Procedure for the elaboration, revision and omission of monographs and other texts for *The International Pharmacopoeia*

2020-01

1. Introduction

Monographs in *The International Pharmacopoeia* (1) are essential standards to ensure the quality of medicines, thus contributing to their safe and efficacious use. They are developed and maintained in an open and transparent process, in line with the principles outlined in the Good Pharmacopoeial Practices (GPhP) (2) and aimed to foster harmonization and convergence of compendial quality standards to ultimately increase access to affordable, quality-assured medicines.

The following procedure describes the life cycle of texts in *The International Pharmacopoeia*: how they are developed, revised and, if appropriate, finally omitted from the compendium. The text also includes steps related to the establishment of the International Chemical Reference Substances (ICRS) referred to in analytical tests. It replaces the previous version of the document (3).

2. Elaboration of monographs

The steps of the development procedure are as follows[1],[2] :

Step 1:	Identify medicines for which pharmacopoeial monographs need to be developed or revised. Set up a biannual work plan prioritizing medicines that are included in the WHO Model List of Essential Medicines (EML) (or are otherwise relevant for WHO health programmes), preferably not already described in pharmacopoeias. Determine whether or not monographs for the corresponding active pharmaceutical ingredients also need to be developed or revised. Confirm the work plan with all WHO parties concerned, including the Department of Essential Medicines and Health Products, specific disease programmes and the Prequalification Team – Medicines (PQT).
Step 2:	Search for relevant information on the product in the public domain, including other pharmacopoeias.
Step 3:	Share the work plan with other pharmacopoeias and identify ways of collaboration to reduce the workload of the monograph development and to promote converged or harmonized quality standards that are globally applicable and recognized.
Step 4:	Contact manufacturers of WHO prequalified medicines and/or of medicines authorized by WHO listed national regulatory systems with an appropriate maturity level [3] to request quality control (QC) specifications and samples of their products.
Step 5:	Assign WHO Collaborating Centres, collaborating laboratories and/or specific experts, if appropriate, to participate in the establishment or revision of the monograph.
Step 6:	Set up a first version of the monograph based on the available information and on discussions with the partners involved. Perform laboratory investigations to develop, adapt, optimize, verify or validate the proposed analytical procedures. Verify the suitability of the proposed specifications by analyzing medicines from different regions or markets of the world. Identify which of the required reference substances would need to be newly established or are already available either as ICRS or as reference substances established by another pharmacopoeia. In case reference is made to already established ICRS or reference substances established by other pharmacopoeias, include these reference substances in the laboratory investigations and advise on their suitability for the new intended use(s). Issue a laboratory report describing the tests performed and the results obtained. Based on mutual agreements, share the laboratory report with other pharmacopoeias with a view to foster harmonization and convergence of compendial quality standards.
Step 7:	Follow the consultative process of the WHO Expert Committee on Specifications for Pharmaceutical Preparations (ECSPP). Circulate the draft text for comments and provide the document on the website of *The International Pharmacopoeia*.
Step 8:	Collate the comments received during the public consultation and review them with the partners involved. If necessary, arrange with the laboratories involved for additional laboratory investigations.
Step 9:	Discuss the comments received and, if applicable, the results of the additional investigations at an informal consultation with experts. Revise the draft text based on the discussions, as appropriate.
Step 10:	Repeat steps 7 to 9 until the text is deemed suitable for adoption.
Step 11:	Identify and contact manufacturers (or other potential donors of candidate materials) to ascertain the availability of candidate materials for the establishment of the ICRSs described in the text. Discuss with the organization for the establishment, storage and distribution of ICRS, the EDQM, the strategy to establish the proposed ICRSs and its impact on the analytical provisions of the monograph.
Step 12:	Submit the draft monograph (together with the laboratory report and a compilation of the comments received during the public consultation) to the ECSPP for information, discussion and/or possible adoption, depending on the maturity of the monograph. If the text is adopted, proceed with step 13. If not, repeat steps 7 to 11.
Step 13:	Incorporate all changes agreed during the final discussions leading to adoption, together with any editorial changes.
Step 14:	Confirm the final text with the experts and laboratories involved in the final discussions and publish the adopted monograph in a new edition or supplement of *The International Pharmacopoeia*. [4]
Step 15:	Identify already established ICRS referred to in the monograph. Review the ICRS establishment report(s) to evaluate if the intended uses and the quantity per vial are still valid and appropriate or need to be amended or revised in view of the analytical provisions of the new standard.
Step 16:	Identify newly to-be-established ICRS referred to in the monograph. Revert to potential donors of candidate material

(Step 11) and initiate the shipment of the material to the organization in charge of ICRSs.

Step 17: Perform laboratory investigations to characterize the candidate material and/or to ensure the suitability of the material for its new or revised intended uses. Issue an ICRS establishment or re-establishment report. If information in the ICRS leaflet of already established ICRS has to be revised, assign a new batch number to the ICRS.

Step 18: Submit the establishment report to the ICRS Board. Start the distribution of the ICRS after the reference substance is released by the ICRS Board and the corresponding new monograph is published.

Step 19: Submit the ICRS report to the ECSPP to confirm the release of the reference standard and/or the change(s) in the leaflets.

3. Omission of monographs

Step 1: Identify monographs on medicines (or other pharmaceutical products) that are described in *The International Pharmacopoeia* but are no longer included in the EML or otherwise relevant for WHO health programmes.

Step 2: Submit the list of monographs (and other texts) proposed for omission to the ECSPP for possible approval.

Step 3: Transfer omitted texts to a publicly accessible archive section on the WHO website, together with the following note: "*These monographs will neither be updated or revised, nor will the corresponding International Chemical Reference Substances be further monitored. Users will need to ensure that the product complies with current rules and regulations governing medicines and related products in their respective territories*."

Step 4: Remove the ICRS referred to in omitted monographs from the ICRS catalogue one year after the monograph has been transferred to the archive page on the WHO website.

4. Elaboration, revision and omission of other pharmacopoeial texts

In principle, the steps outlined above apply to all texts. Some specific texts may, however, necessitate deviations. The steps in the development of pharmacopoeial texts, however, shall always include public consultation, consideration of comments received, if appropriate, and adoption of the texts by the ECSPP.

References

1. The International Pharmacopoeia, 9th ed. Geneva: World Health Organization; 2019.

2. Good pharmacopoeial practices. In: WHO Expert Committee on Specifications for Pharmaceutical Preparations, Fiftieth report. Geneva: World Health Organization; 2016: Annex 1 (WHO Technical Report Series, No. 996).

3. Procedure for the development of monographs and other texts for The International Pharmacopoeia. In: WHO Expert Committee on Specifications for Pharmaceutical Preparations, Forty-ninth report. Geneva: World Health Organization; 2015: Annex 1 (WHO Technical Report Series, No. 992).

[1] The procedure for the elaboration, revision and omission of monographs and other texts for *The International Pharmacopoeia* was developed by the Secretariat of *The International Pharmacopoeia* in consultation with the partners involved: Expert, WHO Collaborating Centre, collaborating laboratories and the organization for the establishment, storage and distribution of ICRS, the European Directorate for the Quality of Medicines & HealthCare (EDQM). The steps are therefore described from the perspective of all partners involved.

[2] The steps are listed in their chronological order. However, certain steps may overlap during the development of monographs and other compendial texts.

[3] It is intended to refer in a future version of the document to the WHO Global Benchmarking Tool (GBT), which is currently under discussion.

[4] Subject to the availability of the necessary resources, the Secretariat aims to publish adopted texts for inclusion in *The International Pharmacopoeia* after each meeting of the ECSPP.

Levomethorphan limit test for dextromethorphan containing finished pharmaceutical products

2016-01

Dextromethorphan-containing medicines shall contain Dextromethorphan hydrobromide which complies with all the requirements of the respective monograph and other applicable chapters of *The International Pharmacopoeia*. In particular, the concentration of impurity E (levomethorphan) shall not exceed the limit of 0.1% (see monograph on Dextromethorphan hydrobromide).

The following tests allow control laboratories (e.g. national quality control laboratories) to test suspicious dextromethorphan-containing medicines to establish whether or not an active pharmaceutical ingredient (API) meeting the limit for impuritiy E (levomethorphan) had been used to manufacture the product under examination.

In many cold and cough medicines dextromethorphan is used in combination with other active ingredients, for example, chlorpheniramine, doxylamine, ephedrine, paracetamol, phenylpropanolamine, pseudoephedrine, promethazine or triprolidine. Due to the diversity of these substances the selectivity of the test procedures described below may not be sufficient for all products under investigation. If the chromatogram obtained provides evidence that other active ingredients or excipients interfere with the levomethorphan determination the analyst shall modify the analytical procedure, e.g. by adding further extraction steps.

Also depending on the additional active ingredients or the excipients in the product to be examined it may be necessary to flush the column with a mobile phase consisting of 950 volumes of 2-propanol R, 50 volumes of n-hexane R and 1 volume of diethylamine R after each run.

Limit test for levomethorphan in dextromethorphan containing oral solutions

Carry out the test as described under *1.14.1 Chromatography*, High-performance liquid chromatography using a stainless steel column (25 cm × 4.6 mm) packed with particles of silica gel coated with cellulose tris(4-methylbenzoate) groups (5 µm). As the mobile phase use a mixture of 940 volumes of n-hexane R, 60 volumes of 2-propanol R and 1 volume of diethylamine R.

Operate with a flow rate of 0.5 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 285 nm. Maintain the column at 30 °C.

Prepare the following solutions. For solution (1) transfer a quantity of the oral solution containing the equivalent of 50 mg of Dextromethorphan hydrobromide to a separating funnel. Add sodium hydroxide (~40 g/L) TS until the solution has a pH value greater than 11 (check the value using pH-indicator paper). Extract the solution with three 50 mL volumes of hexane R. Dry the combined extracts over 3 g anhydrous sodium sulphate R, filter, wash the residue with 30 mL of hexane R, combine the hexane extracts in a round-bottom flask and evaporate to dryness. Add 2.0 mL of 2-propanol R to dissolve the residue and transfer the solution to a 10.0 mL volumetric flask, wash the round-bottom flask with further 2.0 mL of 2-propanol R and also transfer the solution to the 10.0 mL flask. Dilute to volume with mobile phase. For solution (2) dilute 5.0 mL of solution (1) to 100.0 mL with mobile phase. Dilute 2.0 mL of this solution to 100.0 mL with mobile phase. For solution (3) transfer 2 mg of dextromethorphan for system suitability RS (containing a mixture of dextromethorphan and impurity E (levomethorphan)) in a 25.0 mL volumetric flask, add 20 mL of mobile phase, sonicate for about 5 minutes and make up to volume with mobile phase.

Inject 20 µL of solution (3). The test is not valid unless the resolution factor between the peaks due to levomethorphan (retention time about 9 minutes) and dextromethorphan (retention time of about 12 minutes) is at least 3.

Inject alternately 20 µL each of solutions (1) and (2).

In the chromatogram obtained with solution (1) the area of any peak corresponding to levomethorphan is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

Limit test for levomethorphan in dextromethorphan containing capsules and lozenges

Carry out the test as described under *1.14.1 Chromatography*, High-performance liquid chromatography using the chromatographic conditions given under “Limit test for levomethorphan in dextromethorphan oral solutions”.

For solution (1) transfer a quantity of the contents of the capsules (hard gelatin capsules)/transfer a number of capsules (soft gelatin capsules) or lozenges, containing the equivalent of about 50 mg of Dextromethorpan hydrobromide to a 100 mL conical flask, add about 50 mL of water and heat and shake on a steam bath for about 15 minutes. Allow to cool, filter and transfer the eluate to a separating funnel. Wash the flask and the filtrate with 2 times 10 mL of water. Combine the aqueous solutions and add sodium hydroxide (~40 g/L) TS until the solution has a pH value greater than 11 (check the value using pH-indicator paper). Extract with three 50 mL volumes of hexane R. Dry the combined extracts over 3 g anhydrous sodium sulphate R, filter, wash the residue with 30 mL of hexane R, combine the hexane extracts in a round-bottom flask and evaporate to dryness. Add 2.0 mL of 2-propanol R to dissolve the residue and transfer the solution to a 10.0 mL volumetric flask, wash the round-bottom flask with further 2.0 mL of 2-propanol and also transfer the solution to the 10.0 mL flask. Dilute to volume with mobile phase. For solution (2) dilute 5.0 mL of solution (1) to 100.0 mL with mobile phase. Dilute 2.0 mL of this solution to 100.0 mL with mobile phase. Prepare solution (3) as indicated in the leaflet of Dextromethorphan for system suitability RS (containing a mixture of dextromethorphan and levomethorphan).

Inject 20 µL of solution (3). The test is not valid unless the resolution factor between the peaks due to levomethorphan (retention

time about 9 minutes) and dextromethorphan (retention time of about 12 minutes) is at least 3.

Inject alternately 20 µL each of solutions (1) and (2).

In the chromatogram obtained with solution (1) the area of any peak corresponding to levomethorphan is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

Recommendations for quality requirements when plant-derived artemisinin is used as a starting material in the production of antimalarial active pharmaceutical ingredients [1]

2014-01

[1] WHO Expert Committee on Specifications for Pharmaceutical Preparations, Forty-sixth report. Geneva, World Health Organization, 2012, Annex 6 (WHO Technical Report Series, No. 970).

1. Introduction

The harmonized good manufacturing practices (GMP) (*1*,*2*) describe requirements for the production of active pharmaceutical ingredients (APIs). The applicability of these requirements begins with a defined starting material as follows:

"An API starting material is a raw material, intermediate or an API that is used in the production and that is incorporated as a significant structural fragment into the structure of the API. An API starting material can be an article of commerce, a material purchased from one or more suppliers under contract or commercial agreement or produced in-house. API starting materials normally have defined chemical properties and structure."

The focus of GMP for APIs is for field inspector use rather than in applications for marketing authorization. It defines what may be considered as a starting material and provides guidance on where GMP is applied. The GMP guidelines do not apply to steps taken prior to the first introduction of the defined starting material. The manufacturer should designate and document the rationale for the point at which production of the API begins. For a synthesis process this is known as the point at which the starting materials are entered into processes.

From a regulatory standpoint the use of API starting materials marks the beginning of the detailed description of the process. The applicant for marketing authorization should propose and justify which substance should be considered as the API starting material, e.g. incorporated as a significant structural fragment into the structure of the active substance.

In practice the designation of a starting material may be difficult. The number of steps separating the starting material from the final API is an issue to be decided on a case-by-case basis, subject to the manufacturer's proposal and assessors' evaluation. Since a designated starting material may be obtained from multiple sources, it is necessary to have well-defined quality requirements to ensure that the APIs produced meet specifications. Establishing these requirements may involve a compromise between the desire for a pure starting material and the impact of this on cost of API production. Impurities can be tolerated in the starting material if the API manufacturing process has been shown to efficiently remove them. Redundant purification steps may reduce the yield of the final API and thus further increase its cost.

Artemisinin derivatives used in artemisinin-based combination therapy (ACT) are synthesized from artemisinin in one or two synthetic steps. Artemisinin is typically produced as an isolate from *Artemisia annua* L. Artemisinin complies with the definition of a "starting material", as defined above and described in certain national, regional and international guidelines. It is:

- -a material used in the production of the API that is incorporated into the API as a significant structural element;
- -commercially available;
- -a compound whose name, chemical structure, chemical and physical characteristics, properties and impurity profile are well defined;
- -obtained by commonly known procedures.

As artemisinin is extracted from plant material and prior intermediates are thus not available it is logical to designate this compound as the starting material for its derivatives.

A monograph appears in *The International Pharmacopoeia* for artemisinin used as an API. However, at present, artemisinin is mainly used as a starting material for artemisinin-derived APIs and not as an API.

The level of quality of the artemisinin should be acceptable for its intended use as the starting material for the production of artemisinin derivatives. The specifications presented below take into account an acceptable balance of benefit versus risk between the quality of artemisinin used as a starting material and the quality required for artemisinin derivatives for use as APIs.

However, competent authorities may accept other impurity profile levels depending on the capability of the manufacturing process to lead to artemisinin-derived APIs at least compliant with the relevant monographs of *The International Pharmacopoeia*.

The purpose of this document is to offer a global approach to defining the level of quality requirements of artemisinin when used as a starting material for the production of its API derivatives used in ACT formulations. It does not apply to cases where artemisinin is used as an API. It is intended that the recommendations for requirements outlined in this document will apply to artemisinin extracted from *Artemisia annua* L. regardless of variations in agricultural environment or variations in extraction and purification steps. In addition, in order to ensure appropriate quality of the derived APIs, the manufacturer may add additional tests, such as tests for residual solvents and heavy metals, among others, and/or require tighter specifications. For artemisinin produced using synthetic chemical processes or by fermentation other requirements may be applicable.

2. Characterization of artemisinin

Provided that artemisinin intended for use as a starting material has been correctly identified the major quality concern is the presence and level of impurities with the potential to affect the purity of subsequent API derivatives. Impurities may originate from the plant extracts or arise from the purification process or from degradation. Different biosynthetic routes may be used at different stages in the plant's development and there are claims of variability between growing regions and environments. Despite a lack of consensus on a single biosynthetic route several potential impurities are common to different routes. These include artemisinic acid, dihydroartemisinic acid, arteannuin B and artemisitene. Of these only artemisitene has been reported in isolated artemisinin. Recent work (*3*, *4*) has contributed towards a clearer understanding of existing impurities and their analysis.

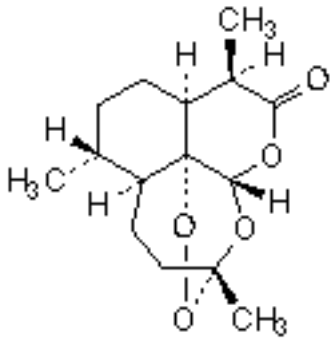
Examination of a wide variety of artemisinin samples produced in various regions indicated the consistent presence of two impurities: artemisitene and an artemisinin diastereomer with the stereochemistry inverted at C-9 (9-epi-artemisinin). A possible concern is that artemisinin impurities may not be detected with high-performance liquid chromatography analysis using ultraviolet detection, as used in the majority of testing laboratories. Recent work (*5*) using more sensitive general detection by mass spectrometry, however, demonstrated that additional impurities occur only in trace amounts. Isolated artemisinin is very stable. The potential degradants proposed on the basis of mechanistic studies do not occur at temperatures below 100 °C. These degradants are not observed in isolated artemisinin.

In the chemical conversion of the artemisinin starting material to its API derivatives (e.g. artesunate), the artemisinin diastereomeric impurity may be converted to a corresponding diastereomer at the C-9 position in the API derivative. However, these resulting diastereomers have not been observed in isolated APIs. The fate of artemisitene is less clear as it may be converted to the same intermediate as artemisinin.

Artemisitene-derived impurities have not been observed in artemisinin derivative APIs. Proposed limits for these impurities are based on historical results. The specifications for artemisinin starting material are based on experience with artemether and artesunate. For a new artemisinin-derived API the suitability of the specifications to control potential impurities arising during its synthesis should be demonstrated.

As the artemisinin extraction processes use solvents like dichloromethane, chloroform, ether and others, residual solvents should be indicated on the certificate of analysis issued by the supplier.

3. Tests and specifications for artemisinin starting material



$C_{15}H_{22}O_5$

Relative molecular mass: 282.3

Chemical name: (3R,5aS,6R,8aS,9R,12S,12aR)-3,6,9-trimethyloctahydro-3,12-epoxypyrano[4,3-j]-1,2-benzodioxepin-10(3H)-one; CAS Reg. No. 63968-64-9.

Description: Colourless needles or a white to almost white to slightly yellow, crystalline powder.

Category: Starting material for the synthesis of artemisinin derivative APIs.

Storage : Artemisinin should be kept in a well-closed container, protected from light.

Requirements

Artemisinin contains not less than 95.0% and not more than the equivalent of 102.0% of $C_{15}H_{22}O_5$ calculated with reference to the dried substance.

Identity tests

Carry out the examination as described under 1.7 Spectrophotometry in the infrared region of *The International Pharmacopoeia* (*6*). The infrared absorption spectrum is concordant with the spectrum obtained from artemisinin RS or with the *reference spectrum* of artemisinin in *The International Pharmacopoeia.*

Specific optical rotation. Use a 10 mg/mL solution in dehydrated ethanol R;

$[\alpha]_D^{20\,°C}$ = + 75° to +78°

Loss on drying. Dry to constant mass at 80 °C; it loses not more than 10.0 mg/g.

Related substances

Note : It may be possible to justify other limits when artemisinin as a starting material is used in a particular synthesis and manufacturing process, by validation of the levels and limits of the impurities in the final API.

Carry out the test as described under *1.14.1 Chromatography*, High-performance liquid chromatography of *The International Pharmacopoeia* (*6*). Use the chromatographic conditions and prepare solutions (1) and (2) as described below under Assay. For solution (3) dilute 1 mL of solution (1) to 100 mL with the mobile phase.

Inject separately 20 µl of solutions (1), (2) and (3). Record the chromatograms for about 1.5 times the retention time of artemisinin. In the chromatogram obtained with solution (2) artemisitene (impurity A) is eluted at the relative retention of about 0.79 with reference to artemisinin (retention time about 10 minutes). The test is not valid unless the resolution between the peak of artemisitene and the peak of artemisinin is at least 4. The chromatogram obtained with solution (1) may show a peak due to impurity B eluting at a retention of about 0.85 with reference to artemisinin.

