storage**. Store protected from light and moisture.

## Mepyramine Tablets

Mepyramine MaleateTablets; Pyrilamine Maleate Tablets; Pyrilamine Tablets

Mepyramine Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of mepyramine maleate, $C_{17}H_{23}N_3O,C_4H_4O_4$.

**Usual strength**. 50 mg.

### Identification

A. Shake a quantity of the powdered tablets containing 0.1 g of Mepyramine Maleate with 10 ml of *dichloromethane*, filter and evaporate the filtrate to dryness.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mepyramine maleate RS* or with the reference spectrum of mepyramine maleate.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

C. Dissolve a quantity of the powdered tablets containing 0.2 g of Mepyramine Maleate, freed as far as possible from any sugar coating, in 3 ml of *water*, add 2 ml of *5 M sodium hydroxide* and shake with three quantities, each of 3 ml, of *ether*. Warm the aqueous layer in a water-bath for 10 minutes with 2 ml of *bromine solution*, heat to boiling, cool and add 0.2 ml to a solution of 10 mg of *resorcinol* in 3 ml of *sulphuric*



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*acid*; a bluish black colour is produced on heating for 15 minutes in a water-bath.

### Tests

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase.* A mixture of 100 volumes of *ethyl acetate* and 2 volumes of *diethylamine*.

*NOTE—Prepare the following solutions immediately before use.*

*Test solution (a).* Shake a quantity of the powdered tablets containing 0.4 g of Mepyramine Maleate with 10 ml of *chloroform* and filter.

*Test solution (b).* Dilute 1 ml of test solution (a) to 10 ml with *chloroform.*

*Reference solution (a).* A 4.0 per cent w/v solution of *mepyramine maleate RS* in *chloroform.*

*Reference solution (b).* A 0.4 per cent w/v solution of *mepyramine maleate RS* in *chloroform.*

*Reference solution (c).* A 0.008 per cent w/v solution of *mepyramine maleate RS* in *chloroform.*

*Reference solution (d).* A 0.004 per cent w/v solution of *mepyramine maleate RS* in *chloroform.*

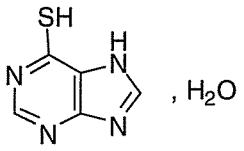
Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (c). The test is not valid unless the $R_f$ values of the principal spots in the chromatograms obtained with test solutions (a) and reference solution (a) are at least 0.2 and unless the spot in the chromatogram obtained with reference solution (d) is clearly visible. Ignore the spot due to maleic acid on the line of application.

**Other tests.** Comply with the tests stated under Tablets.

**Assay.** Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.1 g of Mepyramine Maleate, add 75 ml of *water* and 5 ml of *2 M hydrochloric acid*, shake vigorously for 15 minutes and dilute to 100.0 ml with *water*. Centrifuge and dilute 10.0 ml of the clear, supernatant liquid to 100.0 ml with *water*. To 10.0 ml add 10 ml of *0.1 M hydrochloric acid* and dilute to 50.0 ml with *water*. Measure the absorbance of the resulting solution at the maximum at about 316 nm (2.4.7). Calculate the content of $C_{17}H_{23}N_3O,C_4H_4O_4$ taking 206 as the specific absorbance at 316 nm.

**Storage.** Store protected from light and moisture.

## Mercaptopurine



$C_5H_4N_4S,H_2O$ Mol. Wt. 170.2

Mercaptopurine is purine-6-thiol monohydrate.

Mercaptopurine contains not less than 98.5 per cent and not more than 101.0 per cent of $C_5H_4N_4S$, calculated on the anhydrous basis.

**Category.** Anticancer.

**Dose.** 100 to 200 mg daily, in divided doses.

**Description.** A yellow, crystalline powder.

### Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mercaptopurine RS.*

B. Dissolve 20 mg in 5 ml of *dimethyl sulphoxide* and add sufficient *0.1 M hydrochloric acid* to produce 100 ml. Dilute 5 ml to 200 ml with *0.1 M hydrochloric acid.*

When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum only at about 325 nm.

C. Dissolve 20 mg in 20 ml of *ethanol (95 per cent)* heated to 60° and add 1 ml of a saturated solution of *mercuric acetate* in *ethanol (95 per cent);* a white precipitate is produced.

D. Dissolve 20 mg in 20 ml of *ethanol (95 per cent)* heated at 60° and add 1 ml of a 1 per cent w/v solution of *lead acetate* in *ethanol (95 per cent)*; a yellow precipitate is produced.

### Tests

**Hypoxanthine.** Determine by thin layer chromatography (2.4.17), coating the plate with the *silica gel GF254.*

*Mobile phase.* A mixture of 3 volumes of *concentrated ammonia*, 7 volumes of *water* and 90 volumes of *acetone.*

*Test solution.* Dissolve 50 mg of the substance under examination in 1 ml of *dimethyl sulphoxide* and dilute to 10 ml with *methanol.*

*Reference solution.* Dilute 10 mg of *hypoxanthine* in 10 ml of *dimethyl sulphoxide* and diluted to 100 ml with *methanol.*



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Apply separately to the plate 5 µl of each solution. Allow the mobile phase to rise 10 cm. After development, dry the plate in air and examine at 254 nm. Any secondary spot corresponding to hypoxathine in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (2.0 per cent).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). 10.0 to 12.0 per cent, determined on 0.25 g.

**Assay**. Weigh accurately about 0.15 g, dissolve in 50 ml of *dimethylformamide*. Titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.01522 g of $C_5H_4N_4S$.

**Storage**. Store protected from light and moisture.

# Mercaptopurine Tablets

Mercaptopurine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of mercaptopurine, $C_5H_4N_4S,H_2O$.

**Usual strength**. 50 mg.

## Identification

Shake a quantity of the powdered tablets containing 50 mg of Mercaptopurine with a mixture of 20 ml of *water* and 0.5 ml of *5 M sodium hydroxide* for about 3 minutes, add sufficient *water* to produce 100 ml, mix and filter. Dilute a suitable aliquot of the filtrate with sufficient *0.1 M hydrochloric acid* to give a solution containing 5 µg of Mercaptopurine per ml. The resulting solution shows an absorption maximum at about 325 nm (2.4.7).

## Tests

**Dissolution** (2.5.2).

Apparatus. No 1,
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 50 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

*Test solution.* Use the filtrate as the test solution.

*Reference solution*. A solution containing 0.0055 per cent w/v of *mercaptopurine RS* in the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 3.9 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm),
- mobile phase: 0.1 per cent v/v solution of *acetic acid* in *water*,
- flow rate. 2.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the retention time for mercaptopurine is not less than 4 minutes and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_5H_4N_4S$ in the medium.

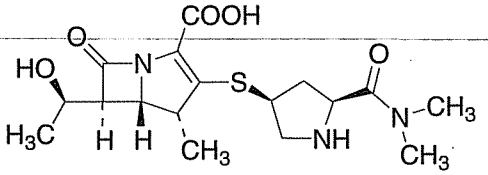
D. Not less than 75 per cent of the stated amount of $C_5H_4N_4S$.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 50 mg of Mercaptopurine, dissolve as completely as possible in 5 ml of *dimethyl sulphoxide* and add sufficient *0.1 M hydrochloric acid* to produce 500.0 ml. Dilute 5.0 ml to 100.0 ml with *0.1 M hydrochloric acid*, filter if necessary and measure the absorbance of the resulting solution at the maximum at about 325 nm (2.4.7). Calculate the content of $C_5H_4N_4S$, $H_2O$ taking 1165 as the specific absorbance at 325 nm.

**Storage**. Store protected from light and moisture.

# Meropenem




$C_{17}H_{25}N_3O_5S,3H_2O$ Mol. Wt. 437.5 (hydrated)
383.5 (anhydrous)

Meropenam is (1*R*,5*S*,6*S*)-2-[(3*S*,5*S*)-5-(dimethylamino-carbonyl)pyrrolidin-3-ylthio-6-[(*R*)-1-hydroxyethyl]-1-methylcarbapen-2-en-3-carboxylic acid.

Meropenem contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{17}H_{25}N_3O_5S$, calculated on the anhydrous basis.

**Category**. Antibiotic.

**Description**. A white to off-white crystalline powder.

## Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *meropenem RS* or with the reference spectrum of meropenem.



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## Tests

**pH** (2.4.24). 4.0 to 6.0, determined in 1.0 per cent w/v solution in *water*.

**Specific optical rotation** (2.4.22). –17.0° to -21.0°, determined in a 0.5 per cent w/v solution.

**Acetone** (5.4). Not more than 0.05 per cent.

Determine by gas chromatography (2.4.13).

*Test solution*. Dissolve 100 mg of the substance under examination in 0.2 ml of *dimethylformamide* and 2.0 ml of internal standard solution.

*Reference solution*. Weigh accurately about 50 mg of *acetone*, dissolve in a small quantity of *dimethylformamide*, dilute to 100.0 ml with *dimethylformamide* and mix. To 1.0 ml of this solution, add 10.0 ml of the internal standard solution, and mix.

*Internal standard solution*. A 0.000005 per cent w/v solution of *ethyl acetate* in *dimethylformamide*.

Chromatographic system
- a glass column 2 m × 3 mm, packed with styrenedivinylbenzene copolymer (Such as Chromosorb 101),
- temperature:
  column. 150°,
  inlet port and detector 170°,
- flow rate adjusted so that the retention time for acetone is about 3 minutes of the carrier gas.

Inject 1 µl of the test solution and the reference solution.

Calculate the percentage of acetone.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. To 900 ml of *water* add 1.0 ml of *triethylamine*, adjust the pH to 5.0 with *dilute phosphoric acid*, dilute to 1000 ml with *water* and mix.

*NOTE — Prepare the solutions immediately before use.*

*Test solution*. Dissolve 0.5 g of the substance under examination in the solvent mixture and dilute to 100 ml of the solvent mixture.

*Reference solution*. A 0.0025 per cent w/v solution of *meropenem RS* in the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature 40°,
- mobile phase: mix 1.0 ml of *triethylamine* and 900 ml of *water*, adjust the pH to 5.0 with *dilute phosphoric acid*, dilute with *water* to 1000 ml, add 70 volumes of *acetonitrile* and mix,
- flow rate. 1.6 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5, the column efficiency in not less than 2500 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution. Any individual impurity is not more than 0.5 per cent and the sum of all impurities found is not more than 2.0 per cent.

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1per cent, igniting at 500 ± 50°, instead of at 800 ± 25°. Use a desiccator containing *silica gel*.

**Water** (2.3.43). 11.4 per cent to 13.4 per cent, determined on 0.5 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. To 900 ml of *water* add 1.0 ml of *triethylamine*, adjust the pH to 5.0 with *dilute phosphoric acid*, dilute to 1000 ml with *water* and mix.

*NOTE — Prepare the solutions immediately before use.*

*Test solution*. Dissolve 50.0 mg of the substance under examination in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

*Reference solution*. A 0.05 per cent w/v solution of *meropenem RS* in the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 30°,
- mobile phase: a mixture of 50 volumes of the solvent mixture and 10 volumes of *methanol*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 300 nm,
- injection volume. 5 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5, the column efficiency is not less than 2500 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_{17}H_{25}N_3O_5S$.

*Meropenem intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.*

**Bacterial endotoxins** (2.2.3). Not more than 0.125 Endotoxin Unit per mg of meropenem.

*Meropenem intended for use in the manufacture of parenteral preparations without a further appropriate sterilisaion*



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*procedure complies with the following additional requirement.*

**Sterility** (2.2.11). Complies with the test for sterility.

**Storage**. Store in airtight containers, at a temperature not exceeding 25°.

**Labelling**. The label states whether or not the contents are intended for use in the manufacture of parenteral preparations.

## Meropenem Injection

Meropenem Injection is a sterile material consisting of Meropenem and Sodium Carbonate.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

*The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).*

**Storage**. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Meropenem Injection contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of meropenem, $C_{17}H_{25}N_3O_5S$.

*The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.*

### Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

### Tests

**pH** (2.4.24). 7.3 to 8.3, determined in 5.0 per cent w/v solution.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. Dissolve 1.0 ml of *triethylamine* in 900 ml of *water*. Adjust the pH to 5.0 with *dilute phosphoric acid* and dilute to 1000 ml with *water*.

*NOTE — Prepare the solutions immediately before use.*

*Test solution*. Determine the weight of the contents of 10 containers. Dissolve an accurately weighed quantity of the mixed contents of the 10 containers containing about 50 mg of Meropenem in 10 ml of the solvent mixture, and mix.

*Reference solution*. A 0.0025 per cent w/v solution of *meropenem RS* in solvent mixture.

Chromatographic system
- a stainless steel column 25 cm × 4.0 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- column temperature 40°,
- mobile phase: mix 1.0 ml of *triethylamine* and 900 ml of *water*, adjust the pH to 5.0 with *dilute phosphoric acid*, dilute to 1000 ml with *water*, filter and mix with 60 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5 and the column efficiency is not less than 2500 theoretical plates.

Inject the test solution and the reference solution. Any individual impurity is not more than 0.8 per cent and the sum of all impurities found is not more than 2.0 per cent.

**Bacterial endotoxins** (2.2.3). Not more than 0.125 Endotoxin Unit per mg of meropenem.

**Sterility** (2.2.11). Complies with the test for sterility.

**Loss on drying** (2.4.19). 9.0 per cent to 12.0 per cent, determined on 1.0 g by drying it in vacuum oven at 65° for 6 hours.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Determine the weight of the contents of 10 containers. Dissolve an accurately weighed quantity of the mixed contents of the 10 containers containing about 10 mg of Meropenem in the mobile phase and dilute to 100.0 ml with the mobile phase.

*Reference solution*. A 0.01 per cent w/v solution of *meropenem RS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: dilute 153 volumes of buffer solution prepared by dissolving 20 ml of 25 per cent w/v of *tetrabutylammonium hydroxide* to 1000 ml with *water*. Adjust the pH to 7.5 with *dilute phosphoric acid*, add 30 volumes of *acetonitrile* and 20 volumes of *methanol* and mix,
- flow rate. 1 ml per minute,
- spectrophotometer set at 300 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5, the column efficiency in not less than 2500 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_{17}H_{25}N_3O_5S$ in the injection.



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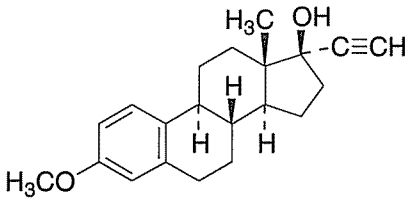
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**Storage**. Store protected from moisture.

**Labelling**. The label states the quantity in mg, of meropenem in a suitable dose-volume.

# Mestranol




$C_{21}H_{26}O_2$ Mol. Wt. 310.4

Mestranol is 3-methoxy-19-nor-17α-pregna-1,3,5(10)-trien-20-yn-17β–ol.

Mestranol contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{21}H_{26}O_2$, calculated on the dried basis.

**Category**. Estrogen.

**Dose**. 12.5 to 50 µg daily, as the oestrogenic component of a combined oral contraceptive preparation.

**Description**. A white or almost white, crystalline powder.

## Identification

*Test B may be omitted if tests A* and *C are carried out. Test A may be omitted if tests B and C are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mestranol RS*.

B. In the test for Related substances the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (c).

C. Dissolve about 5 mg in 1 ml of *sulphuric acid*; a red colour is produced which appears greenish-yellow in ultraviolet light at 365 nm. On adding the solution to 10 ml of *water* and mixing, the solution becomes pink and on standing a pink to violet precipitate is produced.

## Tests

**Specific optical rotation** (2.4.22). –20.0° to –24.0°, determined in a 1.0 per cent w/v solution in *anhydrous pyridine*.

**Light absorption** (2.4.7). Dissolve about 25 mg in sufficient *ethanol (95 per cent)* to produce 25 ml and dilute 10 ml of the solution to 100 ml with *ethanol (95 per cent)*. When examined in the range 230 nm to 360 nm, the resulting solution shows absorption maxima at about 279 nm and 288 nm and a minimum at about 286 nm. Absorbance at about 279 nm is 0.062 to 0.068 and at about 288 nm is 0.059 to 0.064, both calculated on the dried basis.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 90 volumes of *toluene* and 10 volumes of *ethanol (95 per cent)*.

*Test solution (a)*. Dissolve 0.1 g of the substance under examination in 10 ml of *chloroform*.

*Test solution (b)*. Dissolve 0.1 g of the substance under examination in 100 ml of *chloroform*.

*Reference solution (a)*. A 0.01 per cent w/v solution of the substance under examination in *chloroform*.

*Reference solution (b)*. A 0.005 per cent w/v solution of the substance under examination in *chloroform*.

*Reference solution (c)*. A 0.1 per cent w/v solution of *mestranol RS* in *chloroform*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air until the odour of the solvent is no longer detectable, heat it at 110° for 10 minutes and spray the hot plate with *ethanolic sulphuric acid* (20 per cent). Heat again at 110° for 10 minutes and examine in daylight and in ultraviolet light at 365 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a), and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

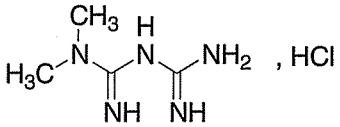
**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 0.5 g by drying in an oven at 105° for 3 hours.

**Assay**. Weigh accurately about 0.2 g, dissolve in 40 ml of *tetrahydrofuran* and add 5 ml of 10 per cent w/v solution of *silver nitrate*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25).

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.03104 g of $C_{21}H_{26}O_2$.

**Storage**. Store protected from light and moisture.

# Metformin Hydrochloride




$C_4H_{11}N_5,HCl$ Mol. Wt. 165.6

Metformin Hydrochloride is 1,1-dimethylbiguanide hydrochloride.



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Metformin Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of $C_4H_{11}N_5$,HCl, calculated on the dried basis.

**Category**. Hypoglycaemic.

**Dose**. 0.5 to 3 g daily, in divided doses.

**Description**. A white, crystalline powder; hygroscopic.

### Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *metformin hydrochloride RS* or with the reference spectrum of metformin hydrochloride.

B. Dissolve 25 mg in 5 ml of *water*, add 1.5 ml of *5 M sodium hydroxide*, 1 ml of *1-naphthol solution* and, dropwise with shaking, 0.5 ml of *sodium hypochlorite solution (3 per cent Cl)*; an orange-red colour is produced which darkens on keeping.

C. Dissolve 10 mg in 10 ml of *water* and add 10 ml of a solution prepared by mixing equal volumes of a 10 per cent w/v solution of *sodium nitroprusside*, a 10 per cent w/v solution of *potassium ferricyanide* and a 10 per cent w/v solution of *sodium hydroxide* and allowing to stand for 20 minutes; a wine red colour develops within 3 minutes.

D. Gives reaction A of chlorides (2.3.1).

### Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 50 mg of the substance under examination in 10 ml of *water*.

*Reference solution (a)*. A 0.0005 per cent w/v solution of the substance under examination in *water*.

*Reference solution (b)*. A 0.0001 per cent w/v solution of *dicyandiamide* in *water*.

Chromatographic system

- a stainless steel column 30 cm x 4 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a solution containing 0.087 per cent w/v of *sodium pentanesulphonate* and 0.12 per cent w/v of *sodium chloride*, adjusted to pH 3.5 using 1 per cent v/v solution of *orthophosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 218 nm,
- injection volume. 20 µl.

For the test solution record the chromatogram for three times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any peak corresponding to dicyandiamide is not greater than that obtained with reference solution (b) and the area of any other secondary peak is not greater than that obtained with reference solution (a).

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 60 mg, dissolve in 4 ml of *anhydrous formic acid*, add 50 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.008281 g of $C_4H_{11}N_5$,HCl.

**Storage**. Store protected from light and moisture.

## Metformin Tablets

### Metformin Hydrochloride Tablets

Metformin Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of metformin hydrochloride, $C_4H_{11}N_5$,HCl.

**Usual strengths**. 500 mg; 850 mg.

### Identification

A. Shake a quantity of the powdered tablets containing 20 mg of Metformin Hydrochloride with 20 ml of *ethanol*, filter, evaporate the filtrate to dryness on a water-bath and dry the residue at 105° for 1 hour.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *metformin hydrochloride RS* or with the reference spectrum of metformin hydrochloride.

B. Triturate a quantity of the powdered tablets containing 50 mg of Metformin Hydrochloride with 10 ml of *water* and filter. To 5 ml of the filtrate, add 1.5 ml of *5 M sodium hydroxide*, 1 ml of *1-naphthol solution* and, dropwise with shaking, 0.5 ml of *dilute sodium hypochlorite solution*; an orange-red colour is produced which darkens on keeping.

C. The filtrate obtained in test B gives reaction A of chlorides (2.3.1).

### Tests

**Related substances**. Determine by liquid chromatography (2.4.14).



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*Test solution*. Shake a quantity of the powdered tablets containing 0.5 g of Metformin Hydrochloride with 100 ml of *water* and filter.

*Reference solution (a)*. Dilute 0.1 ml of the test solution to 100 ml with *water*.

*Reference solution (b)*. A 0.0001 per cent w/v solution of *dicyandiamide* in *water*.

Chromatographic system
- a stainless steel column 30 cm x 4 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a solution containing 0.087 per cent w/v of *sodium pentanesulphonate* and 0.12 per cent w/v of *sodium chloride*, adjusted to pH 3.5 using 1 per cent v/v solution of *orthophosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 218 nm,
- injection volume. 20 µl.

For the test solution record the chromatogram for three times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any peak corresponding to dicyandiamide is not greater than that obtained with reference solution (b) and the area of any other secondary peak is not greater than that obtained with reference solution (a).

**Dissolution** (2.5.2).

Apparatus. No. 2,
Medium. 900 ml of a 0.68 per cent w/v solution of *potassium dihydrogen phosphate*, adjusted to pH 6.8 by the addition of *1 M sodium hydroxide*,
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter, dilute suitably with *water* and measure the absorbance of the resulting solution at the maximum at about 233 nm (2.4.7). Calculate the content of $C_4H_{11}N_5$,HCl, in the medium taking 806 as the specific absorbance at 233 nm.

D. Not less than 70 per cent of the stated amount of $C_4H_{11}N_5$, HCl.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.1 g of Metformin Hydrochloride, shake with 70 ml of *water* for 15 minutes, dilute to 100.0 ml with *water* and filter. Dilute 10.0 ml of the filtrate to 100.0 ml with *water*. Further dilute 10.0 ml to 100.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 232 nm (2.4.7). Calculate the content of $C_4H_{11}N_5$, HCl taking 798 as the specific absorbance at 232 nm.

# Metformin Hydrochloride Sustained-release Tablets

Metformin Hydrochloride Sustained-release Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of metformin hydrochloride, $C_4H_{11}N_5$,HCl.

## Identification

A. Shake a quantity of the powdered tablets containing about 20 mg of Metformin Hydrochloride with 20 ml of *dehydrated alcohol* and filter, evaporate the filtrate to dryness on a water-bath and dry the residue at 105° for 1 hour. The residue complies with the following test. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *metformin hydrochloride RS* or with the reference spectrum of metformin hydrochloride.

B. Triturate a quantity of the powdered tablets containing about 50 mg of Metformin Hydrochloride with 10 ml of *water* and filter. To 5 ml of the filtrate, add 1.5 ml of *5 M sodium hydroxide*, 1 ml of *1-naphthol solution* and, dropwise with shaking, 0.5 ml of *dilute sodium hypochlorite solution;* an orange-red colour is produced which darkens on keeping.

## Tests

**Dissolution** (2.5.2).

Apparatus No. 2,
Medium. 1000 ml of *phosphate buffer pH 6.8* prepared by dissolving 27.22 g of *monobasic potassium phosphate* in 1000 ml of *water*. Take 250 ml of this solution, add 112 ml of *0.2 M sodium hydroxide solution*, then dilute to 1000 ml with *water*,
Speed and time. 100 rpm and 1 hour, 3 hours and 10 hours.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted if necessary, at the maximum at about 233 nm (2.4.7). Calculate the content of $C_4H_{11}N_5$,HCl in the medium from the absorbance obtained from a solution of known concentration of *metformin hydrochloride RS* in the same medium.

D. Not less than 25 per cent and not more than 50 per cent in 1 hour, not less than 45 per cent and not more than 75 per cent in 3 hours and not less than 80 per cent in 10 hours of $C_4H_{11}N_5$HCl in the medium.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. A 2.5 per cent v/v solution of *acetonitrile* in *water*.



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*Test solution.* Disperse a quantity of the powdered tablets containing about 0.5 g of Metformin Hydrochloride in 100 ml of the solvent mixture. Take 10 ml of this solution and centrifuge at 3500 rpm for 15 minutes. Dilute 5 ml of this supernatant to 100 ml with the solvent mixture.

*Reference solution (a).* A 0.0005 per cent w/v solution of *dicyandiamide* in the solvent mixture.

*Reference solution (b).* A 0.0025 per cent w/v solution of *metformin hydrochloride RS* in the solvent mixture.

*Reference solution (c).* Dilute 1 ml each of reference solution (a) and reference solution (b), to 100 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 90 volumes of buffer solution prepared by dissolving 1 g each of *sodium heptane sulphonate* and *sodium chloride* to 1800 ml of *water*, adjusted to pH 3.85 with *0.06 M orthophosphoric acid* and dilute to 2000 ml with *water* and 10 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 218 nm,
- injection volume. 20 µl.

Inject reference solution (c). The test is not valid unless the tailing factor of the peak due to metformin hydrochloride is not more than 2.0. The resolution between the peaks due to dicyandiamide and metformin hydrochloride is not less than 2.0. The relative standard deviation for replicate injections is not more than 2.0 per cent for metformin hydrochloride and not more than 10 per cent for dicyandiamide.

Inject reference solution (c) and the test solution. In the chromatogram obtained with the test solution the area of peak corresponding to dicyandiamide is not more than the area of the peak due to dicyandiamide in the chromatogram obtained with reference solution (c) (0.02 per cent) and the area of any other secondary peak is not more than the area of peak due to metformin hydrochloride in the chromatogarm obtained with reference solution (c) (0.1 per cent).

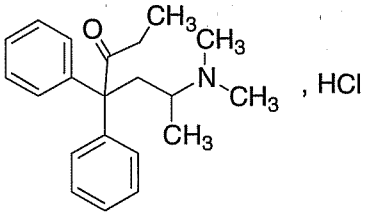
**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Shake a quantity of the powder containing about 0.1 g of Metformin Hydrochloride with 70 ml of *water* for 15 minutes, dilute to 100 ml with *water* and filter. Dilute 5 ml of the filtrate to 50.0 ml with *water*. Further dilute 5.0 ml to 50.0 ml with *water*. Measure the absorbance of the resulting solution at the maximum at about 232 nm (2.4.7).

Calculate the content of $C_4H_{11}N_5HCl$ from the absorbance obtained by carrying out the Assay simultaneously using *metformin hydrochloride RS*.

# Methadone Hydrochloride

Amidone Hydrochloride




$C_{21}H_{27}NO,HCl$ Mol. Wt. 345.9

Methadone Hydrochloride is (*RS*)-dimethyl-(1-methyl-4-oxo-3,3-diphenylhexyl)amine hydrochloride.

Methadone Hydrochloride contains not less than 98.5 per cent and not more than 100.5 per cent of $C_{21}H_{27}NO,HCl$, calculated on the dried basis.

**Category**. Narcotic analgesic.

**Dose**. Orally or by subcutaneous or intramuscular injection, 5 to 10 mg every 6 to 8 hours.

**Description**. A white, crystalline powder.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *methadone hydrochloride RS* or with the reference spectrum of methadone hydrochloride.

B. To 2 ml of a 5 per cent w/v solution in *carbon dioxide-free water* add 1 ml of *0.1 M hydrochloric acid* and 6 ml of *ammonium thiocyanate solution*; a white precipitate is produced which becomes crystalline on stirring for a few minutes. The precipitate, after drying at 105° melts at 143° to 148° (2.4.21).

C. Dissolve 50 mg in 5 ml of *carbon dioxide-free water*, add 1 ml of *6 M ammonia*, mix, allow to stand for 5 minutes and filter; the filtrate gives reaction A of chlorides (2.3.1).

D. Optical rotation of a 2-dm layer of a 5 per cent w/v solution in *carbon dioxide-free water*, is – 0.05° to +0.05° (2.4.22).

## Tests

**Appearance of solution**. A 5.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and colourless (2.4.1).

**Acidity or alkalinity**. To 10 ml of a 2.0 per cent w/v solution in *carbon dioxide-free water* add 0.2 ml of *methyl red solution* and 0.2 ml of *0.01 M sodium hydroxide*; the solution is yellow. Add 0.4 ml of *0.01 M hydrochloric acid*; the solution is red.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.



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*Mobile phase*. A mixture of 60 volumes of *ethanol (95 per cent)*, 30 volumes of *glacial acetic acid* and 10 volumes of *water*.

*Test solution*. Dissolve 0.5 g of the substance under examination in 10 ml of *ethanol (95 per cent)*.

*Reference solution*. A 0.005 per cent w/v solution of the substance under examination in *ethanol (95 per cent)*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with *dilute potassium iodobismuthate solution*. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.5 g, dissolve in 50 ml of *anhydrous glacial acetic acid*, add 5 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator and continuing the titration until the colour changes from violet-blue to green. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03459 g of $C_{21}H_{27}NO,HCl$.

**Storage**. Store protected from light and moisture.

# Methadone Injection

Methadone Hydrochloride Injection; Amidone Hydrochloride Injection; Amidone Injection

Methadone Injection is a sterile solution of Methadone Hydrochloride in Water for Injections.

Methadone Injection contains not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of methadone hydrochloride, $C_{21}H_{27}NO,HCl$.

**Usual strengths**. 5 mg per ml; 10 mg per ml.

## Identification

Make a volume containing 0.1 g of Methadone Hydrochloride alkaline with *5 M sodium hydroxide*, stir with a glass rod until the precipitate solidifies, filter, wash with *water* and dry over *phosphorus pentoxide* at room temperature at a pressure of 2 kPa. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *methadone hydrochloride RS* treated in the same manner or with the reference spectrum of methadone.

B. To 5 mg add 0.05 ml of *dinitrobenzene solution* and 0.05 ml of a 50 per cent w/v solution of *sodium hydroxide*; a purple colour is produced which changes slowly to dark brown.

## Tests

**pH** (2.4.24). 5.0 to 6.5.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. To an accurately measured volume containing about 10 mg of Methadone Hydrochloride add 1 ml of *glacial acetic acid* and dilute to 100.0 ml with *water*. To 10.0 ml of this solution add 10 ml of a 0.4 per cent w/v solution of *picric acid* and 10 ml of *phosphate buffer pH 4.9*, extract with three quantities, each of 15 ml, of *chloroform*, dilute the combined chloroform extracts to 50.0 ml with *chloroform*. To 10.0 ml add sufficient *chloroform* to produce 20.0 ml and measure the absorbance of the resulting solution at the maximum at about 350 nm (2.4.7), using as the blank a solution prepared in the same manner but omitting the substance under examination. Calculate the content of $C_{21}H_{27}NO,HCl$ taking 448 as the specific absorbance at 350 nm.