In the chromatogram obtained with solution (1):

- -the area of any peak corresponding to impurity A, when multiplied by a correction factor of 0.027, is not greater than 0.2 times the area of the peak in the chromatogram obtained with solution (3) (0.2%);
- -the area of any peak corresponding to impurity B is not greater than the area of the peak in the chromatogram obtained with solution (3) (1.0%);
- -the area of any peak other than the principal peak is not greater than 0.5 times the area of the peak in the chromatogram obtained with solution (3) (0.5%);
- -the sum of the corrected area of any peak corresponding to impurity A and the areas of all the peaks, apart from the principal peak, is not greater than 3 times the area of the peak obtained with solution (3) (3.0%). Disregard any peak with an area less than 0.1 times the area of the principal peak obtained with solution (3) (0.1%).

Assay

Carry out the test as described under *1.14.1 Chromatography*, High-performance liquid chromatography of *The International Pharmacopoeia* (*6*) using a stainless steel column (15 cm × 4.6 mm) packed with 5 µm particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl groups. The mobile phase consists of a 50:50 mixture of acetonitrile and water, pumped at a flow rate of 1.0 mL/minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 210 nm.

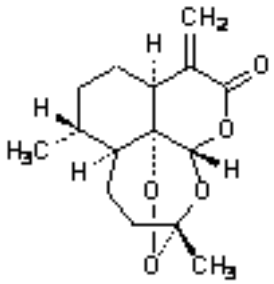
Prepare the following solutions. For solution (1) prepare a 5.0 mg/mL solution of the test substance in the mobile phase. For solution (2) prepare a 5.0 mg/mL solution of artemisinin RS (containing artemisinin and impurity A) in the mobile phase.

Inject separately 20 µl of solutions (1) and (2). Record the chromatograms for about 1.5 times the retention time of artemisinin. In the chromatogram obtained with solution (2) artemisitene (impurity A) is eluted at the relative retention of 0.79 with reference to artemisinin (retention time about 10 minutes). The test is not valid unless the resolution between the peak of artemisitene and the peak of artemisinin is at least 4. The chromatogram obtained with solution (1) may show a peak due to impurity B eluting at a retention of about 0.85 with reference to artemisinin.

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2), and calculate the content of $C_{15}H_{22}O_5$ with reference to the dried substance.

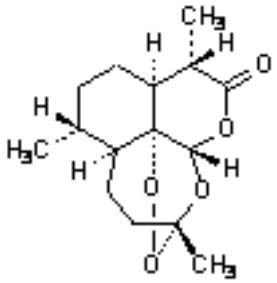
Impurities

A.



(3*R*,5a*S*,6*R*,8a*S*,12*S*,12a*R*)-3,6-dimethyl-9-methylideneoctahydro-3,12-epoxypyrano[4,3-*j*]-1,2-benzodioxepin-10(3*H*)-one (artemisitene)

B.



(3*R*,5a*S*,6*R*,8a*S*,9*S*,12*S*,12a*R*)-3,6,9-trimethyloctahydro-3,12-epoxypyrano[4,3-*j*]-1,2-benzodioxepin-10(3*H*)-one (9-*epi*-artemisinin)

References

1. WHO good manufacturing practices for active pharmaceutical ingredients. In: *WHO Expert Committee on Specifications for Pharmaceutical Preparations. Forty-fourth report*. Geneva, World Health Organization (WHO Technical Report Series, No. 957), Annex 2, 2010.
2. International Conference on Harmonisation (ICH) Topic Q7: Note for guidance on good manufacturing practice for active pharmaceutical ingredients. London, EMEA, 2000 (CPMP/ICH/4106/00); http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002825.pdf.
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6. *The International Pharmacopoeia*, 4th ed., Vol. 1: General notices; monographs for pharmaceutical substances (A–O) and Vol. 2: Monographs for pharmaceutical substances (P–Z); monographs for dosage forms and radiopharmaceutical preparations; methods of analysis; reagents. Geneva, World Health Organization, 2006 online; *The International Pharmacopoeia*, 4th ed., First, Second and Third Supplements, 2013, available on CD-ROM.

Tablet friability

This text is based on the internationally-harmonized texts developed by the Pharmacopoeial Discussion Group (PDG). Some editorial modifications have been made in order to be in line with the style used in The International Pharmacopoeia.

This method text provides guidance for the friability determination of compressed, uncoated tablets. The test procedure presented is generally applicable to most compressed tablets. Measurement of tablet friability supplements other physical strength measurements such as tablet-breaking force.

Use a drum with an internal diameter between 283–291 mm and a depth between 36–40 mm, of transparent synthetic polymer with polished internal surfaces and subject to minimum static build-up (see Figure 1 for a typical apparatus). One side of the drum is removable. The tablets are tumbled at each turn of the drum by a curved projection with an inside radius between 75.5–85.5 mm that extends from the middle of the drum to the outer wall. The outer diameter of the central ring is between 24.5–25.5 mm. The drum is attached to the horizontal axis of a device that rotates at 25 ± 1 rpm. Thus, at each turn the tablets roll or slide and fall onto the drum wall or on to each other.

For tablets with a unit weight equal to or less than 650 mg, take a sample of whole tablets *n* corresponding as near as possible to 6.5 g. For tablets with a unit weight of more than 650 mg, take a sample of 10 whole tablets. The tablets should be carefully dedusted prior to testing. Accurately weigh the tablet sample and place the tablets in the drum. Rotate the drum 100 times and remove the tablets. Remove any loose dust from the tablets as before and accurately weigh.

Generally the test is run once. If obviously cracked, cleaved or broken tablets are present in the tablet sample after tumbling the sample fails the test. If the results are difficult to interpret or if the weight loss is greater than the targeted value the test should be repeated twice and the mean of the three tests determined. A maximum weight loss (obtained from a single test or from the mean of three tests) of not more than 1.0% is considered acceptable for most products.

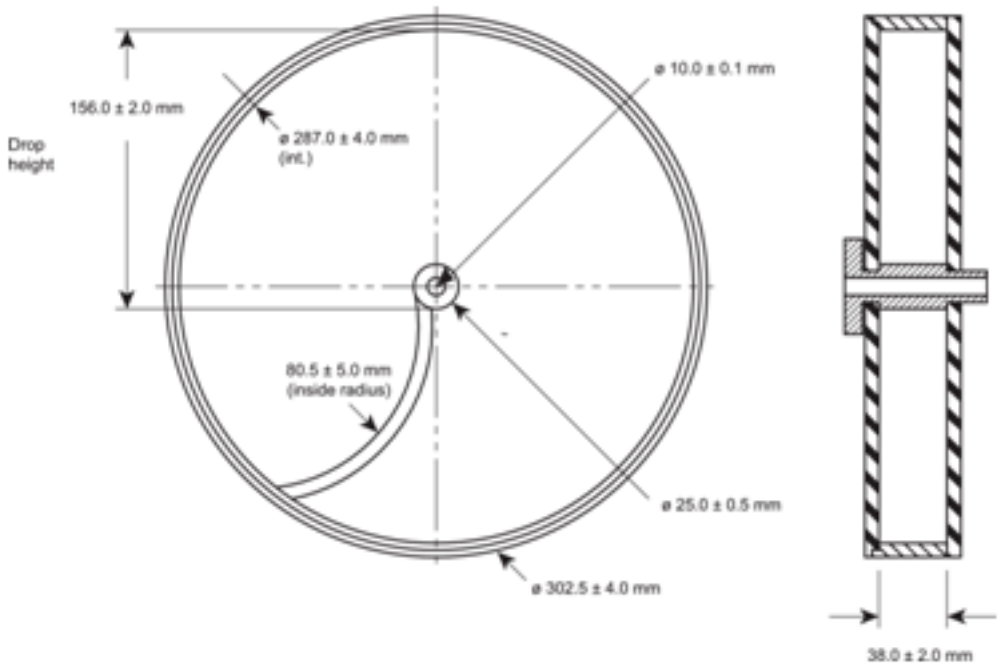

Figure 1. Tablet friability apparatus [1]

[1] Reproduced with the kind permission of the European Directorate for the Quality of Medicines & HealthCare, Council of Europe, Strasbourg, France.

If tablet size or shape causes irregular tumbling adjust the drum base so that the base forms an angle of about 10º with the horizontal and the tablets no longer bind together when lying next to each other, which prevents them from falling freely.

Effervescent tablets and chewable tablets may have different specifications as far as friability is concerned. In the case of hygroscopic tablets an appropriate humidity-controlled environment is required for testing.

Drums with dual scooping projections, or apparatus with more than one drum for the running of multiple samples at one time, are also permitted.

Softening time determination of lipophilic suppositories

2015-01

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This test is intended to determine, under defined conditions, the time elapsed until a suppository maintained in water softens to the extent that it no longer offers resistance when a defined weight is applied.

Method A

Apparatus

The apparatus (see Figure 1) consists of a glass tube 15.5 mm in internal diameter with a flat bottom and a length of about 140 mm. The tube is closed by a removable plastic cover having an opening 5.2 mm in diameter. The apparatus comprises a rod 5.0 mm in diameter that becomes wider towards the lower end, reaching a diameter of 12 mm. A metal needle 2 mm in length and 1 mm in diameter is fixed on the flat underside.

The rod consists of 2 parts, a lower part made of plastic material and an upper part made of plastic material or metal with a weight disc. The upper and lower parts are either fitted together (manual version) or separate (automated version). The weight of the entire rod is 30 ± 0.4 g. The upper part of the rod carries a sliding mark ring. When the rod is introduced into the glass tube so that it touches the bottom the mark ring is adjusted to coincide with the upper level of the plastic cover.

Procedure

Place the glass tube containing 10 mL of water in a water-bath and equilibrate at 36.5 ± 0.5 °C. Fix the glass tube vertically and immerse to a depth of at least 7 cm below the surface but without touching the bottom of the water-bath. Introduce a suppository, tip first, into the tube followed by the rod with the free gliding plastic cover into the glass tube until the metal needle touches the flat end of the suppository. Put the cover on the tube (beginning of time measurement). Note the time elapsed until the rod sinks down to the bottom of the glass tube and the mark ring reaches the upper level of the plastic cover.

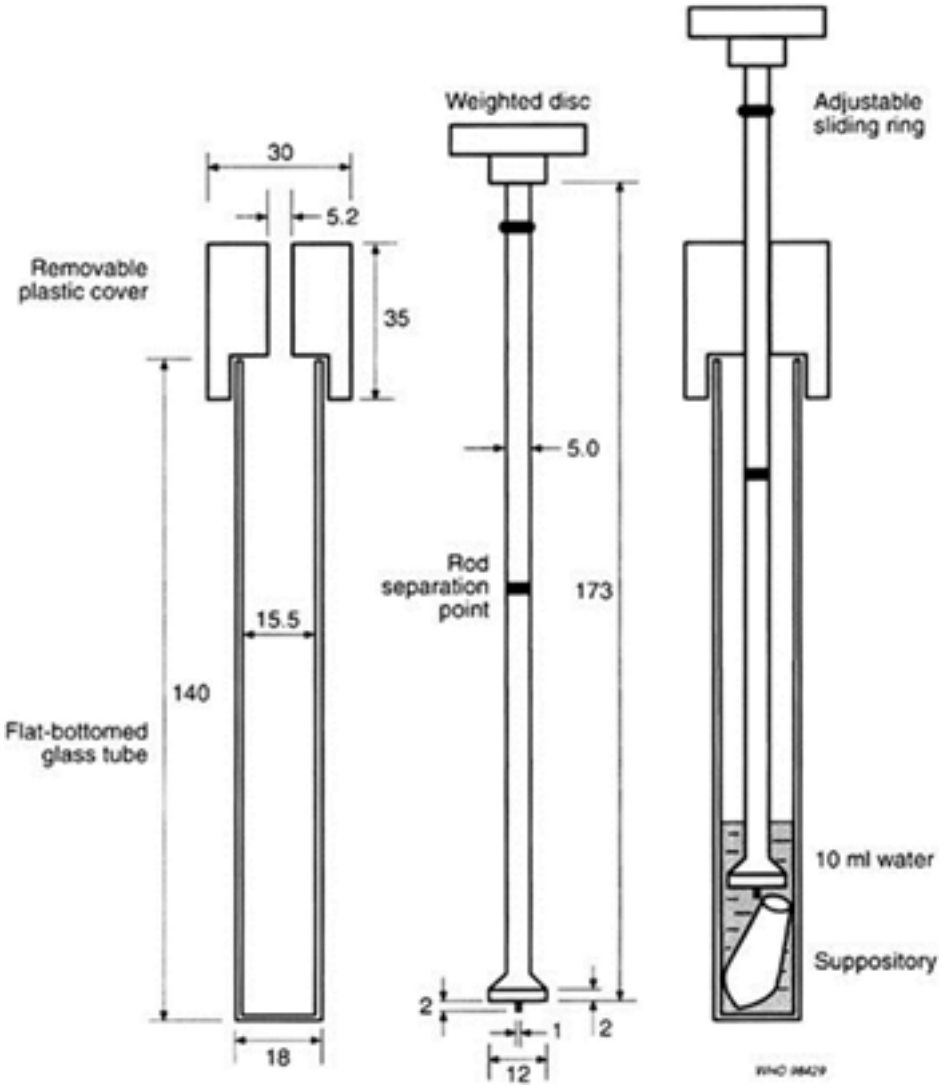

Figure 1. Method A - Apparatus for measuring the softening time of lipophilic suppositories. Dimensions in millimetres

METHOD B

Apparatus

The apparatus (see Figure 2) consists of a water-bath (B) into which an inner tube (A) is inserted and fixed with a stopper. The inner tube is closed by a stopper at the bottom. The apparatus is fitted with a thermometer. Two insets are available:

- a glass rod (C1) in the form of a tube sealed at both ends, carrying a rim at its lower end weighted with lead shot, which has a weight of 30 ± 0.4 g;

- a penetration inset (C2) consisting of a rod (7.5 ± 0.1 g) in a tube which has an enlargement for the suppository, both made of stainless steel.

Procedure

Pour 5 mL of water at 36.5 ± 0.5 °C into the inner tube (A), introduce a suppository with the tip downwards and onto that place the inset (C1 or C2). Note the time elapsed between this moment and the moment when the lower, rimmed end of the glass rod (C1) or the steel rod (C2) reaches the narrowed part of the inner glass tube. Melting or dissolution is then considered as complete.

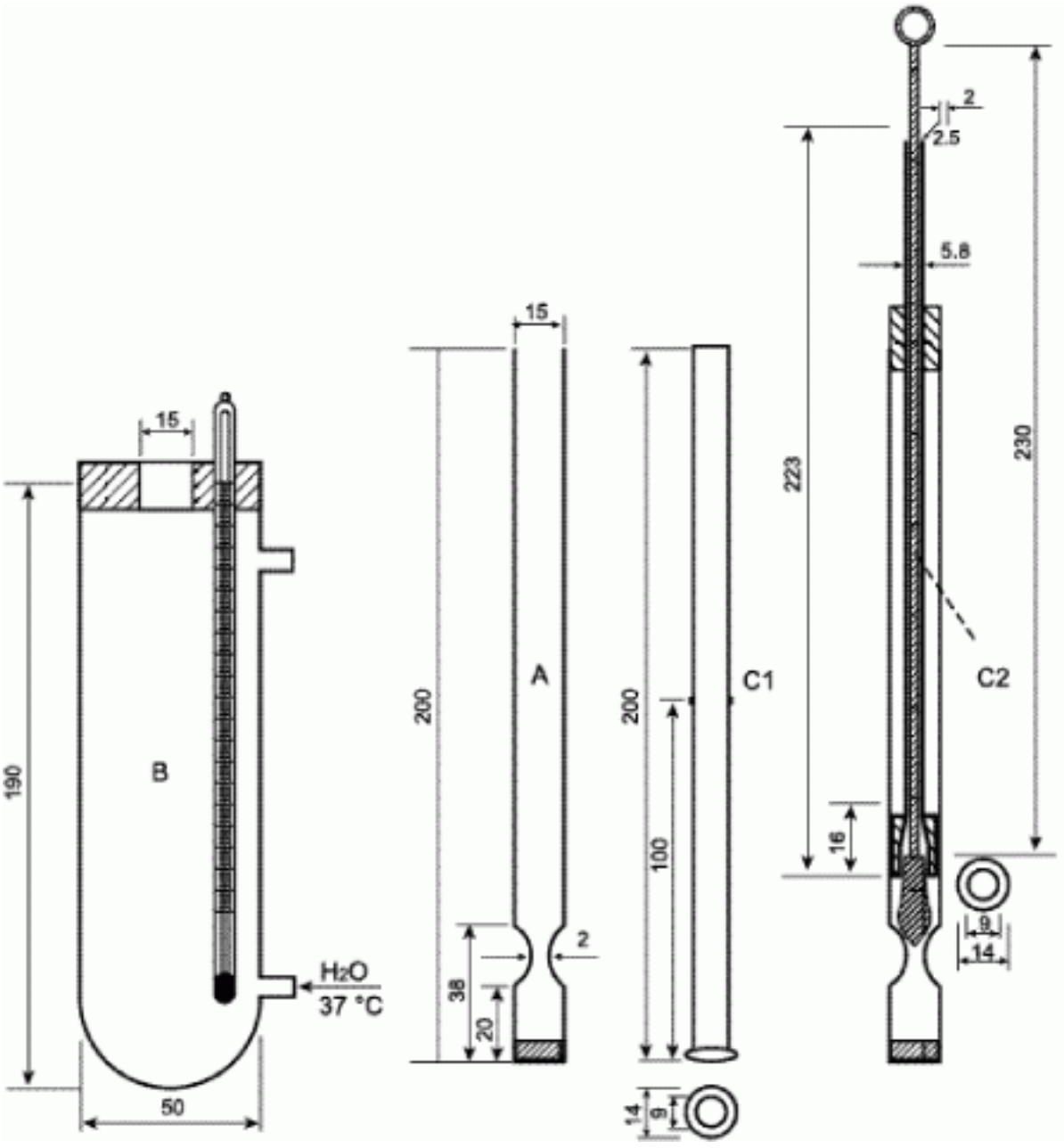

Figure 2. Method B - Apparatus for measuring the softening time of lipophilic suppositories. Dimensions in millimetres

Resistance to crushing of tablets

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This test is intended to determine under defined conditions the resistance to crushing of tablets, measured by the force needed to disrupt them by crushing.

Apparatus

The apparatus consists of 2 jaws facing each other, one of which moves towards the other. The flat surfaces of the jaws are perpendicular to the direction of movement. The crushing surfaces of the jaws are flat and larger than the zone of contact with the tablet. The apparatus is calibrated using a system with a precision of 1 newton.

Procedure

Place the tablet between the jaws, taking into account, where applicable, the shape, the break-mark and the inscription; for each measurement orient the tablet in the same way with respect to the direction of application of the force. Carry out the measurement on 10 tablets, taking care that all fragments of tablets have been removed before each determination.

This procedure does not apply when fully automated equipment is used.

Expression of results

Express the results as the mean, minimum and maximum values of the forces measured, all expressed in newtons.

Indicate the type of apparatus and, where applicable, the orientation of the tablets.

Measurement of consistency by penetrometry

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This test is intended to measure under determined and validated conditions the penetration of an object into the product to be examined in a container with a specified shape and size.

Apparatus

The apparatus consists of a penetrometer made up of a stand and a penetrating object. A suitable apparatus is shown in Figure 1.

The stand is made up of:

-a vertical shaft to maintain and guide the penetrating object;
-a horizontal base;
-a device to ensure that the penetrating object is vertical;
-a device to check that the base is horizontal;
-a device to retain and release the penetrating object;
-a scale or suitable digital analyser showing the depth of penetration, graduated in tenths of a
-millimetre.

The penetrating object, made of a suitable material, has a smooth surface and is characterized by its shape, size and mass (*m*).

Suitable penetrating objects are shown in Figures 2 and 3.

Procedure

Prepare the test samples according to one of the following procedures.

A. Carefully and completely fill 3 containers without forming air bubbles. Level if necessary to obtain a flat surface. Store the samples at 25 ± 0.5 °C for 24 h, unless otherwise prescribed.

B. Store 3 samples at 25 ± 0.5 °C for 24 h, unless otherwise prescribed. Apply a suitable shear to the samples for 5 minutes. Carefully and completely fill 3 containers without forming air bubbles and level if necessary to obtain a flat surface.

C. Melt 3 samples carefully and completely fill 3 containers without forming air bubbles. Store the samples at 25 ± 0.5 °C for 24 h, unless otherwise prescribed.

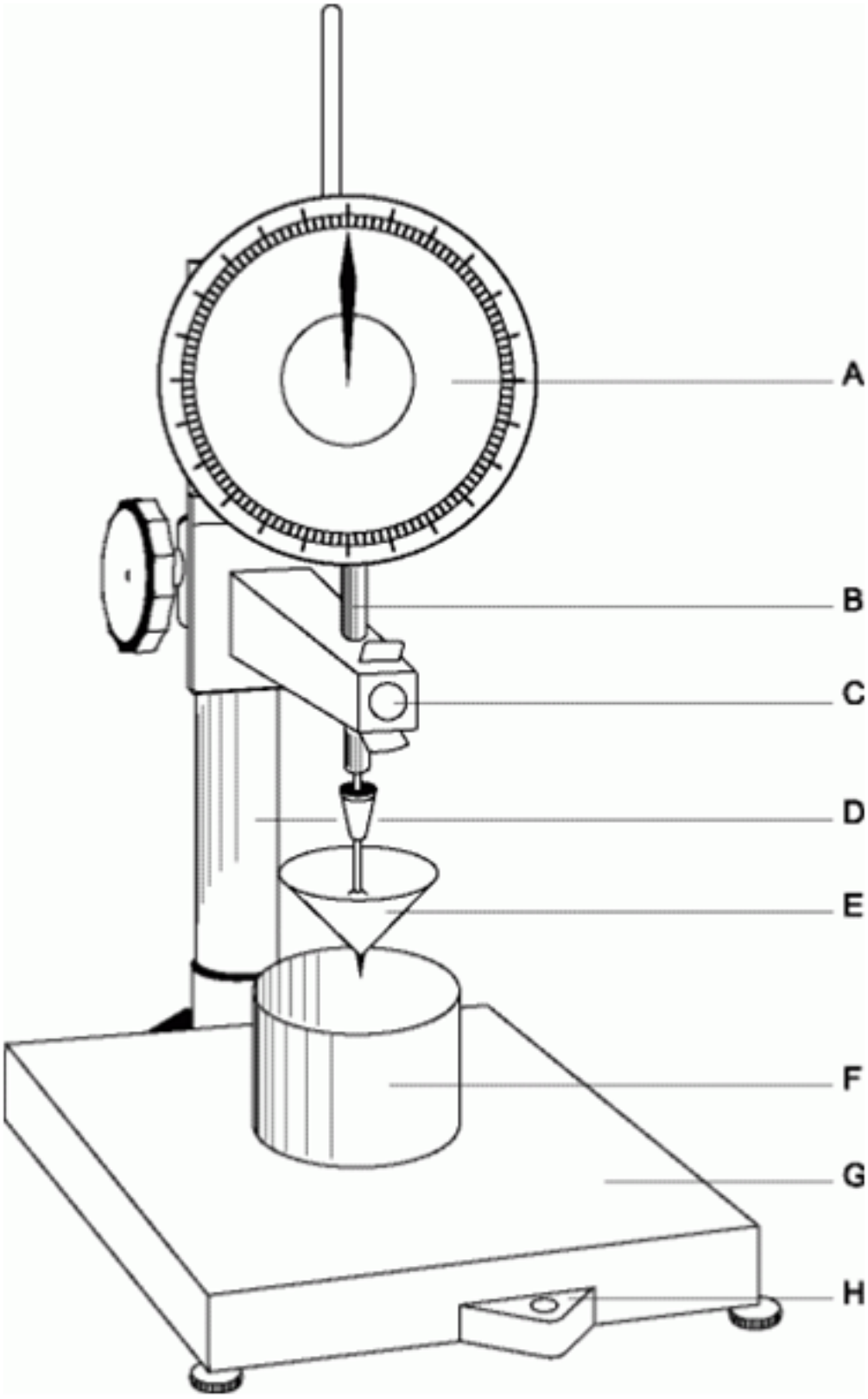

Figure 1. Penetrometer

A. Scale or suitable digital analyzer showing the depth of penetration, graduated in tenths of millimetres.

B. Vertical shaft to maintain and guide the penetrating object.

C. Device to retain and to release the penetrating object automatically and for a constant time.

D. Device to ensure that the penetrating object is vertical and that the base is horizontal.

E. Penetrating object (see Figures 2 and 3).

F. Container.

G. Horizontal base.

H. Control for the horizontal base.

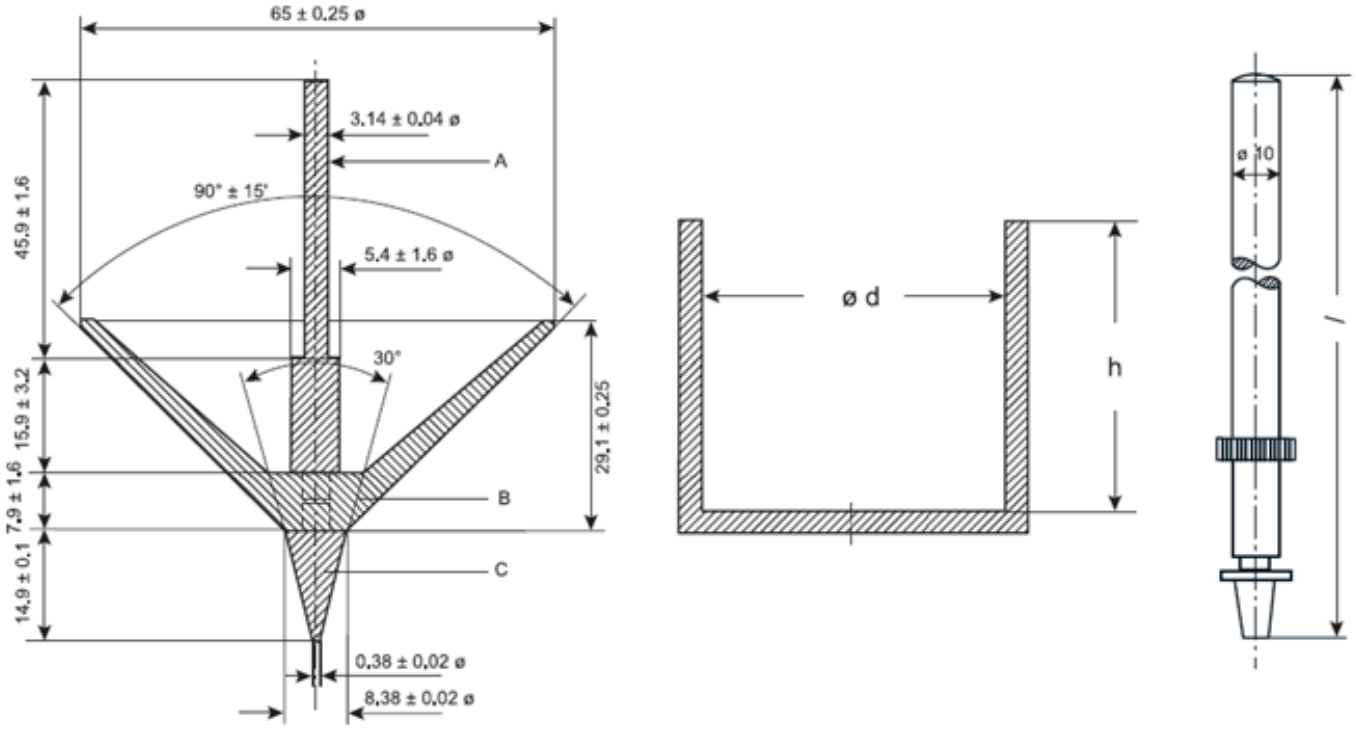

Figure 2.

Cone (m = 102.5 ± 0.05 g), suitable container (d = 102 mm or 75 mm; h ≥ 62 mm) and shaft (l = 162 mm; m = 47.5 ± 0.05 g)

Dimensions in millimetres.

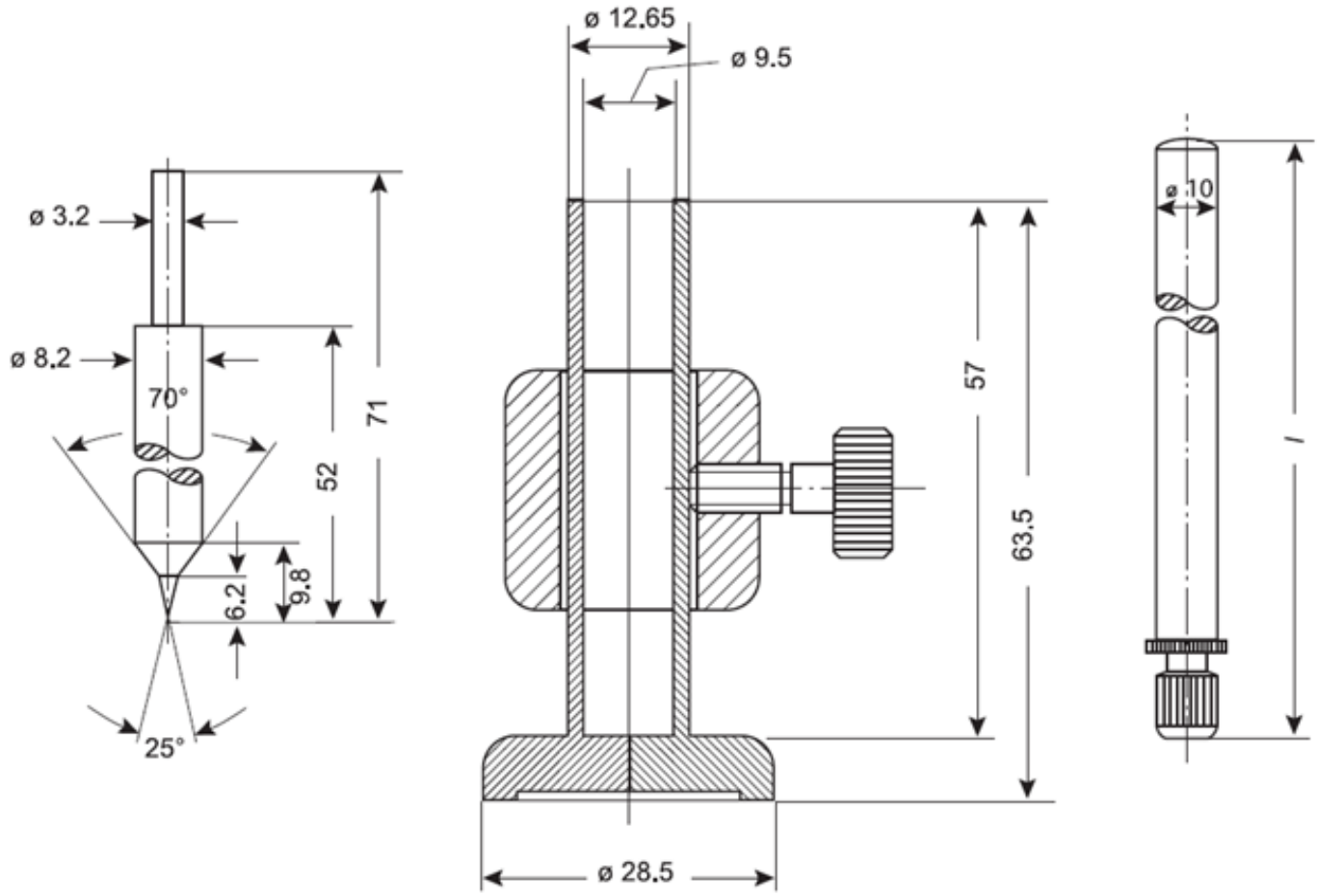

Figure 3.

Micro-cone (m = 7.0 g), suitable container and shaft (l = 116 mm; m = 16.8 g)

Dimensions in millimeters.

Determination of penetration

Place the test sample on the base of the penetrometer. Verify that its surface is perpendicular to the vertical axis of the penetrating object. Bring the temperature of the penetrating object to 25 ± 0.5 °C and then adjust its position such that its tip just touches the surface of the sample. Release the penetrating object and hold it free for 5 seconds. Clamp the penetrating object and measure the depth of penetration. Repeat the test with the 2 remaining containers.

Expression of results

The penetration is expressed in tenths of a millimetre as the arithmetic mean of the 3 measurements. If any of the individual results differ from the mean by more than 3% repeat the test and express the results of the 6 measurements as the mean and the relative standard deviation.

Bulk density and tapped density of powders

The bulk density of a powder is the ratio of the mass of an untapped powder sample and its volume including the contribution of the interparticulate voidvolume. Hence, the bulk density depends on both the density of powder particles and the spatial arrangement of particles in the powder bed. The bulkdensity is expressed in grams per millilitre (g/mL) although the international unit is kilogram per cubic metre (1 g/mL = 1000 kg/m^3) becausethe measurements are made using cylinders.

It may also be expressed in grams per cubic centimetre (g/cm^3).

The bulking properties of a powder are dependent upon the preparation, treatment and storage of the sample, i.e. how it was handled. The particles can bepacked to have a range of bulk densities and, moreover, the slightest disturbance of the powder bed may result in a changed bulk density. Thus, the bulkdensity of a powder is often very difficult to measure with good reproducibility and, in reporting the results, it is essential to specify how thedetermination was made.

The bulk density of a powder is determined by measuring the volume of a known mass of powder sample, that may have been passed through a sieve, into agraduated cylinder (Method A) or by measuring the mass of a known volume of powder that has been passed through a volumeter into a cup (Method B) or ameasuring vessel (Method C).

Method A and Method C are favoured.

Method A. Measurement in a graduated cylinder

Procedure. Pass a quantity of powder sufficient to complete the test through a sieve with apertures greater than or equal to 1.0 mm, if necessary, to break upagglomerates that may have formed during storage; this must be done gently to avoid changing the nature of the material. Into a dry graduated cylinder of250 mL (readable to 2 mL) gently introduce, without compacting, approximately 100 g of the test sample (m) weighed with 0.1% accuracy. Carefullylevel the powder without compacting, if necessary, and read the unsettled apparent volume (V_0) to the nearest graduated unit. Calculatethe bulk density in (g/mL) using the formula m/V_0. Generally, replicate determinations are desirable for the determination ofthis property.

If the powder density is too low or too high, such that the test sample has an untapped apparent volume of either more than 250 mL or less than 150 mL, itis not possible to use 100 g of powder sample. Therefore a different amount of powder has to be selected as test sample, such that its untapped apparentvolume is 150 mL to 250 mL (apparent volume greater than or equal to 60% of the total volume of the cylinder); the mass of the test sample is specified inthe expression of results.

For test samples having an apparent volume between 50 mL and 100 mL a 100 mL cylinder readable to 1 mL can be used; the volume of the cylinder is specifiedin the expression of results.

Method B. Measurement in a volumeter

Apparatus. The apparatus[1] (Figure 1) consists of a top funnel fitted with a 1.0 mm sieve. The funnel is mounted over a baffle box containing four glass baffle plates over which thepowder slides and bounces as it passes. At the bottom of the baffle box is a funnel that collects the powder and allows it to pour into a cup mounteddirectly below it. The cup may be cylindrical (25.00 ± 0.05 mL volume with an inside diameter of 30.00 ± 2.00 mm) or cubical (16.39 ± 0.20 mL volume withinside dimensions of 25.400 ± 0.076 mm).

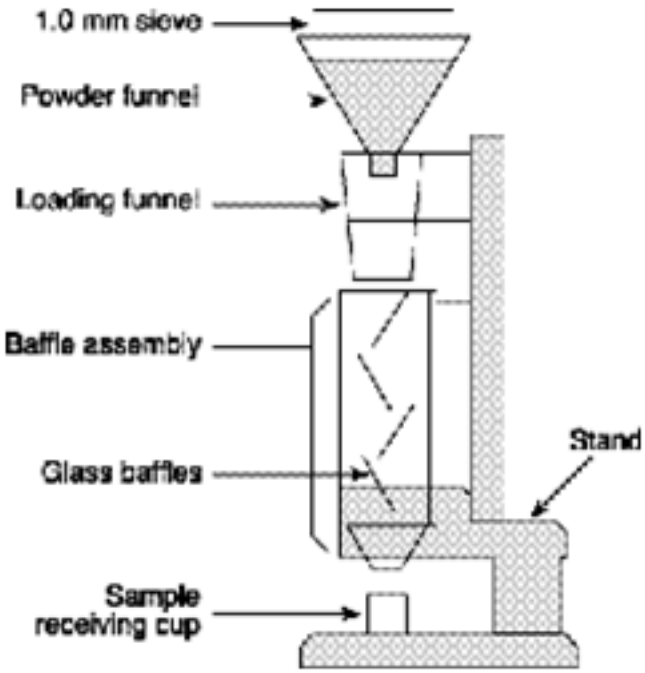

Figure 1. Volumeter

Procedure. Allow an excess of powder to flow through the apparatus into the sample receiving cup until it overflows, using a minimum of 25 cm^3 of powderwith the cubical cup and 35 cm^3 of powder with the cylindrical cup. Carefully, scrape excess powder from the top of the cup by smoothly movingthe edge of the blade of a spatula perpendicular to and in contact with the top surface of the cup, taking care to keep the spatula perpendicular toprevent packing or removal of powder from the cup. Remove any material from the side of the cup and determine the mass (*M*) of the powder to thenearest 0.1%. Calculate the bulk density (g/mL) using the formula M/V_0 in which V_0 is the volume of the cup andrecord the average of three determinations using three different powder samples.

[1]The apparatus (the Scott Volumeter) conforms to the dimensions in ASTM 329 90.

Method C. Measurement in a vessel

Apparatus. The apparatus consists of a 100 mL cylindrical vessel of stainless steel with dimensions as specified in Figure 2.

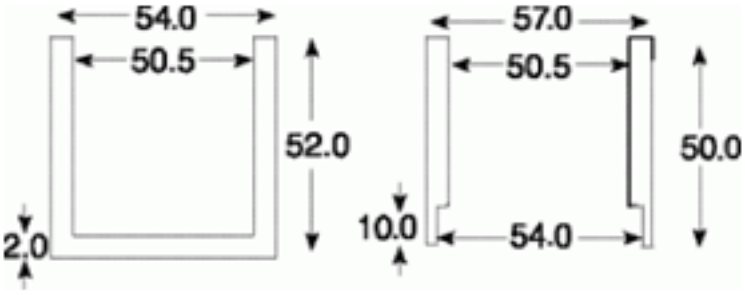

Figure 2. - *Measuring vessel (left) and cap (right) - dimensions in mm*

Procedure. Pass a quantity of powder sufficient to complete the test through a 1.0 mm sieve, if necessary, to break up agglomerates that may have formed duringstorage and allow the obtained sample to flow freely into the measuring vessel until it overflows. Carefully scrape the excess powder from the top of thevessel as described for Method B. Determine the mass (M_0) of the powder to the nearest 0.1% by subtraction of the previously determinedmass of the empty measuring vessel. Calculate the bulk density (g/mL) using the formula $M_0/100$ and record the average of threedeterminations using three different powder samples.

TAPPED DENSITY

The tapped density is an increased bulk density attained after mechanically tapping a container containing the powder sample.

The tapped density is obtained by mechanically tapping a graduated measuring cylinder or vessel containing the powder sample. After observing the initialpowder volume or mass the measuring cylinder or vessel is mechanically tapped and volume or mass readings are taken until little further volume or masschange is observed. The mechanical tapping is achieved by raising the

cylinder or vessel and allowing it to drop, under its own mass, a specified distanceby either of three methods as described below. Devices that rotate the cylinder or vessel during tapping may be preferred to minimize any possibleseparation of the mass during tapping down.

Method A

Apparatus. The apparatus (Figure 3) consists of the following:

-a 250 mL graduated cylinder (readable to 2 mL) with a mass of 220 ± 44 g;

-a settling apparatus capable of producing in 1 minute either nominally 250 ± 15 taps from a height of 3 ± 0.2 mm or nominally 300 ± 15 taps from a heightof 14 ± 2 mm. The support for the graduated cylinder, with its holder, has a mass of 450 ± 10 g.