**Storage**. Store protected from light, in single dose container.

# Methadone Linctus

Methadone Hydrochloride Linctus; Amidone HydrochlorideLinctus; Amidone Linctus

Methadone Linctus is a solution containing 0.04 per cent w/v of Methadone Hydrochloride in a suitable vehicle with a tolu flavour.

Methadone Linctus contains not less than 0.036 per cent and not more than 0.044 per cent w/v of stated amount of methadone hydrochloride, $C_{21}H_{27}NO,HCl$.

**Category**. Opioid analgesic.

## Identification

To 50 ml, add 30 ml of *water* and *1 M sulphuric acid* until the solution is acidic to *litmus paper*. Extract with two 20 ml quantities of *petroleum spirit (boiling range, 40° to 60°)*, discarding the extracts, add *5 M sodium hydroxide* until the solution is alkaline to *litmus paper*. Add 4 g of *sodium chloride*, shake to dissolve, extract with two 25 ml quantities of *ether* and wash the combined ether extracts with five 20 ml quantities of *water*. Dry with *anhydrous sodium sulphate*, filter, evaporate to dryness and dry the residue over *phosphorus pentoxide* at a pressure of 2 kPa. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *methadone RS or* with the reference spectrum of methadone.



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B. To 5 mg add 0.05 ml of *dinitrobenzene solution* and 0.05 ml of a 50 per cent w/v solution of *sodium hydroxide*. A purple colour is produced which changes slowly to dark brown.

### Tests

**Other tests**. Complies with the tests stated under Oral Liquids.

**Assay**. To 30 g add 1 ml of *glacial acetic acid* and dilute to 100 ml with *water*. To 10 ml of the resulting solution add 10 ml of a 0.4 per cent w/v solution of *picric acid* and 10 ml of *phosphate buffer pH 4.9*, extract with three 15 ml quantities of *chloroform* and dilute the combined chloroform extracts to 50 ml with *chloroform*. To 10 ml add sufficient *chloroform* to produce 20 ml and measure the absorbance of the resulting solution (2.4.7), at the maximum at about 350 nm using as the blank a solution prepared in the same manner but using 10 ml of a 1 per cent v/v solution of *glacial acetic acid* and beginning at the words 'add 10 ml of a 0.4 per cent w/v .....'. Calculate the content of $C_{21}H_{27}NO$, HCl taking 448 as the specific absorbance at 350 nm.

Determine the weight per ml (2.4.29) of the linctus, and calculate the content of $C_{21}H_{27}NO$,HCl, weight in volume.

**Storage**. Store protected from light.

**Labelling**. The label states the strength in terms of the equivalent amount of methadone.

## Methadone Tablets

Methadone Hydrochloride Tablets; Amidone Hydrochloride Tablets; Amidone Tablets

Methadone Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of methadone hydrochloride, $C_{21}H_{27}NO$,HCl.

**Usual strengths**. 5 mg; 10 mg.

### Identification

A. Shake a quantity of the powdered tablets containing 0.1 g of Methadone Hydrochloride with 20 ml of *water* and centrifuge. Make the supernatant liquid alkaline with *5 M sodium hydroxide*, stir with a glass rod until the precipitate solidifies, filter, wash with *water* and dry over *phosphorus pentoxide* at room temperature at a pressure of 2 kPa.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *methadone hydrochloride RS* or with the reference spectrum of methadone.

B. Extract a quantity of the powdered tablets containing 0.1 g of Methadone Hydrochloride with 10 ml of *water*, filter and wash the residue with sufficient *water* to bring the volume of the filtrate to 10 ml. Add to the filtrate 0.125 g of *picrolonic acid* dissolved in 50 ml of boiling *water*, stir and allow to stand for 2 hours The residue, after recrystallisation from *ethanol (20 per cent)*, washing with *ethanol (20 per cent)* and drying at 105°, melts at about 160° or 180° (2.4.21).

### Tests

**Uniformity of content**. Comply with the test stated under Tablets.

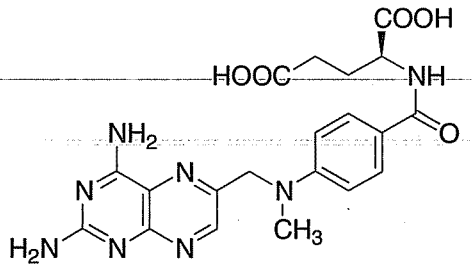
Crush one tablet to a fine powder and transfer to a 25 ml volumetric flask. Add 20 ml of *water*, mix by shaking and dilute to volume with *water*. Mix well and centrifuge. Dilute a suitable volume of the clear, supernatant liquid with *water* to produce a solution containing about 0.2 mg of Methadone Hydrochloride per ml. Measure the absorbance of the resulting solution at the maximum at about 291 nm (2.4.7). Calculate the content of $C_{21}H_{27}NO$,HCl in the tablet from the absorbance obtained by repeating the operation on an accurately weighed quantity of *methadone hydrochloride RS*.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 50 mg of Methadone Hydrochloride, add 60 ml of *water* and 5 ml of *glacial acetic acid*, heat on a water-bath for 5 minutes, mix with the aid of ultrasound for 10 minutes and dilute to 100.0 ml with *water*. Filter, discarding the first 10 ml of the filtrate, and dilute 20.0 ml to 100.0 ml with *water*. To 10.0 ml of the resulting solution, add 10 ml of a 0.4 per cent w/v solution of *picric acid* and 10 ml of *phosphate buffer pH 4.9*, extract with three quantities, each of 15 ml, of *chloroform*, dilute the combined chloroform extracts to 50.0 ml with *chloroform*. To 10.0 ml add sufficient *chloroform* to produce 20.0 ml and measure the absorbance of the resulting solution at the maximum at about 350 nm (2.4.7), using as the blank a solution prepared in the same manner but omitting the substance under examination. Calculate the content of $C_{21}H_{27}NO$,HCl taking 448 as the specific absorbance at 350 nm.

**Storage**. Store protected from light and moisture.

## Methotrexate




$C_{20}H_{22}N_8O_5$ Mol. Wt. 454.4



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Methotrexate is 4-amino-4-deoxy-10-methylpteroyl-L-glutamic acid.

Methotrexate contains not less than 97.0 per cent and not more than 102.0 per cent of $C_{20}H_{22}N_8O_5$, calculated on the anhydrous basis.

**Category**. Anticancer.

**Dose**. For adults, 2.5 to 10 mg; for children, 2.5 to 5 mg.

**Description**. A yellow to orange-brown, crystalline powder.

### Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *methotrexate RS* or with the reference spectrum of methotrexate.

B. When examined in the range 230 nm to 380 nm (2.4.7), a 0.001 per cent w/v solution in *0.1 M sodium hydroxide* shows absorption maxima at about 258 nm, 303 nm and 371 nm; ratio of the absorbance at the maximum at about 303 nm to that at the maximum at about 371 nm, 2.8 to 3.3.

### Tests

**Specific optical rotation** (2.4.22). +19.0° to +24.0°, determined in a 1.0 per cent w/v solution in a solution containing 1.4 per cent w/v of *sodium carbonate*.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 12.0 per cent, determined on 0.25 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 25 mg of the substance under examination in 250.0 ml of the mobile phase.

*Reference solution (a)*. Dissolve 25 mg of *methotrexate RS* in 250.0 ml of the mobile phase.

*Reference solution (b)*. Dissolve 25 mg of *methotrexate RS* and 25 mg of *folic acid* in 250.0 ml of the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 92 volumes of *phosphate buffer pH 6.0* and 8 volumes of *acetonitrile*,
- flow rate. 1.4 ml per minute,
- spectrophotometer set at 302 nm,
- injection volume. 20 µl.

Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (b). The resolution between the peaks due to methotrexate and folic acid is not less than 5.0.

Inject separately the test solution and reference solution (a) and measure the responses for the principal peak.

Calculate the content of $C_{20}H_{22}N_8O_5$.

**Storage**. Store protected from light and moisture.

*CAUTION — Great care should be taken to prevent inhaling particles of Methotrexate and exposing the skin to it.*

## Methotrexate Injection

Methotrexate Injection is a sterile solution of Methotrexate in Water for Injections containing Sodium Hydroxide.

Methotrexate Injection contains not less than 95.0 per cent and not more than 110.0 per cent of the stated amount of methotrexate, $C_{20}H_{22}N_8O_5$.

**Usual strengths**. 2.5 mg per ml; 25 mg per ml.

**Description**. A clear, yellowish solution.

### Identification

When examined in the range of 200 nm to 400 nm (2.4.7), a 0.001 per cent w/v solution in *0.1 M sodium hydroxide* shows absorption maxima at 258, 303 and 371 nm.

### Tests

**pH** (2.4.24). 7.5 to 9.0.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dilute a volume of the injection with the mobile phase to produce a solution containing 0.0025 per cent w/v of Methotrexate.

*Reference solution (a)*. A 0.0025 per cent w/v solution of *methotrexate RS* in the mobile phase.

*Reference solution (b)*. A solution containing 0.0025 per cent w/v each of *methotrexate RS* and *folic acid in* the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: 92 volumes of *phosphate buffer pH 6.0* and 8 volumes of *acetonitrile*,
- flow rate. 1.4 ml per minute,
- spectrophotometer set at 302 nm,
- injection volume. 20 µl.

Inject reference solution (a). The test is not valid unless the relative standard deviation for the replicate injections is not more than 2.0 per cent.



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Inject reference solution (b). The resolution between the peaks due to methotrexate and folic acid is not less than 5.0.

Inject separately the test solution and reference solution (a) and measure the responses for the principal peak.

Calculate the content of $C_{20}H_{22}N_8O_5$ in the injection.

**Storage**. Store protected from light.

**Labelling**. The label states that the injection is not intended for intrathecal injection when an antimicrobial preservative is present.

## Methotrexate Tablets

Methotrexate Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of methotrexate, $C_{20}H_{22}N_8O_5$.

**Usual strength**. 2.5 mg.

### Identification

Extract a quantity of the powdered tablets containing 10 mg of Methotrexate with sufficient *0.1 M sodium hydroxide* to produce 100 ml, filter and dilute 10 ml of the filtrate to 100 ml with *0.1 M sodium hydroxide*.

When examined in the range 230 nm to 380 nm (2.4.7), the resulting solution shows absorption maxima at about 258 nm, 303 nm and 371 nm.

### Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate. Measure the absorbance of the filtrate at the maximum at about 306 nm (2.4.7). Calculate the content of $C_{20}H_{22}N_8O_5$ taking 430 as the specific absorbance at 306 nm.

D. Not less than 75 per cent of the stated amount of $C_{20}H_{22}N_8O_5$.

**Uniformity of content**. Comply with the test stated under Tablets.

Carry out the test as described under Assay, using the following solutions.

*Test solution*. Crush one tablet and mix with 100 ml of the mobile phase with the aid of ultrasound, centrifuge and use the supernatant liquid.

*Reference solution (a)*. A 0.0025 per cent w/v solution of *methotrexate RS* in the mobile phase.

*Reference solution (b)*. A solution containing 0.0025 per cent w/v each of *methotrexate RS* and *folic acid* in the mobile phase.

Calculate the content of $C_{20}H_{22}N_8O_5$ in the tablet.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 2.5 mg of Methotrexate and mix with 100.0 ml of the mobile phase with the aid of ultrasound, centrifuge and use the supernatant liquid.

*Reference solution (a)*. A solution containing 0.0025 per cent w/v each of *methotrexate RS* and *folic acid* in the mobile phase.

*Reference solution (b)*. A 0.0025 per cent w/v solution of *methotrexate RS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 92 volumes of *phosphate buffer pH 6.0* and 8 volumes of *acetonitrile*,
- flow rate. 1.4 ml per minute,
- spectrophotometer set at 302 nm,
- injection volume. 20 µl.

Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

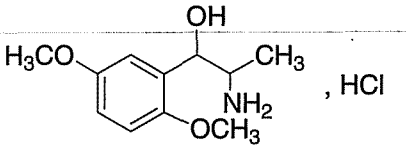
Inject reference solution (b). The resolution between the peaks due to methotrexate and folic acid is not less than 5.0.

Inject separately the test solution and reference solution (a) and measure the responses for the principal peak.

Calculate the content of $C_{20}H_{22}N_8O_5$ in the tablets.

**Storage**. Store protected from light and moisture.

## Methoxamine Hydrochloride




$C_{11}H_{17}NO_3,HCl$ Mol. Wt. 247.7

Methoxamine Hydrochloride is *all-rac*-2-amino-1-(2,5- dimethoxyphenyl)propan-1-ol hydrochloride.



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Methoxamine Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{11}H_{17}NO_3$,HCl, calculated on the dried basis.

**Category**. Sympathomimetic.

**Dose**. By intramuscular injection, 10 to 15 mg; by slow intravenous injection, 5 to 10 mg.

**Description**. Colourless crystals or white, plate-like crystals or a white, crystalline powder; odourless or almost odourless.

### Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *methoxamine hydrochloride RS* or with the reference spectrum of methoxamone hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.005 per cent w/v solution shows an absorption maximum only at about 290 nm; absorbance at about 290 nm, about 0.69.

C. Dissolve 20 mg in 2 ml of *water*, add 5 ml of *diazotised nitroaniline solution* and 1 ml of *dilute sodium carbonate solution*. Allow to stand for 2 minutes and add 1 ml of *1 M sodium hydroxide*; a deep red colour is produced which is extractable with *1-butanol*.

D. A 5 per cent w/v solution gives the reactions of chlorides (2.3.1).

### Tests

**pH** (2.4.24). 4.0 to 6.0, determined in a 2.0 per cent w/v solution.

**Related substances**. Determine by thin-layer chromatography (2.4.14), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 86 volumes of *chloroform*, 12 volumes of *methanol* and 2 volumes of *strong ammonia solution*.

*Test solution*. Dissolve 0.2 g of the substance under examination in 10 ml of *methanol*.

*Reference solution (a)*. A 0.02 per cent w/v solution of the substance under examination in *methanol*.

*Reference solution (b)*. A 0.01 per cent w/v solution of *2,5-dimethoxybenzaldehyde* in *methanol*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 365 nm. Any spot corresponding to 2,5-dimethoxybenzaldehyde in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b). Spray the plate with a 0.3 per cent w/v solution of *ninhydrin* in *1-butanol* containing 3 per cent v/v of *glacial acetic acid* and heat at 105° for 5 minutes. Any other secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a).

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for 2 hours.

**Assay**. Weigh accurately about 0.5 g and dissolve in 30 ml of *anhydrous glacial acetic acid*, 15 ml of *mercuric acetate solution* and 5 ml of *acetic anhydride*, warming if necessary. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02477 g of $C_{11}H_{17}NO_3$,HCl.

**Storage**. Store protected from light and moisture.

## Methoxamine Injection

Methoxamine Hydrochloride Injection

Methoxamine Injection is a sterile solution containing 2 per cent w/v of Methoxamine Hydrochloride in Water for Injections.

Methoxamine Injection contains not less than 1.90 per cent and not more than 2.10 per cent w/v of methoxamine hydrochloride, $C_{11}H_{17}NO_3$, HCl.

### Identification

A. When examined in the range 230 nm to 360 nm (2.4.7), the solution obtained in the Assay shows an absorption maximum only at about 290 nm.

B. Dilute 1 ml with 1 ml of *water*, add 5 ml of *diazotised nitroaniline solution* and 1 ml of *dilute sodium carbonate solution*. Allow to stand for 2 minutes and add 1 ml of *1 M sodium hydroxide*; a deep red colour is produced which is extractable with *1-butanol*.

### Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 86 volumes of *chloroform*, 12 volumes of *methanol* and 2 volumes of *strong ammonia solution*.

*Test solution*. Dilute the injection, if necessary, with *water* so as to contain 2.0 per cent w/v of Methoxamine Hydrochloride.

*Reference solution (a)*. Dilute 1 volume of the test solution to 100 ml with *water*.

*Reference solution (b)*. A 0.01 per cent w/v solution of *2,5-dimethoxybenzaldehyde* in *methanol*.



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Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 365 nm. Any spot corresponding to 2,5-dimethoxybenzaldehyde in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b). Spray the plate with a 0.3 per cent w/v solution of *ninhydrin* in *1-butanol* containing 3 per cent v/v of *glacial acetic acid* and heat at 105° for 5 minutes. Any other secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a).

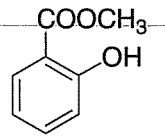
**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. To an accurately measured volume containing about 100 mg of Methoxamine Hydrochloride add sufficient *water* to produce 100.0 ml. Dilute 5.0 ml of this solution to 100.0 ml with *water*. Measure the absorbance of the resulting solution at the maximum at about 290 nm (2.4.7). Calculate the content of $C_{11}H_{17}NO_3$,HCl taking 137 as the specific absorbance at 290 nm.

**Storage**. Store protected from light.

## Methyl Salicylate

Wintergreen Oil




$C_8H_8O_3$ Mol. Wt. 152.2

Methyl Salicylate is 2-hydroxybenzoic acid methyl ester.

Methyl Salicylate contains not less than 99.0 per cent w/w and not more than 100.5 per cent w/w of $C_8H_8O_3$.

**Category**. Counter-irritant.

**Description**. A colourless or slightly yellow liquid; odour, strong, persistent, characteristic and aromatic.

### Identification

A. To 10 ml of a saturated aqueous solution add 0.05 ml of *ferric chloride test solution*; a violet colour develops.

B. Heat 0.25 ml with 2 ml of *2 M sodium hydroxide* on a water-bath for 5 minutes and add 3 ml of *1 M sulphuric acid*. Filter and wash the precipitate with *water*. The precipitate after drying at 105° for 1 hour melts at 156° to 161° (2.4.21).

### Tests

**Appearance of solution**. To 2 ml add 10 ml of *ethanol (95 per cent)*. The resulting solution is clear (2.4.1), and not more intensely coloured than reference solution YS7 (2.4.1).

**Acidity**. Dissolve 5.0 g in 50 ml of *ethanol (95 per cent)*, previously neutralised to a blue colour with *bromocresol green solution* by the addition of *0.1 M sodium hydroxide*. Not more than 0.4 ml of *0.1 M sodium hydroxide* is required to restore the blue colour.

**Refractive index** (2.4.27). 1.534 to 1.538.

**Weight per ml** (2.4.29). 1.175 g to 1.185 g.

**Assay**. Weigh accurately about 0.5 g, dissolve in 25 ml of *ethanol (95 per cent)*, add 0.05 ml of *phenol red solution* and neutralise with *0.1 M sodium hydroxide*. Add 50.0 ml of *0.1 M sodium hydroxide* and heat under a reflux condenser on a water-bath for 30 minutes. Cool and titrate with *0.1 M hydrochloric acid*. Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.01522 g of $C_8H_8O_3$.

**Storage**. Store protected from light.

## Industrial Methylated Spirit

IMS

Industrial Methylated Spirit is a mixture of nineteen volumes of Ethanol of an appropriate strength and one volume of approved wood naphtha.

**Category**. Pharmaceutical aid (solvent).

**Description**. Clear, colourless, mobile, volatile liquid; odour, spirituous and of wood naphtha.

### Identification

Mix 0.1 ml with 0.05 ml of an 11 per cent w/w solution of *phosphoric acid* and 0.25 ml of *dilute potassium permanganate solution*. After 1 minute add a few mg of *sodium metabisulphite* and shake until the mixture is decolorised. Add 1.5 ml of a 50 per cent v/v solution of *sulphuric acid* and a few mg of finely powdered *chromotropic acid sodium salt*, shake well and heat on a water-bath for 5 minutes; a deep violet colour is produced.

### Tests

**Relative density** (2.4.29). Not greater than 0.815.

**Acidity or alkalinity**. 25 ml requires not more than 0.2 ml of *0.1 M sodium hydroxide* to produce a pink colour with *phenolphthalein solution* and not more than 1.0 ml of



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*0.1 M hydrochloric acid* is required to produce a red colour with *methyl red solution*.

**Appearance of solution**. Dilute 5.0 ml to 100 ml with *water*; the solution is clear (2.4.1).

**Aldehydes**. Not more than 50 ppm, determined by the following method. To 5.0 ml add 5 ml of *water* and 1 ml of *decolourised fuchsin solution* and allow to stand for 30 minutes. Any colour produced is not more intense than that obtained by treating in the same manner 5 ml of a 0.005 per cent w/v solution of redistilled *acetaldehyde* in *aldehyde-free ethanol (95 per cent)*.

**Non-volatile matter**. When evaporated and dried at 105°, leaves not more than 0.01 per cent w/v of residue.

**Storage**. Store in tightly-closed containers at a temperature not exceeding 30°.

**Labelling**. The label states that it is inflammable.

# Methylcellulose

Cellulose Methyl Ether

Methylcellulose is a cellulose having some of the hydroxyl groups in the form of the methyl ether. Various grades are available and are distinguished by a number indicative of the apparent viscosity in millipascal seconds of a 2 per cent w/w solution measured at 20°.

Methylcellulose contains not less than 27.5 per cent and not more than 31.5 per cent of methoxyl ($-OCH_3$) groups, calculated on the dried basis.

**Category**. Bulk laxative; pharmaceutical aid (tablet excipient; suspending agent).

**Description**. A white or yellowish white or greyish white powder or granules; practically odourless; hygroscopic after drying.

## Identification

A. With constant stirring add a quantity containing 1.0 g of the dried substance into 50 ml of *carbon dioxide-free water* previously heated to 90°. Allow to cool, dilute to 100 ml with *carbon dioxide-free water* and continue stirring until solution is complete (solution A). Heat 10 ml of solution A on a water-bath with stirring. At temperatures above 40° the solution becomes cloudy or a flocculent precipitate is formed. On cooling, the solution becomes clear.

B. To 10 ml of solution A add 0.3 ml of *2 M acetic acid* and 2.5 ml of a 10.0 per cent w/v solution of *tannic acid*; a yellowish white, flocculent precipitate is produced which dissolves in *6 M ammonia*.

C. Without heating completely dissolve 0.2 g in 15 ml of a 70 per cent w/w solution of *sulphuric acid*, pour the solution with stirring into 100 ml of iced *water*. In a test-tube kept in ice, mix thoroughly 1 ml of the solution with 8 ml of *sulphuric acid*, added dropwise. Heat in a water-bath for exactly 3 minutes and cool immediately in ice. When the mixture is cool, carefully add 0.6 ml of a solution containing 3 g of *ninhydrin* in 100 ml of a 4.55 per cent w/v solution of *sodium metabisulphite*, mix well and allow to stand at 25°; a pink colour is produced immediately which becomes violet within 100 minutes.

D. Place 1 ml of solution A on a glass plate. After evaporation of the *water* a thin film is produced.

## Tests

**Appearance of solution**. Whilst stirring, introduce a quantity containing 1.0 g of the dried substance into 50 g of *carbon dioxide-free water* heated to 90°. Allow to cool, dilute to 100 g with the same solvent and continue stirring until solution is complete. Allow to stand at 2° to 8° for 1 hour. The resulting solution is not more opalescent than opalescence standard OS3 (2.4.1), and is not more intensely coloured than reference solution YS6 (2.4.1).

**pH** (2.4.24). 5.0 to 8.5, determined in solution A.

**Heavy metals** (2.3.13).1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

**Chlorides** (2.3.12). Dilute 5.0 ml of solution A to 15 ml with *water*. The resulting solution complies with the limit test for chlorides (0.5 per cent).

**Apparent viscosity**. Not less than 75 per cent and not more than 140 per cent of the declared value, determined by the following method. To 150 g of *water* heated to 90° add, with stirring, a quantity containing 6.0 g of the dried substance. Stir with a propeller-type stirrer for 10 minutes, place the flask in a bath of iced *water*, continue the stirring and allow to remain in the bath of iced *water* for 40 minutes to ensure that solution is complete. Adjust the weight of the solution to 300 g and centrifuge the solution to expel any trapped air. Determine the viscosity at 20° by Method C (2.4.28), using a shear rate of 10 $s^{-1}$.

**Sulphated ash** (2.3.18). Not more than 1.0 per cent.

**Loss on drying** (2.4.19). Not more than 5.0 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 50 mg in a hard gelatin capsule shell place the capsule and the contents in a 50-ml boiling flask and carry out the determination of methoxyl (2.3.29).

1ml of *0.1 M sodium thiosulphate* is equivalent to 0.0005172 g of methoxyl ($-OCH_3$) groups.

**Storage**. Store protected from light and moisture.

**Labelling**. The label states the apparent viscosity in millipascal seconds of a 2 per cent w/w solution.

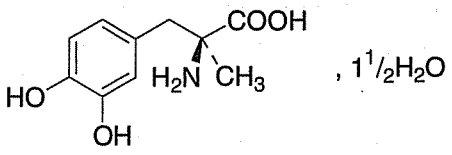


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# Methyldopa




$C_{10}H_{13}NO_4$, $1½\,H_2O$ Mol. Wt. 238.2

Methyldopa is 3-(3,4-dihydroxyphenyl)-2-methyl-L-alanine sesquihydrate.

Methyldopa contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{10}H_{13}NO_4$, calculated on the anhydrous basis.

**Category**. Antihypertensive.

**Dose**. The equivalent of 0.5 to 3 g of anhydrous methyldopa, daily, in divided doses.

**Description**. A white to yellowish white, fine powder which may contain friable lumps.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *methyldopa RS* or with the reference spectrum of methyldopa.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.004 per cent w/v solution in *0.1 M hydrochloric acid* shows an absorption maximum only at about 280 nm; absorbance at about 280 nm, about 0.46.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *microcrystalline cellulose.*

*Mobile phase*. A mixture of 50 volumes of *1-butanol*, 25 volumes of *glacial acetic acid* and 25 volumes of *water*.

*Test solution*. Dissolve 0.1 g of the substance under examination in 10 ml of *1 M hydrochloric acid*.

*Reference solution*. A 1 per cent w/v solution of *methyldopa RS* in *1 M hydrochloric acid*.

Apply to the plate 5 µl of each solution. After development, dry the plate in a current of warm air, and spray with a solution freshly prepared by mixing equal volumes of a 10 per cent w/v solution of *ferric chloride* and a 5 per cent w/v solution of *potassium ferricyanide*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

D. To 10 mg add 3 drops of a 0.4 per cent w/v solution of *ninhydrin* in *sulphuric acid*; a dark purple colour is produced within 5 to 10 minutes. Add 0.15 ml of *water*; the colour changes to pale brownish yellow.

## Tests

**Appearance of solution**. A 4.0 per cent w/v solution in *1 M hydrochloric acid* is not more intensely coloured than reference solution BYS6 or BS6 (2.4.1).

**Acidity**. Dissolve 1.0 g in 100 ml of *carbon dioxide-free water* with the aid of heat, add 0.15 ml of *methyl red solution* and titrate with *0.1 M sodium hydroxide*; not more than 0.5 ml is required to produce a pure yellow colour.

**Optical rotation** (2.4.22). –1.10° to –1.23°, determined in a solution prepared by dissolving a quantity containing 2.2 g of the anhydrous substance in 50.0 ml of *aluminium chloride solution*.

**3-Methoxy compound and related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *microcrystalline cellulose.*

*Mobile phase*. A mixture of 65 volumes of *1-butanol*, 25 volumes of *water* and 15 volumes of *glacial acetic acid.*

*Test solution*. Dissolve 0.1 g of the substance under examination in 10 ml of a mixture of 96 volumes of *methanol* and 4 volumes of *7 M hydrochloric acid.*

*Reference solution (a)*. A 0.005 per cent w/v solution of *3-methoxymethyldopa RS* in *methanol.*

*Reference solution (b)*. A mixture of equal volumes of the test solution and reference solution (a).

Apply to the plate 10 µl of each of the test solution and reference solution (a) and 20 µl of reference solution (b). After development, dry the plate immediately in a current of warm air and spray with a mixture of 5 volumes of a 5 per cent w/v solution of *sodium nitrite* and 45 volumes of a 0.3 per cent w/v solution of *4-nitroaniline* in a mixture of 80 volumes of *hydrochloric acid* and 20 volumes of *water*. Dry it in a current of warm air and spray with a 20 per cent w/v solution of *sodium carbonate* and examine immediately. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

**Heavy metals** (2.3.13). Dissolve 2.0 g in 10 ml of *water*, add 2 ml of *dilute acetic acid* and dilute to 25 ml with *water*. The solution complies with the limit test for heavy metals, Method B (10 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). 10.0 to 13.0 per cent, determined on 0.4 g.



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**Assay**. Weigh accurately about 0.4 g and dissolve in 15 ml of *anhydrous formic acid*, 30 ml of *anhydrous glacial acetic acid* and 30 ml of *dioxan*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02112 g of $C_{10}H_{13}NO_4$.

**Storage**. Store protected from light and moisture.

## Methyldopa Tablets

Methyldopa Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of anhydrous methyldopa, $C_{10}H_{13}NO_4$. The tablets are coated.

**Usual strengths**. The equivalent of 125 mg, 250 mg and 500 mg of anhydrous methyldopa.

### Identification

Remove the coating from a suitable quantity of the tablets by washing with *chloroform*. To a quantity of the powdered tablet cores containing 5 g of anhydrous methyldopa add 35 ml of a mixture of equal volumes of *chloroform* and *methanol* and shake for 3 minutes. Centrifuge and discard the supernatant liquid. Repeat the operation with a further 35 ml of a mixture of equal volumes of *chloroform* and *methanol*. Dry the residue in a current of nitrogen, add 20 ml of *methanol* and 15 ml of *2 M hydrochloric acid*, shake for 2 minutes and filter. Adjust the pH of the filtrate to 4.9 with *5 M ammonia*, allow to stand for several hours at 2° to 8° and filter. Wash the precipitate with 15 ml of *water* and dry it at 50° at a pressure not exceeding 0.7 kPa for 3 hours. Reserve a portion of the residue for the test for Specific optical rotation. The remainder of the residue complies with tests A and B.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *methyldopa RS* or with the reference spectrum of methyldopa.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.004 per cent w/v solution in *0.1 M hydrochloric acid* shows an absorption maximum only at about 280 nm; absorbance at about 280 nm, about 0.46.

C. To a quantity of the powdered tablets containing 10 mg of anhydrous methyldopa, add 3 drops of a 0.4 per cent w/v solution of *ninhydrin* in *sulphuric acid*; a dark purple colour is produced within 5 to 10 minutes. Add 0.15 ml of *water*; the colour changes to pale brownish yellow.

D. To 10 mg of the powdered tablets add 2 ml of *0.1 M sulphuric acid*, 2 ml of *ferrous sulphate-citrate solution* and 0.5 ml of *dilute ammonia solution*; a dark purple colour is immediately produced.

### Tests

**Optical rotation** (2.4.22). – 0.98° to –1.09°, determined on a solution prepared by dissolving an accurately weighed quantity of the residue obtained in the Identification test containing 0.39 g of $C_{10}H_{13}NO_4$ in sufficient *aluminium chloride solution* to produce 10.0 ml. The content of $C_{10}H_{13}NO_4$ in the residue used for the test may be determined by titrating with *0.1 M perchloric acid*, using 0.2 g of the residue, *crystal violet solution* as indicator.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02112 g of $C_{10}H_{13}NO_4$.