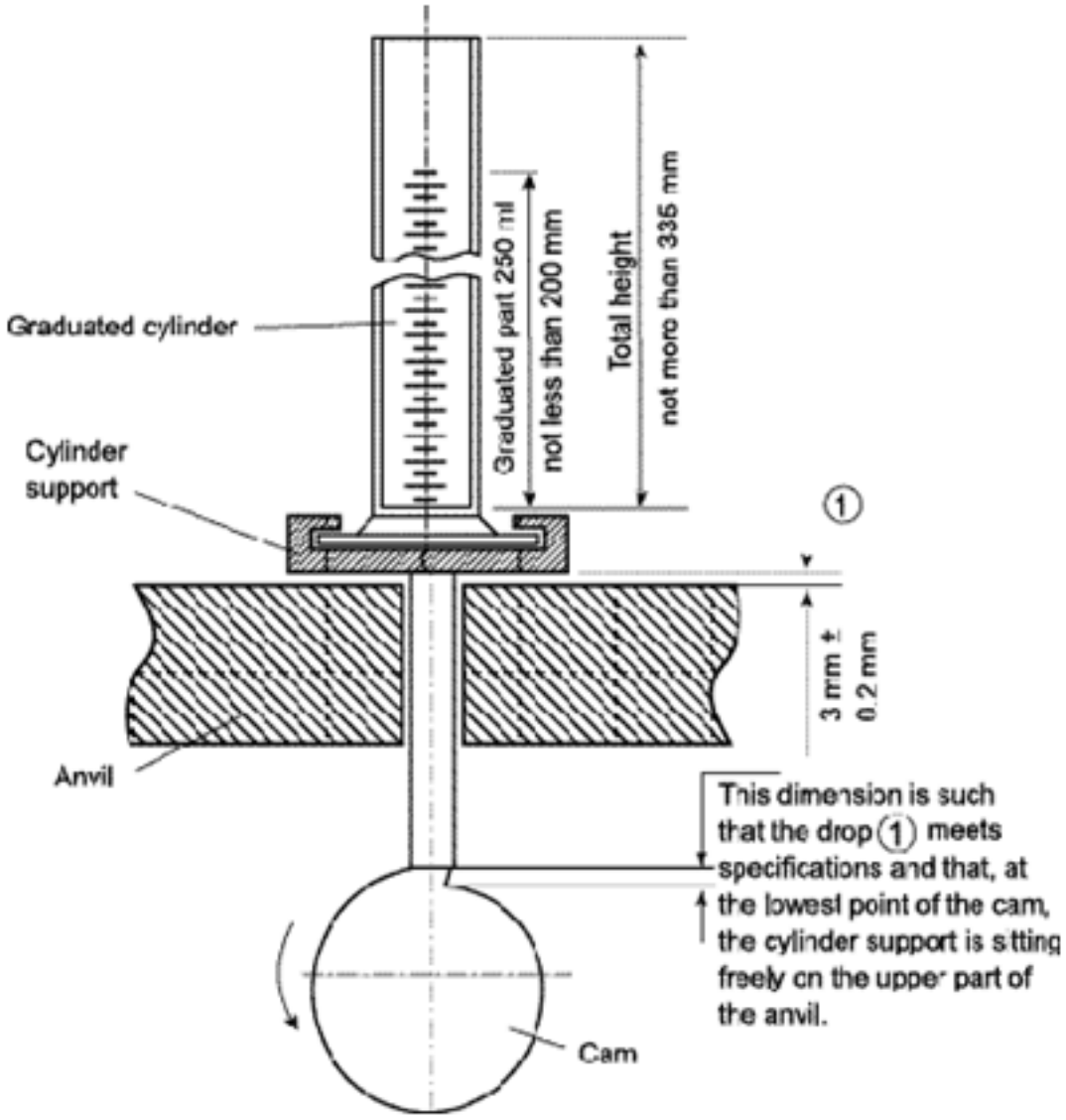

Figure 3

Procedure. Proceed as described above for the determination of the bulk volume (V_0). Secure the cylinder in the holder. Carry out 10, 500 and1250 taps on the same powder sample and read the corresponding volumes V_{10}, V_{500} and V_{1250} to the nearest graduated unit. If the difference between V_{500} and V_{1250} is less than or equal to 2 mL, V_{1250} is the tapped volume. If the difference between V_{500} and V_{1250} exceeds 2 mL repeat in incrementssuch as 1250 taps until the difference between succeeding measurements is less than or equal to 2 mL. Fewer taps may be appropriate for some powders, whenvalidated. Calculate the tapped density (g/mL) using the formula m/V_f in which V_f is the final tappedvolume. Generally, replicate determinations are desirable for the determination of this property. Specify the drop height with the results.

If it is not possible to use a 100 g test sample, use a reduced amount and a suitable 100 mL graduated cylinder (readable to 1 mL) weighing 130 ± 16 g andmounted on a holder weighing 240 ± 12 g. The modified test conditions are specified in the expression of the results.

Method B

Procedure. Proceed as directed under Method A except that the mechanical tester provides a fixed drop of 3 ± 0.2 mm at a nominal rate of 250 taps per minute.

Method C

Procedure. Proceed as described in Method C for measuring the bulk density using the measuring vessel equipped with the cap shown in Figure 2. The measuring vesselwith the cap is lifted 50-60 times per minute by the use of a suitable tapped density tester. Carry out 200 taps, remove the cap and carefully scrapeexcess powder from the top of the measuring vessel as described in Method C for measuring the bulk density. Repeat the procedure using 400 taps. If thedifference between the two masses obtained after 200 and 400 taps exceeds 2% carry out a test using 200 additional taps until the difference betweensucceeding measurements is less than 2%. Calculate the tapped density (g/mL) using the formula $M_f/100$ where M_f isthe mass of powder in the measuring vessel. Record the average of three determinations using three different powder samples. The test conditions includingtapping height are specified in the expression of the results.

MEASURES OF POWDER COMPRESSIBILITY

Because the interparticulate interactions influencing the bulking properties of a powder are also the interactions that interfere with powder flow, acomparison of the bulk and tapped densities can give a measure of the relative importance of these interactions in a given powder. Such a comparison isoften used as an index of the ability of the powder to flow, for example, the Compressibility index or the Hausner ratio.

The Compressibility index and Hausner ratio are measures of the propensity of a powder to be compressed as described above. As such they are measures ofthe powder's ability to settle and they permit an assessment of the relative importance of interparticulate interactions. In a free-flowing powder, suchinteractions are less significant, and the bulk and tapped densities will be closer in value. For poorer flowing materials there are frequently greaterinterparticulate interactions and a greater difference between the bulk and tapped densities will be observed. These differences are reflected in theCompressibility Index and the Hausner Ratio.

Compressibility index :

$$\frac{100\left(V_0 - V_f\right)}{V_0}$$

V_0 = unsettled apparent volume,

V_f = final tapped volume.

Hausner Ratio:

$$\frac{V_0}{V_f}$$

Depending on the material the compressibility index can be determined using V_{10} instead of V_0. If V_{10} is used it is clearly stated in the results.

Polymorphism

1. INTRODUCTION AND TERMINOLOGY

The aim of this chapter is to provide a brief overview of:

-the terminology associated with crystal polymorphism;

-some analytical techniques commonly used to characterise polymorphs;

-the relevance of polymorphism for active pharmaceutical ingredients (APIs) and finished pharmaceutical products (FPPs); and

-the control strategies for polymorphism employed by *The International Pharmacopoeia*.

APIs and excipients, in the solid phase, can be classified as either crystalline or non-crystalline solids. A crystalline structure implies that the structural units (i.e. the unit cells) are repeated in a long range order (i.e. three dimensional crystal structure). The arrangement of atoms and/or molecules in an amorphous solid is non-ordered (i.e. does not have a long range order), random, and does not possess a distinguishable crystal structure. Amorphous solids are classified as non-crystalline solids.

Variation in the crystallization conditions (temperature, pressure, solvent composition, concentration, rate of crystallization, seeding of the crystallization medium, presence and concentration of impurities, etc.) may cause the formation of different crystalline forms.

When a chemical element (e.g. sulfur) exists in different physical forms, it is referred to as *allotropy,* not polymorphism *(1). Polymorphism* (or *crystal polymorphism*) is a phenomenon related to the solid state; it is the ability of a chemical compound in the solid state to exist in different crystalline forms having the same chemical composition (11). The difference in internal crystal structure could be attributed to differences in molecule packing arrangements and/or different molecular conformations. Different forms often exhibit different solubilities, but as they have identical chemical composition they have the same chemical behaviour once in solution.

Crystals of a given chemical compound with the same internal structure may exhibit different external shapes or *crystal habits*. In addition, variations in crystal habit may indicate the presence of polymorphism but is not necessarily indicative of polymorphic forms (*12*).

Solvates are crystal forms containing stoichiometric or non-stoichiometric quantities of a solvent. When the solvent incorporated into the crystal structure of the compound is water, the molecular adduct formed is referred to as a *hydrate.* Hydrates can be classified as three categories based on different structural aspects: *Class I* represents hydrates where the water molecules exist at isolated sites; *Class II* hydrates are generally referred to as channel hydrates; and *Class III* hydrates are generally referred to as ion-coordinated site hydrates. In such systems, water molecules form ion-water bonds that are usually much stronger than hydrogen bonds (*13*). Solvation and hydration products are also sometimes referred to as *pseudopolymorphs (2, 3, 4)*. However, the term “pseudopolymorphism” is ambiguous because of its use in different circumstances. It is therefore preferable to use only the terms “solvates” and “hydrates”.

A compound of a given hydration/solvation composition may crystallize into more than one crystalline form; an example of such a compound is nitrofurantoin *(5).* Nitrofurantoin can be crystallized as two monohydrate forms (Forms I and II) and two anhydrous forms (designated polymorphs a and b) *(5)*. A solvate may interconvert in the solid state to other forms, e.g. darunavir ethanolate can convert to the amorphous form, the desolvated form, or the hydrate, depending on the environmental conditions.

Crystal forms are said to be *isostructural* (also referred to as *isomorphic*) when they have the same overall crystal packing. Solvates, which have the same overall crystal packing, but differ only in the solvents included in their crystal structures, are termed *isostructural or isomorphic solvates*, e.g. hydrate and isopropanolate of hexakis(2,3,6-tri-O-acetyl)-α-cyclodextrin *(6)*.

The term *desolvated solvate* (or *desolvated hydrates*), also referred to as *isomorphous desolvates*, has been used to describe a solid form obtained by removing solvent from the solvate crystal structure (or water from a hydrate) without significantly changing the crystal structure *(4)*, as in the desolvated monohydrate of terazosin HCl *(7)*.

Amorphous forms of APIs and excipients are of substantial interest because they are usually more soluble (also having a faster dissolution rate) than their crystalline counterparts but are thermodynamically less stable. Solid-state properties of amorphous forms of the same chemical compound (i.e. thermal behaviour, solubility profile, density, etc.) may differ;

Co-crystals are crystalline materials composed of two or more different molecules, typically an API and co-crystal formers (“coformers”) within the same crystal structure that are associated by nonionic and noncovalent bonds. An example of a co-crystal is the succinic acid co-crystal of fluoxetine HCl *(8).* Co-crystals are thus more similar to solvates, in that both contain more than one component in the crystal structure. However, for co-crystals the coformer is non-volatile (i.e. exists as solid material at ambient conditions) *(3)*.

Pharmaceutical co-crystals have gained considerable attention as alternative forms in an attempt to enhance the bioavailability, stability and processability of the API in the manufacturing process. Another advantage of co-crystals is that they generate a

diverse array of solid state forms for APIs that lack ionisable functional groups, which is a prerequisite for salt formation *(3)*. Guidance and reflection papers on the use and classification of pharmaceutical co-crystals have been published *(3, 9)*.

2. CHARACTERIZATION AND THERMODYNAMIC STABILITY OF SOLID FORMS

Crystalline and amorphous forms are characterized based on their physicochemical properties. Table 1 lists some examples of the properties that may differ among different forms *(14)*.

Table 1. Examples of physicochemical properties that may differ among different forms

1. Packing properties

a. Molar volume and density

b. Refractive index

c. Conductivity (electrical and thermal)

d. Hygroscopicity

2. Thermodynamic properties

a. Melting and sublimation temperatures

b. Internal energy (i.e. structural energy)

c. Enthalpy (i.e. heat content)

d. Heat capacity

e. Entropy

f. Free energy and chemical potential

g. Thermodynamic activity

h. Vapour pressure

i. Solubility and dissolution characteristics

3. Spectroscopic properties

a. Electronic state transitions

b. Vibrational state transitions

c. Nuclear spin state transitions

4. Kinetic properties

a. Dissolution rate

b. Rates of solid state reactions

5. Surface properties

a. Surface-free energy

b. Interfacial tensions

c. Habit (i.e. shape)

6. Mechanical properties

a. Hardness

b. Tensile strength

c. Compactibility

d. Flow

Table 2 summarizes some of the most commonly used techniques to study and/or classify different amorphous or crystalline forms. These techniques are often complementary and it is indispensable to use several of them; however, demonstration of a

non-equivalent structure by single crystal X-ray diffraction is currently regarded as the definitive evidence of polymorphism. Furthermore, X-ray powder diffraction and/or solid state NMR can also be used, as bulk techniques, to provide unequivocal proof of polymorphism *(10)*.

Any technique(s) chosen to confirm the identity of the specific form(s) must be proven to be suitably specific for the identification of the desired form(s). Care must be taken in choosing the appropriate sample preparation technique, as heat generation, mechanical stress or exposure to elevated pressure and other environmental conditions (humidity) may trigger conversion between different forms.

Table 2. Examples of some techniques that may be used to study and/or classify different crystalline forms

1. X-ray powder diffraction* *&* Single crystal X-ray diffraction
2. Microcalorimetry
3. Thermal analysis (*1.2.1 Melting point*,* differential scanning calorimetry, thermogravimetry, thermomicroscopy)
4. Moisture sorption analysis
5. Polarized optical microscopy and electronic microscopy with diffraction capability (ex. Transmission Electron Microscopy)
6. Solid-state nuclear magnetic resonance;
7. Solubility and dissolution studies
8. *Spectrophotometry in the infrared region (1.7)** and Raman spectroscopy
9. Intrinsic dissolution rate
10. True density measurement

* Methods currently employed by *The International Pharmacopoeia*

Using suitable analytical techniques, the thermodynamic stability of the forms should be investigated. The form with the lowest free energy is the most thermodynamically stable at a given temperature and pressure. All other forms of the given system are in a metastable state. At standard temperature and pressure, a metastable form may remain unchanged or may change to a thermodynamically more stable form. In general, the more stable the form the less soluble it is. Conversion to a thermodynamically more stable form, may cause changes in some of the physicochemical properties (see Table 1) of the compound that may result in changes to other critical properties such as bioavailability, manufacturability (also referred to as processability), etc.

If there are several crystalline forms, one form is thermodynamically more stable at a given temperature and pressure. A given crystalline form may constitute a phase that can reach equilibrium with other solid phases and with the liquid and gas phases.

If each crystalline form is stable within a given temperature range, the change from one form to another is reversible and is said to be *enantiotropic*. The change from one phase to another is a univariate equilibrium so that at a given pressure this state is characterized by a transition temperature. However, if only one of the forms is stable over the entire temperature range, the change is irreversible or *monotropic (11)*.

3. RELEVANCE OF POLYMORPHISM FOR APIs AND FPPs

Polymorphism (and hydrate, solvate and cocrystal formation) of APIs and excipients are of interest as they may affect bioavailability, toxicity and processability. Also, the thermodynamic stability of the form included in the FPP is considered important as environmental conditions may compromise the stability thereof. For formulations where the API is dissolved, attention has to be paid to supersaturation with regards to different forms. A formulation might not be supersaturated regarding a metastable polymorph but supersaturated with regards to the thermodynamically stable polymorph. Control of the form by the manufacturer may be required during the processing of APIs and excipients and during the manufacturing of a dosage form to ensure the correct physicochemical characteristics thereof. For some APIs, control of the form (identity and/or assay) is required in the FPP itself (examples include mebendazole and ritonavir). The control of a specific form is especially critical in the areas where the bioavailability, stability or processability are directly impacted *(4)*.

The form of a readily soluble API that is incorporated into a solution, for example, an injection, an oral solution or eye drops, is normally non-critical (exceptions to this statement might be if the concentration of the solution is such that it is close to the limit of solubility of one of the possible polymorphs – as mentioned above - or solvate formation is observed with one of the excipients). Similarly, if an API is processed during the manufacturing process to obtain an amorphous form (e.g. hot melt extrusion, spray-dried dispersion, etc.), the original form is considered non-critical, as long as the processability is not influenced.

The form may be critical when the material is included in a solid dosage form or as a suspension in a liquid dosage form. In such

cases, the characteristics of the different polymorphs may affect the bioavailability or dissolution of the material. The polymorphic form of a biopharmaceutics classification system (BCS) class I or III API in a solid oral- or suspension dosage form is normally non-critical in terms of dissolution rate or bioavailability as by definition it would be readily soluble, but confirmation thereof by the manufacturer that the relevant solid forms meet BCS class I or III solubility criteria is recommended. The ICH Harmonised Tripartite Guideline on Specifications: Test procedures and acceptance criteria for new drug substances and new drug products: Chemical substances Q6A, provides guidance on when and how polymorphic forms should be controlled and monitored *(4)*.

The inclusion of potentially harmful solvents in the crystal structure, which may render APIs or excipients to be toxic or harmful to patients (i.e. solvates), should also be suitably regulated and monitored by the manufacturer.

4. POLYMORPHISM IN *THE INTERNATIONAL PHARMACOPOEIA*

Where a monograph indicates that a compound shows polymorphism this may be true crystal polymorphism, occurrence of solvates/hydrates or occurrence of the amorphous form.

The International Pharmacopoeia controls the polymorphic or crystalline forms (hereafter referred to as form) of a limited number of substances by restricting it to either:

a single form, for example, carbamazepine API (Anhydrous Form III), mebendazole API (Form C); or

by limiting the presence of unwanted forms, for example, chloramphenicol palmitate API (should contain at least 90% of polymorph B).

The identification and control of forms specified in *The International Pharmacopoeia* may be achieved by:

permitting no deviation from the infrared absorption spectrum of the reference substance prescribed (or reference spectrum supplied) – when the infrared absorption spectrum has been proven to be specific to the preferred form and able to distinguish the undesired form(s), for example, indomethacin API;

restricting the melting point range, when the melting properties of the forms are clearly distinguishable, for example, phenobarbital API;

recommending the use of any other suitable methods such as X-ray powder diffractometry, for example, carbamazepine tablets; and

limiting the incorporated solvent (in the case of solvates/hydrates) with a specific limit test, for example, nevirapine hemihydrate API.

The specific control to be used will be indicated in the applicable monograph.

When the infrared identification test is able to detect differences in forms for a specific compound (i.e. polymorphism may be present for this compound), but the control of a specific form is not required by the monograph, the user may be instructed to manipulate both sample and standard to achieve a common specific crystal form prior to IR identification testing, for example:

recrystallize both the test substance and the specified reference substance, in the event where the infrared spectra are found to be not concordant, for example, fluconazole API; and/or

dry the API and/or specified reference substance to ensure that both forms are in the anhydrous or dehydrated state, for example, nevirapine hemihydrate API.

Whenever the choice of a specific form is critical with regard to bioavailability and/or stability, the method of the manufacturer of the product must be validated to consistently yield the desired polymorph in the final product at release and over its shelf life. In some cases, the identification of the form is sufficient (i.e. assuring the predominance of a specific form). In other cases, control of the amount (i.e. quantitation) of a form may be additionally required. The monograph will include a statement under the heading “Manufacturing” to draw attention to the control of a specified form during manufacturing where specific form control is known to be critical, for example, carbamazepine oral suspension.

It is the intention of *The International Pharmacopoeia* to extend the inclusion of explicit statements in monographs, where appropriate, as information on the occurrence of polymorphism becomes available. The Secretariat thus cordially invites the users of *The International Pharmacopoeia* and manufacturers to share any relevant information that could be included in the monographs.

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Note for guidance on organic impurities in active pharmaceutical ingredients and finished pharmaceutical products

2017

1. Scope

Purity is a critical attribute of active pharmaceutical ingredients (APIs) and finished pharmaceutical products (FPPs), which potentially affects their safety and efficacy. Therefore, API and FPP monographs in *The International Pharmacopoeia* (Ph.Int.) shall contain specifications for purity which include requirements for the control of impurities, wherever possible.

Impurities in APIs and FPPs may include starting materials, by-products, intermediates, degradation products, reagents, ligands, residual catalysts and residual solvents. They can be classified as either organic, inorganic or biological.

This guidance note covers requirements for controlling organic process-related impurities and degradation products in APIs and FPPs, and provides guidance on how to assess compliance with Ph.Int. requirements.

Statements in this document are applicable to monographs included in Ph.Int. after the publication of this guidance note. Compliance with monographs published before this updated guidance shall be evaluated against the previous text *Related substances in dosage form monographs*[1] unless required otherwise by the competent authority. A list of all monographs included in Ph.Int. before the publication of this guidance note is presented in the document titled *Monographs to be evaluated against the text Related substances in dosage form monographs* which can be found in *The International Pharmacopoeia* under Supplementary information.

[1] The replaced text can be found on the homepage of *The International Pharmacopoeia* under “Omitted texts”.

Excluded from this guidance note are biological/biotechnological products, peptides, oligonucleotides, radiopharmaceuticals, herbal products, fermentation products and semisynthetic products derived therefrom and crude products of animal and plant origin.

Further excluded are the following:

- -extraneous contaminants that should not occur in APIs and FPPs and are more appropriately addressed as good manufacturing practices (GMP) issues;
- -crystallographic modifications (“polymorphic forms”);
- -impurities that arise from printing inks or excipients (reaction products between excipients and APIs are not excluded);
- -organic impurities that are leached from container-closure systems;
- -highly toxic (e.g. genotoxic) impurities or highly toxic degradation products and residual solvents (volatile organic impurities) are addressed using separate applicable guidance.

2. Defining the purity of APIs and FPPs

To control relevant organic impurities individual monographs will contain a stability-indicating test entitled “Related substances”. This test may be supplemented by a specific test where a given impurity is not adequately controlled by the related substances test or where there are particular reasons (for example, safety reasons a genotoxic/mutagenic or an enantiomeric impurity) requiring specific control.

Monographs of APIs shall include specification limits for any impurities (i.e. process-related impurities that result from the manufacturing process and degradation products) observed at levels above the identification threshold and – when appropriate – specification limits for the total. Monographs on FPPs must include appropriate limits for degradation products and, if possible to be detected by the method, impurities from the manufacturing process. This approach provides, in conjunction with the monograph on the API, the means for an independent control laboratory without access to manufacturer’s data to establish whether or not an API of pharmacopoeial quality has been used to manufacture the FPP under examination.

It is recognized that limits for degradation impurities given in FPP monographs may need to be higher than the limits for the same impurities that appear in the monograph for the corresponding API to take into account any degradation which may occur during the manufacture and/or storage of the FPP. If the test for impurities in the FPP also limits impurities arising during the API synthesis, the reporting threshold as normally determined for the dosage form degradation products (not as for the API) will apply.

Instruction for control of impurities may also be included in the manufacture section of a monograph, for example, where the only analytical method appropriate for the control of a given impurity is to be performed by the manufacturer since the method is technically too complex for general use. The production process (including the purification steps) should be validated to give sufficient control so that the product, if tested, would comply with the specified limits using a suitable analytical method.

Under the section on “Impurities” in the monographs for APIs and FPPs, known impurities are listed (transparency list) that are able to be separated and detected by the described test method(s). In FPP monographs reference may also be made to the list in the monograph of the corresponding API if the test is able to detect these known impurities. Whenever possible, the impurities

are identified as degradation products and/or synthesis-related impurities.

Tests for related substances are intended to provide control of known potential or actual impurities rather than to control all possible impurities. The tests are not designed to detect any adventitious contaminants or adulteration. APIs or FPPs found to contain an impurity not detectable by means of the prescribed tests are not of pharmaceutical quality if the nature or amount of the impurity found is incompatible with GMP or applicable regulatory standards.

3. Setting acceptance criteria for organic impurities

Limits in Ph.Int. are usually set based on:

- -the evaluation of information, provided by manufacturers, concerning the nature of impurities, the reason for their presence, the concentrations that may be encountered in material produced under GMP, the manner in which the API or FPP may change during storage and when subjected to stress conditions (e.g. light, heat, moisture, acid, base or oxygen), and information on the toxicity of any organic impurity in relation to that of the substance itself;
- -justified limits accepted when the marketing authorization was granted or when the product was included in the WHO list of prequalified APIs or prequalified FPPs. Such acceptance includes establishing the qualification of limits by scientific principles, *inter alia*, ICH Q3 guidelines;
- -limits published by other pharmacopoeias applying good pharmacopoeial practices (GPhP);[2]
- -principles published in current regulatory guidance documents, such as those published by the International Council on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH).

[2] Fiftieth report of the WHO Expert Committee on Specifications for Pharmaceutical Preparations. Geneva, World Health Organization, 2016. WHO Technical Report Series, No. 996, Annex 1.

Safety considerations are of particular importance in establishing acceptance criteria for impurities.

The historical safety record, the route of administration, the type of dosage form, the maximum daily dose, the duration of treatment, the patient population, the need for and the availability of the medicine are also to be taken into consideration when setting limits for impurities.

Also, comments received on the draft monographs from Member States, stakeholders and other interested parties during the public consultation phase are reviewed and considered.

4. Claiming compliance with the requirements by a manufacturer

In the event of monographs that were published prior to the publication of this guidance note[1] which have no related substances test (or equivalent) or where the existing test does not comply with the requirements of the applicable regulatory standards the manufacturer shall nevertheless ensure that there is suitable control of organic impurities.

When an API contains impurities other than those mentioned in the Impurities section (for example, because it was manufactured using an alternative method of synthesis) the manufacturer must ascertain that these impurities can be controlled by the analytical method(s) and limits described in the monograph; otherwise a new analytical method and specifications shall be developed and submitted to the competent authority for approval, while a revision of the monograph of Ph.Int. shall be proposed by the manufacturer.

When a chromatographic peak (at a level greater than the applicable identification threshold) cannot be assigned unambiguously to an impurity in the transparency list using the means described in the monograph (e.g. by means of retention times, relative retentions or comparison to reference substances mentioned in the monograph) the manufacturer has to apply additional measures in order to identify the impurity. These measures may include, for example, ensuring that the response is not due to the chromatographic solvent system or excipients used in the formulation and the identification of potential impurities not referred to in the monograph by the use of additional analytical techniques, e.g. so-called hyphenated analytical techniques, e.g. GC- or LC-mass spectrometric methods. If identification by structure is initially not possible the impurity could be listed as an unidentified specified impurity until identification has been achieved.

When an impurity not listed in the transparency list is found in an API or in an FPP (at a level above the identification threshold) it is the responsibility of the manufacturer to demonstrate that it is identified and a qualified limit is set, in accordance with the applicable regulatory standards, and to communicate this to Ph.Int..

Glossary

degradation product. An impurity resulting from a chemical change in the active pharmaceutical ingredient (API) brought about during manufacture and/or storage of the API or the dosage form by the effect of, for example, light, oxygen, temperature, pH, water or by reaction with an excipient or another API (in fixed dose combination dosage form) and/or the immediate container-closure system.

extraneous contaminant. An impurity arising from any source extraneous to the manufacturing process.

identified impurity. An impurity for which a structural characterization has been achieved.

identification threshold. A limit above (>) which an impurity should be identified, based on the applicable regulatory standards.

impurity (API). Any component of an API that is not the chemical entity defined as the API.

impurity (FPP). Any component of the FPP that is not the API or an excipient in the FPP.

intermediate. A material produced during steps of the synthesis of an API that undergoes further chemical transformation before it becomes an API.

ligand. An agent with a strong affinity to a metal ion.

polymorphic forms. Different crystalline forms of the API. These can include solvation or hydration products (also known as pseudopolymorphs) and amorphous forms.

qualification threshold. A limit above which an impurity should be qualified.

reporting threshold. A limit above which an impurity is to be reported.

specified impurity. An impurity that is individually listed and limited with a specific acceptance criterion in the monograph. A specified impurity can be either identified or unidentified.

starting material. A raw material, intermediate or an API that is used in the production of an API and that is incorporated as a significant structural fragment into the structure of the API. An API starting material can be an article of commerce, a material purchased from one or more suppliers under contract or commercial agreement or produced in-house. API starting materials normally have defined chemical properties and structure.

unidentified impurity. An impurity for which a structural characterization has not been achieved and that is defined solely by qualitative analytical properties (e.g. chromatographic retention time).

unspecified impurity. An impurity that is limited by a general acceptance criterion, but not individually listed with its own specific acceptance criterion.

Microbiological quality of non-sterile products: recommended acceptance criteria for pharmaceutical preparations

This text is based on the internationally-harmonized texts developed by the Pharmacopoeial Discussion Group (PDG). Some editorial modifications have been made in order to be in line with the style used in The International Pharmacopoeia *. It should be noted, however, that acceptance criteria for oral dosage forms, other than herbal medicines, containing raw material of natural origin for which antimicrobial pretreatment is not feasible and for which the competent authority accepts TAMC of the raw material exceeding 103 CFU/g or CFU/mL (see Table 1), were exempted from the PDG harmonization.*

The text is provided to give information and guidance and is not regarded as an analytical requirement. The acceptance criteria do not apply to herbal medicines (i.e. herbs, herbal materials, herbal preparations and finished herbal products). For such preparations reference should be made to Quality control methods for herbal materials: Determination of microorganisms (World Health Organization, 2011), and WHO guideline on assessing quality of herbal medicines with reference to contaminants and residues (Determination of microbial contaminants; Annex 5 – Determination of microorganism) (World Health Organization, 2007).

The presence of certain microorganisms in non-sterile preparations may have the potential to reduce or even inactivate the therapeutic activity of the product and has a potential to adversely affect the health of the patient. Manufacturers have, therefore, to ensure a low bioburden of finished dosage forms by implementing current guidelines on good manufacturing practice (GMP) during the manufacture, storage and distribution of pharmaceutical preparations.

Microbial examination of non-sterile products is performed according to the methods given in the texts on 3.3.1 Microbial enumeration tests and 3.3.2 Tests for specified microorganisms. Acceptance criteria for non-sterile pharmaceutical products based upon the total aerobic microbial count (TAMC) and the total combined yeasts/moulds count (TYMC) are given in Table 1. Acceptance criteria are based on individual results or on the average of replicate counts when replicate counts are performed (e.g. direct plating methods).

Table 1 includes a list of specified microorganisms for which acceptance criteria are set. The list is not necessarily exhaustive and for a given preparation it may be necessary to test for other microorganisms depending on the nature of the starting materials and the manufacturing process.

When an acceptance criterion for microbiological quality is prescribed it is interpreted as follows:

- 10^1 CFU: maximum acceptable count = 20,
- 10^2 CFU: maximum acceptable count = 200,
- 10^3 CFU : maximum acceptable count = 2000, and so forth.

Table 1. Recommended acceptance criteria for microbiological quality of non-sterile dosage forms

Route of administration	Total aerobic microbial count (CFU/g or CFU/ mL)	Total combined yeasts/moulds count (CFU/g or CFU/ mL)	Specified microorganism
Non-aqueous preparations for oral use	10^3	10^2	Absence of *Escherichia coli* (1 g or 1 mL)
Aqueous preparations for oral use	10^2	10^1	Absence of *Escherichia coli* (1 g or 1 mL)
Rectal use	10^3	10^2	–
Oromucosal use Gingival use Cutaneous use Nasal use Auricular use	10^2	10^1	Absence of *Staphylococcus aureus* (1 g or 1 mL) Absence of *Pseudomonas aeruginosa* (1 g or 1 mL)
Vaginal use	10^2	10^1	Absence of *Pseudomonas aeruginosa* (1 g or 1 mL) Absence of *Staphylococcus aureus* (1 g or 1 mL)

			Absence of *Candida albicans* (1 g or 1 mL)
Transdermal patches (limits for one patch including adhesive layer and backing)	10^2	10^1	Absence of *Staphylococcus aureus* (1 patch) Absence of *Pseudomonas aeruginosa* (1 patch)
Inhalation use (special requirements apply to liquid preparations for nebulization)	10^2	10^1	Absence of *Staphylococcus aureus* (1 g or 1 mL) Absence of *Pseudomonas aeruginosa* (1 g or 1 mL) Absence of bile-tolerant gram-negative bacteria (1g or 1 mL)
Oral dosage forms, other than herbal medicines, containing raw materials of natural (animal, vegetal or mineral) origin for which antimicrobial pretreatment is not feasible and for which the relevant national or regional authority accepts TAMC of the raw material exceeding 10^3 CFU/g or CFU/mL.	10^4	10^2	Not more than 10^2 CFU of bile-tolerant gram-negative bacteria (1 g or 1 mL) Absence of *Salmonella* (10 g or 10 mL) Absence of *Escherichia coli* (1 g or 1 mL) Absence of *Staphylococcus aureus* (1 g or 1 mL)

If it has been shown that none of the prescribed tests will allow valid enumeration of microorganisms at the level prescribed, a validated method with a limit of detection as close as possible to the indicated acceptance criterion is used.

In addition to the microorganisms listed in Table 1, the significance of other microorganisms recovered should be evaluated in terms of:

-the use of the product: hazard varies according to the route of administration (eye, nose, respiratory tract);
-the nature of the product: its ability to support growth, the presence of adequate antimicrobial preservation;
-the method of application;
-the intended recipient – risk may differ for neonates, infants, the debilitated;
-use of immunosuppressive agents, corticosteroids;
-presence of disease, wounds, organ damage.

Where warranted a risk-based assessment of the relevant factors is conducted by personnel with specialized training in microbiology and the interpretation of microbiological data. For raw materials the assessment takes account of processing to which the product is subjected, the current technology of testing and the availability of materials of the desired quality.

Monographs to be evaluated against the text "Related substances in dosage form monographs"

2018

A list of all monographs included in *The International Pharmacopoeia* before the publication of the guidance note: " *Guidance on organic impurities in active pharmaceutical ingredients and finished pharmaceutical products* " is presented in this document. Compliance of the monographs listed hereunder shall be evaluated against the previous text " *Related substances in dosage form monographs*" unless required otherwise by the competent authority. This text can be found on the website of The International Pharmacopoeia under "*Omitted texts*".

In the event that a revision of any of the specified texts are to be published in *The International Pharmacopoeia*, the revised text will be removed from this list and compliance of the revised text will be evaluated against the " *Guidance on organic impurities in active pharmaceutical ingredients and finished pharmaceutical products* " guidance note.