**Other tests**. Comply with the tests stated under Tablets.

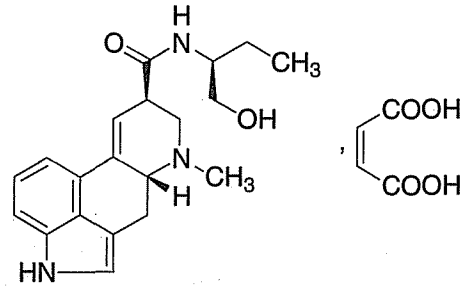
**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.1 g of anhydrous methyldopa, dissolve as completely as possible in sufficient *0.05 M sulphuric acid* to produce 100.0 ml and filter. To 5.0 ml of the filtrate add 2 ml of *ferrous sulphate-citrate solution*, 8 ml of *glycine buffer solution* and sufficient *water* to produce 100.0 ml. Measure the absorbance of the resulting solution at the maximum at about 545 nm (2.4.7). Calculate the content of $C_{10}H_{13}NO_4$ taking 89 as the specific absorbance at 545 nm.

**Storage**. Store protected from light and moisture.

**Labelling**. The label states the strength in terms of the equivalent amount of anhydrous methyldopa.

## Methylergometrine Maleate

Methylergonovine Maleate



$C_{20}H_{25}N_3O_2,C_4H_4O_4$ Mol. Wt. 455.5

Methylergometrine Maleate is 9,10-didehydro-*N*-[(*S*)-1-(hydroxymethyl)propyl]-6-methylergoline-8β-carboxamide hydrogen maleate.

Methylergometrine Maleate contains not less than 95.0 per cent and not more than 105.0 per cent of $C_{20}H_{25}N_3O_2,C_4H_4O_4$, calculated on the dried basis.

**Category**. Uterine stimulant.

**Dose**. Orally, 250 µg to 500 µg; by subcutaneous, intramuscular or intravenous injection, 100 µg to 200 µg.



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**Description**. A white or faintly yellow, crystalline powder; odourless.

### Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *methylergometrine maleate RS*.

B. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

C. A 1 per cent w/v solution shows a blue fluorescence.

D. Dissolve 0.25 mg in 1 ml of *glacial acetic acid* containing a trace of *ferric chloride solution* and add carefully 1 ml of *sulphuric acid* and shake well; a deep blue colour is produced.

### Tests

**pH** (2.4.24). 4.4 to 5.2, determined in a 0.02 per cent w/v solution.

**Specific optical rotation** (2.4.22). +44.0° to +50°, determined at 20° in a 0.5 per cent w/v solution.

**Related substances**. *Protect the solutions from light throughout the test.*

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 10 volumes of *chloroform* and 1 volume of *methanol*.

*Test solution*. Dissolve 40 mg of the substance under examination in 10 ml of *methanol*.

*Reference solution (a)*. A 0.4 per cent w/v solution of *methylergometrine maleate RS* in *methanol*.

*Reference solution (b)*. A 0.012 per cent w/v solution of *methylergometrine maleate RS* in *methanol*.

Place a beaker containing 25 ml of *strong ammonia solution* in the developing chamber, cover the chamber and allow to equilibrate for 30 minutes. Apply to the plate 25 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Spray the plate with a solution containing 0.8 g of *4-dimethylaminobenzaldehyde* in a mixture of 90 ml of *ethanol* and 10 ml of *sulphuric acid*. Dry in a current of warm air for about 2 minutes. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 2.0 per cent, determined on 1.0 g by drying at 80° at a pressure not exceeding 2.7 kPa for 3 hours.

**Assay**. Weigh accurately about 20 mg and dissolve in sufficient *water* to produce 100.0 ml; dilute 20.0 ml of this solution to 100.0 ml with *water*. To 3.0 ml add 6.0 ml of *dimethylaminobenzaldehyde reagent*, mix, cool in running water for 5 minutes and add sufficient reagent to produce 10.0 ml. Measure the absorbance of the resulting solution at the maximum at about 550 nm (2.4.7), using as the blank a solution prepared in the same manner omitting the substance under examination. Calculate the content of $C_{20}H_{25}N_3O_2$, $C_4H_4O_4$ from the absorbance obtained by repeating the Assay using *ergometrine maleate RS* in place of the substance under examination.

1 mg of *ergometrine maleate RS* is equivalent to 1.032 mg of $C_{20}H_{25}N_3O_2$, $C_4H_4O_4$.

**Storage**. Store protected from light, in an atmosphere of nitrogen, at a temperature between 2° to 8°.

## Methylergometrine Injection

Methylergometrine Maleate Injection; Methylergonovine Maleate Injection; Methylergonovine Injection

Methylergometrine Injection is a sterile solution of Methylergometrine Maleate in Water for Injections free from dissolved air.

Methylergometrine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of methylergometrine maleate, $C_{20}H_{25}N_3O_2$,$C_4H_4O_4$.

**Usual strength**. 200 µg per ml.

### Identification

A. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

B. It exhibits a blue fluorescence.

C. To a volume containing 0.1 mg of Methylergometrine Maleate add 0.5 ml of *water* and 2 ml of *4-dimethylaminobenzaldehyde reagent*; after a few minutes a deep blue colour is produced.

### Tests

**pH** (2.4.24). 2.7 to 3.5.

**Related substances**. *Protect the solutions from light throughout the test.*

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 10 volumes of *chloroform* and 1 volume of *methanol*.



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*Test solution*. Transfer a volume containing 1 mg of Methylergometrine Maleate to a separating funnel, add 1 ml of *sodium bicarbonate solution* and extract with three quantities, each of 5 ml, of *chloroform*. Evaporate the combined extracts to dryness at room temperature at a pressure not exceeding 0.7 kPa. Dissolve the residue in 0.25 ml of *methanol* and centrifuge, if necessary.

*Reference solution (a)*. A 0.4 per cent w/v solution of *methylergometrine maleate RS* in *methanol*.

*Reference solution (b)*. A 0.012 per cent w/v solution of *methylergometrine maleate RS* in *methanol*.

Place a beaker containing 25 ml of *strong ammonia solution* in the developing chamber, cover the chamber and allow to equilibrate for 30 minutes. Apply to the plate 25 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Spray the plate with a solution containing 0.8 g of *4-dimethylaminobenzaldehyde* in a mixture of 90 ml of *ethanol* and 10 ml of *sulphuric acid*. Dry in a current of warm air for about 2 minutes. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b).

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. *Protect the solutions from light throughout the Assay.*

To 1.0 ml add sufficient *water* to produce a solution containing 0.04 mg of Methylergometrine Maleate per ml. To 3.0 ml add 6.0 ml of *dimethylaminobenzaldehyde reagent*, mix, cool in running water for 5 minutes and add sufficient reagent to produce 10.0 ml. Measure the absorbance of the resulting solution at the maximum at about 550 nm (2.4.7), using as the blank a solution prepared in the same manner omitting the substance under examination. Calculate the content of $C_{20}H_{25}N_3O_2,C_4H_4O_4$ from the absorbance obtained by repeating the Assay using *ergometrine maleate RS* in place of the substance under examination.

1 mg of *ergometrine maleate RS* is equivalent to 1.032 mg of $C_{20}H_{25}N_3O_2,C_4H_4O_4$.

**Storage**. Store protected from light.

# Methylergometrine Tablets

Methylergometrine Maleate Tablets; Methylergonovine Maleate Tablets; Methylergonovine Tablets

Methylergometrine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of methylergometrine maleate, $C_{20}H_{25}N_3O_2,C_4H_4O_4$.

**Usual strength**. 125 µg.

## Identification

A. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

B. Extract a quantity of the powdered tablets containing 1 mg of Methylergometrine Maleate with 10 ml of *water*, filter and wash the residue with sufficient *water* to produce 10 ml; the solution has a blue fluorescence.

C. To 2 ml of the solution obtained in test B add 4 ml of *4-dimethylaminobenzaldehyde reagent*; a deep blue colour is produced after a few minutes.

## Tests

**Related substances**. *Protect the solutions from light throughout the test.*

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 10 volumes of *chloroform* and 1 volume of *methanol*.

*Test solution*. To a quantity of the powdered tablets containing 1 mg of Methylergometrine Maleate add 5 ml of *water*, 1 ml of *sodium bicarbonate solution* and 2 ml of *chloroform*. Shake, allow to separate and filter the chloroform layer through a plug of cotton moistened with *chloroform*. Repeat the extraction with a further 2 ml of *chloroform* and filter. Evaporate the combined extracts to dryness at room temperature at a pressure not exceeding 0.7 kPa, dissolve the residue in 0.25 ml of *methanol* and centrifuge, if necessary.

*Reference solution (a)*. A 0.4 per cent w/v solution of *methylergometrine maleate RS* in *methanol*.

*Reference solution (b)*. A 0.012 per cent w/v solution of *methylergometrine maleate RS* in *methanol*.

Place a beaker containing 25 ml of *strong ammonia solution* in the developing chamber, cover the chamber and allow to equilibrate for 30 minutes. Apply to the plate 25 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Spray the plate with a solution containing 0.8 g of *4-dimethylaminobenzaldehyde* in a mixture of 90 ml of *ethanol* and 10 ml of *sulphuric acid*. Dry in a current of warm air for about 2 minutes. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b).

**Uniformity of content**. Comply with the test stated under Tablets.

Crush one tablet and transfer to a separating funnel with the aid of not more than 5 ml of *water* and add 3 ml of a 5 per cent w/v solution of *sodium carbonate*. Extract with four quantities, each of 5 ml, of *chloroform*. Filter the extracts through a plug



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of cotton moistened with *chloroform* into a 100-ml separating funnel. Add 2.0 ml of *water* and 10.0 ml of *4-dimethylaminobenzaldehyde reagent*.and shake vigorously for at least 90 seconds. Allow to stand for 30 minutes and discard the chloroform layer. Transfer the aqueous layer to a stoppered-tube and allow to stand for 60 minutes. Measure the absorbance of the resulting solution at the maximum at about 550 nm (2.4.7), using as the blank a mixture of 2.0 ml of *water* and 10.0 ml of *4-dimethylaminobenzaldehyde reagent*. Calculate the content of $C_{19}H_{23}N_3O_2,C_4H_4O_4$ in the tablet from the absorbance obtained by carrying out the following operation simul-taneously. Weigh accurately about 12 mg of *ergometrine maleate RS* and dissolve in sufficient *water* to produce 200.0 ml. To 2.0 ml add 10.0 ml of *4-dimethylaminobenzaldehyde reagent*, mix and cool in running water for 5 minutes. Measure the absorbance of the resulting solution at the maximum at about 550 nm, using as the blank a mixture of 2.0 ml of *water* and 10.0 ml of *4-dimethylaminobenzaldehyde reagent*.

1 mg of *ergometrine maleate RS* is equivalent to 1.032 mg of $C_{20}H_{25}N_3O_2,C_4H_4O_4$.

**Other tests**. Comply with the tests stated under Tablets.

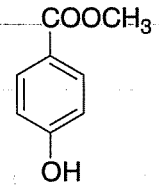
**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 2 mg of Methylergometrine Maleate, dissolve in 50 ml of a 1 per cent w/v solution of *tartaric acid*. To 3.0 ml add 6.0 ml of *dimethylaminobenzaldehyde reagent*, mix, cool in running water for 5 minutes and add sufficient reagent to produce 10.0 ml. Measure the absorbance of the resulting solution at the maximum at about 550 nm (2.4.7), using as the blank a solution prepared in the same manner omitting the substance under examination. Calculate the content of $C_{20}H_{25}N_3O_2,C_4H_4O_4$ from the absorbance obtained by repeating the Assay using *ergometrine maleate RS* in place of the substance under examination.

1 mg of *ergometrine maleate RS* is equivalent to .001032 g of $C_{20}H_{25}N_3O_2,C_4H_4O_4$.

**Storage**. Store protected from light and moisture.

# Methylparaben

Methyl Hydroxybenzoate




$C_8H_8O_3$ Mol. Wt. 152.2

Methylparaben is methyl 4-hydroxybenzoate.

Methylparaben contains not less than 99.0 per cent and not more than 101.0 per cent of $C_8H_8O_3$.

**Category**. Pharmaceutical aid (antimicrobial preservative).

**Description**. Colourless crystals or white, crystalline powder.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *methylparaben RS*.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.0005 per cent w/v solution in *ethanol (95 per cent)* shows an absorption maximum at about 258 nm; absorption at about 258 nm, 0.52 to 0.56.

C. Boil 10 mg with 10 ml of *water*, cool and add 0.05 ml of *ferric chloride solution*; a reddish violet colour is produced.

D. Dissolve 0.1 g in 2 ml of *ethanol (95 per cent)*, boil and add 0.5 ml of *mercuric nitrate solution*; a precipitate is formed and the supernatant liquid becomes red.

## Tests

**Appearance of solution**. A 10.0 per cent w/v solution in *ethanol (95 per cent)* is clear (2.4.1), and not more intensely coloured than reference solution BYS6 (2.4.1).

**Acidity**. Dissolve 1.0 g in sufficient *ethanol (95 per cent)* to produce 10 ml. To 2 ml of the solution add 3 ml of *ethanol (95 per cent)*, 5 ml of *carbon dioxide-free water* and 0.1 ml of *bromocresol green solution*. Not more than 0.1 ml of *0.1 M sodium hydroxide* is required to change the colour of the solution.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 1 volume of *glacial acetic acid*, 30 volumes of *water* and 70 volumes of *methanol*.

*Test solution*. Dissolve 0.1 g of the substance under examination in 10.0 ml of *acetone*.

*Reference solution (a)*. Dilute 0.5 ml of test solution to 100 ml with *acetone*.

*Reference solution (b)*. Dissolve 10 mg of *ethyl parahydroxybenzoate RS* in 1 ml of the test solution and dilute to 10.0 ml with *acetone*.

Apply 2 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. In the chromatogram obtained with the test solution, any secondary spot is not more intense than the corresponding spot in the chromatogram obtained with



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reference solution (a) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated principal spots.

**Chlorides** (2.3.12). Heat 2.0 g with 100 ml of *water*, cool, add sufficient *water* to restore the original volume, and filter. 25 ml of the filtrate complies with the limit test for chlorides (500 ppm).

**Sulphates**. To 10 ml of the filtrate obtained in the test for Chloride add 0.15 ml of *dilute hydrochloric acid* and 0.1 ml of *barium chloride solution*; no turbidity is produced within 10 minutes.

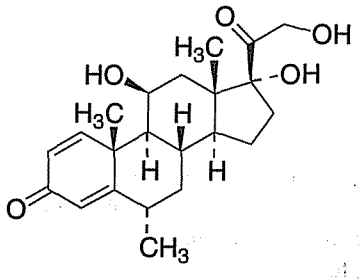
**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Assay**. To 1.0 g add 20.0 ml of *1 M sodium hydroxide*. Heat at about 70° for 1 hour. Cool rapidly in an ice bath. Titrate the excess *sodium hydroxide* with *0.5 M sulphuric acid*, continuing the titration until the second point of inflexion and determining the end-point potentiometrically (2.4.25).

1 ml of *1 M sodium hydroxide* is equivalent to 0.1521 g of $C_8H_8O_3$.

**Storage**. Store protected from light and moisture.

# Methylprednisolone




$C_{22}H_{30}O_5$ Mol. Wt. 374.5

Methylprednisolone is 11β,17α,21-trihydroxy-6α-methylpregna-1,4-diene-3,20-dione.

Methylprednisolone contains not less than 96.0 per cent and not more than 104.0 per cent of $C_{22}H_{30}O_5$, calculated on the dried basis.

**Category**. Adrenocortical steroid.

**Dose**. 4 to 48 mg daily, in divided doses.

**Description**. A white or almost white, crystalline powder.

## Identification

*Test A may be omitted if tests B and C are carried out. Test C may be omitted if tests A and B are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *methylprednisolone RS* or with the reference spectrum of methylprednisolone.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Solvent mixture*. A mixture of 90 volumes of *acetone* and 10 volumes of *formamide*.

*Mobile phase*. *Chloroform*.

*Test solution*. Dissolve 25 mg of the substance under examination in 10 ml of the solvent mixture.

*Reference solution (a)*. Dissolve 25 mg of *methylprednisolone RS* in 10 ml of the solvent mixture.

*Reference solution (b)*. Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v)*. Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

C. Dissolve about 2 mg in 2 ml of *sulphuric acid* by shaking and allow to stand for 5 minutes; an intense red colour is produced and the solution exhibits a reddish brown fluorescence when examined in ultraviolet light at 365 nm. Add the solution to 10 ml of *water* and mix; the colour fades and the solution exhibits a yellowish green fluorescence in ultraviolet light at 365 nm.

## Tests

**Specific optical rotation** (2.4.22). +79.0° to +86.0°, determined in a 1.0 per cent w/v solution in *dioxan*.

**Light absorption** (2.4.7). Absorbance of a 0.001 per cent w/v solution in *ethanol (95 per cent)* at the maximum at about 243 nm, 0.38 to 0.40.

**Related substances**. Determine by liquid chromatography (2.4.14).



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Test solution. *Dissolve 25 mg of the substance under examination in a mixture of equal volumes of* acetonitrile *and* methanol *and dilute to 10 ml with the same solvent mixture.*

*Reference solution (a).* Dissolve 2.0 mg of *methylprednisolone RS* and 2.0 mg of *betamethasone RS* in mobile phase A and dilute to 200 ml with the same mobile phase.

*Reference solution (b).* Dilute 1 ml of the test solution to 100 ml with mobile phase A

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane chemically bonded to silica gel (5 µm),
- column temperature. 45°,
- mobile phase A. 250 volumes of *acetonitrile* and 700 volumes of *water* mixed, allowed to equilibrate and adjusted to 1000 volumes with *water* and mixed,
  B. *acetonitrile*,
- flow rate. 2.5 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

| Time (min) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) | Comment |
|---|---|---|---|
| 0 | 100 | 0 | Isocratic |
| 15 | 100 | 100 | begin linear gradient |
| 40 | 0 | 100 | end chromatogram, return to 100 A |
| 41 | 100 | 0 | begin equilibration with A |
| 46=0 | 100 | 0 | end equilibration, begin next chromatogram |

Equilibrate the column for at least 30 minutes with mobile phase B and then with mobile phase A for 5 minutes. For subsequent operations use the conditions described from 40 to 46 minutes.

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (b) is at least 50 per cent of the full scale of the recorder.

Inject reference solution (a). When the chromatograms are recorded, the retention times are; methylprednisolone about 11.5 minutes, and betamethasone about 12.5 minutes. The test is not valid unless the resolution between the peaks corresponding to methylprednisolone and betamethasone is at least 1.5; if necessary, adjust the concentration of acetonitrile in mobile phase A.

Inject separately a mixture of equal volumes of *acetonitrile* and *methanol* as a blank, the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any peak other than the principal peak, is not greater than half the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent); the sum of the areas of all the peaks other than the principal peak, is not greater than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2 per cent). Ignore any peak due to the blank and any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b).

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

**Assay**. Weigh accurately about 0.1 g and dissolve in sufficient *ethanol (95 per cent)* to produce 100.0 ml and mix. Dilute 2.0 ml of this solution to 100.0 ml with *ethanol (95 per cent)* and mix well. Determine the absorbance of the resulting solution (2.4.7) at the maximum at about 243 nm. Calculate the content of $C_{22}H_{30}O_5$ taking 395 as the specific absorbance at 243 nm.

**Storage**. Store protected from light and moisture.

## Methylprednisolone Tablets

Methylprednisolone Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of methylprednisolone, $C_{22}H_{30}O_5$.

**Usual strengths**. 2 mg; 4 mg; 16 mg.

### Identification

Extract a quantity of the powdered tablets containing 50 mg of Methylprednisolone with 100 ml of *chloroform*, filter and evaporate the filtrate to dryness. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *methylprednisolone RS* or with the reference spectrum of methylprednisolone.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Solvent mixture*. A mixture of 90 volumes of *acetone* and 10 volumes of *formamide*.

*Mobile phase*. A mixture of 30 volumes of *toluene* and 10 volumes of *chloroform*.

*Test solution*. Dissolve 25 mg of the residue in 10 ml of the solvent mixture.

*Reference solution (a)*. Dissolve 25 mg of *methylprednisolone acetate RS* in 10 ml of the solvent mixture.



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*Reference solution (b).* Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v).* Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

## Tests

**Dissolution** (2.5.2).

Apparatus No. 2,
Medium. 900 ml of *water,*
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of a layer of suitable thickness of the filtered solution at the maximum at about 246 nm (2.4.7). Calculate the content of $C_{22}H_{30}O_5$ in the medium taking 400 as the specific absorbance at 246 nm.

D. Not less than 70 per cent of the stated amount of $C_{22}H_{30}O_5$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Diluting solution.* A filtered mixture of 72 volumes of *water,* 25 volumes of *tetrahydrofuran* and 3 volumes of *glacial acetic acid.*

*Test solution.* Extract a quantity of the powdered tablets containing 25 mg of Methylprednisolone with the diluting solution and dilute to 25 ml with the same solvent. Filter and centrifuge if necessary.

*Reference solution.* A 0.001 per cent w/v solution of *methylprednisolone RS* in the diluting solution.

Chromatographic system
- a stainless steel column 20 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a mixture of 149 volumes of *water,* 40 volumes of *tetrahydrofuran,* 10 volumes of *dimethylsulfoxide* and 1 volume of *butanol,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Inject the reference solution. The column efficiency is not less than 800 theoretical plates and the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the test solution and the reference solution. In the chromatogram obtained with the test solution, the area of any peak other than the principal peak is not greater than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent). The sum of the areas of all the peaks other than the principal peak is not greater than twice the area of the principal peak in the chromatogram obtained with the reference solution (2.0 per cent).

**Uniformity of content**. Comply with the test stated under Tablets.

To one tablet add 0.5 ml of *water* (in the case of tablets containing 10 mg or less) or 1.0 ml of *water* (in the case of tablets containing more than 10 mg). Allow the tablet to stand for about 2 minutes, then swirl to disperse the tablet. Add 5.0 ml of the internal standard used in the assay for each mg of methylprednisolone, shake for 15 minutes, filter and centrifuge. Use the filtrate as the test solution.

Determine by liquid chromatography (2.4.14) using the chromatographic system and the reference solution described under Assay.

Calculate the content of $C_{22}H_{30}O_5$ in the tablet.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Internal standard solution.* Weigh accurately a suitable quantity of prednisone in a 3 per cent v/v solution of *glacial acetic acid* in *chloroform* to obtain a solution having a known concentration of about 0.2 mg per ml of prednisone.

*Test solution.* Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 10 mg of Methylprednisolone transfer to a suitable container and add 2.5 ml of *water.* Swirl to form a slurry. Add 50.0 ml of the internal standard solution, and shake for 15 minutes. Filter and centrifuge a portion of the filtrate if necessary and use this as the test solution.

*Reference solution.* Weigh accurately a suitable quantity of *methylprednisolone RS* in the internal standard solution to obtain a solution having a known concentration of about 0.2 mg per ml of methylprednisolone.

Chromatographic system
- stainless steel column 25 cm x 4 mm, packed with porous silica particles (3 to 10 µm),
- mobile phase: a mixture of 475 volumes of *butyl chloride,* 475 volumes of *water-saturated butyl chloride,*



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70 volumes of *tetrahydrofuran*, 35 volumes of *methanol*, and 30 volumes of *glacial acetic acid*,
- flow rate 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

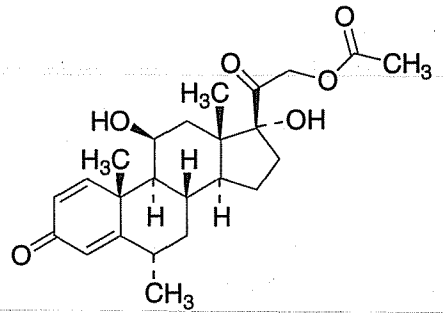
Inject the reference solution. The resolution between methylprednisolone and prednisone is not less than 4.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution. The relative retention time with reference to methylprednisolone for prednisone is about 0.7.

Calculate the content of $C_{22}H_{30}O_5$ in the tablets.

**Storage**. Store protected from light and moisture.

# Methylprednisolone Acetate



$C_{24}H_{32}O_6$ Mol. Wt. 416.5

Methylprednisolone Acetate is 11β,17α-dihydroxy-6α-methyl-3, 20-dioxopregna-1,4-dien-21-yl acetate.

Methylprednisolone Acetate contains not less than 96.0 per cent and not more than 104.0 per cent of $C_{24}H_{32}O_6$, calculated on the dried basis.

**Category**. Adrenocortical steroid.

**Dose**. By deep intramuscular injection, 40 to 120 mg.

**Description**. A white or almost white, crystalline powder; odourless or almost odourless.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *methylprednisolone acetate RS* or with the reference spectrum of methylprednisolone acetate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Solvent mixture*. A mixture of 90 volumes of *acetone* and 10 volumes of *formamide*.

*Mobile phase*. A mixture of 30 volumes of *toluene* and 10 volumes of *chloroform*.

*Test solution*. Dissolve 25 mg of the substance under examination in 10 ml of the solvent mixture.

*Reference solution (a)*. Dissolve 25 mg of *methylprednisolone acetate RS* in 10 ml of the solvent mixture.

*Reference solution (b)*. Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v)*. Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

## Tests

**Specific optical rotation** (2.4.22). +97.0° to +105°, determined in a 1.0 per cent w/v solution in *dioxan*.

**Light absorption** (2.4.7). Absorbance of a 0.001 per cent w/v solution in *ethanol (95 per cent)* at the maximum at about 240 nm, 0.34 to 0.37. The ratio of the absorbance at the maximum at about 240 nm to that at about 263 nm is 1.50 to 1.70.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 20 mg of the substance under examination in 5 ml of *tetrahydrofuran* and dilute to 10 ml with *water*.

*Reference solution (a)*. Dissolve 4.0 mg of *methylprednisolone acetate RS* and 4.0 mg of *dexamethasone acetate RS* in the mobile phase and dilute to 20 ml with the mobile phase.

*Reference solution (b)*. Dilute 1 ml of the test solution to 50 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm),



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- mobile phase: a mixture of 260 ml of *tetrahydrofuran* and 760 ml of *water*, allowed to equilibrate, diluted to 1000 ml with *water* and mixed,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Equilibrate the column with the mobile phase for about 45 minutes.

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (b) is at least 50 per cent of the full scale of the recorder.

Inject reference solution (a). The retention times are: methylprednisolone acetate, about 43 minutes and dexamethasone acetate about 57 minutes. The test is not valid unless the resolution between the peaks corresponding to methylprednisolone acetate and dexamethasone acetate is not less than 6.5. If necessary, adjust the concentration of water in the mobile phase.

Inject the test solution and reference solution (b). Continue the chromatography for 1.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the sum of the areas of all the peaks other than the principal peak is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore any peak due to the solvent and any peak with an area less than 0.025 times the area of the principal peak in the chromatogram obtained with reference solution (b).

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Loss on drying** (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

**Assay**. Weigh accurately about 0.1 g and dissolve in sufficient *ethanol* to produce 100.0 ml and mix. Dilute 1.0 ml of this solution to 100.0 ml with *ethanol* and mix well. Determine the absorbance of the resulting solution at the maximum at about 243 nm (2.4.7). Calculate the content of $C_{24}H_{32}O_6$, taking 355 as the specific absorbance at 243 nm.

**Storage**. Store protected from light and moisture.

# Methylprednisolone Acetate Injection

Methylprednisolone Acetate Injection is a sterile suspension of Methylprednisolone Acetate in Water for Injections.

Methylprednisolone Acetate Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of methylprednisolone acetate, $C_{24}H_{32}O_6$.

**Usual strength**. 40 mg per ml.

**Description**. A white suspension which settles on standing but readily disperses on shaking. On examination under a microscope, the particles are seen to be crystalline and rarely exceed 20 µm in diameter.

## Identification

Dilute a volume containing 0.1 g of Methylprednisolone Acetate to 5 ml with *water*, centrifuge and discard the supernatant liquid. Wash the residue with five quantities, each of 5 ml, of *water*, resuspending the residue in *water* each time. Centrifuge and discard the washings. The residue, after drying at 105° for 3 hours, complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *methylprednisolone acetate RS* or with the reference spectrum of methylprednisolone acetate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Solvent mixture*. 90 volumes of *acetone* and 10 volumes of *formamide*.

*Mobile phase*. A mixture of 30 volumes of *toluene* and 10 volumes of *chloroform*.

*Test solution*. Dissolve 25 mg of the residue in 10 ml of the solvent mixture.

*Reference solution (a)*. Dissolve 25 mg of *methylprednisolone acetate RS* in 10 ml of the solvent mixture.

*Reference solution (b)*. Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v)*. Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

## Tests

**pH** (2.4.24). 3.5 to 7.0.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).



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**Assay**. Determine by liquid chromatography (2.4.14).

Mix 0.12 g of *prednisone RS* (internal standard) with 0.6 ml of *glacial acetic acid*, slowly add *chloroform* with the aid of ultrasound, shake to dissolve and dilute with sufficient *chloroform* to produce 20 ml (solution A).

*Test solution*. Add 10 ml of solution A to an accurately measured quantity of the injection containing about 40 mg of Methylprednisolone Acetate, add sufficient *chloroform* to produce 25.0 ml and shake for 5 minutes or until the aqueous layer is clear; to 4.0 ml of the chloroform layer, add 30 ml of chloroform and 0.4 g of *anhydrous sodium sulphate*, shake for 5 minutes, and use the clear solution.

*Reference solution*. Dissolve 20.0 mg of *methylprednisolone acetate RS* in 5 ml of solution A and add sufficient *chloroform* to produce 100.0 ml.

Chromatographic system
- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane chemically bonded to porous silica (5 to 10 µm),
- mobile phase: a mixture of 30 volumes of *glacial acetic acid* and 35 volumes of *methanol*, 75 volumes of *tetrahydrofuran*, 475 volumes of water-saturated *1-chlorobutane* and 475 volumes of *1-chlorobutane*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

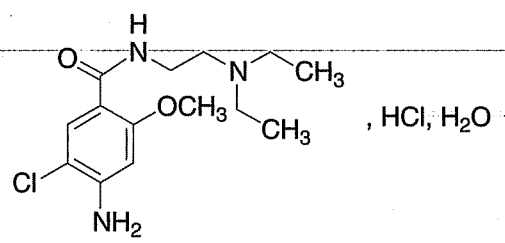
The assay is not valid unless the resolution between the peaks due to methylprednisolone and the internal standard is at least 2.5.

Calculate the content of $C_{22}H_{29}FO_5$ in the injection.

**Storage**. Store protected from light at a temperature not exceeding 30°. The injection should not be allowed to freeze.

**Labelling**. The label states (1) that the preparation is not to be given by intravenous injection; (2) that the container should be shaken gently before a dose is withdrawn.

# Metoclopramide Hydrochloride




$C_{14}H_{22}ClN_3O_2,HCl,H_2O$ Mol. Wt. 354.3

Metoclopramide Hydrochloride is 4-amino-5-chloro-*N*-(2-diethylaminoethyl)-2- methoxybenzamide hydrochloride monohydrate.

Metoclopramide Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{14}H_{22}ClN_3O_2,HCl$, calculated on the anhydrous basis.