Pharmaceutical substances monographs

Abacavir sulfate

Acacia

Acetazolamide

Acetic acid

Acetylsalicylic acid

Aciclovir

Albendazole

Alcohol

Alcuronium chloride

Alginic acid

Allopurinol

Aluminium hydroxide

Aluminium magnesium silicate

Aluminium sulfate

Amidotrizoic acid

Amikacin sulfate

Amikacin

Amiloride hydrochloride

Aminophylline

Amitriptyline hydrochloride

Amodiaquine

Amodiaquine hydrochloride

Amphotericin B

Ampicillin

Ampicillin sodium

Anaesthetic Ether

Antimony sodium tartrate

Arachis oil

Artemether

Artemisinin

Artemotil

Artenimol

Artesunate

Ascorbic acid

Atazanavir sulfate

Atropine sulfate

Azathioprine

Bacitracin

Bacitracin zinc

Barium sulfate

Beclometasone dipropionate

Bentonite

Benzalkonium chloride

Benzathine benzylpenicillin

Benznidazole

Benzocaine

Benzoic acid

Benzyl alcohol

Benzyl benzoate

Benzyl hydroxybenzoate

Benzylpenicillin potassium

Benzylpenicillin sodium

Bephenium hydroxynaphthoate

Betamethasone

Betamethasone valerate

Biperiden

Biperiden hydrochloride

Bleomycin hydrochloride

Bleomycin sulfate

Bupivacaine hydrochloride

Busulfan

Butylated hydroxyanisole

Butylated hydroxytoluene

Caffeine

Calamine

Calcium carbonate

Calcium folinate

Calcium gluconate

Calcium hydrogen phosphate

Calcium phosphate

Calcium stearate

Calcium sulfate

Capreomycin sulfate

Captopril

Carbamazepine

Carbidopa

Carbomer

Carmellose sodium

Carnauba wax

Cellacefate

Cetomacrogol 1000

Cetostearyl alcohol

Cetrimide

Cetyl alcohol

Cetyl esters wax

Charcoal, activated

Chloral hydrate

Chlorambucil

Chloramphenicol

Chloramphenicol palmitate

Chloramphenicol sodium succinate

Chlorhexidine diacetate

Chlorhexidine dihydrochloride

Chlormethine hydrochloride

Chlorobutanol

Chlorocresol

Chloroquine phosphate

Chloroquine sulfate

Chlorphenamine hydrogen maleate

Chlorpromazine hydrochloride

Chlortalidone

Chlortetracycline hydrochloride

Cimetidine

Ciprofloxacin

Ciprofloxacin hydrochloride

Cisplatin

Citric acid

Clindamycin phosphate

Clofazimine

Clomifene citrate

Cloxacillin sodium

Coal tar

Codeine monohydrate

Codeine phosphate

Colchicine

Colecalciferol

Cyanocobalamin

Cyclophosphamide

Cycloserine

Cytarabine

Dactinomycin

Dapsone

Deferoxamine mesilate

Dehydroemetine dihydrochloride

Dexamethasone

Dexamethasone acetate

Dexamethasone sodium phosphate

Dextromethorphan hydrobromide

Diazepam

Diazoxide

Dicloxacillin sodium

Dicoumarol

Didanosine

Diethylcarbamazine dihydrogen citrate

Diethyltoluamide

Digitoxin

Digoxin

Diloxanide furoate

Dilute hydrochloric acid

Diluted Isosorbide dinitrate

Dimercaprol

Dinitrogen oxide

Diphenoxylate hydrochloride

Disodium edetate

Dithranol

Dopamine hydrochloride

Doxorubicin hydrochloride

Doxycycline hyclate

Edrophonium chloride

Efavirenz

Emetine hydrochloride

Emtricitabine

Ephedrine

Ephedrine hydrochloride

Ephedrine sulfate

Epinephrine

Epinephrine hydrogen tartrate

Ergocalciferol

Ergometrine hydrogen maleate

Ergotamine tartrate

Erythromycin

Erythromycin ethylsuccinate

Erythromycin lactobionate

Erythromycin stearate

Ethambutol hydrochloride

Ethanol

Ethinylestradiol

Ethionamide

Ethosuximide

Ethyl hydroxybenzoate

Ethylcellulose

Etoposide

Ferrous fumarate

Ferrous sulfate

Fluconazole

Flucytosine

Fludrocortisone acetate

Fluorescein sodium

Fluorouracil

Fluphenazine decanoate

Fluphenazine enantate

Fluphenazine hydrochloride

Folic acid

Furosemide

Gallamine triethiodide

Gelatin

Gentamicin sulfate

Glibenclamide

Glucose

Glycerol

Glycerol 85% m/m

Glyceryl monostearate

Griseofulvin

Haloperidol

Halothane

Hard fat

Hard paraffin

Heparin calcium

Heparin sodium

Homatropine hydrobromide

Homatropine methylbromide

Hydralazine hydrochloride

Hydrochloric acid

Hydrochlorothiazide

Hydrocortisone

Hydrocortisone acetate

Hydrocortisone sodium succinate

Hydrous Benzoyl peroxide

Hydroxocobalamin

Hydroxocobalamin chloride – Hydroxocobalamin sulfate

Hydroxyethylcellulose

Hydroxypropylcellulose

Hypromellose

Ibuprofen

Idoxuridine

Imipramine hydrochloride

Indinavir sulfate

Indometacin

Insulin

Iodine

Iohexol

Iopanoic acid

Iotroxic acid

Ipecacuanha root

Isoniazid

Isoprenaline hydrochloride

Isoprenaline sulfate

Kanamycin acid sulfate

Kanamycin monosulfate

Kaolin

Ketamine hydrochloride

Ketoconazole

Lactic acid

Lactose

Lamivudine

Levamisole hydrochloride

Levodopa

Levonorgestrel

Levothyroxine sodium

Lidocaine

Lidocaine hydrochloride

Lindane

Lithium carbonate

Loperamide hydrochloride

Lopinavir

Lumefantrine

Magnesium hydroxide

Magnesium oxide

Magnesium stearate

Magnesium sulfate heptahydrate

Mannitol

Mebendazole

Medroxyprogesterone acetate

Mefloquine hydrochloride

Meglumine

Mercaptopurine

Mercuric oxycyanide

DL-Methionine

Methotrexate

Methyl hydroxybenzoate

Methylcellulose

Methyldopa

Methylrosanilinium chloride

Methyltestosterone

Methylthioninium chloride

Metoclopramide hydrochloride

Metrifonate

Metronidazole

Metronidazole benzoate

Miconazole nitrate

Microcrystalline cellulose

Morphine hydrochloride

Morphine sulfate

Naloxone hydrochloride

Nelfinavir mesilate

Neomycin sulfate

Neostigmine bromide

Neostigmine metilsulfate

Nevirapine

Niclosamide

Nicotinamide

Nicotinic acid

Nifedipine

Nifurtimox

Niridazole

Nitrazepam

Nitrofurantoin

Nonoxinol 9

Norethisterone acetate

Norethisterone

Norethisterone enantate

Noscapine

Noscapine hydrochloride

Nystatin

Oseltamivir phosphate

Oxamniquine

Oxygen

Oxytetracycline dihydrate

Oxytetracycline hydrochloride

Oxytocin

Papaverine hydrochloride

Paracetamol

Paromomycin sulfate

Penicillamine

Pentamidine isetionate

Pentamidine mesilate

Pethidine hydrochloride

Phenobarbital

Phenobarbital sodium

Phenoxymethylpenicillin

Phenoxymethylpenicillin calcium

Phenoxymethylpenicillin potassium

Phenylmercuric nitrate

Phenytoin

Phenytoin sodium

Physostigmine salicylate

Phytomenadione

Pilocarpine hydrochloride

Pilocarpine nitrate

Piperazine adipate

Piperazine citrate

Podophyllum resin

Polysorbates 20, 60, 80

Potassium chloride

Potassium citrate

Potassium iodide

Povidone

Praziquantel

Prednisolone

Prednisolone acetate

Prednisolone sodium phosphate

Primaquine diphosphate

Probenecid

Procainamide hydrochloride

Procaine benzylpenicillin

Procaine hydrochloride

Procarbazine hydrochloride

Progesterone

Proguanil hydrochloride

Promethazine hydrochloride

2-Propanol

Propranolol hydrochloride

Propylene glycol

Propyliodone

Propyl hydroxybenzoate

Propylthiouracil

Protamine sulfate

Purified water

Pyrantel embonate

Pyrazinamide

Pyridostigmine bromide

Pyridoxine hydrochloride

Pyrimethamine

Quinidine sulfate

Quinine bisulfate

Quinine dihydrochloride

Quinine hydrochloride

Quinine sulfate

Reserpine

Retinol concentrate, oily form

Riboflavin

Rifampicin

Ritonavir

Saccharin sodium

Salbutamol

Salbutamol sulfate

Salicylic acid

Saquinavir mesilate

Saquinavir

Selenium disulfide

Senna fruit

Senna leaf

Silver nitrate

Sodium amidotrizoate

Sodium calcium edetate

Sodium chloride

Sodium citrate

Sodium cromoglicate

Sodium fluoride

Sodium hydrogen carbonate

Sodium hydroxide

Sodium nitrite

Sodium nitroprusside

Sodium salicylate

Sodium stibogluconate

Sodium sulfate

Sodium sulfate, anhydrous

Sodium thiosulfate

Sodium valproate

Spectinomycin hydrochloride

Spironolactone

Starches

Stavudine

Sterile water for injections

Streptomycin sulfate

Sulfacetamide

Sulfacetamide sodium

Sulfadiazine silver

Sulfadimidine

Sulfadimidine sodium

Sulfadoxine

Sulfamethoxazole

Sulfamethoxypyridazine

Sulfasalazine

Suramin sodium

Suxamethonium chloride

Talc

Tamoxifen citrate

Tenofovir disoproxil fumarate

Testosterone enantate

Testosterone propionate

Tetracaine hydrochloride

Tetracycline hydrochloride

Thiamine hydrobromide

Thiamine hydrochloride

Thiamine mononitrate

Thioacetazone

Thiopental sodium

Tiabendazole

Timolol maleate

Tolbutamide

Trihexyphenidyl hydrochloride

Trimethadione

Trimethoprim

Tropicamide

Tubocurarine chloride

Verapamil hydrochloride

Vinblastine sulfate

Vincristine sulfate

Warfarin sodium

Water for injections

White, soft paraffin; Yellow soft paraffin

Wool fat

Zidovudine

Zinc acetate

Zinc gluconate

Zinc oxide

Zinc sulfate

Dosage form monographs

Abacavir oral solution

Abacavir tablets

Acetylsalicylic acid tablets

Aciclovir for injection

Aciclovir tablets

Albendazole chewable tablets

Allopurinol tablets

Amikacin for injection

Amodiaquine tablets

Amoxicillin oral suspension

Amphotericin B for injection

Ampicillin capsules

Ampicillin sodium for injection

Artemether and lumefantrine oral suspension

Artemether and lumefantrine tablets

Artemether capsules

Artemether injection

Artemether tablets

Artemotil injection

Artenimol tablets

Artesunate for injection

Artesunate tablets

Atazanavir capsules

Atropine sulfate tablets

Benzylpenicillin potassium for injection

Capreomycin for injection

Carbamazepine tablets

Chewable mebendazole tablets

Chloroquine phosphate tablets

Chloroquine sulfate oral solution

Chloroquine sulfate tablets

Chlorphenamine hydrogen maleate tablets

Cloxacillin sodium capsules

Cloxacillin sodium for injection

Codeine phosphate tablets

Colchicine tablets

Cycloserine capsules

Dapsone tablets

Dexamethasone phosphate injection

Dexamethasone tablets

Didanosine oral powder

Didanosine tablets

Diethylcarbamazine dihydrogen citrate tablets

Diloxanide furoate tablets

Doxycycline capsules

Doxycycline tablets

Efavirenz capsules

Efavirenz oral solution

Efavirenz tablets

Efavirenz, emtricitabine and tenofovir tablets

Emtricitabine and tenofovir tablets

Emtricitabine capsules

Ephedrine sulfate injection

Ergometrine hydrogen maleate tablets

Ergometrine injection

Erythromycin ethylsuccinate tablets

Erythromycin stearate tablets

Ethambutol hydrochloride tablets

Fluconazole capsules

Fluconazole injection

Glyceryl trinitrate tablets

Griseofulvin tablets

Ibuprofen tablets

Indinavir capsules

Indometacin tablets

Isoniazid and ethambutol hydrochloride tablets

Isoniazid tablets

Kanamycin for injection

Lamivudine oral solution

Lamivudine tablets

Levamisole tablets

Levonorgestrel and ethinylestradiol tablets

Levonorgestrel tablets

Lopinavir and ritonavir tablets

Magnesium sulfate injection

Medroxyprogesterone injection

Mefloquine tablets

Melarsoprol injection

Metronidazole injection

Metronidazole oral suspension

Metronidazole tablets

Morphine sulfate tablets

Nelfinavir mesilate oral powder

Nelfinavir mesilate tablets

Nevirapine oral suspension

Nevirapine tablets

Niclosamide tablets

Nitrofurantoin tablets

Nystatin tablets

Oral rehydration salts

Oseltamivir capsules

Oxytocin injection

Paediatric didanosine liquid for oral use

Paediatric zinc sulfate oral solution

Paediatric zinc sulfate tablets

Paracetamol oral solution

Paracetamol oral suspension

Paracetamol tablets

Pentamidine isetionate for injection

Pethidine hydrochloride tablets

Phenobarbital tablets

Phenoxymethylpenicillin potassium tablets

Phenytoin sodium tablets

Piperazine adipate tablets

Piperazine citrate tablets

Praziquantel tablets

Prednisolone phosphate injection

Prednisolone sodium succinate for injection

Prednisolone tablets

Primaquine diphosphate tablets

Probenecid tablets

Procaine benzylpenicillin for injection

Pyrantel chewable tablets

Pyrantel oral suspension

Pyrantel tablets

Pyrazinamide tablets

Quinine bisulfate tablets

Quinine dihydrochloride injection

Quinine sulfate tablets

Retinol oral solution

Rifampicin and isoniazid dispersible tablets

Rifampicin and isoniazid tablets

Rifampicin capsules

Rifampicin tablets

Rifampicin, isoniazid and ethambutol hydrochloride tablets

Rifampicin, isoniazid and pyrazinamide dispersible tablets

Rifampicin, isoniazid and pyrazinamide tablets

Rifampicin, isoniazid, pyrazinamide and ethambutol hydrochloride tablets

Ritonavir tablets

Saquinavir mesilate capsules

Saquinavir tablets

Sodium bicarbonate intravenous infusion

Stavudine capsules

Streptomycin for injection

Sulfadoxine and pyrimethamine tablets

Sulfamethoxazole and trimethoprim intravenous infusion

Sulfamethoxazole and trimethoprim oral suspension

Sulfamethoxazole and trimethoprim tablets

Tenofovir tablets

Zidovudine and lamivudine tablets

Zidovudine capsules

Zidovudine intravenous infusion

Zidovudine, lamivudine and abacavir tablets

Zidovudine, lamivudine and nevirapine tablets

Zidovudine oral solution

Dissolution testing of tablets and capsules

2016-01

1. Introduction

Chapter *5.5 Dissolution test for solid oral dosage forms* is based on the internationally-harmonized dissolution test developed by the Pharmacopoeial Discussion Group (PDG), which comprises representatives from the European Pharmacopoeia, the Japanese Pharmacopoeia and the United States Pharmacopeia. The general method presents the Paddle and Basket methods for dissolution testing. Two other general methods contained in the PDG text, namely the Reciprocating-cylinder method and the Flow-through cell, have not so far been adopted for *The International Pharmacopoeia*.

It is not the intention of *The International Pharmacopoeia* to apply retrospectively the test conditions and acceptance criteria of the revised dissolution test or to change specifications for existing products. Table 1 lists monographs with dissolution tests, which were developed applying previous versions of chapter 5.5 and which are thus not subject to the internationally-harmonized provision. In the elaboration of new monographs and revision of individual monographs in *The International Pharmacopoeia* the principles of the revised test, e.g. to base acceptance criteria on “Q” values (dissolution limits), will be applied.

Amodiaquine tablets
Artemether capsules
Artemether tablets
Artenimol tablets
Artesunate tablets
Chloroquine phosphate tablets
Chloroquine sulfate tablets
Doxycycline capsules
Doxycycline tablets
Efavirenz, emtricitabine and tenofovir tablets
Emtricitabine and tenofovir tablets
Emtricitabine capsules
Erythromycin ethylsuccinate tablets
Erythromycin stearate tablets
Ethambutol hydrochloride tablets
Griseofulvin tablets
Ibuprofen tablets
Indinavir capsules
Indometacin tablets
Isoniazid tablets
Isoniazid and ethambutol hydrochloride tablets
Levonorgestrel and ethinylestradiol tablets
Lopinavir and ritonavir tablets
Metronidazole tablets
Phenoxymethylpenicillin potassium tablets
Phenytoin sodium tablets
Pyrazinamide tablets
Quinine bisulfate tablets
Quinine sulfate tablets
Rifampicin capsules
Rifampicin tablets
Ritonavir tablets
Saquinavir tablets
Sulfadoxine and pyrimethamine tablets
Sulfamethoxazole and trimethoprim tablets
Tenofovir tablets

Table 1. Monographs on solid, oral dosage forms with dissolution test conditions and specifications elaborated before chapter *5.5 Dissolution test for solid oral dosage forms* were revised to encompass the internationally-harmonized procedure.

2. Objective of dissolution testing

While the ultimate objective of dissolution testing is to ensure adequate and reproducible bioavailability, the objective of the

dissolution tests prescribed in the individual monographs of *The International Pharmacopoeia* is to obtain information about the drug-release characteristics of a particular formulation or batch of a product under standardized test conditions. Compliance with the test provides an assurance that most of the active ingredient will be dissolved in an aqueous medium within a reasonable amount of time when the preparation is subject to a mild agitation. Compliance with the dissolution test does not by itself guarantee bioavailability.

Standardized conditions and limits are considered appropriate for a pharmacopoeial test that is intended to apply to a monograph covering multisource products.

3. Policy of *The International Pharmacopoeia*

Monographs on tablet and capsule preparations listed in Table 1 include a dissolution test, either with or without further information on the test conditions. Spectrophotometry is typically employed as an analytical test method. In the case where a dissolution test is prescribed an additional disintegration test is not required.

In the elaboration of new tablet and capsule monographs and revision of existing monographs, decisions on dissolution and disintegration testing will be taken in agreement with the guidance given by the International Conference on Harmonisation (ICH) on the application of dissolution testing to medicinal products (see www.ich.org). The monograph will contain a dissolution test and/or a disintegration test. The choice of applying disintegration or dissolution for a given product should follow ICH Q6A and Q6A decision tree 7-1. As per these guidelines consideration to establish disintegration instead of dissolution should include the solubility of the active pharmaceutical ingredient, dissolution characteristics of the product and a demonstration that a relationship has been established between dissolution and disintegration. A demonstration of robustness with respect to the chosen disintegration method may substitute for the demonstration that a relationship has been established between dissolution and disintegration. In such a case where disintegration is found suitable for control of the performance of a product the disintegration specifications should be based on the results of the validation exercise.

Chewable tablets are intended to be chewed before being swallowed; however, they may be swallowed whole and therefore performance-testing requirements for conventional-release tablets should be applied to chewable tablets.

4. Test conditions

Following a decision made at the forty-fifth meeting of the Expert Committee on Specifications for Pharmaceutical Preparations a standardized dissolution test is applied to conventional-release tablet and capsule formulations containing highly soluble active ingredients (Class I and III of the Biopharmaceutics Classification System (BCS)[1]). The following conditions for a single-time test using the Paddle method are preferred:

- dissolution medium: dissolution buffer pH 6.8;
- volume of medium: 500 mL;
- rotation speed: 75 rpm;
- sampling time: 30 min.

When test conditions are not specified in the individual monograph it is recommended to apply similar test conditions. If the Basket method is used a rotation speed of 100 rpm is recommended.

For conventional-release tablet and capsule formulations containing poorly water-soluble active ingredients (Class II and IV of the BCS) decisions on the appropriate test conditions are taken on a case-by-case basis. A single-point dissolution test is normally applied. Because of the low aqueous solubility dissolution medium of volume 900 mL and addition of a surfactant may be needed. The concentration of active ingredient at 100% dissolution should not exceed approximately 35% saturation.

For delayed-release dosage forms two-stage testing according to the procedure in *5.5 Dissolution test for solid oral dosage forms* is applied. It is important to consider the population of individuals who will be taking the dosage form when designing the test, e.g. administration of the dosage form to achlorhydric patients may require testing for resistance of the product against gastric juice at elevated pH, for example, pH 3.5.

For sustained-release dosage forms the appropriate test conditions and sampling procedures are specified in the monograph. Three time-points are applied.

5. Acceptance criteria

The revised dissolution test contains acceptance criteria for conventional-release, delayed-release and sustained-release dosage forms. The acceptance criteria are expressed according to the principles stated in the internationally-harmonized dissolution test. The harmonized dissolution limits (Q-values) are applied in new and revised monographs (i.e. monographs on solid, oral dosage forms containing a dissolution test but not listed in Table 1).

The three-level acceptance criteria, i.e. S_1, S_2 and S_3 for conventional-release dosage forms, are not applied in monographs

listed in Table 1; acceptance criteria for a two-stage test (6 + 6 dosage units) are specified in some monographs. For dosage forms for which the monograph require compliance with *5.5 Dissolution test for solid oral dosage forms*, but without specification of test conditions, it is recommended to apply a test using Q = 75% and the three-level acceptance criteria.

[1] Classification of active ingredients, included in the WHO Model List of Essential Medicines, is provided in the WHO Technical Report Series, No. 937, Annex 7 (2006).

Recommendations on Risk of Transmitting Animal Spongiform Encephalopathy Agents via Medicinal Products

Products with risk of transmitting agents of animal spongiform encephalopathies are those derived from tissues or secretions of animals susceptible to transmissible spongiform encephalopathies other than by experimental challenge. This definition applies to all substances or preparations obtained from such animals and to all substances or preparations where products obtained from such animals are included as active substances or excipients or have been used during production, e.g. as raw or source materials, starting materials or reagents.

Materials of animal origin should be avoided whenever possible. However, if used, manufacturers should be aware of the risk and have a system in place to minimize it, especially since international trading patterns often include the processing and re-export of products, so that their origin may not be traceable. In order to minimize the risk of transmitting animal spongiform encephalopathy agents via medicinal products, manufacturers should follow the current WHO Guidelines on Transmissible Spongiform Encephalopathies in Relation to Biological and Pharmaceutical Products[1] and the recommendations of the Joint Technical Consultation on Bovine Spongiform Encephalopathy, public health, animal health and trade, convened by the WHO, the Food and Agriculture Organization of the United Nations and the Office International des Epizooties 11-14 June 2001 in Paris.[2]

[1] Note: the current guidelines are published on the WHO web site [http://www.who.int/bloodproducts/tse]. These guidelines are kept under review and are regularly updated.

[2] Proceedings - Joint WHO/FAO/OIE Technical Consultation on BSE: public health, animal health and trade. Paris: Office International des Epizooties, World Health Organization, Food and Agriculture Organization, World Organisation for Animal Health, 2002.

General guidelines for the establishment, maintenance and distribution of chemical reference substances[1]

[1] *WHO Expert Committee on Specifications for Pharmaceutical Preparations, Forty-first report*. Geneva, World Health Organization, 2007, Annex 3 (WHO Technical Report Series, No. 943)

Part B. Secondary chemical reference substances

This new Part B is intended to apply to secondary reference substances supplied as “official”, e.g. regional or national standards. In principle, secondary reference standards prepared by manufacturers can be prepared as “working standards” using the same procedures.

B.9 Distribution and supply

The distribution of secondary reference substances is carried out in such a manner as to maintain the integrity of the substance and avoid unnecessary delay in delivery to the users. The following factors are taken into account:

– conformity with safety and transport requirements;
– export and import procedure when the substance is to be delivered outside the country of the issuing body;
– customs regulations, e.g. special requirements applicable to substances under international control; and
– means of transportation.

B.8 Period of use

The expiry date is not indicated for secondary reference substances because the substances comply, where applicable, with the requirement of the pharmacopoeial monograph and are monitored regularly according to the re-testing programme. The issuing body should have effective means of communication to inform users of the validity of reference substances. It is recommended that only an amount sufficient for immediate use be purchased, and that the substances are used as soon as possible. Once the container has been opened efforts should be made to avoid possible degradation, contamination and/or introduction of moisture and/or exposure to air.

B.6 Retesting programme

See also Section 6.3 in Part A of these guidelines.

A system must be in place to ensure the continued fitness for use of the reference substances. Normally, a re-test programme is applied.

Reference substances are regularly tested for stability during their storage. A testing programme is applied which is designed to detect any sign of decomposition at an early stage using appropriate analytical techniques. The methods employed are suitable for small quantities, are both rapid and sensitive, and will have been performed during the establishment phase.

The frequency and extent of re-testing reference substances depends on a number of factors including stability, container and closure system, storage conditions, hygroscopicity, physical form and intended use. The frequency of testing and the testing methods to be employed for each reference substance must be documented.

Reference substances should preferably be subdivided and presented as single-use units. However, if the reference substance is kept in a multiuse container then re-testing will need to be more frequent because there is a greater risk of the uptake of moisture and/or decomposition of the reference substance. The testing methods should include the determination of water content and decomposition products. The maximum permitted variation from the assigned value should be predefined and if exceeded the batch should be re-established or replaced.

If the batch of primary reference substance used to calibrate the secondary reference substance is replaced, the secondary reference substance must be recalibrated against the new batch of the primary reference substance.

B.7 Information to be supplied with secondary chemical reference substances

For details of the information to be supplied see Section 6.4 of Part A of these guidelines: “Information to be supplied with chemical reference substances”.

B.5 Adoption of the secondary reference substance

The report of the collaborative trial to establish the secondary reference standard is submitted to the appropriate national or regional body to approve the secondary standard for the uses described.

B.4 Interlaboratory testing to establish the assigned content

Having demonstrated the suitability of the substance, the content value is assigned on the basis of the results generated by an interlaboratory trial. At least three laboratories participate in testing the proposed substance (*10*).

B.4.1 Competence of the participating laboratories Participating laboratories will have demonstrated their adherence to the concepts of an appropriate quality management system (*9*–*12*).

B.4.2 Dispatch of the candidate materials The proposed secondary reference substance is packaged in appropriate unit quantities. The quantity of each unit is dependent on the intended use. The proposed substance and the primary reference substance are dispatched to the participating laboratories in sufficient amounts for replicate analysis as required by the test protocol. The participating laboratories are instructed to record any abnormalities observed with the proposed substance. The packaging facilities are adequate and environmental conditions are controlled to ensure the integrity of the material throughout the packaging process.

The following documents should be supplied with the material:

– test protocol;
– test result report form;
– health and safety information; and
– information on the primary chemical reference substance.

B.4.3 Test protocol While the testing of primary chemical reference substances employs different analytical methods in a collaborative study, an alternative approach is normally applied to the testing of a secondary chemical reference substance. Since most secondary reference substances are established to determine the content of the drug substance itself (for which a pharmacopoeial monograph exists) and/or the amount of the drug substance contained in a pharmaceutical preparation, it is essential to use the method specifi ed in the relevant pharmacopoeia to obtain the assigned value.

The coordinating laboratory prepares the testing protocol, including predefined acceptance criteria of the results. The protocol clearly describes each step of the procedure and includes data reporting sheets The experimental design of the interlaboratory study is such that the results are statistically evaluated to assign a content with an acceptable confi dence interval in relation to the permitted limits of content as set in the defi nition. Both the number of independent replicate determinations to be performed and acceptance criteria to be applied are predefi ned.

B.4.4 Evaluation of test results Test results submitted by the participating laboratories are evaluated in accordance with the criteria set out in the protocol. The data submitted by each laboratory are tested statistically for “outliers” and for conformity with the system suitability criteria. Apparent “outliers” are investigated by the laboratory concerned, remedial action taken, and the analysis repeated. If a valid reason is discovered for the “outliers” then these are excluded from the statistical evaluation.

The mean and confidence interval are then calculated. The reference value is assigned using the mean of the laboratory means.

B.4.5 Traceability The term for “traceability”, for the purposes of this document, is defined as the property of a result of measurement which can be related to the appropriate standards, generally international or national standards, through an unbroken chain of comparison. In other words, when the result of a measurement is described as traceable, it is essential to specify to what (value of) “appropriate standards” traceability has been established.

The assigned value of a secondary chemical reference substance is traceable to the relevant primary reference substance. In the context of WHO quality specifications the relevant primary chemical reference substance is usually the ICRS established for use with *The International Pharmacopoeia*. In other contexts the relevant primary chemical reference substance will be the reference substance established for use with another internationally recognized pharmacopoeia (e.g. the European Pharmacopoeia chemical reference substances (Ph.Eur CRS), British Pharmacopoeia chemical reference substances (BPCRS), or the United States Pharmacopeia reference substances (USPRS)).

B.3 Packaging

See Section 6.1 in Part A of these guidelines.

B.2 Obtaining source material

B.2.1 Selection of candidate substance When it is intended to establish a secondary reference substance for use as an assay standard for the determination of the content of the drug substance itself or in a drug formulation, a source(s) of pharmaceutical grade substance(s) is (are) identified. Availability of the required quantity is assured. The guidelines given in Section 2 of Part A also apply in this case. If a substance is intended to be used as an impurity standard, the candidate material may be obtained from commercial suppliers, provided that the percentage purity is more than 95% (or 90% if for use in TLC).

B.2.2 Documentation to be supplied with the candidate reference substance The supplier of the candidate reference substance is requested to supply the same documentation as required for a candidate primary reference substance (see Section 2, Part A).

B.2.3 Initial testing for compliance with the requirements of the monograph The coordinating laboratory is responsible for verifying that the candidate reference substance complies with the requirements of the monograph, where applicable. In such a case compliance is a prerequisite to proceeding to the interlaboratory study to assign the content of the secondary standard.

A.6 Handling and distribution of chemical reference substances

The handling, distribution and use of established chemical reference substances must ensure that their integrity is safeguarded and maintained throughout their period of use.

A.6.1 Packaging operations Appropriate GMP requirements (*6*) should be observed. The various stages in packaging chemical reference substances should be clearly defined and controlled, to avoid contamination of the sample, mislabelling of containers, or any other event which might result in mishandling or mismanagement.

Containers for chemical reference substances should protect their contents from moisture, light and oxygen and must be tested for permeability to moisture. Additional measures may be necessary to ensure long-term integrity and stability. Most chemical reference substances, however, are conveniently supplied in moisture-proof containers which should be uniform in type and size to facilitate distribution. The lack of permeability to moisture and the compatibility of the material of which the closure is made with the chemical reference substance are important factors in determining the suitability of container closure systems. The best containers for chemical reference substances from the point of view of stability are sealed glass ampoules, but these have certain disadvantages. There is a risk of contaminating the substance with glass particles when the ampoules are opened.

It is preferable to restrict the quantity of reference substance held in each container to that required to perform the test(s). The use of multidose containers is not excluded, but is not recommended.

Before undertaking any packaging operations, the health hazards of the item to be packaged should be assessed using suitable information sources, e.g. the material safety data sheet. Appropriate precautions should be taken to protect the person(s) handling the chemical reference substance.

The packaging of a batch of a chemical reference substance into containers is a small-scale operation for which suitable equipment is not always available to the manufacturer of the material. Therefore, the packaging of chemical reference substances is usually undertaken by the responsible issuing body. Screw-type feeders have been constructed, but generally the packaging of chemical reference substances is carried out manually. Substances which are expensive or available only in very small quantities may have to be divided between containers in solution and then lyophilized, or evaporated to dryness.

Some chemical reference substances must be packaged under an inert gas or in conditions of controlled humidity. Therefore, the use of a glovebox or an air-tight cabinet is necessary. Single-use vials can be used for hygroscopic materials.

A.6.2 Storage Information about suitable storage conditions can often be obtained from the manufacturer of the source material and should be requested routinely when a new chemical reference substance is established. Theoretically, the stability of the substances should be enhanced by keeping them at low temperatures but, for substances that contain water, storage below 0 °C may impair the stability. It should also be remembered that the relative humidity in normal refrigerators or cold rooms may be high and, unless ampoules or other tightly closed containers are used, the improvement in stability may be more than offset by degradation due to the absorption of moisture. Storage at about 5 °C, with precautions to prevent such absorption, has proved satisfactory for most chemical reference substances. Vials should, however, not be opened until they have attained room temperature to prevent ingress of moisture by condensation.

A.6.3 Stability A chemical reference substance is an integral part of the drug specification. Thus, if the reference substance deteriorates, this will change the specification of the drug. It is, therefore, of the utmost importance that the stability of chemical reference substances is monitored by regular re-examination and that they should be replaced as soon as a significant change in a property is noted.

The definition of a “significant change” differs according to the intended use of the chemical reference substance. Several per cent of degradation products found in a substance may not impair the usefulness of the material in identification tests. For chemical reference substances that are used in chromatographic assays, however, even small amounts of impurities may be unacceptable. When establishing a chemical reference substance, consideration must be given to its intended use and to the performance characteristics of the analytical methods in which it will be used. The tolerable degree of degradation will differ from case to case but should be predefined.

Laboratories in charge of collections of chemical reference substances should have a system for regular re-examination of the materials in stock. The frequency of re-testing may be modified according to need. It must be borne in mind that the stability of a specially prepared chemical reference substance may not always be the same as that of commercial samples of the same material.

The selection of suitable analytical methods for monitoring the stability of chemical reference substances depends on the nature and intended use of the substance. A substance used solely for identification purposes will normally only require demonstration that it is still suitable for this use, e.g. that the IR spectrum is identical to that obtained during establishment. If substances are employed for other purposes, the testing must be more extensive, but should use methods which are rapid and sensitive so as not to consume too much of the existing stock. In many cases it is important to check that there has been no significant uptake of moisture, which could result in degradation by hydrolysis and/or a decrease in the assigned content of the substance. Chromatography is employed extensively, as are absolute methods such as differential scanning calorimetry where applicable.

Changes in the impurity profile or purity determination usually mean that the batch must be replaced. Changes which compromise the integrity of the batch indicate that it should immediately be withdrawn from use. Sometimes a batch of a chemical reference substance will discolour or otherwise change in appearance. Steps should be taken to replace this substance whether or not the results of subsequent analyses indicate significant degradation. Such changes in physical appearance reduce the confidence of the user in the suitability of the chemical reference substance. Appropriate testing of active bulk substance should be carried out before further dispensing into vials or ampoules.

A.6.4 Information to be supplied with chemical reference substances The labels on chemical reference substances should give the following information:

– the appropriate name of the substance: the international nonproprietary name (INN) should be used wherever possible;
– the name of the issuing body;
– the approximate quantity of material in the container; and
– the batch or control number.

Where associated documents are provided they should incorporate relevant items from the list above. The following information should be given, as necessary, on the labels and/or in associated documents:

– the name and address of the issuing body;
– the recommended storage conditions (if special conditions apply);
– the intended use of the chemical reference substance;
– directions for use (e.g. storage and handling);
– information about the assigned analytical value of the chemical reference substance (needed for calculation of the results of tests in which the substance will be used);
– a disclaimer of responsibility in cases where chemical reference substances are misused, or stored under inappropriate conditions, or used for purposes other than those intended by the issuing body; and
– health hazard information or warnings in conformity with national and regional regulations or international agreements.

If analytical data are to be supplied with the chemical reference substances, it is recommended that the data provided be limited to what is necessary for the proper use of the substances in the tests and assays.

A.6.5 Distribution and supply Distribution of chemical reference substances within the same country usually does not present problems. However, when samples are to be sent to other countries, both the sender and the receiver of the goods may encounter difficulties because of the vagaries of postal and customs regulations, e.g. the application of special procedural requirements applicable to substances under international control. Distributors of chemical reference substances waste considerable resources in seeking information on different international import regulations, and in completing the required forms. A way of reducing such difficulties and barriers to effective distribution of chemical reference substances should be sought. There should be a minimum of delay in providing the chemical reference substances to the users, and the most speedy means of transport should be chosen.

A.6.6 Period of use Chemical reference substances do not carry an “expiry date” in the conventional sense. To avoid the unnecessary discarding of satisfactory substances, a mechanism for general control of the batch of a chemical reference substance may be used by the issuing body. If the issuing body applies stability considerations and a monitoring procedure to its collection based on its experience, this should be a guarantee to the user of the acceptability of the chemical reference substance for its intended use.

Whenever a batch of primary reference standards needs to be replaced, the issuing body should, wherever practical, allow for a transition period.

If, exceptionally, it is considered necessary to specify an expiry or re-test date, this should be stated on the label and/or in a document accompanying the chemical reference substances. Adequate shipping records should be kept to enable contact to be made with the purchaser of a batch for recall or other notification.

The storage and maintenance of unopened containers of the chemical reference substance in accordance with the information provided are integral to its suitability for use. To avoid potential doubts concerning the integrity of opened containers, it is suggested that potential users obtain only the quantities of substances necessary to meet their short-term needs and to obtain fresh stocks (held under controlled and known conditions) when required. Long-term storage of substances in opened containers should be avoided. Similarly, efforts should be made to avoid possible degradation, contamination and/or introduction of moisture during the repeated use of portions of a substance from the same container.

B.1 Assessment of need

The establishment of a secondary chemical reference substance, calibrated against a primary reference standard substance, may be desirable for various practical reasons, e.g. the primary standard may not be available in adequate quantities to supply all local needs. Moreover, the availability of such secondary chemical reference substances (for example, on a regional basis) would reduce the cost and the delay in receiving the reference material.

The body that establishes a secondary chemical reference substance for national or regional use should be clearly defined by the appropriate regional or national drug regulatory authority. The traceability between the secondary and the primary chemical reference substance must be documented.

A.5 Assignment of content

If a content is to be assigned to a chemical reference substance, it should be borne in mind that the value is based on the results of a collaborative interlaboratory programme using different analytical methods. This experimentally obtained value represents the best estimate of the true value. In general, the value must be further corrected for the fraction of impurity. Sometimes the chemical reference substances must be dried before use, in which case the content is expressed on the basis of the dried material.

A.4 Chemical and physical methods used in evaluating chemical reference substances

It is important to establish by individual testing that a substance proposed for use as a chemical reference is suitable for that purpose.

The methods used to establish the suitability of such a substance fall into two broad groups: those intended primarily to identify the substance and those used to establish its purity. With most methods, the percentage purity of a chemical reference substance cannot be expressed as an absolute value if the impurities have not been identified. The quoted purity is then an estimate based upon the data obtained by the various analytical methods.

A.4.1 Methods used to verify the identity of chemical reference substances Where a proposed reference substance is a substance whose structure has been satisfactorily defined, its identity may be confirmed by matching the IR spectrum of the substance to that of an authentic specimen. Particular care should be taken when polymorphism exists (*8*). Other highly specific techniques, such as NMR spectroscopy, MS, or X-ray diffraction crystallography, may also be used for such comparisons. The identity of a substance that is intended to replace an established chemical reference substance of the same molecular constitution must be verified, to determine that the characteristic properties of the two specimens are identical. For this purpose it is often sufficient to compare their IR absorption spectra.

However, where no authentic specimen of the proposed substance is available for comparison, and definitive data about its properties are lacking, it may be necessary to verify its identity by applying several of the analytical techniques currently used to characterize new compounds. Such analytical methods may include elemental analyses, crystallographic studies, MS, NMR spectroscopy, functional group analyses, and IR or UV spectrophotometry, as well as other supplementary tests, as required, to establish that the proposed substance is fully characterized.

A.4.2 Methods used to determine the purity of chemical reference substances The analytical methods to be employed in examining a substance should be considered in relation to its intended use. These analytical methods may be divided into three broad categories:

– those that require comparison with an external chemical reference substance (e.g. chromatographic or spectrophotometric methods);
– those that depend solely on an intrinsic dynamic property (e.g. phase solubility analysis and differential scanning calorimetry); and
– other methods.

A.4.2.1 Separation techniques

The methods used for the determination of purity should be established and validated with system suitability requirements as appropriate.

Chromatographic methods. Methods of analysis based on chromatographic separation are especially useful for detecting and determining impurities in chemical reference substances. High-performance liquid chromatography (HPLC) is the most widely used chromatographic method, but TLC and GC are also used. The individual components separated by chromatographic methods may sometimes be recovered for characterization.

The selectivity of HPLC and of GC usually exceeds that of TLC. Both of the first two methods also have the advantage of being readily applicable on a quantitative basis, but they require more complex equipment. HPLC, employing a spectrophotometric method of detection, is of particular value in the examination of chemical reference substances intended for use in UV spectrophotometric assays. The UV wavelength of detection employed for determining the impurity content of the chemical reference substance should be chosen so that the detection responses of the substance and its impurities are similar. When the response factors are signifi cantly different at the optimal wavelength of detection, appropriate corrections must be made to estimate the content of impurities. LC with diode-array detection is very useful for recording the UV spectra of both the main peak and the impurities. LC with MS detection is used for identification of separated impurities as well as for the main component, and is particularly important for use with chemical reference substances for which no other reference standards or IR reference spectra are available.

In a GC method used for an assay, as with LC, the detection responses of the impurities are determined. Generally, monograph methods using GC are of particular value in detecting and determining volatile impurities, including solvent residues, in chemical reference substances.

TLC uses apparatus that is simple and inexpensive; the technique is easy to carry out and is readily applicable even in the microgram range. It can separate closely related compounds, such as geometric isomers and the members of a homologous series. All the constituents of a substance subjected to chromatography appear somewhere on the chromatogram. However, some constituents may remain on the starting line, some may move with the solvent front, some may migrate at the same rate as the main component and some may remain undetected. For this reason, the usefulness of the method may be greatly enhanced by performing two-dimensional chromatography and by using a number of different solvent systems and a variety of detection methods. In some cases the method may be used quantitatively with acceptable accuracy by using a densitometer.

Capillary electrophoresis. Capillary electrophoresis (CE) is an increasingly common method. It may be considered as complementary to LC for detecting impurities.

A.4.2.2 Methods based on intrinsic thermodynamic properties

Methods in this group measure total impurity levels in absolute terms.

Differential scanning calorimetry. This technique is used to check for the presence of different polymorphic forms and to determine the total amount of solid impurities. Purity estimation is based on determination of the heat of fusion of the sample and of the change in its melting point caused by the presence of impurities. This analytical method can be performed rapidly and with high precision. However, it is not applicable if the substance decomposes on melting. This limits its value as a general procedure for estimating the purity of chemical reference substances. It is also inapplicable if solid solutions are formed.

Phase solubility analysis. The method has occasionally been used but its value is limited and the procedure is time consuming. It may be employed to detect contaminating substances, including isomeric species, and to estimate their concentration. Some factors that may make the method inapplicable are degradation of the substance during the course of analysis, formation of a solid solution and polymorphism in the main component.

A.4.2.3 Other methods

Spectrophotometric methods. UV spectrophotometry is occasionally used to determine purity. Since it depends upon the presence of a characteristic chromophore, it can detect impurities that contribute excessively to the absorbance value and may indicate the presence of impurities that have a negligible or distinctive absorbance.

However, the utility of the method is limited by the small number of absorption maxima in the UV range, the large numbers of compounds containing similar characteristic chromophores, and the need for an external chemical reference substance.

IR spectrophotometry may be used to identify and determine the proportions of geometric isomers. NMR spectroscopy, a powerful spectroscopic identification tool, is also occasionally useful in the determination of purity.

Titrimetric methods. Titrimetric methods provide a valuable means of confirming the identity and purity of a proposed chemical reference substance and are useful in confirming purity values obtained by other methods.

Optical rotation methods. Many chemical reference substances are optically active and the relative proportion of optical isomers can sometimes be determined by an optical rotation method, but generally such methods lack specificity and sensitivity. However, the quantitative use of these techniques is well established and can yield results of high precision, depending on the solvent and the wavelength chosen for measurement, provided that pure substances of individual isomers are available. Chiral chromatography, NMR and CE are becoming increasingly important.

Determination of water and organic volatiles. It is essential that an accurate assessment of the moisture content and the content of volatile substances be made. These total values may often be obtained by drying under defined conditions that are appropriate to the proposed substance. Sometimes this may not be possible or may yield misleading results. In such cases, thermogravimetric analysis may be used to determine the content of water and organic volatiles. Alternatively, the water content may be determined by Karl Fischer titration and the content of volatile solvents by GC. Without an accurate assessment of these values at the time that other determinations are being made, judgements of the acceptability of the proposed chemical reference substance will be invalid.

A.3 Evaluation of chemical reference substances

The suitability of a substance proposed for use as a chemical reference requires careful evaluation by the issuing body. It is necessary to consider all data obtained from testing the material by a wide variety of analytical methods. When taken as a whole, this will ensure that the substance is suitable for its intended use. The extent of the analyses required depends on the purpose(s) for which the chemical reference substance is to be employed, and may involve a number of independent laboratories.

A.3.1 Use in identification tests For use in identification tests (IR spectrophotometry and/or chromatographic methods), a batch of good quality material selected from the normal production process is satisfactory if it is of acceptable purity. Additional purification by the supplier may be necessary. The most important check is the application of the test(s) for which the substance is intended. It is usual for at least one laboratory to apply all the chemical and physical tests described in the relevant monograph; some tests, such as those for sterility or for bacterial endotoxins, may not be necessary for materials intended as reference standards.

A.3.2 Use in purity tests The characterization of a chemical reference substance for use in the determination of a specific impurity is more extensive, especially when used in a limit test. If the technique employed is thin-layer chromatography (TLC), an acceptable minimum purity is recommended (normally at least 90%), but purer material (at least 95%) may be required for liquid chromatography (LC) or gas chromatography (GC). It is usually enough to involve only one laboratory when the reference substance is used in purity tests. If the proposed reference substance is being prepared or isolated for the first time, appropriate chemical and physicochemical tests, such as nuclear magnetic resonance (NMR), mass spectrometry (MS) and elemental analysis, must be applied to characterize it.

A.3.3 Use in assays If the chemical reference substance is to be used in an assay (colorimetry, LC, GC or UV spectrophotometry), the extent of testing is much greater. Several (a minimum of three) laboratories should collaborate in testing the proposed substance, using a variety of established and validated techniques, including the method used in the pharmacopoeial specification. The relative reactivity or relative absorbance of the impurities present must be checked when a nonspecific assay method is employed, e.g. by colorimetry or UV spectrophotometry. When a selective assay method is employed, it is particularly important to determine the quantity of impurities. In such a case, it is best to examine the proposed reference substance by as many methods as practicable including, where possible, absolute methods. For substances that are acidic or basic a titration with alkali or acid is simple, but other reactions which are known to be stoichiometric may be used. Phase solubility analysis and differential scanning calorimetry may also be employed in certain cases.

The total of the determinations of water content, organic solvents, mineral impurities and organic components should amount to 100%. For most chemical reference substances intended for assays, the content may be expressed “as is”. When establishing the chemical reference substance it is, therefore, essential to determine the content of water and residual solvents for a non-specific assay, and also to determine the content of impurities for a selective assay.

A.3.4 Use in the calibration of an instrument Where the chemical reference substance is to be employed as calibration material, the extent of testing is similar to that for a chemical reference substance used in assays. Several laboratories should collaborate in testing the proposed substance using a variety of techniques to check that its purity is adequate. An appropriate number of collaborating laboratories should also participate, after the reference substance has been deemed suitable, to establish a value for the essential property of the substance using an appropriate instrument.

A.2 Obtaining source material

Source material of satisfactory quality can be selected from a batch (lot) of the substance originating from the normal production process, if the purity is acceptable. Further purification techniques may be needed to render the material acceptable for use as a chemical reference substance.

The purity requirements for a chemical reference substance depend upon its intended use. A chemical reference substance proposed for an identification test does not require meticulous purification, since the presence of a small percentage of impurities in the substance often has no noticeable effect on the test.

On the other hand, chemical reference substances that are to be used in assays should possess a high degree of purity. As a guiding principle, a purity of 99.5% or higher is desirable, calculated on the basis of the material in its anhydrous form or free of volatile substances. However, where the selectivity of the analytical procedure for which the chemical reference substance is required is low, such a degree of purity may not be necessary. In making a decision about the suitability of a chemical reference substance, the most important consideration is the influence of the impurity on the attribute measured in the assay when used in a nonspecific assay procedure. Impurities with physicochemical characteristics similar to those of the main component will not diminish the usefulness of a chemical reference substance, whereas even traces of impurities with significantly different properties may render a substance unsuitable for use as a chemical reference substance.

When source material to be used as a chemical reference substance is obtained from a supplier, the following should be supplied with the material:

– certificate of analysis with complete information on test methods employed, values found and number of replicates used, where applicable, and relevant spectra and/or chromatograms;
– results of any accelerated stability studies;
– information on optimal storage conditions required to ensure stability (temperature and humidity considerations);
– results of any hygroscopicity study and/or statement of the hygroscopicity of the source material;
– identification of impurities detected and/or specific information on the relative response factor as determined in compendial methods concerning the principal component, and/or the percentage mass of the impurity;
– updated material safety data sheet outlining any health hazards associated with the material.

For new drug substances, manufacturers should be aware that elaboration of pharmacopoeial monographs will be necessary, and a batch of the new substance should be set aside to be used if necessary as the chemical reference substance. It is desirable for bodies that issue chemical reference substances to share the same batch of material, even if the substance will be employed for different test methods. This will require exchange of information concerning the establishment process, supplier(s), availability and conditions of supply.

A.1 Assessment of need for the establishment of chemical reference substances

The production, validation, maintenance and distribution of chemical reference substances is a costly and time-consuming undertaking. It is, therefore, crucial to determine for certain whether a need for a given substance exists. Requests for new chemical reference substances usually arise when a particular approach to developing a specification for a new substance or product has been adopted. Methods may have been proposed in a specification that require the establishment of a chemical reference substance for use as a comparative standard. Therefore, the first matter that should be assessed is whether an alternative, equally satisfactory, procedure could be adopted that does not require a comparative standard.

Analytical procedures currently used in specifications for pharmaceutical substances and products that may require a chemical reference substance are:

– infrared (IR) spectrophotometry, whether for identification or quantitative purposes;
– quantitative methods based on ultraviolet (UV) absorption spectrophotometry;
– quantitative methods based on the development of a colour and the measurement of its intensity, whether by instrumental or visual comparison;
– methods based on chromatographic separation for identification or quantitative purposes;
– quantitative methods (including automated methods) based on other separation techniques that depend on partition of the substance to be determined between solvent phases, where the precise efficiency of the extraction procedure might depend upon ambient conditions that occasionally vary and from laboratory to laboratory;
– quantitative methods, often titrimetric but sometimes gravimetric, that are based on non-stoichiometric relationships;
– assay methods based on measurement of optical rotation; and
– methods that might require a chemical reference substance consisting of a fixed ratio of known components (for example, *cis/trans* isomers, spiked samples).

Introduction

In 1975 the WHO Expert Committee on Specifications for Pharmaceutical Preparations recommended the “General guidelines for the establishment, maintenance and distribution of chemical reference substances” (*1*). At that time these general guidelines were aimed at fostering greater collaboration and harmonization among various national and regional authorities responsible for collections of chemical reference substances. This aim is still relevant. The guidelines were initially drawn up specifically for use by the WHO Collaborating Centre for Chemical Reference Substances in Sweden, which supplies International Chemical Reference Substances (ICRS). These substances are primarily intended for use with pharmacopoeial monographs included in *The International Pharmacopoeia* (*2*).