**Category**. Antiemetic.

**Dose**. Orally, the equivalent of 10 to 30 mg of anhydrous metoclopramide hydrochloride daily, in divided doses; by intramuscular or intravenous injection, the equivalent of 10 mg of anhydrous metoclopramide hydrochloride.

**Description**. White or almost white crystals or crystalline powder.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B and D may be omitted if tests A and C are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *metoclopramide hydrochloride RS* or with the reference spectrum of metoclopramide hydrochloride.

B. Examine the chromatograms obtained in the test for Related substances in ultraviolet light before spraying with the *4-dimethylaminobenzaldehyde reagent*. The principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

C. A 5 per cent w/v solution in *carbon dioxide-free water* gives reaction A of chlorides (2.3.1).

D. Dissolve about 2 mg in 2 ml of *water*. The solution gives the reaction of primary aromatic amines (2.3.1).

## Tests

**Appearance of solution**. A 10.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and colourless (2.4.1).

**pH** (2.4.24). 4.5 to 6.0, determined in a 10.0 per cent w/v solution.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254*.

*Mobile phase*. A mixture of 90 volumes of *dichloromethane*, 14 volumes of *methanol*, 10 volumes of *dioxan* and 2 volumes of *strong ammonia solution*.

*Test solution (a)*. Dissolve 0.4 g of the substance under examination in 10 ml of *methanol*.

*Test solution (b)*. Dissolve 0.4 g of the substance under examination in 100 ml of *methanol*.

*Reference solution (a)*. A 0.02 per cent w/v solution of the substance under examination in *methanol*.



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*Reference solution (b)*. A 0.4 per cent w/v solution of *metoclopramide hydrochloride RS* in *methanol*.

*Reference solution (c)*. A 0.02 per cent w/v solution of *N,N-diethyl-ethylenediamine* in *methanol*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a). Spray the plate with *dimethylaminobenzaldehyde reagent* and allow it to dry in air. Any secondary spot in the chromatogram obtained with test solution (a) that has not been visualised in ultraviolet light at 254 nm is not more intense than the spot in the chromatogram obtained with reference solution (c).

**Heavy metals** (2.3.13). 12 ml of a 10 per cent w/v solution in *carbon dioxide-free water* complies with the limit test for heavy metals, Method D ( 20 ppm). Use *lead standard solution (2 ppm Pb)* to prepare the standard.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). 4.5 to 5.5 per cent, determined on 0.5 g.

**Assay**. Weigh accurately about 0.25 g, dissolve in a mixture of 50 ml of *ethanol (95 per cent)* and 5.0 ml of *0.01 M hydrochloric acid*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25). Note the volume added between the two inflections.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.03363 g of $C_{14}H_{22}ClN_3O_2$,HCl.

**Storage**. Store protected from light and moisture.

# Metoclopramide Injection

Metoclopramide Hydrochloride Injection

Metoclopramide Injection is a sterile solution of Metoclopramide Hydrochloride in Water for Injections free from dissolved air. It contains suitable buffering and stabilising agents.

Metoclopramide Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of anhydrous metoclopramide hydrochloride, $C_{14}H_{22}ClN_3O_2$,HCl.

**Usual strength**. The equivalent of 10 mg of anhydrous metoclopramide hydrochloride in 2 ml.

**Description**. A clear, colourless solution.

## Identification

A. Dilute a volume containing 10 mg of anhydrous metoclopramide hydrochloride to 500 ml with *0.01 M hydrochloric acid*.

When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows absorption maxima at about 273 nm and 309 nm.

B. To a volume containing 50 mg of anhydrous metoclopramide hydrochloride add 5 ml of *water* and 5 ml of a 1 per cent w/v solution of *4-dimethylaminobenzaldehyde* in *1 M hydrochloric acid*; a yellow-orange colour is produced.

C. Gives reaction A of chlorides (2.3.1).

## Tests

**pH** (2.4.24). 3.0 to 5.0.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254*.

*Mobile phase*. A mixture of 90 volumes of *dichloromethane*, 14 volumes of *methanol*, 10 volumes of *dioxan* and 2 volumes of *strong ammonia solution*.

*Test solution*. Dilute a volume of the injection with *methanol*, if necessary, to contain the equivalent of 0.5 per cent w/v of anhydrous metoclopramide hydrochloride.

*Reference solution (a)*. Dilute 1 volume of the test solution to 200 volumes with *methanol*.

*Reference solution (b)*. A 0.0025 per cent w/v solution of *N,N-diethylethylenediamine* in *methanol*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a). Spray the plate with *dimethylaminobenzaldehyde reagent* and allow it to dry in air. Any secondary spot in the chromatogram obtained with the test solution that has not been visualised in ultraviolet light at 254 nm is not more intense than the spot in the chromatogram obtained with reference solution (b).

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. Dilute an accurately measured volume containing about 10 mg of anhydrous metoclopramide hydrochloride to 100.0 ml with *water*. To 20.0 ml of this solution add 15 ml of *1.25 M sodium hydroxide* and extract with three quantities, each of 30 ml, of *chloroform*, dry each extract with *anhydrous sodium sulphate* and filter. Dilute the combined extracts to 100.0 ml with *chloroform* and mix. Measure the absorbance of the resulting solution at the maximum at about 305 nm (2.4.7). Calculate the content of $C_{14}H_{22}ClN_3O_2$,HCl, taking 265 as the specific absorbance at 305 nm.

**Storage**. Store protected from light.



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**Labelling.** The label states the strength in terms of the equivalent amount of anhydrous metoclopramide hydrochloride in a suitable dose-volume.

## Metoclopramide Syrup

Metoclopramide Hydrochloride Syrup

Metoclopramide Syrup contains Metoclopramide Hydrochloride in a suitable flavoured vehicle.

Metoclopramide Syrup contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of anhydrous metoclopramide hydrochloride, $C_{14}H_{22}ClN_3O_2$,HCl.

**Usual strength.** The equivalent of 5 mg of anhydrous metoclopramide hydrochloride in 5 ml.

### Identification

To 50 ml add *5 M sodium hydroxide* till the solution becomes alkaline and extract with three quantities, each of 40 ml, of *chloroform*, dry each extract with *anhydrous sodium sulphate*. Evaporate the combined extracts to dryness on a water-bath. The residue complies with the following tests.

A. Dissolve 10 mg of the residue in *0.01 M hydrochloric acid* and dilute to 500 ml with *0.01 M hydrochloric acid*.

When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows absorption maxima at about 273 nm and 309 nm.

B. To 25 mg of the residue add 2.5 ml of *water* and 2.5 ml of a 1 per cent w/v solution of *4-dimethylaminobenzaldehyde* in *1 M hydrochloric acid*; a yellow-orange colour is produced.

C. Dissolve about 2 mg in 2 ml of *water*. The solution gives the reaction of primary aromatic amines (2.3.1).

### Tests

**pH** (2.4.24). 2.0 to 4.0.

**Other tests.** Complies with the tests stated under Oral Liquids.

**Assay.** Dilute an accurately measured volume containing about 10 mg of anhydrous metoclopramide hydrochloride to 100.0 ml with *water*. To 20.0 ml of this solution add 15 ml of *1.25 M sodium hydroxide* and extract with three quantities, each of 30 ml, of *chloroform*, dry each extract with *anhydrous sodium sulphate* and filter. Dilute the combined extracts to 100.0 ml with *chloroform* and mix. Measure the absorbance of the resulting solution at the maximum at about 305 nm (2.4.7). Calculate the content of $C_{14}H_{22}ClN_3O_2$,HCl, taking 265 as the specific absorbance at 305 nm.

**Storage.** Store protected from light and moisture.

**Labelling.** The label states the strength in terms of the equivalent amount of anhydrous metoclopramide hydrochloride in a suitable dose-volume.

## Metoclopramide Tablets

Metoclopramide Hydrochloride Tablets

Metoclopramide Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of anhydrous metoclopramide hydrochloride, $C_{14}H_{22}ClN_3O_2$,HCl.

**Usual strength.** The equivalent of 10 mg of anhydrous metoclopramide hydrochloride.

### Identification

A. Shake a quantity of the powdered tablets containing 10 mg of anhydrous metoclopramide hydrochloride with 50 ml of *0.01 M hydrochloric acid* and heat at 70° for 15 minutes with frequent shaking. Cool, dilute to 100 ml with *0.01 M hydrochloric acid*, filter and dilute 10 ml of the filtrate to 50 ml with the same solvent.

When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows absorption maxima at about 273 nm and 309 nm.

B. Shake a quantity of the powdered tablets containing 50 mg of anhydrous metoclopramide hydrochloride with 5 ml of *water*, filter and add to the filtrate 5 ml of a 1 per cent w/v solution of *4-dimethylaminobenzaldehyde* in *1 M hydrochloric acid*; a yellow-orange colour is produced.

### Tests

**Related substances.** Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254*.

*Mobile phase.* A mixture of 90 volumes of *dichloromethane*, 14 volumes of *methanol*, 10 volumes of *dioxan* and 2 volumes of *strong ammonia solution*.

*Test solution.* Shake a quantity of the powdered tablets containing 0.1 g of anhydrous metoclopramide hydrochloride with 20 ml of *methanol* for 5 minutes and filter.

*Reference solution (a).* Dilute 1 volume of the test solution to 200 volumes with *methanol*.

*Reference solution (b).* A 0.0025 per cent w/v solution of *N,N-diethylethylenediamine* in *methanol*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution



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(a). Spray the plate with *dimethylaminobenzaldehyde reagent* and allow it to dry in air. Any secondary spot in the chromatogram obtained with the test solution that has not been visualised in ultraviolet light at 254 nm is not more intense than the spot in the chromatogram obtained with reference solution (b).

**Uniformity of content**. Comply with the test stated under Tablets.

Powder one tablet and carry out the Assay beginning at the words "add 50 ml of *0.1 M hydrochloric acid*,....".

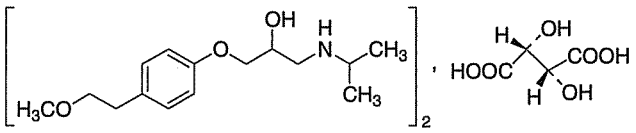
**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 10 mg of anhydrous metoclopramide hydrochloride, add 50 ml of *0.1 M hydrochloric acid*, heat on a water-bath at 70° for 15 minutes, cool, dilute to 100.0 ml with *water* and filter. To 20.0 ml of this solution add 15 ml of *1.25 M sodium hydroxide* and extract with three quantities, each of 30 ml, of *chloroform*, dry each extract with *anhydrous sodium sulphate* and filter. Dilute the combined extracts to 100.0 ml with *chloroform* and mix. Measure the absorbance of the resulting solution at the maximum at about 305 nm (2.4.7). Calculate the content of $C_{14}H_{22}ClN_3O_2,HCl$, taking 265 as the specific absorbance at 305 nm.

**Storage**. Store protected from light and moisture.

**Labelling**. The label states the strength in terms of the equivalent amount of anhydrous metoclopramide hydrochloride.

# Metoprolol Tartrate




$(C_{15}H_{25}NO_3)_2,C_4H_6O_6$ Mol. Wt. 684.8

Metoprolol Tartrate is (*RS*)-1-isopropylamino-3-*p*-(2-methoxyethyl)phenoxypropan-2-ol tartrate.

Metoprolol Tartrate contains not less than 99.0 per cent and not more than 101.0 per cent of $(C_{15}H_{25}NO_3)_2,C_4H_6O_6$, calculated on the dried basis.

**Category**. Beta-adrenoceptor antagonist.

**Dose**. 100 to 450 mg daily, in divided doses; the initial dose should not exceed 100 mg daily.

**Description**. A white, crystalline powder or colourless crystals.

## Identification

A. To 25 ml of a 0.4 per cent w/v solution add 2 ml of *5 M ammonia*, extract with 20 ml of *dichloromethane*, filter the lower layer through *anhydrous sodium sulphate* and evaporate to dryness. Place in a freezer for a few minutes to congeal the residue and allow to warm to room temperature.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *metoprolol tartrate RS* treated in the same manner or with the reference spectrum of metoprolol.

B. A 5 per cent w/v solution gives reaction B of tartrates (2.3.1).

## Tests

**Appearance of solution**. A 2.0 per cent w/v solution is clear (2.4.1), and not more intensely coloured than reference solution BS8 (2.4.1).

**pH** (2.4.24). 6.0 to 7.0, determined in a 2.0 per cent w/v solution.

**Specific optical rotation** (2.4.22). +7.0° to +10.0°, determined at 20° in a 2.0 per cent w/v solution.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase. Chloroform*. Pour 200 ml of *chloroform* in the developing chamber containing several beakers, each containing 45 ml of *strong ammonia solution* and saturate for 1½ hours by lining the walls with absorbent paper.

*Test solution*. Dissolve 0.2 g of the substance under examination in 10 ml of *chloroform*.

*Reference solution (a)*. A 0.01 per cent w/v solution of the substance under examination in *chloroform*.

*Reference solution (b)*. A 0.004 per cent w/v solution of the substance under examination in *chloroform*.

Apply to the plate 20 µl of each solution. After development, dry the plate in air. Place the plate in a chamber of chlorine gas prepared by adding 5 ml of *5 M hydrochloric acid* to a beaker containing 0.5 g of *potassium permanganate*, and let the plate remain in the chamber for about a minute. Remove the plate from the chamber, allow to stand for a few minutes and spray it with a 0.5 per cent w/v solution of *potassium iodide* in *starch solution*. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals, Method A (10 ppm).



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**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.3 g, dissolve in 30 ml *glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03424 g of $(C_{15}H_{25}NO_3)_2,C_4H_6O_6$.

**Storage**. Store protected from light.

# Metoprolol Tablets

Metoprolol Tartrate Tablets

Metoprolol Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of metoprolol tartrate, $(C_{15}H_{25}NO_3)_2, C_4H_6O_6$.

**Usual strengths**. 50 mg; 100 mg.

## Identification

Transfer a quantity of the powdered tablets containing about 40 mg of Metoprolol Tartrate to a separator, add 25 ml of *water* and 4 ml of *5 M ammonia*, extract with 20 ml of *dichloromethane*, filter the lower layer through *anhydrous sodium sulphate* and evaporate to dryness. Place in a freezer for a few minutes to congeal the residue and allow to warm to room temperature.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *metoprolol tartrate RS* treated in the same manner or with the reference spectrum of metoprolol.

## Tests

**Dissolution** (2.5.2).

Apparatus No. 2,
Medium. 900 ml of *simulated gastric fluid (without enzyme)*,
Speed and time. 100 rpm and 30 minutes.

Withdraw a suitable volume of the solution and filter. Dilute appropriately with the dissolution medium if necessary. Measure the absorbance (2.4.7) of the solution at the maximum at about 275 nm.

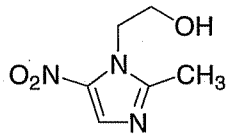
Calculate the content of $(C_{15}H_{25}NO_3)_2$, $C_4H_6O_6$ in the medium from the absorbance obtained from a solution of known concentration of *metoprolol tartrate RS*.

D. Not less than 80 per cent of the stated amount of $(C_{15}H_{25}NO_3)_2, C_4H_6O_6$.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.12 g of Metoprolol Tartrate, transfer to a 100-ml volumetric flask, add about 75 ml of *ethanol (95 per cent)* and shake for 15 minutes. Dilute to volume with *ethanol (95 per cent)*, mix and filter. Dilute 5.0 ml of the filtrate to 50.0 ml with *ethanol (95 per cent)*. Measure the absorbance of the resulting solution at the maximum at about 275 nm (2.4.7). Calculate the content of $(C_{15}H_{25}NO_3)_2$, $C_4H_6O_6$ from the absorbance obtained by repeating the operation using *metoprolol tartrate RS* in place of the substance under examination.

# Metronidazole




$C_6H_9N_3O_3$ Mol. Wt. 171.2

Metronidazole is 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl) ethanol.

Metronidazole contains not less than 99.0 per cent and not more than 101.0 per cent of $C_6H_9N_3O_3$, calculated on the dried basis.

**Category**. Antiamoebic.

**Dose**. For trichomoniasis, 200 mg thrice daily, for 7 days. For amoebiasis, 400 mg thrice daily, for 5 to 10 days. For giardiasis, 2 g daily for 3 successive days for adults, 1 g for children and 400 mg daily for infants. Initial dose, by intravenous infusion 15 mg per kg of body weight; subsequent doses, 7.5 mg per kg of body weight upto a maximum of 1 g every six hours, for 7 days or longer.

**Description**. A white or yellowish, crystalline powder.

## Identification

*Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *metronidazole RS* or with the reference spectrum of metronidazole.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *0.1 M hydrochloric acid* shows an absorption maximum at about 277 nm and a minimum at about 240 nm; absorbance at about 277 nm, between 0.365 and 0.395.



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C. Heat about 10 mg in a water-bath with 10 mg of *zinc powder*, 1 ml of *water* and 0.25 ml of *2 M hydrochloric acid* for 5 minutes and cool. The solution gives the reaction of primary aromatic amines (2.3.1).

### Tests

**Appearance of solution**. A 5.0 per cent w/v solution in *1 M hydrochloric acid* is not more opalescent than opalescence standard OS2 (2.4.1), and not more intensely coloured than reference solution GYS4 (2.4.1).

**Related substances.** Determine by liquid chromatography (2.4.14).

*NOTE—Prepare the solutions protected from light.*

*Test solution*. Dissolve 50 mg of the substance under examination in 100 ml of the mobile phase.

*Reference solution (a).* Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

*Reference solution (b).* Dissolve 5 mg of *2-methyl-4-nitromidazole RS (metronidazole impurity A RS)* in the mobile phase, add 10.0 ml of the test solution and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture 30 volumes of *methanol* and 70 volumes of a 0.14 per cent w/v solution of *potassium dihydrogen phosphate,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 315 nm,
- injection volume. 10 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peak due to metronidazole and metronidazole impurity A is not less than 2.0.

Inject the test solution and reference solution (a). In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent), the sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.01 per cent).

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

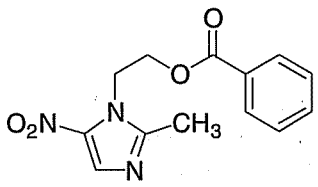
**Assay**. Weigh accurately about 0.15 g, dissolve in 50 ml of *anhydrous glacial acetic acid.* Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01712 g of $C_6H_9N_3O_3$.

**Storage**. Store protected from light and moisture.

## Metronidazole Benzoate

Benzoylmetronidazole



$C_{13}H_{13}N_3O_4$ Mol. Wt. 275.3

Metronidazole Benzoate is 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethyl benzoate.

Metronidazole Benzoate contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{13}H_{13}N_3O_4$, calculated on the dried basis.

**Category**. Antiamoebic; antiprotozoal; antibacterial.

**Dose**. For amoebiasis, the equivalent of 400 mg of metronidazole thrice daily, for 5 to 10 days.

(200 mg of metronidazole benzoate is approximately equivalent to 125 mg of metronidazole).

**Description**. A white or cream-coloured, crystalline powder or flakes.

### Indentification

A. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *ethanol* shows an absorption maximum only at about 309 nm; absorbance at about 309 nm, about 0.3.

B. Gives reaction B of benzoates (2.3.1).

C. Melting range 98° to 102° (2.4.21).

### Tests

**pH** (2.4.24). 5.0 to 7.0, determined in a 2.0 per cent w/v suspension.



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**Free benzoic acid**. Not more than 0.2 per cent, determined by the following method. Dissolve 0.5 g in 25 ml of *ethanol (95 per cent)* and titrate with *0.01 M sodium hydroxide* using *phenol red solution* as indicator. Carry out a blank titration.

1 ml of *0.01 M sodium hydroxide* is equivalent to 0.001221 g of $C_7H_6O_2$.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254*.

*Mobile phase*. A mixture of 80 volumes of *chloroform* and 20 volumes of *acetone*.

*Test solution*. Dissolve 0.6 g of the substance under examination in 10 ml of equal volumes of *chloroform* and *methanol*.

*Reference solution*. A 0.02 per cent w/v solution of the substance under examination in a mixture of equal volumes of *chloroform* and *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine it in ultraviolet light at 254 nm. Any secondary spots in the chromatogram obtained with the test solution are not more intense than the spot in the chromatogram obtained with the reference solution.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying at in an oven at 60° at a pressure not exceeding 0.7 kPa.

**Assay**. Weigh accurately about 0.25 g and dissolve in 50 ml of *acetone*. Add 10 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02753 g of $C_{13}H_{13}N_3O_4$.

**Storage**. Store protected from light and moisture.

# Metronidazole Benzoate Oral Suspension

Benzoylmetronidazole Oral Suspension

Metronidazole Benzoate Oral Suspension is a suspension of Metronidazole Benzoate in a suitable aqueous vehicle. It may contain suitable colouring, flavouring, sweetening, buffering, suspending and antimicrobial agents.

Metronidazole Benzoate Oral Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of metronidazole, $C_6H_9N_3O_3$.

**Usual strengths**. 4 mg per ml; 5 mg per ml.

## Identification

Extract a quantity of the suspension containing 0.5 g of metronidazole with *chloroform*, filter and evaporate the filtrate to dryness. The residue complies with the following tests.

A. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *ethanol (95 per cent)* shows an absorption maximum only at about 309 nm; absorbance at about 309 nm, about 0.3.

B. Gives reaction B of benzoates (2.3.1).

## Tests

**Other tests**. Complies with the tests stated under Oral Liquids.

**Assay**. Weigh accurately a quantity containing about 200 mg of metronidazole, add 10 ml of *water* and extract with four quantities, each of 25 ml, of *chloroform*. Combine the chloroform extracts and wash with two quantities, each of 5 ml, of *water*. Wash the aqueous solution with 5 ml of *chloroform* and evaporate the combined extracts and washing to dryness on a water-bath. Add two successive quantities, each of 25 ml, of *acetone* and 10 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01712 g of $C_6H_9N_3O_3$.

Determine the weight per ml of the suspension (2.4.29), and calculate the content of metronidazole, weight in volume.

**Storage**. Store protected from light and moisture.

**Labelling**. The label states the strength in terms of the equivalent amount of metronidazole in a suitable dose-volume.

# Metronidazole Injection

Metronidazole Intravenous Infusion

Metronidazole Injection is a sterile isotonic solution of Metronidazole in Water for Injections. It may contain suitable buffering agents.

Metronidazole Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of metronidazole, $C_6H_9N_3O_3$.

**Usual strength**. 5 mg per ml.

**Description**. An almost colourless to pale yellow solution.

## Identification

A. Shake a volume of the injection containing about 0.1 g of Metronidazole with 9 g of *sodium chloride* for 5 minutes. Add 20 ml of *acetone*, shake for further 5 minutes and allow to separate. Evaporate the upper layer to dryness. On the residue,



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determine by infrared absorption spectrophotometery (2.4.6). Compare the spectrum with that obtained with *metronidazole RS* or with the reference spectrum of metronidazole.

B. Heat 2 ml of the injection in a water-bath for 5 minutes with 10 mg of *zinc powder* and 0.25 ml of *2 M hydrochloric acid* for 5 minutes and cool in ice. The solution gives the reaction of primary aromatic amines (2.3.1).

### Tests

**pH** (2.4.24). 4.5 to 7.0.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dilute a volume of injection containing about 100 mg of metronidazole in 100 ml with the mobile phase.

*Reference solution (a)*. A 0.0005 per cent w/v solution of *2-methyl-5-nitroimidazole RS* in the mobile phase.

*Reference solution (b)*. A 0.0005 per cent w/v solution of *2-methyl-5-nitroimidazole RS* in the test solution.

Chromatographic system

- a stainless steel column 20 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (10 µm) (Such as Spherisorb ODS1),
- mobile phase: a mixture of 30 volumes of *methanol* and 70 volumes of a *0.1 M potassium dihydrogen orthophosphate* prepared by dissolving 1.4 g of *potassium dihydrogen orthophosphate* with 1000 ml of *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 315 nm,
- injection volume. 20 µl.

Inject reference solution (b). Adjust the sensitivity so that the height of the peak due to *2-methyl-5-nitroimidazole* is about 50 per cent of full scale deflection. Measure the height (a) of the peak due to *2-methyl-5-nitroimidazole* and the height (b) of the lowest part of the curve separating this peak from the principal peak. The test is not valid unless a is greater than 10b.

Inject the test solution and reference solution (a). Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the peak due to *2-methyl-5-nitroimidazole* in the chromatogram obtained with reference solution (a) (0.5 per cent).

**Bacterial endotoxins** (2.2.3). Not more than 0.35 Endotoxin Unit per mg of metronidazole.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Intravenous Infusions).

**Assay**. Dilute a suitable volume with sufficient *0.1 M hydrochloric acid* to produce a solution containing 0.001 per cent w/v of Metronidazole. Measure the absorbance of the resulting solution at the maximum at about 277 nm (2.4.7), using as the blank a solution prepared in the same manner omitting the substance under examination.

Calculate the content of $C_6H_9N_3O_3$ from the absorbance obtained by repeating the operation using *metronidazole RS* in place of the substance under examination.

**Storage**. Store protected from light, in single dose containers.

**Labelling**. The label states that the contents should not be used if they contain any visible solid particles.

## Metronidazole Sterile Suspension

Metronidazole Sterile Suspension is a sterile suspension of Metronidazole in Water for Injections. The suspension is prepared by suspending Metronidazole for Injection in the requisite amount of Water for Injections immediately before use.

*The sterile suspension complies with the tests stated under Parenteral Preparations.*

**Storage**. The suspension should be used immediately after preparation but in any case within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

### Metronidazole for Injection

Metronidazole for Injection is a sterile material consisting of Metronidazole with or without excipients. It is filled in a sealed container.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Metronidazole Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of metronidazole, $C_6H_9N_3O_3$.

### Identification

Shake a quantity of the powder containing 0.1 g of Metronidazole with 40 ml of *chloroform* for 15 minutes, filter and evaporate the filtrate to dryness. The residue complies with the following test. Determine by infrared spectrophotometry (2.4.6). Compare the spectrum with that obtained with *metronidazole RS* or with the reference spectrum of metronidazole.

### Tests

**Related substances**. Determine by liquid chromatography (2.4.14).



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*Test solution.* Determine the weight of the contents of 10 containers. Transfer an accurately weighed quantity of the mixed contents of 10 containers containing about 0.1 g of Metronidazole with 40 ml of *chloroform* for 15 minutes, filter, evaporate the filtrate to dryness and dissolve the residue in 100 ml of mobile phase.

*Reference solution (a).* A 0.0005 per cent w/v solution of *2-methyl-5-nitroimidazole RS* in the mobile phase.

*Reference solution (b).* A 0.0005 per cent w/v solution of *2-methyl-5-nitroimidazole RS* in the test solution.

Chromatographic system
- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (10 µm) ( Such as Spherisorb ODS 1),
- mobile phase: a mixture of 30 volumes of *methanol* and 70 volumes of *0.01M potassium dihydrogen orthophosphate,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 315 nm,
- injection volume. 20 µl.

Inject reference solution (b). Adjust the sensitivity so that the height of the peak due to 2-methyl-5-nitroimidazole is about 50 per cent of full-scale deflection. Measure the height (a) of the peak due to 2-methyl-5-nitroimidazole and the height (b) of the lowest part of the curve separating this peak from the principal peak. The test is not valid unless *a* is more than *10b*.

Inject reference solution (a) and the test solution. Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the peak due to 2-methyl-5-nitroimidazole in the chromatogram obtained with reference solution (a) (0.5 per cent).

**Assay**. Determine the weight of the contents of 10 containers. Transfer an accurately weighed quantity of the mixed contents of the 10 containers containing about 50 mg of Metronidazole, dissolve in 100 ml of *0.1 M hydrochloric acid*. Dilute 10 ml of the solution to 250 ml with *0.1 M hydrochloric acid* and measure the absorbance of the resulting solution at the maximum at about 277 nm (2.4.7). Calculate the content of $C_6H_9N_3O_3$ taking 375 as the specific absorbance at 277 nm.

**Storage**. Store protected from light.

# Metronidazole Tablets

Metronidazole Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of metronidazole, $C_6H_9N_3O_3$.

**Usual strengths**. 200 mg; 400 mg.

## Identification

A. Shake a quantity of the powdered tablets containing 0.1 g of Metronidazole with 40 ml of *chloroform* for 15 minutes, filter and evaporate the filtrate to dryness.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *metronidazole RS* or with the reference spectrum of metronidazole.

B. Heat a quantity of the powdered tablets containing 10 mg of Metronidazole in a water-bath with 10 mg of *zinc powder*, 1 ml of *water* and 0.25 ml of *2 M hydrochloric acid* for 5 minutes and cool in ice. The solution gives the reaction of primary aromatic amines (2.3.1).

C. Shake a quantity of the powdered tablets containing about 0.2 g of Metronidazole with 4 ml of *0.5 M sulphuric acid* and filter. To the filtrate add 10 ml of *picric acid solution* and allow to stand for 1 hour; the precipitate, after washing with cold *water* under suction and drying at 105°, melts at about 150° (2.4.21).

## Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 100 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc with an average pore diameter not greater than 1.0 µm. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate with the same solvent. Measure the absorbance of the resulting solution at the maximum at about 277 nm (2.4.7). Calculate the content of $C_6H_9N_3O_3$ from the absorbance obtained by repeating the operation using *metronidazole RS* instead of the substance under examination.

D. Not less than 85 per cent of the stated amount of $C_6H_9N_3O_3$.

**Related substances.** Determine by liquid chromatography (2.4.14).

*Test solution.* Shake a quantity of powdered tablets containing about 100 mg of Metronidazole in 100 ml of the mobile phase,

*Reference solution (a).* A 0.0005 per cent w/v solution of *2-methyl-5-nitroimidazole RS* in the mobile phase.

*Reference solution (b).* A 0.0005 per cent w/v solution of *2-methyl-5-nitroimidazole RS* in the test solution.

Chromatographic system
- a stainless steel column 20 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (10 µm) (Such as Spherisorb ODS1),



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- mobile phase: a mixture of 30 volumes of *methanol* and 70 volumes of a *0.1 M potassium dihydrogen orthophosphate* prepared by dissolving 1.4 g of *potassium dihydrogen orthophosphate* with 1000 ml of *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 315 nm,
- injection volume. 20 µl.

Inject reference solution (b). Adjust the sensitivity so that the height of the peak due to *2-methyl-5-nitroimidazole* is about 50 per cent of full scale deflection. Measure the height (a) of the peak due to *2-methyl-5-nitroimidazole* and the height (b) of the lowest part of the curve separating this peak from the principal peak. The test is not valid unless a is greater than 10b.

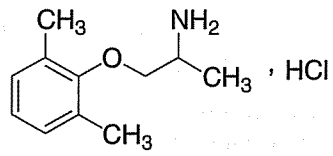
Inject the test solution and reference solution (a). Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the peak due to *2-methyl-5-nitroimidazole* in the chromatogram obtained with reference solution (a).

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.2 g of Metronidazole, transfer to a sintered-glass crucible and extract with six quantities, each of 10 ml, of hot *acetone*. Cool, add to the combined extracts 50 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid*, using 0.1 ml of a 1 per cent w/v solution of *brilliant green* in *anhydrous glacial acetic* acid as indicator to a yellowish-green end-point. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01712 g of $C_6H_9N_3O_3$.

# Mexiletine Hydrochloride




$C_{11}H_{17}NO,HCl$ Mol. Wt. 215.7

Mexiltine Hydrochloride is (*RS*)-1-methyl-2-(2,6-xylyloxy) ethylamine hydrochloride.

Mexiletine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{11}H_{17}NO,HCl$, calculated on the anhydrous basis.

**Category**. Antiarrhythmic.

**Dose**. Orally, initial loading dose 400 mg, followed by 200 to 250 mg three to four times daily; by slow intravenous injection, 100 to 200 mg in 4 to 8 minutes, followed by infusion at a rate of 250 mg over 1 hour, 250 mg over the next 2 hours, and then about 0.5 mg per minute for maintenance, according to the patient's response.

**Description**. A white or almost white, crystalline powder.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mexiletine hydrochloride RS*.

B. When examined in the range 220 nm to 360 nm (2.4.7), a 0.04 per cent w/v solution in *0.01 M hydrochloric* acid shows an absorption maximum at about 260 nm; absorbance at 260 nm, about 0.46.

C. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

D. Dissolve 0.1 g in 3 ml of *0.02 M hydrochloric acid* and add a few crystals of *sodium nitrite*. Nitrogen is evolved and a yellow colour may be produced slowly.

E. Gives reaction A of chlorides (2.3.1).

## Tests

**Appearance of solution**. A 5.0 per cent w/v solution is clear (2.4.1), and colourless (2.4.1).

**pH** (2.4.24). 4.0 to 5.5, determined in a 10.0 per cent w/v solution.

**2,6-Dimethylphenol**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 85 volumes of *chloroform*, 14 Volumes of *methanol* and 1 volume of *strong ammonia solution*.

*Test solution*. Dissolve 0.5 g of the substance under examination in 10 ml of *methanol*.

*Reference solution*. A 0.01 per cent w/v solution of *2, 6-dimethylphenol* in *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with a 0.1 per cent w/v solution of *fast blue BB salt* in *methanol*, dry at about 90° and then spray with *3 M methanolic potassium hydroxide*. Any spot corresponding to *2,6-dimethylphenol* in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.



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*Mobile phase*. A mixture of 85 volumes of *chloroform*, 14 volumes of *methanol* and 1 volume of *strong ammonia solution*.

*Test solution (a)*. Dissolve 0.5 g of the substance under examination in 10 ml of *methanol*.

*Test solution (b)*. Dissolve 0.2 g of the substance under examination in 100 ml of *methanol*.

*Reference solution (a)*. A 0.025 per cent w/v solution of the substance under examination in *methanol*.

*Reference solution (b)*. A 0.2 per cent w/v solution of *mexiletine hydrochloride RS* in *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with *ninhydrin solution* and heat at 110° for 15 minutes. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a).

**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals, Method C (10 ppm). Use 2 ml of *lead standard solution (10 ppm Pb)* to prepare the standard.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.4.19). Not more than 0.5 per cent, determined on 5 g.

**Assay**. Weigh accurately about 0.15 g, dissolve in 50 ml of a mixture of equal volumes of *anhydrous glacial acetic acid* and *acetic anhydride*, add 10 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02157 g of $C_{11}H_{17}NO,HCl$.

**Storage**. Store protected from light and moisture.

# Mexiletine Capsules

Mexiletine Hydrochloride Capsules

Mexiletine Capsules contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of mexiletine hydrochloride $C_{11}H_{17}NO,HCl$.

**Usual strengths**. 50 mg; 100 mg.

## Identification

A. Shake a quantity of the contents of the capsules containing about 0.5 g of Mexiletine Hydrochloride with 10 ml of *methanol*, filter, evaporate to dryness and dry the residue at 105°.

When examined in the range 230 nm to 360 nm (2.4.7), a 0.04 per cent w/v solution of the residue in *0.01 M hydrochloric acid* shows an absorption maximum at 260 nm.

B. Dissolve 0.1 g of the residue obtained in test A in 3 ml of *0.02 M hydrochloric acid* and add a few crystals of *sodium nitrite*; nitrogen is evolved and a yellow colour may be produced slowly.

C. In the test of Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

D. A 1 per cent w/v solution of the residue obtained in test A gives the reactions of chlorides (2.3.1).

## Tests

**2,6-Dimethylphenol**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 85 volumes of *chloroform*, 14 volumes of *methanol* and 1 volume of *strong ammonia solution*.

*Test solution*. A 5 per cent w/v solution of the residue obtained in Identification test A in *methanol*.

*Reference solution*. A 0.01 per cent w/v solution of *2, 6-dimethylphenol* in *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with a 0.1 per cent w/v solution of *fast blue BB salt* in *methanol*, dry at about 90° and then spray with *3 M methanolic potassium hydroxide*. Any spot corresponding to *2,6-dimethylphenol* in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 85 volumes of *chloroform*, 14 volumes of *methanol* and 1 volume of *strong ammonia solution*.

*Test solution (a)*. A 5 per cent w/v solution of the residue obtained in Identification test A in *methanol*.

*Test solution (b)*. Dissolve 20 mg of the residue obtained in Identification test A in 10 ml of *methanol*.

*Reference solution (a)*. A 0.025 per cent w/v solution of the residue obtained in Identification test A in *methanol*.

*Reference solution (b)*. A 0.2 per cent w/v solution of *mexiletine hydrochloride RS* in *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with *ninhydrin solution* and heat at 110° for 15 minutes. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a).



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**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *water,*
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter with an average pore size of 1.0 µm. Measure the fluorescence intensities using the maximum excitation wavelength at about 265 nm and the maximum emission wavelength at about 295 nm (2.4.5). Calculate the content of $C_{11}H_{17}NO$, HCl by comparing the fluorescence intensities obtained with a standard solution of a known concentration of *mexiletine hydrochloride RS* in *water.*

D. Not less than 80 per cent of the stated amount of $C_{11}H_{17}NO$, HCl.

**Assay**. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 0.05 g of Mexiletine Hydrochloride, mix with 50 ml of *0.01 M hydrochloride acid,* shake for 30 minutes, dilute to 100.0 ml with *0.01 M hydrochloric acid* and centrifuge. Measure the absorbance of the supernatant liquid at the maximum at about 260 nm (2.4.7). Calculate the content of $C_{11}H_{17}NO$,HCl taking 11.6 as the specific absorbance at 260 nm.

**Storage**. Store protected from light.

## Mexiletine Injection

Mexiletine Hydrochloride Injection

Mexiletine Injection is a sterile solution of Mexiletine Hydrochloride in Water for Injections.

Mexiletine Injection contains not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of Mexiletine Hydrochloride $C_{11}H_{17}NO$, HCl.

**Usual strength**. 250 mg per 10 ml.

### Identification

A. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (b).

B. A volume containing 2.5 mg of Mexiletine Hydrochloride diluted to 2 ml gives reaction A of chlorides (2.3.1).

### Tests

**pH** (2.4.24). 5.0 to 6.0.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

*Mobile phase*. A mixture of 85 volumes of *chloroform,* 14 volumes of *methanol* and 1 volume of *strong ammonia solution.*

*Test solution*. Dilute a volume of the injection with sufficient mobile phase to produce a solution containing 0.2 per cent w/v of Mexiletine Hydrochloride

*Reference solution (a)*. A 0.025 per cent w/v solution of the injection in *methanol.*

*Reference solution (b)*. A 0.2 per cent w/v solution of *mexiletine hydrochloride RS* in *methanol.*

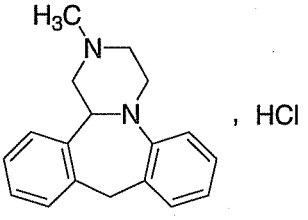
Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with *ninhydrin solution* and heat at 110° for 15 minutes. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a).

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. To an accurately measured volume containing about 0.125 g of Mexiletine Hydrochloride add sufficient *0.01 M hydrochloric acid* to produce 250.0 ml and measure the absorbance of the resulting solution at the maximum at about 260 nm (2.4.7). Calculate the content of $C_{11}H_{17}NO$, HCl taking 11.6 as the specific absorbance at 260 nm.

**Storage**. Store in single dose containers.

## Mianserin Hydrochloride




$C_{18}H_{20}N_2$,HCl Mol. Wt. 300.8

Mianserin Hydrochloride is (*RS*)-1,2,3,4,10, 14b-hexahydro-2-methyldibenzo[*c,f*] pyrazino[1,2-*a*]azepine hydrochloride.

Mianserin Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{18}H_{20}N_2$,HCl, calculated on the dried basis.

**Category**. Antidepressant

**Dose**. 30 to 90 mg daily, in divided doses.

**Description**. A white or almost white crystals or crystalline powder; odourless or almost odourless.



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### Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mianserin hydrochloride RS* or with the reference spectrum of mianserin hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.01 per cent w/v solution shows an absorption maximum only at about 279 nm; absorbance at about 279 nm, 0.64 to 0.72.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 75 volumes of *cyclohexane*, 20 volumes of *ether* and 5 volumes of *diethylamine*.

*Test solution*. Dissolve 0.2 g of the substance under examination in 100 ml of *dichloromethane*.

*Reference solution (a)*. A 0.2 per cent w/v solution of *mianserin hydrochloride RS* in *dichloromethane*.

*Reference solution (b)*. A solution containing 0.2 per cent w/v each of *mianserin hydrochloride RS* and *cyproheptadine hydrochloride RS in dichloromethane*.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in a current of cold air for 5 minutes and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated principal spots.

D. Gives the reactions of chlorides (2.3.1).

### Tests

**pH** (2.4.24). 4.0 to 5.5, determined in a 1.0 per cent w/v solution.

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 90 volumes of *dichloromethane* and 10 volumes of *methanol*.

*Test solution*. Dissolve 0.2 g of the substance under examination in 10 ml of a mixture of 4 volumes of *methanol* and 1 volume of *10 M ammonia*.

*Reference solution (a)*. A 0.01 per cent w/v solution of the substance under examination in a mixture of 4 volumes of *methanol* and 1 volume of *10 M ammonia*.

*Reference solution (b)*. A 0.002 per cent w/v solution of the substance under examination in a mixture of 4 volumes of *methanol* and 1 volume of *10 M ammonia*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in a current of cold air for 5 minutes and expose to iodine vapour for 20 minutes. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 65° over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 5 hours.

**Assay**. Weigh accurately about 0.2 g, dissolve in a mixture of 50 ml of *ethanol (95 per cent)* and 5 ml of *0.01 M hydrochloric acid*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25). Note the volume added between the two points of inflection.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.03008 g of $C_{18}H_{20}N_2,HCl$.

**Storage**. Store protected from light and moisture.

## Mianserin Tablets

Mianserin Hydrochloride Tablets

Mianserin Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of mianserin hydrochloride, $C_{18}H_{20}N_2$, HCl.

**Usual strengths**. 10 mg; 20 mg; 30 mg.

### Identification

A. Shake a quantity of the powdered tablets containing about 20 mg of Mianserin Hydrochloride with 10 ml of *methanol*, filter and evaporate the filtrate to dryness.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mianserin hydrochloride RS* or with the reference spectrum of mianserin hydrochloride.

B. In the Assay, the principal peak in the chromatogram obtained with test solution (b) corresponds to the peak due to mianserin in the chromatogram obtained with the reference solution.

C. The residue obtained in test A gives the reactions of chlorides (2.3.1).

### Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.



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*Mobile phase*. A mixture of 90 volumes of *dichloromethane* and 10 volumes of *methanol*.

*Test solution*. Triturate a quantity of the powdered tablets containing 40 mg of Mianserin Hydrochloride with 2 ml of a mixture of 4 volumes of *methanol* and 1 volume of *strong ammonia solution* and centrifuge.

*Reference solution (a)*. Dilute 1 volume of the test solution to 200 volumes with the same solvent mixture.

*Reference solution (b)*. Dilute 1 volume of the test solution to 500 volumes with the same solvent mixture.

Apply to the plate 5 µl of each solution. After development, dry the plate in a current of cold air for 5 minutes and expose to iodine vapour for 20 minutes. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by gas chromatography (2.4.13).

*Test solution (a)*. Weigh and powder 20 tablets. Shake a quantity of the powdered tablets containing about 60 mg of Mianserin Hydrochloride with 30.0 ml of *0.2 M hydrochloric acid* for 1 hour and filter. To 10.0 ml of the filtrate add 3.0 ml of *1 M sodium hydroxide* and 10.0 ml of *toluene*, mix thoroughly, centrifuge and use the clear upper layer.

*Test solution (b)*. To 10.0 ml of the filtrate obtained in test solution (a), add 3.0 ml of *1 M sodium hydroxide* and 10.0 ml of *toluene* containing 0.2 per cent w/v of *triphenylamine* (internal standard), mix thoroughly, centrifuge and use the clear upper layer.

*Reference solution*. Add 3.0 ml of *1 M sodium hydroxide* and 10.0 ml of *toluene* containing 0.2 per cent w/v of *triphenylamine* (internal standard) to 10.0 ml of a solution containing 0.2 per cent w/v of *mianserin hydrochloride RS* in *0.2 M hydrochloric acid*, mix thoroughly, centrifuge and use the clear upper layer.

Chromatographic system

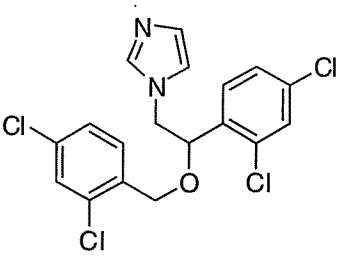
- a glass column 1.0 m × 4 mm, packed with acid-washed, silanised diatomaceous support (80 to 100 mesh) coated with 3 per cent w/w of phenyl methyl silicone fluid (50 per cent phenyl) (Such as OV-17),
- temperature:
  column 255°,
  inlet port and detector at 240°,
- flow rate. 30 ml per minute of the carrier gas.

Inject 1 µl of test solution (a), (b) and the reference solution.

Calculate the content of $C_{18}H_{20}N_2$, HCl in the tablets.

**Storage**. Store protected from light and moisture.

# Miconazole




$C_{18}H_{14}Cl_4N_2O$ Mol. Wt. 416.1

Miconazole is 1-[2-(2,4-dichlorophenyl)-2-[(2,4-dichlorophenyl)methoxy]ethyl-1*H*-imidazole.

Miconazole contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{18}H_{14}Cl_4N_2O$, calculated on the dried basis.

**Category**. Antifungal.

**Description**. A white or almost white powder.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *miconazole RS* or with the reference spectrum of miconazole.

B. Determine by thin-layer chromatography (2.4.17), using *silica gel G*.

*Mobile phase*. A mixture of 20 volumes of *ammonium acetate solution*, 40 volumes of *dioxin* and 40 volumes of *methanol*.

*Test solution*. Dissolve 30 mg of the substance under examination in 5 ml of the mobile phase.

*Reference solution (a)*. Dissolve 30 mg of *miconazole RS* in 5 ml of the mobile phase.

*Reference solution (b)*. Dissolve 30 mg each of *miconazole RS* and *econazole nitrate RS* in 5 ml of the mobile phase.

Apply 5 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in a current of warm air for 15 minutes. Expose to iodine vapour until the spots appear and examine in daylight. The principal spot in the chromatogram obtained with the test solution corresponds to that obtained in the chromatogram with reference solution (a). The test is not valid



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unless the chromatogram obtained with reference solution (b) shows two distinct spots.

C. To about 30 mg in a porcelain crucible add 0.3 g of *anhydrous sodium carbonate*. Heat on flame for 10 minutes, allow to cool. Take up the residue with 5 ml of *dilute nitric acid* and filter. To 1 ml of the filtrate add 1 ml of *water*. The solution gives reaction A of chlorides (2.3.1).

D. Melting range (2.4.21). 83° to 87°.

### Tests

**Appearance of solution**. A 1.0 per cent w/v solution in *methanol* (Solution A) is clear (2.4.1) and not more intensely coloured than the reference solution YS5 (2.4.1).

**Optical rotation** (2.4.22). – 0.10° to + 0.10°, determined in solution A.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 0.1 g of the substance under examination in 10.0 ml of the mobile phase.

*Reference solution (a)*. Dissolve 2.5 mg each of *miconazole RS* and *econazole nitrate RS* in 100 ml of the mobile phase.

*Reference solution (b)*. Dilute 1.0 ml of the test solution to 100 ml with the mobile phase. Dilute 5.0 ml of this solution to 20 ml with the mobile phase.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- mobile phase: dissolve 6.0 g of *ammonium acetate* in a mixture of 30 volumes of *acetonitrile*, 32 volumes of *methanol* and 38 volumes of *water*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume. 10 µl.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to econazole and miconazole is not less than 10.

Inject reference solution (b) and the test solution. Run the chromatogram 1.2 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of secondary peak corresponding to (1*RS*)-1-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-yl)ethanol (miconazole impurity A), 1-[(2*RS*)-2-[(4-chlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole (miconazole impurity B), (2*RS*)-2-[(2,4-dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethanamine (miconazole impurity C), 1-[(2*RS*)-2-[(2,6-dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole (miconazole impurity D), 2-[1-[(2*RS*)-2-[(2,4-dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazol-3-io]-2-methylpropanoate (miconazole impurity E), 1-[(2*RS*)-2-[(3,4-dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole (miconazole impurity F), and 1-[(2*RS*)-2-[(2,5-dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole (miconazole impurity G) is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent). The sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

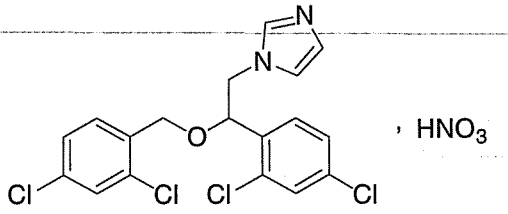
**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in vacuum at 60° for 4 hours.

**Assay**. Weigh accurately about 0.3 g, dissolve in 50 ml of a mixture of 1 volume of *anhydrous acetic acid* and 7 volumes of *methyl ethyl ketone*. Titrate with *0. 1 M perchloric acid* using 0.2 ml of *naphtholbenzein solution* as indicator, until the colour changes from orange-yellow to green. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04161 g of $C_{18}H_{14}Cl_4N_2O$.

**Storage**. Store protected from light.

## Miconazole Nitrate




$C_{18}H_{14}Cl_4N_2O,HNO_3$ Mol. Wt. 479.2

Miconazole Nitrate is (*RS*)-1-[2-(2,4-dichlorophenylmethoxy)-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole nitrate.

Miconazole Nitrate contains not less than 98.5 per cent and not more than 101.5 per cent of $C_{18}H_{14}Cl_4N_2O,HNO_3$, calculated on the dried basis.

**Category**. Antifungal.

**Description**. A white or almost white, crystalline or microcrystalline powder.

### Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.*



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A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *miconazole nitrate RS* or with the reference spectrum of miconazole nitrate.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.04 per cent w/v solution in a mixture of 90 volumes of *2-propanol* and 10 volumes of *0.1 M hydrochloric acid* shows absorption maxima at about 264 nm, 272 nm and 280 nm; ratio of the absorbance at the maximum at about 272 nm to that at the maximum at about 280 nm, 1.18 to 1.22.

C. In the test for Related substances, examine the chromatograms obtained in ultraviolet light at 254 nm before exposure to iodine vapour. The principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

D. Gives the reactions of nitrates (2.3.1).

### Tests

**Appearance of solution**. A 1.0 per cent w/v solution in *methanol* is clear (2.4.1), and not more intensely coloured than reference solution YS7 (2.4.1).

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 60 volumes of *hexane*, 30 volumes of *chloroform*, 10 volumes of *methanol* and 1 volume of *strong ammonia solution*.

*Test solution (a)*. Dissolve 0.5 g of the substance under examination in 10 ml of a mixture of 90 volumes of *methanol* and 10 volumes of *strong ammonia solution*.

*Test solution (b)*. Dissolve 0.5 g of the substance under examination in 100 ml of a mixture of 90 volumes of *methanol* and 10 volumes of *strong ammonia solution*.

*Reference solution (a)*. A 0.5 per cent w/v solution of *miconazole nitrate RS* in a mixture of 90 volumes of *methanol* and 10 volumes of *strong ammonia solution*.

*Reference solution (b)*. A 0.0125 per cent w/v solution of *miconazole nitrate RS* in a mixture of 90 volumes of *methanol* and 10 volumes of *strong ammonia solution*.

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of air for 15 minutes and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b). Expose the plate to iodine vapour until a brown spot appears in the chromatogram obtained with reference solution (b) and examine immediately in daylight. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 2 hours.

**Assay**. Weigh accurately about 0.4 g, dissolve in 50 ml of *anhydrous glacial acetic acid*, with slight heating if necessary. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04791 g of $C_{18}H_{14}Cl_4N_2O,HNO_3$.

**Storage**. Store protected from light and moisture.

## Miconazole Cream

Miconazole Nitrate Cream

Miconazole Cream contains Miconazole Nitrate in a suitable basis.

Miconazole Cream contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of miconazole nitrate, $C_{18}H_{14}Cl_4N_2O,HNO_3$.

**Usual strength**. 2 per cent w/w.

### Identification

A. Mix a quantity containing 40 mg of Miconazole Nitrate with 20 ml of a mixture of 4 volumes of *methanol* and 1 volume of *1 M sulphuric acid* and shake with two quantities, each of 50 ml, of *carbon tetrachloride*, discarding the organic layers. Make the aqueous phase alkaline with *2 M ammonia* and extract with two quantities, each of 40 ml, of *chloroform*. Combine the chloroform extracts, shake with 5 g of *anhydrous sodium sulphate*, filter and dilute the filtrate to 100 ml with *chloroform*. Evaporate 50 ml to dryness and dissolve the residue in 50 ml of a mixture of 90 volumes of *methanol* and 10 volumes of *0.1 M hydrochloric acid*.

When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows absorption maxima at about 264 nm, 272 nm and 280 nm.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to miconazole nitrate in the chromatogram obtained with reference solution (a).

### Tests

**Other tests**. Complies with the tests stated under Creams.

**Assay**. Determine by gas chromatography (2.4.13).

*Test solution*. Weigh accurately a quantity of the cream containing about 40 mg of Miconazole Nitrate, mix with 20 ml



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of a mixture of 4 volumes of *methanol* and 1 volume of *0.5 M sulphuric acid* and shake with two quantities, each of 50 ml, of *carbon tetrachloride*. Wash each organic layer in turn with the same 10-ml quantity of a mixture of 4 volumes of *methanol* and 1 volume of *0.5 M sulphuric acid*. Combine the aqueous phase and the washings, make alkaline with *2 M ammonia* and extract with two quantities, each of 50 ml, of *chloroform*. To the combined extracts add 10.0 ml of a 0.3 per cent w/v solution of *cetyl palmitate* (internal standard) in *chloroform* and 5 g of *anhydrous sodium sulphate*, shake, filter, evaporate the filtrate to a low volume and add sufficient *chloroform* to produce 10.0 ml.

*Reference solution (a).* Weigh accurately about 40 mg of *miconazole nitrate RS* and mix with 10.0 ml of a 0.3 per cent w/v solution of the internal standard in *chloroform* and 0.2 ml of *strong ammonia solution*, add 1 g of *anhydrous sodium sulphate*, shake again and filter.

*Reference solution (b).* Prepare the solution in the same manner as reference solution (a) but omitting the addition of the internal standard solution.

Chromatographic system

- a glass column 1.5 m × 2 mm, packed with acid-washed, silanised diatomaceous support (80 to 100 mesh) coated with 3 per cent w/w of phenyl methyl silicone fluid (50 per cent phenyl) (such as 5 per cent OV-101),
- temperature:
  column 270°,
  inlet port and detector at 270°,
- flow rate. 30 ml per minute of the carrier gas.

Inject 1 µl of the test solution, reference solution (a) and reference solution (b).

Calculate the content of $C_{18}H_{14}Cl_4N_2O,HNO_3$ in the cream.

**Storage**. Store protected from light and moisture at a temperature not exceeding 30°. If it is packed in aluminium tubes, the inner surfaces of the tubes should be coated with a suitable lacquer.

# Miconazole Pessaries

Miconazole Nitrate Pessaries; Miconazole Nitrate Vaginal Tablets; Miconazole Tablets

Miconazole Pessaries contain Miconazole Nitrate in a suitable basis.

Miconazole Pessaries contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of miconazole nitrate, $C_{18}H_{14}Cl_4N_2O, HNO_3$.

**Usual strength**. 150 mg.

## Identification

A. Mix a quantity of the crushed pessaries containing 40 mg of Miconazole Nitrate with 20 ml of a mixture of 4 volumes of *methanol* and 1 volume of *1 M sulphuric acid* and shake with two quantities, each of 50 ml, of *carbon tetrachloride*, discarding the organic layers. Make the aqueous phase alkaline with *2 M ammonia* and extract with two quantities, each of 40 ml, of *chloroform*. Combine the chloroform extracts, shake with 5 g of *anhydrous sodium sulphate*, filter and dilute the filtrate to 100 ml with *chloroform*. Evaporate 50 ml to dryness and dissolve the residue in 50 ml of a mixture of 90 volumes of *methanol* and 10 volumes of *0.1 M hydrochloric acid*.

When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows absorption maxima at about 264 nm, 272 nm and 280 nm.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to miconazole nitrate in the chromatogram obtained with reference solution (a).

## Tests

**Other tests**. Comply with the tests stated under Pessaries.

**Assay**. Determine by gas chromatography (2.4.13).

*Test solution.* Weigh accurately a quantity of the crushed pessaries containing about 40 mg of Miconazole Nitrate, mix with 20 ml of a mixture of 4 volumes of *methanol* and 1 volume of *0.5 M sulphuric acid* and shake with two quantities, each of 50 ml, of *carbon tetrachloride*. Wash each organic layer in turn with the same 10-ml quantity of a mixture of 4 volumes of *methanol* and 1 volume of *0.5 M sulphuric acid*. Combine the aqueous phase and the washings, make alkaline with *2 M ammonia* and extract with two quantities, each of 50 ml, of *chloroform*. To the combined extracts add 10.0 ml of a 0.3 per cent w/v solution of *cetyl palmitate* (internal standard) in *chloroform* and 5 g of *anhydrous sodium sulphate*, shake, filter, evaporate the filtrate to a low volume and add sufficient *chloroform* to produce 10.0 ml.

*Reference solution (a).* Weigh accurately about 40 mg of *miconazole nitrate RS* and mix with 10.0 ml of a 0.3 per cent w/v solution of the internal standard in *chloroform* and 0.2 ml of *strong ammonia solution*, add 1 g of *anhydrous sodium sulphate*, shake again and filter.

*Reference solution (b).* Prepare the solution in the same manner as reference solution (a) but omitting the addition of the internal standard solution.

Chromatographic system

- a glass column 1.5 m × 2 mm, packed with acid-washed, silanised diatomaceous support (80 to 100 mesh) coated with 3 per cent w/w of phenyl methyl silicone fluid (50 per cent phenyl) (Such as 5 per cent OV-101),



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– temperature:
  column 270°,
  inlet port and detector at 300°,
– flow rate. 30 ml per minute of the carrier gas.

Inject 1 µl of the test solution, reference solution (a) and reference solution (b).

Calculate the content of $C_{18}H_{14}Cl_4N_2O$, $HNO_3$ in the pessaries.

**Storage**. Store protected from light and moisture.

## Microcrystalline Cellulose

Microcrystalline Cellulose is purified, partially depolymerised cellulose prepared from alpha cellulose.

Microcrystalline Cellulose contains not less than 97.0 per cent and not more than 102.0 per cent of cellulose, calculated on the dried basis.

**Category**. Pharmaceutical aid (suspending agent; tablet and capsule adjuvant).

**Description**. A fine or granular, white or almost white powder; odourless.

### Identification

A. To about 1 mg add 1 ml of *phosphoric acid*, heat on a water-bath for 30 minutes, add 4 ml of a 0.2 per cent w/v solution of *catechol* in *phosphoric acid* and heat for further 30 minutes; a red colour is produced.

B. To 50 mg add 2 ml of *iodine solution*, allow to stand for 5 minutes and remove the excess reagent with the aid of a filter paper and add 1 or 2 drops of *sulphuric acid (66 per cent v/v)*; a blue-purple colour is produced.

C. Mix 30 g with 270 ml of *water*, mix in a blender at 18,000 rpm for 5 minutes, transfer 100 ml of the mixture to a 100-ml graduated cylinder and allow to stand for 3 hours. A white, opaque, bubble-free dispersion is obtained that does not produce a supernatant liquid.

### Tests

**pH** (2.4.24). 5.0 to 7.5, determined on the supernatant liquid obtained by shaking 2.0 g with 100 ml of *carbon dioxide-free water* for 5 minutes and centrifuging.

**Starch and dextrins**. Mix 0.1 g with 5 ml of *water* by vigorous shaking and add 2 to 3 drops of *iodine solution*; no blue or brownish-red colour is produced.

**Organic impurities**. Place 10 mg on a watch-glass and add 0.05 ml of a freshly prepared solution of 0.1 g of *phloroglucinol* in 5 ml of *hydrochloric acid*; no red colour is produced.

**Water-soluble substances**. Shake 5.0 g with about 80 ml of *water* for 10 minutes, filter through a filter paper (Whatman No 42 or equivalent) into a tared beaker and evaporate the filtrate to dryness and dry the residue at 105° for 1 hour. The residue weighs not more than 10 mg (0.2 per cent).

**Arsenic** (2.3.10). Mix 5.0 g with 3 g of *anhydrous sodium carbonate*, add 10 ml of *bromine solution* and mix thoroughly. Evaporate to dryness on a water-bath, gently ignite and dissolve the cooled residue in a mixture of 15 ml of *hydrochloric acid* containing 0.15 ml of *bromine solution* and 45 ml of *water*. Add 2 ml of *stannous chloride solution AsT*. The resulting solution complies with the limit test for arsenic (2 ppm).

**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Loss on drying** (2.4.19). Not more than 6.0 per cent, determined on 0.5 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.125 g and transfer to a 300-ml conical flask with the aid of about 25 ml of *water*. Add 50.0 ml of *0.083 M potassium dichromate*, mix, carefully add 100 ml of *sulphuric acid* and heat to boiling. Remove from heat, allow to stand at room temperature for 15 minutes, cool and transfer to a 250-ml volumetric flask. Dilute with *water* almost to volume, cool to 25°, dilute with *water* to volume and mix. Titrate 50.0 ml of the resulting solution with *0.1 M ferrous ammonium sulphate* using 2 to 3 drops of *ferroin sulphate solution* as indicator. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of ferrous ammonium sulphate required.

1 ml of *0.1 M ferrous ammonium sulphate* is equivalent to 0.000675 g of cellulose.

**Storage**. Store protected from light and moisture.

## Microcrystalline Cellulose and Carboxymethylcellulose Sodium

Microcrystalline Cellulose and Carboxymethylcellulose Sodium is a colloid-forming, attrited mixture of Microcrystalline Cellulose and Carboxymethylcellulose Sodium.

Microcrystalline Cellulose and Carboxymethylcellulose Sodium contains not less than 75.0 per cent and not more than 125.0 per cent of carboxymethylcellulose sodium, calculated on the dried basis. The viscosity of its aqueous dispersion of per cent by weight stated on the label is not less than 60.0 per cent and not more than 140.0 per cent of that stated on the label in centipoises.



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### Identification

Mix 6 g with 300 ml of *water*, mix in a blender at 18,000 rpm for 5 minutes, transfer 100 ml of the mixture to a 100-ml graduated cylinder and allow to stand for 3 hours. A white, opaque, dispersion is produced which does not settle on standing.

A. Add few drops of the dispersion obtained to a 10 per cent w/v solution of *aluminum chloride*, each drop forms a white, opaque globule which does not disperse on standing.

B. Add 3 ml of *iodine solution* to the dispersion obtained; no blue or purplish blue color is produced

### Tests

**pH** (2.4.24). 6.0 to 8.0, determined in the dispersion prepared in the test for Viscosity.

**Viscosity** (2.4.28). Determine the amounts of Microcrystalline Cellulose and Carboxymethylcellulose Sodium needed to prepare 600 g of a suitable dispersion, calculated on the dried basis. Transfer an accurately weighed amount of *water* to a 1000-ml blender bowl. Begin stirring with an 18,000 rpm blender at a reduced speed obtained by adjusting the voltage to 30 volts by means of a suitable transformer, and immediately add the accurately weighed portion of Microcrystalline Cellulose and Carboxymethylcellulose Sodium, taking care to avoid contacting the sides of the bowl with the powder. Continue stirring at this speed for 15 seconds following the addition of the powder, then increase the transformer setting to 115 volts, and mix for 2 minutes, accurately timed, at 18,000 rpm. Stop the blender, and lower the appropriate spindle of a suitable rotational viscosimeter into the dispersion. Thirty seconds after cessation of mixing, start the viscosimeter, and determine the viscosity using the appropriate spindle to obtain a scale reading between 10 per cent and 90 per cent of full-scale at a speed of 20 rpm. Determine the scale reading after 30 seconds of rotation, and calculate the viscosity, in centipoises, by multiplying the scale reading by the constant for the spindle used at 20 rpm.

**Heavy metals** (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

**Sulphated ash** (2.3.18). Not more than 5.0 per cent.

**Loss on drying** (2.4.19). Not more than 8.0 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 2 g of Microcrystalline Cellulose and Carboxymethylcellulose Sodium and transfer to a 250-ml of a glass-stoppered conical flask, add 75 ml of *glacial acetic acid*, attach a condenser, and reflux for 2 hours, cool, transfer the mixture to a 250-ml beaker with the aid of small volumes of *glacial acetic acid*. Titrate with *0.1 M perchloric acid* in *dioxane solution*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.0296 g of carboxymethylcellulose sodium.

**Storage**. Store protected from light and moisture.

**Labelling**. Label it to indicate the percentage content of carboxymethylcellulose sodium and the viscosity of the dispersion in water of the designated weight percentage composition.

## Microcrystalline Wax

Petroleum Wax (microcrystalline); Amorphous Wax

Microcrystalline Wax is a mixture of straight-chain, branched-chain and cyclic hydrocarbons, obtained by solvent fractionation of the still bottom fraction of petroleum by suitable dewaxing or de-oiling means.

**Category**. Pharmaceutical aid (stiffening and coating agent; ointment base).

**Description**. A white or cream-coloured waxy solid; odourless.

### Tests

**Acidity or alkalinity**. Introduce 35.0 g into a 250-ml separating funnel, add 100 ml of boiling *water* and shake vigorously for 5 minutes. Draw off the separated water into a beaker, wash further with two quantities, each of 50 ml, of boiling *water* and add the washings to the liquid in the beaker. To the pooled washings add 0.05 ml of *phenolphthalein solution* and boil; the solution does not acquire a pink colour. Cool, add 0.1 ml of *methyl orange solution*; no red or pink colour is produced.

**Solidifying point** (2.4.10). 54° to 102°. Follow the method with the following modifications. Place in the inner test-tube sufficient of the melted substance to fill the tube to a depth of about 50 mm. Stir the substance gently and steadily, without scraping the side of the tube, while the tube and its contents are allowed to cool. The temperature at which the level of the mercury in the thermometer remains stationary for a short time is taken as the solidifying point.

**Colour**. Melt about 10.0 g on a water-bath and pour 5 ml of the liquid into a clear-glass (15 cm × 16 mm) bacteriological test-tube; the warm, melted liquid is not more intensely coloured than a solution prepared by mixing 3.8 ml of FCS and 1.2 ml of CCS (2.4.1), in a similar tube, the comparison being made in reflected light against a white background, the tubes being held directly against the background at such an angle that there is no fluorescence.

**Organic acids**. To 20.0 g add 100 ml of a 50 per cent v/v solution of *ethanol (95 per cent)* neutralised to *phenolphthalein solution* and titrate rapidly with *0.1 M sodium*



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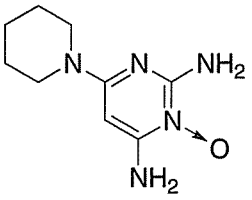
*hydroxide* with vigorous agitation, to a sharp pink end-point. Not more than 0.4 ml of *0.1 M sodium hydroxide* is required.

**Fats, fixed oils and rosin**. Digest 10.0 g with 10 ml of *5 M sodium hydroxide* at 100° for 30 minutes. Separate the water layer and acidify with *sulphuric acid*; no oily or solid matter separates.

**Ash** (2.3.19). Not more than 0.1 per cent, determined on 2.0 g. It volatilises without emitting an acrid odour.

**Storage**. Store protected from light and moisture.

# Minoxidil



$C_9H_{15}N_5O$ Mol. Wt. 209.3

Minoxidil is 2,4-diamino-6-piperidinopyrimidine 3-oxide.

Minoxidil contains not less than 98.5 per cent and not more than 101.0 per cent of $C_9H_{15}N_5O$, calculated on the dried basis.

**Category**. Antihypertensive.

**Dose**. Initially, 5 mg daily, gradually increased at intervals of not less than 3 days to 40 or 50 mg, according to patient's response.

**Description**. A white or almost white, crystalline powder.

## Identification

*Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *minoxidil RS*.

B. Dissolve about 20mg in *0.1 M hydrochloric acid* and dilute to 100 ml with the same solvent (solution A). Dilute 2 ml of solution A to 100 ml with *0.1 M hydrochloric acid* (solution B) and dilute 2 ml of solution A to 100 ml with *0.1 M sodium hydroxide* (solution C).

Measure the light absorption of solutions B and C in the range 220 nm to 360 nm (2.4.7). Solution B shows absorption maxima at about 230 nm and 281 nm; absorbance at about 230 nm, 0.406 to 0.448 and at about 281 nm, 0.424 to 0.468. Solution C shows absorption maxima at about 230 nm, 262 nm and 288 nm; absorbance at about 230 nm, 0.610 to 0.674, at about 262 nm, 0.194 to 0.214 and at about 288 nm, 0.222 to 0.242.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase*. A mixture of 100 volumes of *methanol* and 1.5 volumes of *strong ammonia solution*.

*Test solution*. Dissolve 0.1 g of the substance under examination in 100 ml of *methanol*.

*Reference solution*. A 0.1 per cent w/v solution of *minoxidil RS* in *methanol*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

D. Dissolve about 10 mg in 1 ml of *methanol*, add 0.1 ml of *cupric sulphate solution*; a green colour develops. The solution becomes greenish-yellow on addition of 0.1ml of *2 M hydrochloric acid*.

## Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 25 mg of the substance under examination in 100 ml of the mobile phase.

*Reference solution (a)*. Dilute 1 ml of the test solution to 100 ml with the mobile phase.

*Reference solution (b)*. Dissolve 5 mg of *deoxyminoxidil RS* in the mobile phase and dilute to 20 ml with the mobile phase. To 2 ml of this solution add 2 ml of the test solution and dilute to 10 ml with the mobile phase.

Chromatographic system

- a stainless steel column 10 cm × 3 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: dissolve 3.0 g of *dioctyl sodium sulphosuccinate* in a mixture of 10 ml of *glacial acetic acid* and 300 ml of *water*, adjusted to pH 3.0 with *perchloric acid* and add 700 ml of *methanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume. 10 µl.

Inject the test solution, reference solution (a) and reference solution (b). Continue the chromatography for twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the sum of the areas of any secondary peaks is not greater than 1.5 times the area of reference solution (a) The test is not valid unless, in the chromatogram obtained with reference solution (b), the resolution between the peaks corresponding to minoxidil and deoxyminoxidil is at least 2.0. Ignore any peak with an area



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less than 0.1 per cent of that of the peak in the chromatogram obtained with reference solution (a) (0.001 per cent).

**Heavy metals** (2.3.13).1.0 g complies with the limit test for heavy metals, Method C (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

**Assay**. Weigh accurately about 0.15 g, dissolve in 50 ml of *anhydrous glacial acetic acid.* Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02093 g of $C_9H_{15}N_5O$.

**Storage**. Store protected from light and moisture.

# Minoxidil Tablets

Minoxidil Tablets contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of minoxidil, $C_9H_{15}N_5O$.

**Usual strengths**. 2.5 mg; 5 mg; 10 mg.

## Identification

A.Transfer a portion of the finely powdered tablets containing about 10 mg of Minoxidil to a separator, add 25 ml of *water*, and extract with three quantities, each of 15 ml, of *chloroform*. Combine the chloroform extracts and evaporate with the aid of stream of nitrogen. Wash the inside of the container with about 5 ml of *ethanol (95 per cent)*, add 0.3 g of *potassium bromide IR* and evaporate under vacuum at 50° until dry. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *minoxidil RS*.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

*Mobile phase*. A mixture of 100 volumes of *methanol* and 1.5 volumes of *strong ammonia solution.*

*Test solution*. Shake a quantity of the powdered tablets containing 10 mg of Minoxidil with 10 ml of *methanol*, centrifuge and use the supernatant liquid.

*Reference solution*. A 0.1 per cent w/v solution of *minoxidil RS* in *methanol.*

Apply to the plate 2 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

## Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *phosphate buffer pH 7.2,*
Speed and time. 75 rpm and 15 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc with an average pore diameter not greater than 1.0 µm. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate with the same solvent. Measure the absorbance of the resulting solution at the maximum at about 231 nm (2.4.7), for tablets containing up to 10 mg Minoxidil; for tablets containing more than 10 mg of Minoxidil the wavelength used is about 287 nm. Similarly measure the absorbance of a solution of known concentration of *minoxidil RS*. Calculate the content of $C_9H_{15}N_5O$.

D. Not less than 75 per cent of the stated amount of $C_9H_{15}N_5O$.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Weigh and powder 20 tablets. Shake a quantity of the powder containing about 5 mg of Minoxidil with 20 ml of a solution of *medroxyprogesterone acetate* (internal standard) in the mobile phase having a concentration of about 0.2 mg per ml (solution A) for 5 minutes and centrifuge.

*Reference solution*. Dissolve an accurately weighed quantity of *minoxidil RS* in solution A to obtain a solution having a known concentration of about 0.25 mg per ml.

Chromatographic system
- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a solution prepared by adding 3.0 g of *docusate sodium* per litre to a mixture of 700 volumes of *methanol*, 300 volumes of *water* and 10 volumes of *glacial acetic acid*, adjusting to pH 3.0 with *perchloric acid*, filtering and degassing,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Chromatograph not less than 4 replicate injections of reference solution and record the peak response as mentioned below. The relative standard deviation is not more than 2.0 per cent and the resolution between the internal standard and minoxidil is not less than 2.0.

Inject separately the test solution and the reference solution and measure the responses for the major peaks. The relative retention times are about 0.8 for the internal standard and 1.0 for minoxidil.



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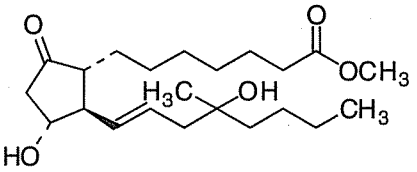
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Calculated the content of minoxidil, $C_9H_{15}N_5O$ in the tablets.

**Storage**. Store protected from light.

# Misoprostol



$C_{22}H_{38}O_5$ Mol. Wt. 382.5

Misoprostol is (*RS*)-methyl (13*E*)-11,16-dihydroxy-16-methyl-9-oxoprost-13-enoate.

Misoprostol contains not less than 96.5 per cent and not more than 102.0 per cent of $C_{22}H_{38}O_5$, calculated on the anhydrous basis.

**Category**. Abortifacient; gastric cytoprotector.

**Description**. A clear, colourless or yellowish oily liquid.

## Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *misoprostol RS* or with the reference spectrum of misoprostol.

## Tests

**Related substances.** Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 10 mg of the substance under examination in 5.0 ml of *acetonitrile.*

*Reference solution (a).* Dilute 1.0 ml of the test solution to 100.0 ml with *acetonitrile* .

*Reference solution (b).* Dilute 1.0 ml of reference solution (a) to 10.0 ml with *acetonitrile*.

*Reference solution (c).* A 0.0025 per cent w/v solution of *misoprostol impurity A RS* in reference solution (a).

*Reference solution (d).* A 0.2 per cent w/v solution of *misoprostol RS* in *acetonitrile.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm),
- column temperature. 40°,
- mobile phase: a mixture of 45 volumes of *acetonitrile*, 55 volumes of *water* and 0.05 volume of 2.45 per cent w/v solution of *orthophosphoric acid*,
- flow rate. 0.75 ml per minute,
- spectrophotometer set at 200 nm,
- injection volume. 10 µl.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to misoprostol impurity A and misoprostol is not less than 1.9. The relative retention time with reference to misoprostol for 8-epimisoprostol (misoprostol impurity A) is about 0.9, for 11-epi misoprostol (misoprostol impurity E) is about 0.9, for 12-epimisoprostol (misoprostol impurity B) (1st peak) is about 0.9 and for misoprostol impurity B (2nd peak) is about 0.95.

Inject reference solution (a), (b) and the test solution. Run the chromatogram 3 times the retention time of the principal peak. The sum of areas of the peaks due to misoprostol impurity A, B and E is not more than 1.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.3 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The sum of areas of all the secondary peaks is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

**Diastereoisomers**. Determine by liquid chromatography (2.4.14).

*Test solution.* Dissolve about 20 mg of the substance under examination in 1.0 ml of the mobile phase.

*Reference solution.* Dilute 0.1 ml of the test solution to 10.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with silica (5 µm),
- column temperature. 40°,
- mobile phase: a mixture of 5 volumes of *2-propanol* , 95 volumes of *heptane* and 0.01 volume of *glacial acetic acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 205 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the resolution between the 1st peak and the 2nd peak of misoprostol is not less than 2.3. The retention time of Misoprostol 1st peak is about 19 minutes and misoprostol 2nd peak about 21 minutes.

Inject the reference solution and the test solution. Run the chromatogram 1.5 times the retention time of the 1st peak of misoprostol. In the chromatogram obtained with the test solution, the area of the 1st peak of misoprostol is 50 per cent



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to 55 per cent of the sum of the areas of the 2 peaks due to misoprostol.

**Water** (2.3.43). Not more than 1.0 per cent, determined on 1 g.

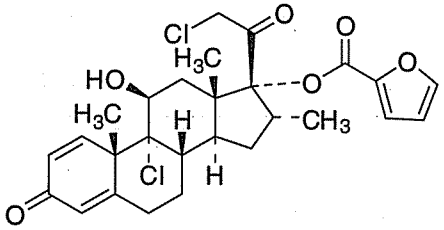
**Assay**. Determine by liquid chromatography (2.4.14), as described under Related substances.

Inject reference solution (d) and the test solution.

Calculate the content of $C_{22}H_{38}O_5$.

**Storage**. Store protected from moisture, at a temperature of about – 20°.

# Mometasone Furoate



$C_{27}H_{30}Cl_2O_6$ Mol. Wt. 521.4

Mometasone Furoate is 9α,21-Dichloro-11β-hydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-17-yl furan-2-carboxylate.

Mometasone Furoate contains not less than 97.0 per cent and not more than 102.0 per cent of $C_{27}H_{30}Cl_2O_6$, calculated on the dried basis.

**Category**. Glucocorticoid.

**Description**. A white or almost white powder.

## Identification

*Test A may be omitted if tests B, C and D are carried out and tests B, C and D may be omitted if test A is carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mometasone furoate RS* or with the reference spectrum of mometasone furoate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

*Mobile phase.* a mixture of 1.2 volumes of *water*, 8 volumes of *methanol*, 15 volumes of *ether* and 77 volumes of *dichloromethane*.

*Test solution.* Dissolve 10 mg of the substance under examination in 10.0 ml of *dichloromethane*.

*Reference solution (a).* A 0.1 per cent w/v solution of *mometasone furoate RS* in *dichloromethane*.

*Reference solution (b).* Dissolve 10 mg of *beclometasone dipropionate RS* in 10.0 ml of reference solution (a).

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with reference solution (a). Spray with *ethanolic sulphuric acid*. Heat at 120° for 10 minutes or until the spots appear. Allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with reference solution (a). In the chromatogram obtained with reference solution (b), shows 2 spots which, when examined in ultraviolet light at 365 nm, may not be completely separated.

C. Shake about 2 mg with 2 ml of *sulphuric acid* for 15 minutes, a light yellow colour develops. When examined in ultraviolet light at 365 nm, no fluorescence is seen. Add this solution to 10 ml of *water* and mix. The colour fades and there is no fluorescence.

D. Mix 80 mg with 0.3 g of *anhydrous sodium carbonate* and ignite in a crucible until an almost white residue is obtained. Allow to cool and dissolve the residue in 5 ml of *dilute nitric acid*, filter. To 1 ml of the filtrate, add 1 ml of *water*. The solution gives reaction (a) of chlorides (2.3.1).

## Tests

**Specific optical rotation** (2.4.22). + 50° to + 55°, determined in 0.5 per cent w/v solution in *ethanol (95 per cent)*.

**Related substances.** Determine by liquid chromatography (2.4.14).

*NOTE—Prepare the solutions immediately before use.*

*Solvent mixture.* 50 volumes of *acetonitrile*, 50 volumes of *water* and 0.1 volume of *acetic acid*.

*Test solution.* Dissolve 25 mg of the substance under examination in 4 ml of *acetonitrile* and dilute to 25.0 ml with the solvent mixture.

*Reference solution (a).* Dissolve 2 mg of *mometasone furoate RS* and 6 mg of *beclometasone dipropionate RS* in 10.0 ml of the solvent mixture. Dilute 2.0 ml of this solution to 100.0 ml with the solvent mixture.

*Reference solution (b).* Dilute 1.0 ml of the test solution to 25.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Chromatographic system

– a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),



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- mobile phase: a mixture of 50 volumes of *acetonitrile* and 50 volumes of *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to mometasone furoate and beclometasone dipropionate is not less than 6.0.

Inject reference solution (b) and the test solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent). The sum of all the secondary peaks is not more than 1.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.6 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

**Heavy metals** (2.3.13). 0.67 g complies with the limit test for heavy metals, Method B (30 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 5.0 per cent, determined on 1.0 g.

**Assay**. Dissolve 50 mg in 100.0 ml of *ethanol (95 per cent)*. Dilute 2.0 ml of this solution to 100.0 ml with *ethanol (95 per cent)* and measure the absorbance of the resulting solution at the maximum at about 249 nm (2.4.7). Calculate the content of $C_{27}H_{30}Cl_2O_6$ taking 481 as the specific absorbance at 249 nm.

# Mometasone Aqueous Nasal Spray

**Category**. Glucocorticoid.

Mometasone Aqueous Nasal Spray is an aqueous suspension of Mometasone Furoate in a suitable pressurised container fitted with an appropriate nasal delivery system.

*The nasal spray complies with the requirements stated under Nasal Preparations and with the following requirements.*

Mometasone Aqueous Nasal Spray contains not less than 80.0 per cent and not more than 120.0 per cent of the stated amount of mometasone furoate, $C_{27}H_{30}Cl_2O_6$.

**Usual Strength** . 0.05 per cent w/v.

## Identification

A. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (d).

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

## Tests

**Related substances**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase*. A mixture of 3 volumes of *methanol* and 97 volumes of *1, 2-dichloroethane*.

*Test solution*. Dissolve 1 mg of Mometasone Furoate in 4 ml of *acetone*, sonicate and filter. Evaporate the filtrate to dryness and dissolve in 1.0 ml of *acetone*.

*Reference solution (a)*. A 0.002 per cent w/v of solution of *mometasone furoate RS* in *acetone*.

*Reference solution (b)*. Dilute 1.0 ml of reference solution (a) with 2.0 ml of *acetone*.

*Reference solution (c)*. Dilute 1.0 ml of reference solution (b) with 4.0 ml of *acetone*.

*Reference solution (d)*. A 0.1 per cent w/v solution of *mometasone furoate RS* in *acetone*.

Apply to the plate 50 µl of each solution. After development, dry the plate in air and spray with *alkaline tetrazolium blue solution* and heat at 50° for 5 minutes and allow to cool, again spray with *alkaline tetrazolium blue solution*. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) (2.0 per cent). Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b) (1.0 per cent), any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.5 per cent).

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dilute a quantity of nasal spray containing1 mg of Mometasone Furoate in 20 ml of hot *methanol (90 per cent)*, and add 25 ml of *2,2,4- trimethylpentane*, cool, shake the mixture and filter the lower methanol layer through a small plug of absorbent cotton previously washed with *methanol (80 per cent)*. Repeat the extraction of the *2,2,4-trimethylpentane* layer with two further 10 ml quantities of *methanol (80 per cent)*, filtering the extracts through the absorbent cotton. Combine the extracts and add sufficient *methanol (80 per cent)* to produce 50 ml. Filter through a 0.45 µm nylon filter.

*Reference solution*. A 0.002 per cent w/v solution of *mometasone furoate RS* in *methanol (80 per cent)*.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with base-deactivated end-capped octadecylsilane bonded to porous silica (5 µm),



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- column temperature 60°,
- mobile phase: a mixture of 45 volumes of *water* and 55 volumes of *methanol,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 238 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor of the peak due to mometasone furoate is not more than 1.2.

Inject the reference solution and the test solution.

Calculate the content of $C_{27}H_{30}Cl_2O_6$.

**Labelling**. The label states the amount of active ingredient delivered by each actuation of the valve and the number of deliveries available from the container.

# Mometasone Cream

Mometasone Furoate Cream

Mometasone Cream contains Mometasone Furoate in a suitable base.

Mometasone Cream contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of mometasone furoate, $C_{27}H_{30}Cl_2O_6$.

**Usual strength**. 0.1 per cent w/w.

## Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

*Mobile phase.* A mixture of 5 volumes of *absolute ethanol*, 10 volumes of *acetone* and 100 volumes of *dichloromethane*.

*Test solution.* Disperse a quantity of the cream containing about 0.5 mg of Mometasone Furoate in 20 ml of *methanol (80 per cent)* by heating on a water-bath until the solution begins to boil. Shake vigorously, cool in ice for 30 minutes and centrifuge. Mix 10 ml of the supernatant liquid with 3 ml of *water* and 5 ml of *dichloromethane*, shake vigorously, allow the layers to separate, evaporate the *dichloromethane* layer to dryness in a current of nitrogen with gentle heating and dissolve the residue in 1 ml of *dichloromethane*.

*Reference solution (a).* A 0.025 per cent w/v solution of *mometasone furoate RS* in *dichloromethane*.

*Reference solution (b).* A solution containing 5 ml each of the test solution and reference solution (a).

Apply to the plate 10 µl of each solution. After development, dry the plate in air, heat at 105° for 5 minutes and, while hot, spray with *alcoholic sulphuric acid solution* and heat at 105° for a further 5 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single compact spot.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

## Tests

**Other tests**. Complies with the tests stated under Creams.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Disperse a quantity of the cream containing about 1 mg of Mometasone Furoate in 20 ml of hot *methanol (90 per cent)*, add 25 ml of *2,2,4-trimethylpentane*, cool, shake the mixture and filter the lower methanol layer through a small plug of absorbent cotton previously washed with *methanol (80 per cent)*. Repeat the extraction of the *2,2,4-trimethylpentane* layer with two further 10 ml quantities of *methanol (80 per cent)*, filter the extracts and dilute to 50 ml with *methanol (80 per cent)*, filter.

*Reference solution.* A 0.002 per cent w/v solution of *mometasone furoate RS* in *methanol (80 per cent)*.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with base deactivated endcapped octadecylsilane bonded to porous silica (5 µm) (such as Hypersil BDS C18),
- column temperature. 60°,
- mobile phase: a mixture of 45 volumes of *water* and 55 volumes of *methanol,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 238 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor of the principal peak is not more than 1.2.

Inject the reference solution and the test solution.

Calculate the content of $C_{27}H_{30}Cl_2O_6$ in the cream.

**Storage**. Store protected from light.

# Mometasone Ointment

Mometasone Furoate Ointment

Mometasone Ointment contains Mometasone Furoate in a suitable base.

Mometasone Ointment contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of mometasone furoate, $C_{27}H_{30}Cl_2O_6$.

**Usual strength**. 0.1 per cent w/w.



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### Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

*Mobile phase.* A mixture of 5 volumes of *absolute ethanol*, 10 volumes of *acetone* and 100 volumes of *dichloromethane*.

*Test solution.* Disperse a quantity of the ointment containing about 0.5 mg of Mometasone Furoate in 20 ml of *methanol (80 per cent)* by heating on a water-bath until the solution begins to boil. Shake vigorously, cool in ice for 30 minutes and centrifuge. Mix 10 ml of the supernatant liquid with 3 ml of *water* and 5 ml of *dichloromethane*, shake vigorously, allow the layers to separate, evaporate the *dichloromethane* layer to dryness in a current of nitrogen with gentle heating and dissolve the residue in 1 ml of *dichloromethane*.

*Reference solution (a).* A 0.025 per cent w/v solution of *mometasone furoate RS* in *dichloromethane*.

*Reference solution (b).* A solution containing 5 ml each of the test solution and reference solution (a).

Apply to the plate 10 µl of each solution. After development, dry the plate in air, heat at 105° for 5 minutes and, while hot, spray with *alcoholic sulphuric acid solution* and heat at 105° for a further 5 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single compact spot.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

### Tests

**Other tests**. Complies with the tests stated under Ointments.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Disperse a quantity of the ointment containing about 1 mg of Mometasone Furoate in 20 ml of hot *methanol (90 per cent)*, add 25 ml of *2,2,4-trimethylpentane*, cool, shake the mixture and filter the lower methanol layer through a small plug of absorbent cotton previously washed with *methanol (80 per cent)*. Repeat the extraction of the *2,2,4-trimethylpentane* layer with two further 10 ml quantities of *methanol (80 per cent)*, filter the extracts and dilute to 50 ml with *methanol (80 per cent)*, filter.

*Reference solution.* A 0.002 per cent w/v solution of *mometasone furoate RS* in *methanol (80 per cent)*.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm) (such as Hypersil BDS C18),
- column temperature. 60°,
- mobile phase: a mixture of 45 volumes of *water* and 55 volumes of *methanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 238 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor of the principal peak is not more than 1.2.

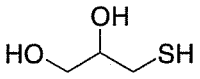
Inject the reference solution and the test solution.

Calculate the content of $C_{27}H_{30}Cl_2O_6$ in the ointment.

**Storage**. Store protected from light.

## Monothioglycerol

Thioglycerol




$C_3H_8O_2S$ Mol. Wt. 108.2

Monothioglycerol is 3-mercaptopropane-1,2-diol.

Monothioglycerol contains not less than 97.0 per cent and not more than 101.0 per cent of $C_3H_8O_2S$, calculated on the anhydrous basis.

**Category**. Pharmaceutical aid.

**Description**. A colourless to pale yellow, viscous liquid; odour resembling that of sulphides; hygroscopic.

### Tests

**pH** (2.4.24). 3.5 to 7.0, determined in a 10.0 per cent w/v solution.

**Relative density** (2.4.29). 1.241 to 1.250.

**Refractive index** (2.4.27). 1.521 to 1.526.

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 5.0 per cent, determined by Method C on 50.0 g.

**Assay**. Weigh accurately about 0.2 g, dissolve in 50 ml of *water* and titrate with *0.05 M iodine* using 3 ml of *starch solution*, added towards the end of the titration, as indicator.

1 ml of *0.05 M iodine* is equivalent to 0.01082 g of $C_3H_8O_2S$.

**Storage**. Store protected from light and moisture.

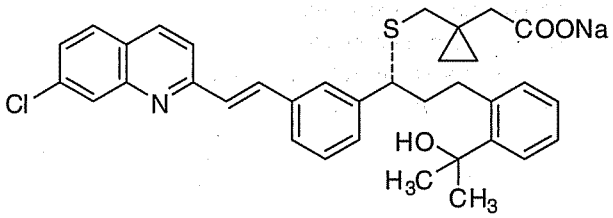


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# Montelukast Sodium




$C_{35}H_{35}ClNNaO_3S$ Mol. Wt. 608.2

Montelukast sodium is monosodium salt of 1-[[[(1*R*)-1-[3-[(1*E*)-2-(7-chloro-2-quinolinyl)ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]thio]methyl]cyclopropaneacetic acid.

Montelukast Sodium contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{35}H_{35}ClNNaO_3S$, calculated on the anhydrous basis.

**Category.** Antiasthmatic (add-on therapy for mild to moderate asthma).

**Description.** A white to pale yellow powder.

## Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum obtained with *montelukast sodium RS* or with the reference spectrum of montelukast sodium.

B. Gives reaction A of sodium salt (2.3.1).

## Tests

**Specific optical rotation** (2.4.22). + 95.0° to + 106.0°, determined on 1.0 per cent w/v solution in *methanol*.

**Related substances**. Determine by liquid chromatography (2.4.14).

*NOTE—Use freshly prepared solution, sonicate in ice-cold water.*

*Solvent mixture*. 20 volumes of *water* and 80 volumes of *methanol*.

*Test solution*. Dissolve about 100 mg of the substance under examination in 100.0 ml of the solvent mixture.

*Reference solution (a)*. A 0.1 per cent w/v solution of *montelukast sodium RS* in the solvent mixture.

*Reference solution (b)*. Dilute 10.0 ml of reference solution (a) to 100.0 ml with the solvent mixture. Dilute 3.0 ml of this solution to 100.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Hypersil ODS),
- mobile phase: A. dissolve 3.85 g of *ammonium acetate* in 1000 ml of *water*, add 1 ml of *triethylamine*, adjusted to pH 5.5 with *glacial acetic acid*,
  B. *methanol*,
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume. 20 µl.

| Time (in min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0 | 40 | 60 |
| 20 | 30 | 70 |
| 45 | 20 | 80 |
| 55 | 15 | 85 |
| 60 | 15 | 85 |
| 65 | 40 | 60 |
| 70 | 40 | 60 |

Inject reference solution (b). The test is not valid unless the relative standard deviation for replicate injections is not more than 5.0 per cent. The relative retention time with reference to montelukast for montelukast sulphoxide isomers is about 0.66 and 0.69 and for montelukast styrene is about 1.38.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution the sum of areas of montelukast sulphoxide isomers is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent), the area of the peak due to montelukast styrene is not more than area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent). The sum of areas of all the secondary peaks is not more than 3.3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent).

**Heavy Metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

**Water** (2.3.43). Not more than 3.0 per cent, determined on 1.0 g.

**Assay**. Determine by liquid chromatography (2.4.14).

*NOTE—Use freshly prepared solution, sonicate in ice-cold water.*

*Solvent mixture*. 20 volumes of *water* and 80 volumes of *methanol*.

*Test solution*. Dissolve about 50 mg of the substance under examination in 100.0 ml of the solvent mixture.

Dilute 5.0 ml of this solution to 50.0 ml with the solvent mixture.



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*Reference solution (a).* A 0.05 per cent w/v solution of *montelukast sodium RS* in the solvent mixture.

*Reference solution (b).* Dilute 5.0 ml of reference solution (a) to 50.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Hypersil ODS),
- mobile phase: a mixture of 22 volumes of buffer solution prepared by dissolving 3.85 g of *ammonium acetate* in 1000 ml of *water*, add 1 ml of *triethylamine*, adjusted to pH 5.5 with *glacial acetic acid* and 78 volumes of *methanol*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the theoretical plates is not less than 2000, tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (b) and the test solution.

Calculate the content of $C_{35}H_{35}ClNNaSO_3$.

**Storage**. Store protected from light and moisture, at a temperature not exceeding 30°.

# Montelukast Tablets

Montelukast Sodium Tablets

Montelukast Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of montelukast, $C_{35}H_{35}ClNO_3S$

## Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

## Tests

**Dissolution** (2.5.2).

Apparatus No. 1,
Medium. 900 ml of 0.5 per cent w/v solution of *sodium dodecyl sulphate* in *water*,
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

*Test solution.* Use the filtrate.

*Reference solution.* A 0.023 per cent w/v solultion of *montelukast sodium RS* in the dissolution medium. Dilute 5.0 ml of this solution to 100.0 ml with the dissolution medium.

Use the chromatographic system as described under Assay.

Inject the reference solution. The test is not valid unless the theoretical plates is not less than 2000, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{35}H_{35}ClNO_3S$ in the tablet.

D. Not less than 70 per cent of the stated amount of $C_{35}H_{35}ClNO_3S$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture.* A 0.1 per cent v/v solution of *triethylamine* in *methanol*.

*Test solution.* Weigh and transfer intact tablets containing about 100 mg of montelukast in 200-ml volumetric flask. Add about 20 ml of *water*, sonicate. Add 150 ml of the solvent mixture and sonicate for 20 minutes at a temperature not exceeding 10°. Dilute to 200.0 ml with the solvent mixture, filter.

*Reference solution (a).* A 0.0025 per cent w/v solution of *montelukast sodium RS* in the solvent mixture

*Reference solution (b).* Dilute 5.0 ml of reference solution (a) to 50.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil ODS),
- column temperature. 40°,
- mobile phase: A. a solution containing 6.0 g of *ammonium acetate* in 1000 ml of *water*, adjusted to pH 5.5 with *acetic acid*,
  B. *methanol*,
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume. 10 µl.

| Time (in min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0 | 50 | 50 |
| 15 | 35 | 65 |
| 40 | 30 | 70 |
| 60 | 20 | 80 |
| 65 | 20 | 80 |
| 70 | 50 | 50 |
| 75 | 50 | 50 |



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Inject reference solution (b). The test is not valid unless the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of the peak corresponding to sulphoxide impurity at relative retention time 0.63 and styrene impurity at relative retention time 1.37 is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than 4 times the area of the peak in the chromatogram obtained with reference solution (b) (2.0 per cent).

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Solvent mixture*. A 0.1 per cent v/v solution of *triethylamine* in *methanol*.

*Test solution*. Weigh and transfer intact tablets containing about 100 mg of montelukast in 200-ml volumetric flask. Add about 20 ml of *water*, sonicate. Add 150 ml of the solvent mixture, sonicate for 20 minutes at a temperature not exceeding 10°. Dilute to 200.0 ml with the solvent mixture, filter. Dilute 5.0 ml of this solution to 25.0 ml with the solvent mixture.

*Reference solution*. Dissolve 26 mg of *montelukast sodium RS* in 250.0 ml of the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil ODS),
- column temperature. 40°,
- mobile phase: a mixture of 20 volumes of a buffer solution prepared by dissolving 3.85 g of *ammonium acetate* in 1000 ml of *water*, add 1.0 ml *triethylamine*, adjusted the pH to 5.5 with *acetic acid* and 80 volumes of *methanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 345 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the theoretical plates is not less than 2000, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent

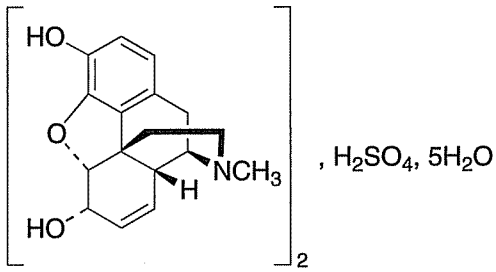
Inject the reference solution and the test solution.

Calculate the content of $C_{35}H_{35}ClNO_3S$ in the tablets.

**Storage**. Store protected from light and moisture.

**Labelling**. The label states the strength in terms of the equivalent amount of montelukast.

# Morphine Sulphate




$(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$ Mol. Wt. 758.8

Morphine Sulphate is 7,8-didehydro-4,5α-epoxy-17-methylmorphinan-3,6α-diol sulphate pentahydrate.

Morphine Sulphate contains not less than 98.0 per cent and not more than 102.0 per cent of $(C_{17}H_{19}NO_3)_2,H_2SO_4$, calculated on the dried basis.

**Category**. Narcotic analgesic.

**Dose**. Orally or by subcutaneous or intramuscular injection, 10 to 20 mg.

**Description**. White, acicular crystals or cubical masses or a white, crystalline powder; odourless.

## Identification

A. When examined in the range 230 nm to 360 nm (2.4.7), a 0.015 per cent w/v solution shows an absorption maximum only at about 285 nm; absorbance at about 285 nm, about 0.65.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.005 per cent w/v solution in *0.1 M sodium hydroxide* shows an absorption maximum only at about 298 nm; absorbance at about 298 nm, about 0.34.

C. Add a few mg of the powdered substance to a mixture of 1 ml of *sulphuric acid* and 0.05 ml of *formaldehyde solution*; a purple colour is produced.

D. Dissolve 5 mg in 5 ml of *water* and add 0.15 ml of *dilute potassium ferricyanide solution* and 0.05 ml of *ferric chloride solution*; a bluish green colour is produced immediately, which changes rapidly to blue.

E. Gives the reactions of sulphates (2.3.1).

## Tests

**Acidity**. Dissolve 0.2 g in 10 ml of freshly boiled and cooled *water* and titrate with *0.02 M sodium hydroxide* using *methyl red solution* as indicator. Not more than 0.2 ml of *0.02 M sodium hydroxide* is required to change the colour of the solution.



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**Related substances**. Determine by liquid chromatography (2.4.14).

*Solvent mixture.* 1.0 per cent v/v solution of *acetic acid.*

*Test solution.* Dissolve 125 mg of the substance under examination in 50 ml of the solvent mixture.

*Reference solution (a).* Dilute 1.0 ml of the test solution to 100 ml with the solvent mixture. Dilute 2.0 ml of this solution to 10.0 ml with the solvent mixture.

*Reference solution (b).* Dissolve 5 mg of *morphine for system suitability RS* (containing morphine impurities B, C, E and F) in 2 ml of the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 35°,
- mobile phase: A. a 0.1 per cent w/v solution of *sodium heptanesulphonate* adjusted to pH 2.6 with *orthophosphoric acid*,
  B. *methanol*,
- a linear gradient programme using the conditions given below,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 10 µl.

| Time (in min.) | Mobile phase A (per cent v/v) | Mobile phase B (per cent v/v) |
|---|---|---|
| 0-2 | 85 | 15 |
| 2-35 | 85-50 | 15-50 |
| 35-40 | 50 | 50 |

Inject reference solution (b). The test is not valid unless the peak-to-valley ratio, where Hp is height above the baseline of the peak due to morphine impurity F, Hv is the height above the baseline of the lowest point of the curve separating this peak from the peak due to morphine is not less than 2.0.

Inject the test solution and reference solution (a). In the chromatogram obtained with the test solution, the area of the peak corresponding to 2,2'-bimorphine (morphine impurity B) is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent). The area of the each peak corresponding to oripavine (morphine impurity C) and morphinone (morphine impurity E) is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent) and sum of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). 9.0 to 12.0 per cent, determined on 0.5 g by drying in an oven at 145° for 1 hour.

**Assay**. Weigh accurately about 0.5 g, dissolve in 30 ml of *glacial acetic acid.* Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.06688 g of $(C_{17}H_{19}NO_3)_2, H_2SO_4$.

**Storage**. Store protected from light and moisture.

# Morphine and Atropine Injection

## Morphine Sulphate and Atropine Sulphate Injection

Morphine and Atropine Injection is a sterile isotonic solution in Water for Injections containing 1.0 per cent w/v of Morphine Sulphate and 0.06 per cent w/v of Atropine Sulphate.

Morphine and Atropine Injection contains not less than 0.90 per cent w/v and not more than 1.10 per cent w/v of morphine sulphate, $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$, and not less than 0.054 per cent w/v and not more than 0.066 per cent w/v of atropine sulphate, $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$.

### Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

*Mobile phase.* A mixture of 100 volumes of *methanol* and 1.5 volumes of *strong ammonia solution.*

*Test solution.* Add 1 ml of *5 M ammonia* to 1 ml of the injection, extract with two quantities, each of 5 ml, of *chloroform*, filter the combined extracts through *anhydrous sodium sulphate*, evaporate to dryness in a current of warm air and dissolve the residue in 0.5 ml of *chloroform.*

*Reference solution (a).* Treat 1 ml of a 0.06 per cent w/v solution of *atropine sulphate RS* in the same manner as for the test solution.

*Reference solution (b).* Treat 1 ml of a 1 per cent w/v solution of *morphine sulphate RS* in the same manner as for the test solution.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with *dilute potassium iodobismuthate solution.* The principal spots in the chromatogram obtained with the test solution correspond to



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the spots in the chromatograms obtained with reference solutions (a) and (b).

### Tests

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. *For atropine sulphate* — To 10.0 ml add 10 ml of *water* and 5 ml of *1 M sodium hydroxide* and extract successively with 15, 10 and 10 ml of *chloroform* and continue the extraction with 10-ml quantities of *chloroform* until complete extraction of alkaloids has been effected (2.6.4). Wash the chloroform extracts with the same 5 ml of *water* (preserve the aqueous solution and the washings for the Assay for morphine sulphate). Evaporate the chloroform, dissolve the residue in 2 ml of *ethanol (95 per cent)*, add 2.0 ml of *0.025 M sulphuric acid*, cool and titrate the excess of acid with *0.05 M sodium hydroxide* using *methyl red solution* as indicator.

1 ml of *0.025 M sulphuric acid* is equivalent to 0.01737 g of $(C_{17}H_{23}NO_3)_2, H_2SO_4, H_2O$.

*For morphine Sulphate* — Combine the aqueous solution and washings obtained in the Assay for atropine sulphate, add 1 g of *ammonium sulphate* and 25 ml of *ethanol (95 per cent)* and extract with 40 ml, followed by successive quantities of 40, 20 and 20 ml, of a mixture of 1 volume of *ethanol (95 per cent)* and 3 volumes of *chloroform*, washing each extract with the same two successive quantities, each of 5 ml, of *water* and continue the extraction until complete extraction of alkaloids has been effected (2.6.4). Evaporate the chloroform, boil the residue with 10.0 ml of *0.05 M sulphuric acid*, cool and titrate the excess of acid with *0.1 M sodium hydroxide* using *methyl red solution* as indicator.

1 ml of *0.05 M sulphuric acid* is equivalent to 0.03794 g of $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$.

**Storage**. Store protected from light.

## Morphine Injection

Morphine Sulphate Injection

Morphine Injection is a sterile solution of Morphine Sulphate in Water for Injections.

Morphine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of morphine sulphate, $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$.

**Usual strength**. 10 mg per ml.

### Identification

A. Evaporate a volume containing 5 mg of Morphine Sulphate to dryness on a water-bath. Dissolve the residue in 5 ml of *water* and add 0.15 ml of *dilute potassium ferricyanide solution*; a bluish green colour is produced immediately, which changes rapidly to blue.

B. Gives reaction A of sulphates (2.3.1).

### Tests

**pH** (2.4.24). 2.5 to 6.0.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution*. Measure accurately a volume containing about 25 mg of Morphine Sulphate and dilute with sufficient of the mobile phase to produce 100.0 ml, freshly prepared.

*Reference solution*. Weigh accurately about 25 mg of *morphine sulphate RS* and dissolve in sufficient of the mobile phase to produce 100.0 ml, freshly prepared.

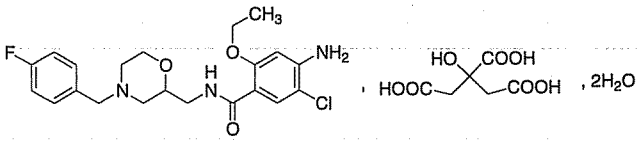
Chromatographic system
- a stainless steel column 40 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: dissolve 0.73 g of *sodium heptane-sulphonate* in 720 ml of *water*, add 280 ml of *methanol* and 10 ml of *glacial acetic acid*, mix and filter,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume. 20 µl.

Inject the test solution and reference solution.

Calculate the content of $(C_{17}H_{19}NO_3)_2$, $H_2SO_4$, $5H_2O$ in the injection.

**Storage**. Store protected from light.

## Mosapride Citrate Dihydrate



$C_{21}H_{25}ClFN_3O_3,C_6H_8O_7,2H_2O$ Mol. Wt. 650.0

Mosapride Citrate Dihydrate is (*RS*)-4-amino-5-chloro-2-ethoxy-*N*-{[4-(4-fluorobenzyl)-2-morpholinyl]methyl} benzamide citrate dihydrate.

Mosapride Citrate Dihydrate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{21}H_{25}ClFN_3O_3,C_6H_8O_7$, calculated on the anhydrous basis.

**Category**. Gastroprokinetic agent.

**Description**. A white or yellowish white crystalline powder.



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### Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mosapride citrate dihydrate RS*.

### Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*Test solution*. Dissolve 20 mg of the substance under examination in 100.0 ml of the mobile phase.

*Reference solution (a)*. Dissolve 6.5 mg of *citric acid monohydrate* in 100.0 ml of the mobile phase.

*Reference solution (b)*. Dissolve 20 mg of *mosapride citrate dihydrate RS* in 100 ml of the mobile phase. Stir with the aid of ultrasound to dissolve, cool and dilute 1 ml of this solution to 200 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: 60 volumes of a buffer solution, prepared by dissolving 1.4 ml of *orthophosphoric acid* in 1000 ml of *water*, adjusting the pH to 3.0 with *triethylamine*, and 40 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 276 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the column efficiency is not less than 4000 theoretical plates and the tailing factor is not more than 2.0.

Inject reference solution (a).

Inject the test solution. Measure the responses for all peaks except the peak due to the analyte, peaks from the blank and the peak corresponding to citric acid. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent).

**Heavy metals** (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.2 per cent.

**Water** (2.3.43). 5.0 per cent to 7.0 per cent, determined on 1.0 g.

**Assay**. Weigh accurately about 0.35 g, dissolve in 70 ml of a mixture of 1 volume of *glacial acetic acid* and 7 volumes of *methyl ethyl ketone*. Cover the beaker and heat on a water-bath at 80° to dissolve. Cool and titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.061405 g of $C_{21}H_{25}ClFN_3O_3,C_6H_8O_7$,.

**Storage**. Store protected from moisture.

## Mosapride Citrate Tablets

Mosapride Citrate Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of mosapride citrate, $C_{21}H_{25}ClFN_3O_3,C_6H_8O_7$.

### Identification

In the Assay, the retention time of the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

### Tests

**Dissolution** (2.5.2).

Apparatus No.1,
Medium. 500 ml of *0.1 M hydrochloric acid,*
Speed and time. 100 rpm and 15 minutes.

Withdraw a suitable volume of the medium and filter. Determine by liquid chromatography (2.4.14).

*Test solution*. Use the filtrate, diluted if necessary, with the dissolution medium

*Reference solution.*

*For 2.5 mg tablets*

Weigh 26.5 mg of *mosapride citrate dihydrate RS* and dissolve in 25 ml of the mobile phase. Dilute 5 ml of this solution to 1000 ml with the medium.

*For other than 2.5mg tablets*

Weigh 26.5 mg of *mosapride citrate dihydrate RS* and dissolve in 25 ml of the mobile phase. Dilute 5 ml of this solution to 500 ml with the medium.

Use the chromatographic system described under Assay

Inject the reference solution. The test is not valid unless the column efficiency is not less than 4000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and the reference solution.

Calculate the content of $C_{21}H_{25}ClFN_3O_3,C_6H_8O_7$.

D. Not less than 70 per cent of the stated amount of $C_{21}H_{25}ClFN_3O_3,C_6H_8O_7$.

**Uniformity of content**. Comply with the test stated under tablets.



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Determine by liquid chromatography (2.4.14) as described under Assay using the following solutions.

*Test solution.* Dissolve 1 tablet in 100 ml of the mobile phase. Centrifuge for 15 minutes. Dilute the clear supernatant liquid if necessary, with the mobile phase to produce a solution containing 0.02 mg of mosapride citrate per ml.

*Reference solution.* Weigh 26.5 mg of *mosapride citrate dihydrate RS* and dissolve in 100 ml of the mobile phase. Dilute with the mobile phase to produce a solution containing of 0.02 mg of mosapride citrate per ml.

**Other tests**. Comply with the tests stated under Tablets.

**Assay**. Determine by liquid chromatography (2.4.14).

*Test solution.* Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 10 mg of mosapride citrate and dissolve in 50 ml of the mobile phase. Centrifuge for 15 minutes. Dilute 5 ml of the clear supernatant liquid to 50 ml with the mobile phase.

*Reference solution.* Weigh 21.2 mg of *mosapride citrate dihydrate RS* and dissolve in 100 ml of the mobile phase. Dilute 5 ml of this solution to 50 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: 60 volumes of a buffer solution, prepared by dissolving 1.4 ml of *orthophosphoric acid* in 1000 ml of *water* and adjusting the pH to 3.0 with *triethylamine*, and 40 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 276 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 4000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and reference solution.

Calculate the content of $C_{21}H_{25}ClFN_3O_3,C_6H_8O_7$ in the tablets.

**Labelling**. The label states the strength in terms of the equivalent amount of anhydrous mosapride citrate.

# Multiple Electrolytes and Dextrose Injection Type I

Multiple Electrolytes and Dextrose Injection Type I is a sterile solution of Dextrose and suitable salts in Water for Injection to provide sodium, potassium, magnesium, acetate, phosphate and chloride ions. It may contain Hydrochloric Acid or Sodium Hydroxide used for adjusting the pH.

**Usual strength.**

| | |
|---|---|
| Sodium acetate | 0.32 g |
| Potassium chloride | 0.13 g |
| Dipotassium hydrogen phosphate | 0.026 g |
| Magnesium chloride | 0.031 g |
| Dextrose | 5.0 g |
| Water for Injections to | 100 ml |

| Concentration of electrolytes in mmol / l | |
|---|---|
| Sodium | 23.0 |
| Potassium | 20.0 |
| Magnesium | 1.5 |
| Acetate | 23.0 |
| Chloride | 20.0 |
| Phosphate | 1.5 |

Multiple Electrolytes and Dextrose Injection Type I contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of sodium, Na, potassium, K, magnesium, Mg, acetate, $C_2H_3O_2$, and phosphate, $PO_4$. It also contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of chloride, Cl and not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of dextrose, $C_6H_{12}O_6$. It contains no antimicrobial agent.

**Description**. A clear, colourless or faintly straw-coloured solution.

## Identification

A. To 1 ml add 0.05 ml of *potassium cupri-tartrate solution*; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.

B. 20 ml gives the reactions of acetates, chlorides phosphates, sodium salts, potassium salts and magnesium salts (2.3.1).

## Tests

**pH** (2.4.24). 3.0 to 7.0.

**5-Hydroxymethylfurfural and Related substances**. Dilute a volume containing 1.0 g of Dextrose to 500.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 284 nm; absorbance at about 284 nm, not more than 0.25 (2.4.7).

**Bacterial endotoxins** (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. *For sodium* — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 589 nm and using *sodium solution FP* or *sodium solution*



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*AAS* respectively, suitably diluted with *water* for the standard solutions.

*For total potassium* — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 767 nm and using *potassium solution FP* or *potassium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

*For magnesium* — To 50.0 ml add 50 ml of *water* and 5 ml of *strong ammonia-ammonium chloride solution* and titrate with *0.005 M disodium edetate* using 50 mg of *eriochrome black T mixture* as indicator.

1 ml of *0.005 M disodium edetate* is equivalent to 0.1215 mg of Mg.

*For acetate* — Determine by liquid chromatography (2.4.14).

*Test solution.* Dilute an accurately measured volume of the preparation under examination quantitatively with *water* to obtain a solution containing about 0.12 per cent w/v of Sodium Acetate.

*Reference solution.* Dissolve an accurately weighed quantity of *sodium acetate* in *water* to obtain a solution having a known concentration of about 0.12 per cent w/v of sodium acetate.

Chromatographic system

- a stainless steel column 30 cm × 7.8 mm, packed with strong cation-exchange resin consisting of sulphonated cross-linked styrene-divinylbenzene copolymer in the hydrogen form (7 µm) and a guard column 4 cm x 7.8 mm packed with the same column material,
- temperature. column 60°,
- mobile phase: *0.1 M sulphuric acid,*
- flow rate. 0.8 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject separately the test solution and reference solution and measure the responses for the major peak and calculate the content of acetate in the preparation under examination.

*For phosphate* — Dilute an accurately measured volume containing about 4 mg of phosphate with sufficient *water* to produce 50.0 ml. Transfer 2.0 ml of this solution to a test-tube. Add 1.0 ml of a 5 per cent w/v solution of *ammonium molybdate* in a cooled mixture of *sulphuric acid* and *water* (15:85) and allow to stand for 3 minutes. Add 1.0 ml of a freshly prepared 0.5 per cent w/v solution of *hydroquinone* containing 1 drop of *sulphuric acid* and 1.0 ml of a freshly prepared 20 per cent w/v solution of *anhydrous sodium sulphite*, mix and allow to stand for 30 minutes. Measure the absorbance of the resulting solution at the maximum at about 640 nm (2.4.7), using as the blank a solution prepared in the same manner by treating 2 ml of *water* instead of the solution of the preparation under examination. Calculate the content of phosphate from the absorbance obtained by simultaneously carrying out the operation using a known concentration of about 0.11 mg per ml of *dipotassium hydrogen phosphate* in *water* instead of the solution of the preparation under examination.

*For total chloride* — To 20.0 ml add 30 ml of *water*, 50.0 ml of *0.1 M silver nitrate* and 2 ml of *nitric acid.* Filter, wash the precipitate with *water* slightly acidified with *nitric acid* and titrate the excess of *silver nitrate* with *0.1 M ammonium thiocyanate* using *ferric ammonium sulphate solution* as indicator until a reddish yellow colour is produced. Carry out a blank titration.

1 ml of *0.1 M silver nitrate* is equivalent to 0.003545 g of total chloride, calculated as Cl.

*For dextrose* — To an accurately measured volume containing 2 g to 5 g of Dextrose, add 0.2 ml of *5 M ammonia* and sufficient *water* to produce 100.0 ml. Mix well, allow to stand for 30 minutes and determine the optical rotation in a 2-dm tube (2.4.22). The observed rotation in degrees multiplied by 0.9477 represents the weight, in g, of dextrose, $C_6H_{12}O_6$ in the volume taken for assay.

**Storage**. Store in single dose containers at a temperature not exceeding 30°.

**Labelling**. The label states (1) the content of each electrolyte in terms of millimoles in a given volume; (2) the amount of each ingredient in 100 ml; (3) the total osmolar concentration in mOsmol per litre; (4) that the preparation should not be used if it contains visible particles.

## Multiple Electrolytes and Dextrose Injection Type II

Multiple Electrolytes and Dextrose Injection Type II is a sterile solution of Dextrose and suitable salts in Water for Injections to provide sodium, potassium, calcium, magnesium and chloride ions. It may contain Hydrochloric Acid or Sodium Hydroxide used for adjusting the pH.

**Usual strength**.

| | |
|---|---|
| Sodium acetate | 0.33 g |
| Sodium chloride | 0.088 g |
| Potassium chloride | 0.12 g |
| Calcium chloride dihydrate | 0.037 g |
| Magnesium chloride | 0.031 g |
| Dextrose | 5.0 g |
| Water for Injections to | 100 ml |



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| Concentration of electrolytes in mmol / l | |
|---|---|
| Sodium | 40.0 |
| Potassium | 16.0 |
| Calcium | 2.5 |
| Chloride | 40.0 |
| Magnesium | 1.5 |
| Acetate | 24.0 |

Multiple Electrolytes and Dextrose Injection Type II contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of sodium, Na, potassium, K, calcium, Ca, magnesium, Mg, and acetate, $C_2H_3O_2$. It contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of chloride, Cl. It also contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of dextrose, $C_6H_{12}O_6$. It contains no antimicrobial agent.

**Description**. A clear, colourless or faintly straw-coloured solution.

## Identification

A. To 1 ml add 0.05 ml of *potassium cupri-tartrate solution*; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.

B. 20 ml gives the reactions of acetates, chlorides sodium salts, potassium salts, calcium salts and magnesium salts (2.3.1).

## Tests

**pH** (2.4.24). 3.0 to 7.0.

**5-Hydroxymethylfurfural and Related substances**. Dilute a volume containing 1.0 g of Dextrose to 500.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 284 nm; absorbance at about 284 nm, not more than 0.25 (2.4.7).

**Bacterial endotoxins** (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. *For total sodium* — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 589 nm and using *sodium solution FP* or *sodium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

*For potassium* — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 767 nm and using *potassium solution FP* or *potassium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

*For calcium* — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 422.7 nm and using *calcium solution FP* or *calcium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

*For magnesium* — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 296 nm and using *magnesium solution FP* or *magnesium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

*For acetate* — Determine by liquid chromatography (2.4.14).

*Test solution*. Dilute an accurately measured volume of the preparation under examination quantitatively with *water* to obtain a solution containing about 0.12 per cent w/v of Sodium Acetate.

*Reference solution*. Dissolve an accurately weighed quantity of *sodium acetate* in *water* to obtain a solution having a known concentration of about 0.12 per cent w/v of sodium acetate.

Chromatographic system

- a stainless steel column 30 cm × 7.8 mm, packed with strong cation-exchange resin consisting of sulphonated cross-linked styrene-divinylbenzene copolymer in the hydrogen form (7 µm) and a guard column 4 cm × 7.8 mm packed with the same column material,
- column. temperature 60°,
- mobile phase: *0.1 M sulphuric acid,*
- flow rate. 0.8 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject separately the test solution and the reference solution and measure the responses for the major peak and calculate the content of acetate in the preparation under examination.

*For total chloride* — To 20.0 ml add 30 ml of *water*, 50.0 ml of *0.1 M silver nitrate* and 2 ml of *nitric acid*. Filter, wash the precipitate with *water* slightly acidified with *nitric acid* and titrate the excess of *silver nitrate* with *0.1 M ammonium thiocyanate* using *ferric ammonium sulphate solution* as indicator until a reddish yellow colour is produced. Carry out a blank titration.

1 ml of *0.1 M silver nitrate* is equivalent to 0.003545 g of total chloride, calculated as Cl.



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*For dextrose* — To an accurately measured volume containing 2 g to 5 g of Dextrose, add 0.2 ml of *5 M ammonia* and sufficient *water* to produce 100.0 ml. Mix well, allow to stand for 30 minutes and determine the optical rotation in a 2-dm tube (2.4.22). The observed rotation in degrees multiplied by 0.9477 represents the weight, in g, of dextrose, $C_6H_{12}O_6$ in the volume taken for assay.

**Storage**. Store in single dose containers at a temperature not exceeding 30°.

**Labelling**. The label states (1) the content of each electrolyte in terms of millimoles in a given volume; (2) the amount of each ingredient in 100 ml; (3) the total osmolar concentration in mOsmol per litre; (4) that the preparation should not be used if it contains visible particles.

# Multiple Electrolytes and Dextrose Injection Type III

Multiple Electrolytes and Dextrose Injection Type III is a sterile solution of Dextrose and suitable salts in Water for Injections to provide sodium, potassium, acetate, chloride and phosphate ions. It may contain Hydrochloric Acid or Sodium Hydroxide used for adjusting the pH.

**Usual strength**.

| | |
|---|---|
| Sodium acetate | 0.28 g |
| Sodium chloride | 0.10 g |
| Potassium chloride | 0.15 g |
| Dipotassium hydrogen phosphate | 0.13 g |
| Dextrose | 5.0 g |
| Water for Injections to | 100 ml |
| Concentration of electrolytes in mmol / l | |
| Sodium | 37.0 |
| Potassium | 35.0 |
| Acetate | 20.0 |
| Chloride | 37.0 |
| Phosphate | 7.5 |

Multiple Electrolytes and Dextrose Injection Type III contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of sodium, Na, potassium, K, acetate, $C_2H_3O_2$, and phosphate, $PO_4$, and not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of chloride, Cl. It also contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of dextrose, $C_6H_{12}O_6$. It contains no antimicrobial agent.

**Description**. A clear, colourless or faintly straw-coloured solution.

## Identification

A. To 1 ml add 0.05 ml of *potassium cupri-tartrate solution*; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.

B. 20 ml gives the reactions of acetates, chlorides phosphates, sodium salts and potassium salts (2.3.1).

## Tests

**pH** (2.4.24). 3.0 to 7.0.

**5-Hydroxymethylfurfural and Related substances**. Dilute a volume containing 1.0 g of Dextrose to 500.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 284 nm; absorbance at about 284 nm, not more than 0.25 (2.4.7).

**Bacterial endotoxins** (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. *For total sodium* — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 589 nm and using *sodium solution FP* or *sodium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

*For potassium* — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 767 nm and using *potassium solution FP* or *potassium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

*For acetate* — Determine by liquid chromatography (2.4.14).

*Test solution*. Dilute an accurately measured volume of the preparation under examination quantitatively with *water* to obtain a solution containing about 0.12 per cent w/v of Sodium Acetate.

*Reference solution*. Dissolve an accurately weighed quantity of *sodium acetate* in *water* to obtain a solution having a known concentration of about 0.12 per cent w/v of sodium acetate.

Chromatographic system

- a stainless steel column 30 cm × 7.8 mm, packed with strong cation-exchange resin consisting of sulphonated cross-linked styrene-divinylbenzene copolymer in the hydrogen form (7 µm) and a guard column 4 cm × 7.8 mm packed with the same column material,
- column. temperature 60°,
- mobile phase: *0.1 M sulphuric acid,*
- flow rate. 0.8 ml per minute,



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– spectrophotometer set at 210 nm,
– injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject separately the test solution and the reference solution and measure the responses for the major peak and calculate the content of acetate in the preparation under examination.

*For phosphate* — Dilute an accurately measured volume containing about 4 mg of phosphate with sufficient *water* to produce 50.0 ml. Transfer 2.0 ml of this solution to a test-tube. Add 1.0 ml of a 5 per cent w/v solution of *ammonium molybdate* in a cooled mixture of *sulphuric acid* and *water* (15:85) and allow to stand for 3 minutes. Add 1.0 ml of a freshly prepared 0.5 per cent w/v solution of *hydroquinone* containing 1 drop of *sulphuric acid* and 1.0 ml of a freshly prepared 20 per cent w/v solution of *anhydrous sodium sulphite*, mix and allow to stand for 30 minutes. Measure the absorbance of the resulting solution at the maximum at about 640 nm (2.4.7), using as the blank a solution prepared in the same manner by treating 2 ml of *water* instead of the solution of the preparation under examination. Calculate the content of phosphate from the absorbance obtained by simultaneously carrying out the operation using a known concentration of about 0.11 mg per ml of *dipotassium hydrogen phosphate* in *water* instead of the solution of the preparation under examination.

*For total chloride* — To 20.0 ml add 30 ml of *water*, 50.0 ml of *0.1 M silver nitrate* and 2 ml of *nitric acid*. Filter, wash the precipitate with *water* slightly acidified with *nitric acid* and titrate the excess of *silver nitrate* with *0.1 M ammonium thiocyanate* using *ferric ammonium sulphate solution* as indicator until a reddish yellow colour is produced. Carry out a blank titration.

1 ml of *0.1 M silver nitrate* is equivalent to 0.003545 g of total chloride, calculated as Cl.

*For dextrose* — To an accurately measured volume containing 2 g to 5 g of Dextrose, add 0.2 ml of *5 M ammonia* and sufficient *water* to produce 100.0 ml. Mix well, allow to stand for 30 minutes and determine the optical rotation in a 2-dm tube (2.4.22). The observed rotation in degrees multiplied by 0.9477 represents the weight, in g, of dextrose, $C_6H_{12}O_6$ in the volume taken for assay.

**Storage**. Store in single dose containers at a temperature not exceeding 30°.

**Labelling**. The label states (1) the content of each electrolyte in terms of millimoles in a given volume; (2) the amount of each ingredient in 100 ml; (3) the total osmolar concentration in mOsmol per litre; (4) that the preparation should not be used if it contains visible particles.

# Multiple Electrolytes and Dextrose Injection Type IV

Multiple Electrolytes and Dextrose Injection Type IV is a sterile solution of Dextrose and suitable salts in Water for Injections to provide sodium, potassium, ammonium and chloride ions. It may contain Hydrochloric Acid or Sodium Hydroxide used for adjusting the pH.

**Usual strength**.

| | | |
|---|---|---|
| Sodium chloride | 0.37 | g |
| Potassium chloride | 0.13 | g |
| Ammonium chloride | 0.37 | g |
| Dextrose | 5.0 | g |
| Water for Injections to | 100 | ml |
| Concentration of electrolytes in mmol / l | | |
| Sodium | 63.0 | |
| Potassium | 17.0 | |
| Ammonium | 70.0 | |
| Chloride | 150.0 | |

Multiple Electrolytes and Dextrose Injection Type IV contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of sodium, Na, potassium, K, and ammonium, $NH_4$ and not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of chloride, Cl. It also contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of dextrose, $C_6H_{12}O_6$. It contains no antimicrobial agent.

**Description**. A clear, colourless or faintly straw-coloured solution.

## Identification

A. To 1 ml add 0.05 ml of *potassium cupri-tartrate solution*; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.

B. 20 ml gives the reactions of chlorides ammonium salts, sodium salts and potassium salts (2.3.1).

## Tests

**pH** (2.4.24). 3.0 to 7.0.

**5-Hydroxymethylfurfural and Related substances**. Dilute a volume containing 1.0 g of Dextrose to 500.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 284 nm: absorbance at about 284 nm, not more than 0.25 (2.4.7).

**Bacterial endotoxins** (2.2.3). Not more than 0.5 Endotoxin Unit per ml.



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**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. *For total sodium* — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 589 nm and using *sodium solution FP* or *sodium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

*For potassium* — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 767 nm and using *potassium solution FP* or *potassium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

*For ammonium* — Transfer an accurately measured volume of the preparation under examination, containing about 63 mg of ammonium, to a 500-ml Kjeldahl flask, dilute to 200 ml with *water*, mix and add 50 ml of 40 per cent w/v solution of *sodium hydroxide*. Connect the flask immediately to a well-cooled condenser through a distillation trap. Let the delivery tube from the condenser dip into 40 ml of a 4.0 per cent w/v solution of *boric acid* contained in a suitable receiver. Heat to boiling and distil about 200 ml. Cool the liquid in the receiver, if necessary, and titrate with *0.05 M sulphuric acid* using *methyl red solution* as indicator. Carry out a blank titration.

1 ml of *0.05 M sulphuric acid* is equivalent to 1.804 mg of ammonium, $NH_4$.

*For total chloride* — To 20.0 ml add 30 ml of *water*, 50.0 ml of *0.1 M silver nitrate* and 2 ml of *nitric acid.* Filter, wash the precipitate with *water* slightly acidified with *nitric acid* and titrate the excess of *silver nitrate* with *0.1 M ammonium thiocyanate* using *ferric ammonium sulphate solution* as indicator until a reddish yellow colour is produced. Carry out a blank titration.

1 ml of *0.1 M silver nitrate* is equivalent to 0.003545 g of total chloride, calculated as Cl.

*For dextrose* — To an accurately measured volume containing 2 g to 5 g of Dextrose, add 0.2 ml of *5 M ammonia* and sufficient *water* to produce 100.0 ml. Mix well, allow to stand for 30 minutes and determine the optical rotation in a 2-dm tube (2.4.22). The observed rotation in degrees multiplied by 0.9477 represents the weight, in g, of dextrose, $C_6H_{12}O_6$ in the volume taken for assay.

**Storage**. Store in single dose containers at a temperature not exceeding 30°.

**Labelling**. The label states (1) the content of each electrolyte in terms of millimoles in a given volume; (2) the amount of each ingredient in 100 ml; (3) the total osmolar concentration in mOsmol per litre; (4) that the preparation should not be used if it contains visible particles.

# Multiple Electrolytes and Dextrose Injection Type V

Multiple Electrolytes and Dextrose Injection Type V is a sterile solution of Dextrose and suitable salts in Water for Injections to provide sodium, potassium, calcium, magnesium, acetate, citrate and chloride ions. It may contain Hydrochloric Acid or Sodium Hydroxide used for adjusting the pH.

**Usual strength**.

| | |
|---|---|
| Sodium acetate | 0.64 g |
| Sodium chloride | 0.50 g |
| Sodium citrate | 0.075 g |
| Potassium chloride | 0.075 g |
| Calcium chloride | 0.035 g |
| Magnesium chloride | 0.031 g |
| Dextrose | 5.0 g |
| Water for Injections to | 100 ml |

| Concentration of electrolytes in mmol / l | |
|---|---|
| Sodium | 140.0 |
| Potassium | 10.0 |
| Calcium | 2.5 |
| Magnesium | 1.5 |
| Acetate | 47.0 |
| Chloride | 103.0 |
| Citrate | 2.5 |

Multiple Electrolytes and Dextrose Injection Type V contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of sodium, Na, potassium, K, and ammonium, $NH_4$ and not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of chloride, Cl. It also contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of dextrose, $C_6H_{12}O_6$. It contains no antimicrobial agent.

**Description**. A clear, colourless or faintly straw-coloured solution.

## Identification

A. To 1 ml add 0.05 ml of *potassium cupri-tartrate solution*; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.

B. 20 ml gives the reactions of acetates, chlorides citrates, sodium salts, potassium salts, calcium salts and magnesium salts (2.3.1).

## Tests

**pH** (2.4.24). 3.0 to 7.0.



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**5-Hydroxymethylfurfural and Related substances**. Dilute a volume containing 1.0 g of Dextrose to 500.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 284 nm; absorbance at about 284 nm, not more than 0.25 (2.4.7).

**Bacterial endotoxins** (2.2.3): Not more than 0.5 Endotoxin Unit per ml.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. *For total sodium* — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 589 nm and using *sodium solution FP* or *sodium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

*For potassium* — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 767 nm and using *potassium solution FP* or *potassium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

*For calcium* — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 422.7 nm and using *calcium solution FP* or *calcium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

*For magnesium* — To 50.0 ml add 50 ml of *water* and 5 ml of *strong ammonia-ammonium chloride solution* and titrate with *0.005 M disodium edetate* using 50 mg of *eriochrome black T mixture* as indicator.

1 ml of *0.005 M disodium edetate* is equivalent to 0.1215 mg of Mg.

*For acetate* — Determine by liquid chromatography (2.4.14).

*Test solution*. Dilute an accurately measured volume of the preparation under examination quantitatively with *water* to obtain a solution containing about 0.12 per cent w/v of Sodium Acetate.

*Reference solution*. Dissolve an accurately weighed quantity of *sodium acetate* in *water* to obtain a solution having a known concentration of about 0.12 per cent w/v of sodium acetate.

Chromatographic system
- a stainless steel column 30 cm × 7.8 mm, packed with strong cation-exchange resin consisting of sulphonated cross-linked styrene-divinylbenzene copolymer in the hydrogen form (7 µm) and a guard column 4 cm × 7.8 mm packed with the same column material,
- column. temperature 60°,
- mobile phase: *0.1 M sulphuric acid,*
- flow rate. 0.8 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject separately the test solution and the reference solution and measure the responses for the major peak and calculate the content of acetate in the preparation under examination.

*For citrate* — Determine by liquid chromatography (2.4.14).

*Test solution*. Preparation under examination.

*Reference solution*. Dissolve an accurately weighed quantity of anhydrous *sodium citrate*, previously dried at 180° for 18 hours, in *water* to obtain a stock solution having a known concentration of about 10 mg per ml. Dilute accurately measured volumes of this solution quantitatively with *water* to obtain three solutions having known concentrations of about 0.5, 1.0 and 2.0 mg, respectively of anhydrous sodium citrate per ml.

Chromatographic system
- a stainless steel column 30 cm × 7.8 mm, packed with strong cation-exchange resin consisting of sulphonated cross-linked styrene-divinylbenzene copolymer in the hydrogen form (7 µm) and a guard column 4 cm × 7.8 mm packed with the same column material,
- column. temperature 60°,
- mobile phase: *0.1 M sulphuric acid,*
- flow rate. 0.8 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

Inject the reference solution containing 1.0 mg of anhydrous sodium citrate per ml. The test is not valid unless the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject separately the test solution and all the three preparations of reference solution and measure the responses for the major peak. Plot the responses of all the three preparations of reference solution versus concentration, in mg of anhydrous sodium citrate per ml, and draw the straight line best fitting the three plotted points. From the graph so obtained, calculate the content of citrate in mg equivalent to anhydrous sodium citrate per litre of the preparation under examination.

*For total chloride* — To 20.0 ml add 30 ml of *water*, 50.0 ml of *0.1 M silver nitrate* and 2 ml of *nitric acid*. Filter, wash the precipitate with *water* slightly acidified with *nitric acid* and titrate the excess of silver nitrate with *0.1 M ammonium thiocyanate* using *ferric ammonium sulphate solution* as indicator until a reddish yellow colour is produced. Carry out a blank titration.



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1 ml of *0.1 M silver nitrate* is equivalent to 0.003545 g of total chloride, calculated as Cl.

*For dextrose* —To an accurately measured volume containing 2 g to 5 g of Dextrose, add 0.2 ml of *5 M ammonia* and sufficient *water* to produce 100.0 ml. Mix well, allow to stand for 30 minutes and determine the optical rotation in a 2-dm tube (2.4.22). The observed rotation in degrees multiplied by 0.9477 represents the weight, in g, of dextrose, $C_6H_{12}O_6$ in the volume taken for assay.

**Storage**. Store in single dose containers at a temperature not exceeding 30°.

**Labelling**. The label states (1) the content of each electrolyte in terms of millimoles in a given volume; (2) the amount of each ingredient in 100 ml; (3) the total osmolar concentration in mOsmol per litre; (4) that the preparation should not be used if it contains visible particles.

# Multiple Electrolytes Injection Type VI

Multiple Electrolytes Injection Type VI is a sterile solution of suitable salts in Water for Injections to provide sodium, potassium, calcium, magnesium, acetate, citrate and chloride ions. It may contain Hydrochloric Acid or Sodium Hydroxide used for adjusting the pH.

**Usual strength.**

| | |
|---|---|
| Sodium acetate | 0.64 g |
| Sodium chloride | 0.5 g |
| Sodium citrate | 0.075 g |
| Potassium chloride | 0.075 g |
| Calcium chloride | 0.035 g |
| Magnesium chloride | 0.031 g |
| Water for Injections to | 100 ml |

| Concentration of electrolytes in mmol / l | |
|---|---|
| Sodium | 140.0 |
| Potassium | 10.0 |
| Calcium | 2.5 |
| Magnesium | 1.5 |
| Acetate | 47.0 |
| Chloride | 103.0 |
| Citrate | 2.5 |

Multiple Electrolytes Injection Type VI contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of sodium, Na, potassium, K, calcium, Ca, magnesium, Mg, acetate, $C_2H_3O_2$, and citrate, $C_6H_5O_7$. It also contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of chloride, Cl. It contains no antimicrobial agent.

**Description**. A clear, colourless or faintly straw-coloured solution.

## Identification

20 ml gives the reactions of acetates, chlorides citrates, sodium salts, potassium salts, calcium salts and magnesium salts (2.3.1).

## Tests

**pH** (2.4.24). 3.0 to 7.0.

**Bacterial endotoxins** (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

**Other tests**. Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**. *For total sodium* — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 589 nm and using *sodium solution FP* or *sodium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

*For potassium* — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 767 nm and using *potassium solution FP* or *potassium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

*For calcium* — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 422.7 nm and using *calcium solution FP* or *calcium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

*For magnesium* — To 50.0 ml add 50 ml of *water* and 5 ml of *strong ammonia-ammonium chloride solution* and titrate with *0.005 M disodium edetate* using 50 mg of *eriochrome black T mixture* as indicator.

1 ml of *0.005 M disodium edetate* is equivalent to 0.1215 mg of Mg.

*For acetate* — Determine by liquid chromatography (2.4.14).

*Test solution*. Dilute an accurately measured volume of the preparation under examination quantitatively with *water* to obtain a solution containing about 0.12 per cent w/v of *Sodium Acetate*.

*Reference solution*. Dissolve an accurately weighed quantity of *sodium acetate* in *water* to obtain a solution having a known concentration of about 0.12 per cent w/v of sodium acetate.



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Chromatographic system
- a stainless steel column 30 cm × 7.8 mm, packed with strong cation-exchange resin consisting of sulphonated cross-linked styrene-divinylbenzene copolymer in the hydrogen form (7 µm) and a guard column 4 cm × 7.8 mm packed with the same column material,
- column. temperature 60°,
- mobile phase: *0.1 M sulphuric acid,*
- flow rate. 0.8 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject separately the test solution and the reference solution and measure the responses for the major peak and calculate the content of acetate in the preparation under examination.

*For citrate* — Determine by liquid chromatography (2.4.14).

*Test solution.* Preparation under examination.

*Reference solution.* Dissolve an accurately weighed quantity of anhydrous *sodium citrate*, previously dried at 180° for 18 hours, in *water* to obtain a stock solution having a known concentration of about 10 mg per ml. Dilute accurately measured volumes of this solution quantitatively with *water* to obtain three solutions having known concentrations of about 0.5, 1.0 and 2.0 mg, respectively of anhydrous sodium citrate per ml.

Chromatographic system
- a stainless steel column 30 cm × 7.8 mm, packed with strong cation-exchange resin consisting of sulphonated cross-linked styrene-divinylbenzene copolymer in the hydrogen form (7 µm) and a guard column 4 cm × 7.8 mm packed with the same column material,
- column. temperature 60°,
- mobile phase: *0.1 M sulphuric acid,*
- flow rate. 0.8 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

Inject the reference solution containing 1.0 mg of anhydrous *sodium citrate* per ml. The test is not valid unless the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject separately the test solution and all the three preparations of reference solution and measure the responses for the major peak. Plot the responses of all the three preparations of reference solution versus concentration, in mg of anhydrous sodium citrate per ml, and draw the straight line best fitting the three plotted points. From the graph so obtained, calculate the content of citrate in mg equivalent to anhydrous sodium citrate per litre of the preparation under examination.

*For total chloride* — To 20.0 ml add 30 ml of *water*, 50.0 ml of *0.1 M silver nitrate* and 2 ml of *nitric acid*. Filter, wash the precipitate with *water* slightly acidified with *nitric acid* and titrate the excess of silver nitrate with *0.1 M ammonium thiocyanate* using *ferric ammonium sulphate solution* as indicator until a reddish yellow colour is produced. Carry out a blank titration.

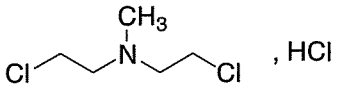
1 ml of *0.1 M silver nitrate* is equivalent to 0.003545 g of total chloride, calculated as Cl.

**Storage.** Store in single dose containers at a temperature not exceeding 30°.

**Labelling.** The label states (1) the content of each electrolyte in terms of millimoles in a given volume; (2) the amount of each ingredient in 100 ml; (3) the total osmolar concentration in mOsmol per litre; (4) that the preparation should not be used if it contains visible particles.

# Mustine Hydrochloride

Nitrogen Mustard




$C_5H_{11}Cl_2N,HCl$ Mol Wt. 192.4

Mustine Hydrochloride is bis(2-chloroethyl)methylamine hydrochloride.

Mustine Hydrochloride contains not less than 98.0 per cent and not more than 101.0 per cent of $C_5H_{11}Cl_2N$, HCl.

**Category.** Anticancer.

**Dose.** By intravenous Injection, infused with normal saline, 0.1 mg per kg of body weight, daily for three to four days. Maximum single dose, 8 mg.

**Description.** A white or almost white, crystalline powder or mass; hydroscopic; vesicant.

## Identification

A. Dissolve 50 mg in 5 ml of *water* and add 1 ml of *5 M sodium hydroxide*; oily globules are produced which dissolve on warming.

B. Dissolve 50 mg in 5 ml of *water* and add 0.02 ml of *potassium mercuri-iodide solution*; a cream-coloured precipitate is produced.

A. Melts at about 108° (2.4.21).



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### Tests

**Assay**. Weigh accurately about 0.2 g, add 15 ml of *1 M ethanolic potassium hydroxide* and 15 ml of *water* and boil under a reflux condenser for 2 hours. Evaporate the solution to half its volume on water-bath, dilute to 150 ml with *water*, add 3 ml of *nitric acid* and 50.0 ml of *0.1 M silver nitrate*. Shake vigorously and filter. Wash the residue with *water* and titrate the excess of silver nitrate in the combined filtrate and washings with *0.1 M ammonium thiocycanate* using 1 ml of *ferric ammonium sulphate solution* as indicator.

1 ml of *0.1 M silver nitrate* is equivalent to 0.006418 g of $C_5H_{11}Cl_2N$, HCl.

**Storage**. Store protected from light and moisture at a temperature not exceeding 30°.

**Labeling**. The label states that the contents of the container are strongly vesicant.

## Mustine Injection

Mustine Hydrochloride Injection

Mustine Injection is a sterile material consisting of Mustine Hydrochloride with or without buffering agents and other excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections or Sodium Chloride Intravenous Infusion, immediately before use.

*The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).*

**Storage**. The constituted solution deteriorates rapidly on storage and should be used immediately after preparation.

Mustine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of the stated amount of mustine hydrochloride, $C_5H_{11}Cl_2N$,HCl.

**Usual strength**. 10 mg.

*The contents of the sealed container complies with the tests stated under Parenteral Preparations (Powders for Injection) and with the following requirements.*

### Identification

Dissolve about 20 mg in 1 ml of *water* and add 0.02 ml of *potassium mercuri-iodide solution*; a cream-coloured precipitate is produced.

### Tests

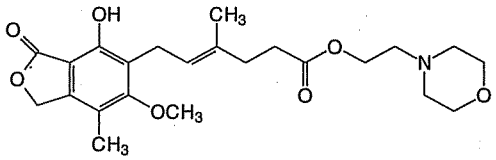
**Assay**. Determine the weight of the contents of 10 containers. Weigh accurately a quantity of the mixed contents of the ten containers, containing 40 mg of Mustine Hydrochloride, dissolve in 10 ml of *ethanol (95 per cent)*, previously neutralised to dilute *phenolphthalein solution*. Titrate with *0.01 M sodium hydroxide* using *dilute phenolphthalein solution* as indicator.

1 ml of *0.01 M sodium hydroxide* is equivalent to 0.001925 g of $C_5H_{11}Cl_2N$, HCl.

**Storage**. Store protected from moisture at a temperature not exceeding 30°.

**Labelling**. The label states (1) that the contents are strongly vesicant; (2) the amount of Mustine Hydrochloride in the container, (3) that the injection should be used immediately after preparation.

## Mycophenolate Mofetil




$C_{23}H_{31}NO_7$ Mol. Wt. 433.5

Mycophenolate Mofetil is 2-(morpholin-4-yl)ethyl (4*E*)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoate.

Mycophenolate Mofetil contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{23}H_{31}NO_7$, calculated on the dried basis.

**Category**. Anticancer.

**Description**. A white or almost white, crystalline powder.

### Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mycophenolate mofetil RS* or with the reference spectrum of mycophenolate mofetil.

### Tests

**Related substances**. Determine by liquid chromatography (2.4.14).

*NOTE—Prepare the solutions immediately before use. Protect the solutions from light.*

*Test solution*. Dissolve 200 mg of the substance under examination in 100.0 ml of *acetonitrile*.



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*Reference solution*. A 0.001 per cent w/v solution of *mycophenolate mofetil RS* in *acetonitrile.*

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (such as Zorbax SB-C8),
- column temperature. 45°,
- sample temperature. 10°,
- mobile phase: a mixture 65 volumes of *water*, 2 volumes of *triethylamine*, adjusted to pH 5.3 with *orthophosphoric acid* and 35 volumes of *acetonitrile*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 250 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 5.0 per cent. The relative retention time with reference to the principal peak for impurity F is about 0.3, for impurity A is about 0.4, for impurity H is about 0.5, for impurity G is about 0.6, for impurity B is about 0.8, for impurity D is about 1.2 and for impurity E is about 1.6.

Inject the reference solution and the test solution. Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of peak due to impurity A, B, D, E, F, G, H or any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent).

**Heavy metals** (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Loss on drying** (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying it at 60° for 3 hours under vacuum.

**Assay**. Weigh accurately about 0.4 g and dissolve in about 50 ml of *glacial acetic acid* and titrate with *0.1 M perchloric acid,* determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04335 g of $C_{23}H_{31}NO_7$.

**Storage**. Store protected from light and moisture.

# Mycophenolate Mofetil Capsules

Mycophenolate Mofetil Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of mycophenolate mofetil, $C_{23}H_{31}NO_7$.

## Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. When examined in the range 200 nm to 400 nm (2.4.7), a 0.05 per cent w/v solution in *methanol* shows an absorption maximum as obtained with *mycophenolate mofetil RS* of the same concentration.

## Tests

**Dissolution** (2.5.2).

Apparatus No. 2,
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

*Test solution*. Dilute the filtrate, if necessary, with the dissolution medium.

*Reference solution*. Dissolve a quantity of *mycophenolate mofetil RS* in *methanol* and dilute with dissolution medium to obtain a solution having a known concentration similar to the test solution.

Chromatographic system as described under Assay.

Inject the test solution and the reference solution.

Calculate the content of $C_{23}H_{31}NO_7$ in the capsule.

D. Not less than 75 per cent of the stated amount of $C_{23}H_{31}NO_7$.

**Related substances**. Determine by liquid chromatography (2.4.14).

*NOTE—Use freshly prepared solutions.*

*Test solution*. Mix the contents of 20 capsules. Disperse a quantity of the mixed content containing about 200 mg of Mycophenolate with *acetonitrile,* sonicate for 15 minutes and dilute to 200.0 ml with *acetonitrile.*

*Reference solution*. A 0.001 per cent w/v solution of *mycophenolate mofetil RS* in *acetonitrile.*

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Inertsil ODS-3),
- mobile phase: a mixture of 40 volumes of *acetonitrile* and 60 volumes of buffer solution prepared by dissolving 13.6 g of *potassium dihydrogen orthophosphate anhydrous* in 1000 ml of *water*, adjusted to pH 3.6 with *orthophosphoric acid* and 4.0 g of *sodium heptane sulphonate*,



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- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the reference solution and the test solution. Run the chromatogram 2.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent); the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (2.0 per cent).

**Water** (2.3.43). Not more than 4.0 per cent, determined on 0.5 g.

**Other tests**. Comply with the tests stated under Capsules.

**Assay**. Determine by liquid chromatography (2.4.14).

*NOTE—Use freshly prepared solutions.*

*Test solution.* Disperse a quantity of the content of the capsules containing about 500 mg of Mycophenolate Mofetil with *methanol*, sonicate for 15 minutes and dilute to 500.0 ml with the *methanol*. Dilute 5.0 ml of this solution to 100.0 ml with the mobile phase.

*Reference solution.* Dissolve 10 mg of *mycophenolate mofetil RS* in sufficient amount of *methanol*, sonicate for 15 minutes and dilute to 20.0 ml with *methanol*. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Inertsil ODS- 3V),
- mobile phase: a mixture of 50 volumes of *acetonitrile* and 50 volumes of buffer solution prepared by dissolving 13.6 g of *potassium dihydrogen orthophosphate anhydrous* in 1000 ml with *water*, adjusted to pH 3.6 with *orthophosphoric acid* and dissolve 4.0 g of *sodium heptane sulphonate,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 305 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the theoretical plates is not less than 2000 and the tailing factor is not more than 2.0. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference sollution and the test solution.

Calculate the content of $C_{23}H_{31}NO_7$ in the capsules.

**Storage**. Store protected from light, at a temperature not exceeding 30°.

# Myristic Acid

$$CH_3(CH_2)_{12}COOH$$

$C_{14}H_{28}O_2$ Mol. Wt. 228.4

Myristic acid is tetradecanoic acid.

Myristic Acid is obtained from coconut oil and other fats.

Myristic Acid contains not less than 97.0 per cent of $C_{14}H_{28}O_2$.

**Category**. Pharmaceutical aid.

## Tests

**Congealing temperature** (2.4.10). 48° to 55.5°.

**Acid value** (2.3.23). 242 to 249.

**Saponification value** (2.3.37). 242 to 251.

**Iodine value** (2.3.28). Not more than 1.0.

**Unsaponifiable matter** (2.3.39). Not more than 1.0 per cent.

**Sulphated ash** (2.3.18). Not more than 0.1 per cent.

**Water** (2.3.43). Not more than 0.2 per cent.

**Lead**. Determine by atomic absorption spectrophotometry (2.4.2), measuring at 283.3 nm using a lead electrodeless discharge lamp, an air acetylene flame, and a suitable burner head, preferably a slit-width of 0.7 nm.

*NOTE—Select reagents having as low a lead content as practicable, and store all solutions in high-density polyethylene containers. Rinse all plastic and glassware thoroughly with warm, 8 M nitric acid followed by deionized water.*

*Test solution.* Weigh accurately about 5 g of Myristic Acid to an evaporating dish, add 5 ml of a 25 per cent *sulphuric acid solution* and distribute the *sulphuric acid* uniformly through the sample. Within a hood, place the dish on a steam bath to evaporate most of the *water*. Place the dish on a burner, and slowly pre-ash the sample by expelling most of the *sulphuric acid*. Place the dish in a muffle furnace that has been set at 525°, and ash the sample until the residue appears free from carbon. Prepare a blank by ashing 5 ml of 10 per cent *sulphuric acid solution*. Cool, and cautiously wash down the inside of each evaporation dish with water. Treat both the sample and the blank as follows. Add 5 ml of *1 M hydrochloric acid*. Place each dish on a steam bath, and evaporate to dryness. To each dish add 1.0 ml of *3 M hydrochloric acid* and approximately 5 ml of *water*, and heat briefly on a steam bath to dissolve any residue. Transfer each solution quantitatively to a 10-ml volumetric flask, dilute with water to volume, and mix.



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*Reference solution (a)*. Weigh accurately about 0.16 g of *lead nitrate* in 1 per cent v/v solution of *nitric acid*. Dilute to 1000 ml with *water* and mix.

*Reference solution (b)*. Dissolve 10.0 ml of reference solution (a) in *water* to a 100-ml volumetric flask and mix. 1 ml of this solution contains the equivalent of about 10 µg of lead. Dilute accurately measured volumes of the diluted reference solution (a) with *water* to obtain solutions having known concentrations of about 1 µg, 2 µg, and 5 µg of lead per ml.

*NOTE—Prepare these solutions on the day of use.*

Calculate the content of lead, in the substance under examination.

**Assay**. Determine by gas chromatography (2.4.13).

*Test solution*. Dissolve 100 mg of the substance to a 50-ml conical flask fitted with a suitable water-cooled reflux condenser and a magnetic stir bar. Add 4 ml of *0.5 M sodium hydroxide solution methanolic*, and reflux until fat globules disappear (usually 5 to 10 minutes). Add 5 ml of a solution prepared by dissolving 14 g of boron trifluoride in *methanol* to make 100 ml, swirl to mix, and reflux for 2 minutes. Add 4 ml of chromatographic *n-heptane* through the condenser, and reflux for 1 minute. Cool, remove the condenser, add about 15 ml of *saturated sodium chloride solution*, shake, and allow the layers to separate. Pass the n-heptane layer through 0.1 g of *anhydrous sodium sulphate* (previously washed with chromatographic *n-heptane*) into a suitable flask. Transfer 1.0 ml of this solution to a 10-ml volumetric flask, dilute with chromatographic *n-heptane* to volume, and mix.

*Reference solution (a)*. Prepare as directed for the test solution using 100 mg of *myristic acid RS* instead of the substance under examination.

*Reference solution (b)*. Dissolve about 20 mg each of stearic acid, palmitic acid and oleic acid to a 25-ml conical flask fitted with a suitable water-cooled reflux condenser and a magnetic stir bar, and proceed as directed for the test solution, beginning with "Add 5.0 ml of a solution prepared by dissolving."

Chromatographic system

- a fused-silica capillary column 30 m x 0.53 mm, packed with bonded with a 1.0 µm layer of phase G16,
- temperature:
  column.70° for 2 minutes and 240° for 5 minutes,
  inlet port. 220°,
- a flame ionisation detector,
- flow rate. 30 ml per minute using nitrogen as the carrier gas.

Inject reference solution (b). The test is not valid unless the resolution between the peaks corresponding to methyl stearate and methyl palmitate is not less than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject 1 µl of reference solution (a) and the test solution.

Calculate the content $C_{14}H_{28}O_2$.

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