It became evident that to ensure ready availability and cost-effectiveness, and in order to meet particular national or regional pharmacopoeial requirements, it was necessary to establish chemical reference substances external to the WHO Collaborating Centre for Chemical Reference Substances. Since the meticulous work of the WHO Collaborating Centre establishing the international collection would have to be duplicated in local or regional laboratories, guidelines were necessary to ensure the integrity of national or regional collections. The 1975 guidelines were reviewed and modified in 1982 (*3*) and subsequently revised in 1999 (*4*).

In 2004, the WHO Expert Committee on Specifications for Pharmaceutical Preparations recommended the development of more detailed guidelines on the establishment of secondary chemical reference substances. This additional guidance forms part B of the present revision and is intended to apply to secondary reference substances supplied as “official”, e.g. regional/national standards, and not to the working standards of manufacturers or other laboratories. However, in principle, secondary reference standards prepared by manufacturers can be prepared as “working standards” using the same procedures.

The purpose of establishing chemical reference substances is to achieve accuracy and reproducibility of the analytical results required by pharmacopoeial testing and pharmaceutical control in general. These substances are normally prepared and issued by the regional or national pharmacopoeia commission or the regional or national quality control laboratory on behalf of the drug regulatory authority. In the context of these guidelines, the general use of a chemical reference substance should be considered an integral part of a compliance-oriented monograph or test procedure used to demonstrate the identity, purity and content of pharmaceutical substances and preparations.

The purpose of establishing secondary reference substances is for use in routine analysis to determine the identity, purity and, in particular, the content of pharmaceutical substances in pharmaceutical preparations. The extent of characterization and testing of a secondary reference substance is less than that for a primary reference substance. It is essential that a secondary reference substance is traceable to a primary reference substance, such as a pharmacopoeial or other officially recognized reference substance. In the cases of doubtful results or dispute when using secondary chemical reference substances, the test should be repeated using the primary standard.

The establishment of a chemical reference substance is based on the evaluation of the results of analytical testing. The report should subsequently be approved and adopted by a certifying body, normally the relevant pharmacopoeial committee or drug regulatory authority. The establishment of the reference substance can be on an international, national or regional basis. Each substance is generally established for a specific analytical purpose, defined by the issuing body. Its use for any other purpose becomes the responsibility of the user and a suitable caution is included in the accompanying information sheet. The present guidelines are concerned with both primary and secondary chemical reference substances as defi ned below.

The preparation of a chemical reference substance should comply with the requirements for quality assurance systems, including applicable principles of good manufacturing practices (GMP) and good control laboratory practices (*5–10*).

Adequate training programmes are also required. Both the WHO Collaborating Centre and other laboratories concerned with the evaluation and establishment of chemical reference substances give assistance in training, subject to the availability of resources.

Glossary

The definitions given below apply to the terms used in these guidelines. They may have different meanings in other contexts.

Chemical reference substance

The term *chemical reference substance*, as used in this text, refers to an authenticated, uniform material that is intended for use in specified chemical and physical tests, in which its properties are compared with those of the product under examination, and which possesses a degree of purity adequate for its intended use.

Primary chemical reference substance

A designated primary chemical reference substance is one that is widely acknowledged to have the appropriate qualities within a specified context, and whose assigned content when used as an assay standard is accepted without requiring comparison with another chemical substance.

Secondary chemical reference substance

A secondary chemical reference substance is a substance whose characteristics are assigned and/or calibrated by comparison with a primary chemical reference substance. The extent of characterization and testing of a secondary chemical reference substance may be less than for a primary chemical reference substance. Although this definition may apply inter alia to some substances termed “working standards”, part B of these guidelines is intended to apply to secondary reference substances supplied as “official”, e.g. regional/national standards, and not to manufacturers’ or other laboratories’ working standards.

International Chemical Reference Substance

International Chemical Reference Substances (ICRS) are primary chemical reference substances established on the advice of the WHO Expert Committee on Specifications for Pharmaceutical Preparations. They are supplied primarily for use in physical and chemical tests and assays described in the specifications for quality control of drugs published in *The International Pharmacopoeia* or proposed in draft monographs. The ICRS may be used to calibrate secondary standards.

Pharmacopoeial reference standards

The specificity of pharmacopoeial reference substances has been addressed in the introduction of *ISO Guide: General requirements for the competence of reference material producers.* “Pharmacopoeial standards and substances are established and distributed by pharmacopoeial authorities following the general principles of this Guide. It should be noted, however, that a different approach is used by the pharmacopoeial authorities to give the user the information provided by certificate of analysis and expiration dates” (*9*).

Release procedure for International Chemical Reference Substances

Background

During its forty-fifth meeting in 2010 the Expert Committee on Specifications for Pharmaceutical Preparations agreed on a release procedure for International Chemical Reference Substances (ICRS) (*1*). Based on this procedure case-reports issued by the custodian centre for ICRS after analytical testing of candidate material were reviewed by the Secretariat with assistance from collaborating laboratories. If the testing was performed according to the *General guidelines for the establishment, maintenance and distribution of chemical reference substances* (*2*) and the candidate material was found suitable, the Secretariat, in cooperation with the collaborating laboratories, released the ICRS provisionally. In accordance with the rules, the case-reports were then submitted to the Expert Committee on Specifications for Pharmaceutical Preparations during its subsequent meeting, for final adoption. After provisional release the custodian centre for ICRS started the distribution of the ICRS.

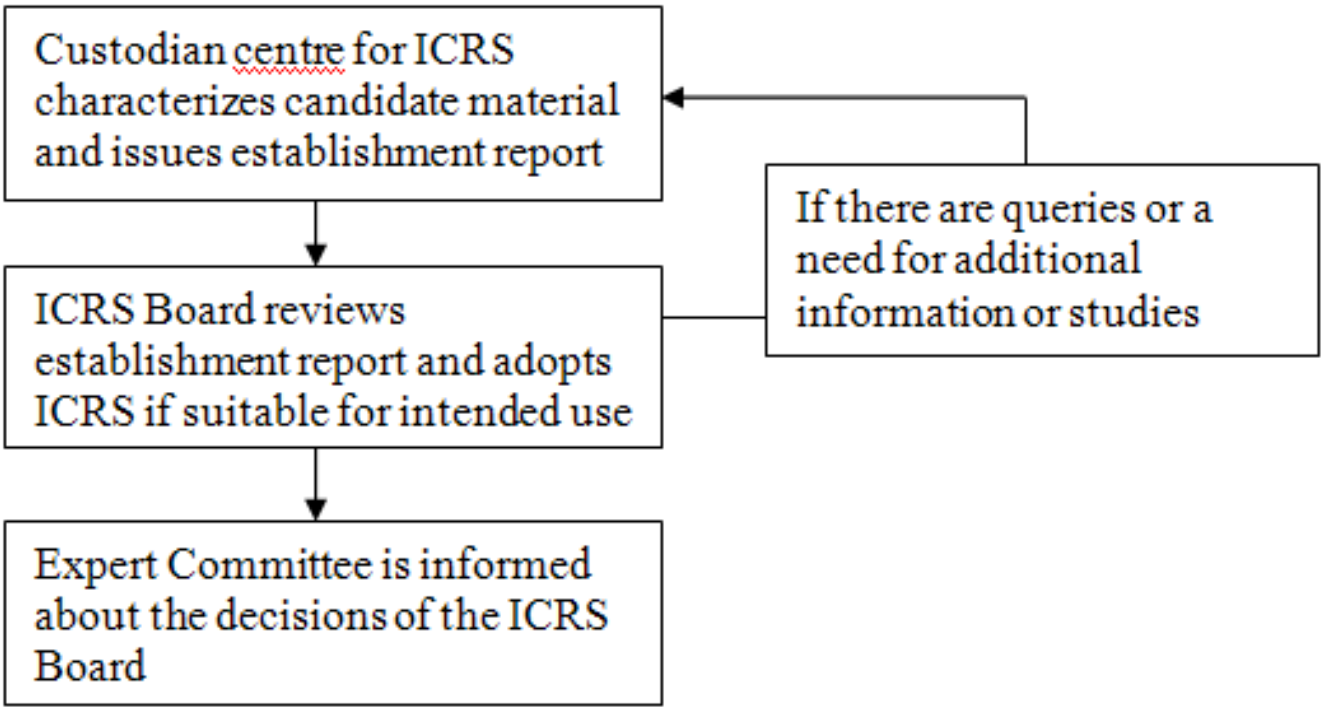
This process expedited the release of ICRS and enabled WHO to react more quickly to urgent demands for ICRS. However, the procedure did not clearly allocate the accountability for the release of ICRS to a single time, person or body. Therefore, the Expert Committee members adopted the following new procedure (Figure 1).

New release procedure

After testing of candidate material, the custodian centre for ICRS will submit analytical case-reports to a newly established ICRS Board, which consists of three experts and a representative of the Secretariat. The Board will decide on the suitability of the reference substance on behalf of the Expert Committee and adopt the ICRS, if found to be suitable for the intended use. In case the Board has queries or considers during its in-depth review that there is a need for additional information and/or studies, the Secretariat will contact the custodian centre accordingly. The feedback will in turn be submitted to the Board for its consideration and final decision.

During the subsequent meeting of the Expert Committee its members will be informed about newly adopted ICRS.

Figure 1. New procedure for the adoption of ICRS



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2. General guidelines for the establishment, maintenance and distribution of chemical reference substances. In: *WHO Expert Committee on Specifications for Pharmaceutical Preparations. Forty-first report.* Geneva, World Health Organization, 2001 (WHO Technical Report Series, No. 943), Annex 3.

Reference substances and reference spectra

2016-01

1. International Chemical Reference Substances

1.1 Introduction

International Chemical Reference Substances (ICRS) are primary chemical reference substances for use in physical and chemical tests and assays described in *The International Pharmacopoeia* or in other World Health Organization (WHO) quality assurance documents adopted by the WHO Expert Committee on Specifications for Pharmaceutical Preparations. ICRS are used to identify, determine the purity or assay of pharmaceutical substances and preparations or to verify the performance of test methods.

This chapter describes principles to be applied during the establishment and use of ICRS, which guarantee that the reference substances are suitable for their intended purpose.

1.2 Terminology

Chemical reference substance

The term chemical reference substance, as used in this text, refers to an authenticated, homogenous material that is intended for use in specified physical and chemical tests, in which one or more of its properties are compared with those of the product under examination and which possesses a degree of purity adequate for its intended use.

Primary chemical reference substance

A designated primary chemical reference substance is one that is widely acknowledged to have the appropriate qualities within a specified context and whose assigned content when used as an assay standard is accepted without requiring comparison with another chemical substance.

Secondary chemical reference substance

A secondary chemical reference substance is a substance whose characteristics are assigned and/or calibrated by comparison with a primary chemical reference substance.

1.3 Purpose of ICRS

The purpose of establishing ICRS is to provide users of *The International Pharmacopoeia* or other WHO quality assurance documents adopted by the WHO Expert Committee on Specifications for Pharmaceutical Preparations with authenticated substances for reference. Many analytical tests and assays are based on comparison of physical or chemical attributes of a sample with those of the reference substance. ICRS serve as such reference substances and thus enable the analyst to achieve accurate and traceable results. Furthermore ICRS may be used to assess system suitability during analyses and to calibrate analytical instruments.

ICRS may also be employed to establish secondary reference substances according to the WHO *General guidelines for the establishment, maintenance and distribution of chemical reference substances*. [1] In cases of doubtful results or dispute, however, the tests performed using ICRS are the only authoritative ones.

> [1] *WHO Expert Committee on Specifications for Pharmaceutical Preparations. Forty- first report.* Geneva, World Health Organization, 2007, Annex 3 (WHO Technical Report Series, No. 943).

1.4 Production of ICRS

All operations related to the establishment and distribution of ICRS should be carried out according to the relevant guidelines. Among these, the WHO *General guidelines for the establishment, maintenance and distribution of chemical reference substances* [1] and International Organization for Standardization (ISO) Guide 34 – *General requirements for the competence of reference material producers* (including related guides) have pre-eminence.

Manufacture

WHO encourages pharmaceutical manufacturers to donate suitable candidate materials and thus to contribute to the availability of ICRS.

Candidate material for the establishment of ICRS may be synthesized and purified for this purpose or may be selected from the pharmaceutical production provided that the purity and homogeneity are suitable. In some cases, for example, in order to improve the stability of the reference substance it may be useful to process the reference substance (e.g. by freeze drying) or to select an alternative salt (or salt vs base), solvate or hydrate. The content assigned to the standard takes into account which substance is selected.

The candidate should comply with the relevant test(s) of the corresponding monograph as published in *The International*

Pharmacopoeia, where applicable.

Reference substances are dispensed into suitable containers under appropriate filling and closure conditions, to ensure the integrity of the substance. The containers employed are preferably single-use in order to minimize the risk of decomposition, contamination and moisture uptake. Where multiple-use containers are employed appropriate use and handling controls should be implemented by the user to assure their suitability.

Analytical characterization

The candidate material should be tested with suitable analytical techniques aiming to characterize all relevant quality attributes. The identity is confirmed and the purity is determined, usually based on results obtained with the methods of the respective monographs. However, the use of further analytical techniques may be appropriate in order to fully characterize the candidate material. Absolute methods (for example, volumetric titrations, differential scanning calorimetry, qNMR) should be employed to complement and verify the results of relative methods where the properties of a sample are compared with those of a reference substance (for example, chromatographic methods). The extent of testing and the number of laboratories involved in characterizing the material depend on the intended use of the reference substance to be established. If required, assay standards are characterized in interlaboratory studies to increase the accuracy of the assigned value.

A thorough purity investigation of the candidate material is performed to verify the presence of all relevant components (i.e. main component, organic and inorganic impurities, water and residual solvents) and to quantify them, if relevant. For standards used for quantitative purposes, the cumulative percentage of all components should yield 100% (mass balance approach).

The purity of a candidate material is calculated on the “as is” basis, so that the analyst can use the substance without pretreatment, for example, drying.

Provided that all components themselves are expressed as a percentage of the mass of sample taken the “as is” content can be calculated as follows:

Purity = 100 – organic impurities [%] – inorganic impurities [%] – water [%] – residual solvents [%]

Formula 1. Formula to calculate the purity of ICRS on an “as is” basis.

When chromatographic methods are used to test for related substances impurity concentrations are often determined in relation to the principal compound. The “as is” content of organic impurities, to be substituted in formula 1, can be calculated as follows:

Organic impurities = chromatographic result x (100 [%] – water [%] – residual solvents [%] – inorganic impurities [%]) / 100

Formula 2. Formula to calculate the percentage of organic impurities, determined by a chromatographic method, on an “as is” basis.

The content assigned to a quantitative ICRS depends on the purity of the candidate material and is specific to the method for which the substance will serve as a reference. If the reference substance is intended to be used with a method that has the same selectivity as the method used to determine its purity the calculated purity will be assigned as the content of the ICRS. However, if the intended method is less discriminative it may be necessary to add to the purity the content of impurities that cannot be discriminated from the detector response of the parent compound. The following example illustrates this:

> *A candidate material is analysed with different analytical methods to identify and quantify all relevant components. The results reveal that, besides the labelled substance, the following components are present: 2.0% water (analysed by Karl Fischer titration, calculated on an “as is” basis); 1.0% enantiomer of the labelled substance (analysed by chiral high-performance liquid chromatography (HPLC), calculated in relation to the sum of the peak areas of both enantiomers); and two organic impurities, each 0.75% (analysed by an achiral HPLC method, calculated in relation to the sum of the peak area of all peaks, ignoring solvent and injection peaks). The purity of the standard is calculated to 95.55% (purity = 100% – (2.5% x 0.98) – 2%). The candidate material is intended to be used as a reference in an assay test, which stipulates the use of the same HPLC method as already applied to determine the organic impurities in the characterization of the candidate material. A content of 96.53% is assigned to the reference substance (assigned content = 100% – (1.5% x 0.98) – 2%). The concentration of the enantiomer is not taken into consideration as the method, for which the reference substance is intended, is not selective for the enantiomer.*

Labelling

The labelling should provide all the necessary information to use the reference substance as intended, i.e. the name of the reference substance, the batch number, storage conditions, etc. If intended for quantification the assigned content is also given. The accompanying leaflet is considered to be part of the labelling.

Release and adoption

ICRS are established and released under the authority of the WHO Expert Committee on Specifications for Pharmaceutical

Preparations. The Committee adopts new ICRS and new lots as being suitable for use as described in *The International Pharmacopoeia* or in other WHO quality assurance documents.

Stability monitoring and distribution

At the WHO custodian centre for ICRS the established reference substances are stored and distributed under conditions suitable to ensure their continuous fitness for purpose.

The fitness-for-purpose of ICRS is monitored by regular re-examinations. Their frequency and extent is based on:

-the stability of the ICRS;
-the container and closure systems;
-the storage conditions;
-the hygroscopicity;
-the physical form;
-the intended use.

The analytical methods employed to verify the stability are chosen among those used during the establishment of the reference standard. The maximum permitted deviation from the assigned value should be predefined and, if exceeded, the batch should be re-established or replaced.

1.5 Use and storage of ICRS by the user

The letters RS after the name of a substance in a test or assay described in *The International Pharmacopoeia* or in other WHO quality assurance documents adopted by the WHO Expert Committee on Specifications for Pharmaceutical Preparations indicate the use of the respective ICRS.

ICRS are suitable for the analytical purpose described in *The International Pharmacopoeia* or other WHO quality assurance documents adopted by the WHO Expert Committee on Specifications for Pharmaceutical Preparations. The analytical specifications and test methods in these documents are regularly revised to stay abreast of advances in analytical science and regulatory requirements. In addition, the intended use of a reference substance may change. For example, an ICRS previously used only for identification shall also be employed in a quantitative test. Information on the actually established intended uses of an ICRS can be found in the leaflet enclosed with the substance when distributed or accessible via the ICRS online database (see http://www.edqm.eu). The information found in the current leaflets is applicable to all standards of the respective batch number.

If used for other purposes the responsibility of assessing the suitability rests with the user or the authority that prescribes or authorizes this use. If reference substances other than ICRS or other than those listed under Annex 1 are used for purposes described in *The International Pharmacopoeia* or in other WHO quality assurance documents adopted by the WHO Expert Committee on Specifications for Pharmaceutical Preparations the suitability of these substances has to be demonstrated by the user.

The user has to apply an assigned content in assay determinations or when it is indicated in the method description.

ICRS are supplied in adequate quantities for immediate use after opening of the container. Users should purchase only sufficient units for short-term use.

It is generally recommended that the user stores ICRS protected from light and moisture and preferably at a temperature of about 5 ± 3 °C. When special storage conditions are required this is stated on the label or in the accompanying leaflet.

If an unopened container is stored under the recommended conditions it remains suitable for use as long as the respective batch is valid. Information on current batch numbers is provided on the website of the WHO custodian centre for ICRS (see under Ordering information).

Reference standards that are normally stored at 5 ± 3 °C are dispatched at ambient temperature since short-term excursions from the storage recommendations are not considered to be deleterious to the reference substance. Reference substances stored at -20 °C are packed on ice or dry ice and dispatched by courier. Reference substances stored at -80 °C or stored under liquid nitrogen are packed on dry ice and dispatched by courier.

1.6 Rational to limit the number of ICRS in *The International Pharmacopoeia*

Specifications and test procedures of *The International Pharmacopoeia* are intended to be applicable in all WHO Member States wishing to implement them. Procuring reference substances may, however, be difficult in certain areas of the world due to delays in their delivery and the cost of purchase. *The International Pharmacopoeia* therefore endeavours to reduce the number of reference substances required to perform the included tests and assays. For this purpose the following strategies and practices may be applied during the elaboration of monographs:

-in situ preparation of impurities for identification of related substances/impurities;

-quantification of impurities by comparing their detector responses with the response of the parent compound in a diluted sample solution along with the establishment of correction factors to compensate for differences in the responses of the impurity and the parent compound;

-provision of International Infrared Reference Spectra (IIRS) for use in identification tests;

-provision of assay methods not requiring reference substances, like titrations and ultraviolet spectrophotometry using absorptivity values. These methods shall be provided as alternatives in particular to chromatographic assays in monographs for pharmaceutical substances.

These strategies, however, shall only be applied when, during the elaboration of the methods, evidence has been obtained demonstrating that the intended measures do not compromise the quality of the analytical results or the ability of the tests to conclusively demonstrate conformance to the applicable standards.

1.7 Analytical information provided in the leaflet of the ICRS

The leaflets of the ICRS may provide analytical information, including, but not limited to:

-the IR spectrum of the substance (together with information on the sample preparation);

-additional analytical information generated at the time of establishment;

-the assigned content.

The section “Additional analytical information generated at the time of establishment” provides data about the purity of the reference substance and the methods used for its determination. The information was valid at the time of the establishment of the standard and will not be monitored or adjusted. The information may help the user to understand the calculation of the content that has been assigned to a standard for quantification. It may further be of value to assess risks associated with an unintended use of an ICRS. This information, however, is not given to authorize such an unintended use. As laid down under section 1.5, ICRS are adopted by the WHO Expert Committee on Specifications for Pharmaceutical Preparations for their intended uses only; the responsibility for an unintended use of an ICRS rests with the user or the authority that prescribes or authorizes this use.

1.8 Ordering information

The European Directorate for the Quality of Medicines & HealthCare (EDQM), Council of Europe, is responsible for the establishment, preparation, storage and distribution of ICRS for *The International Pharmacopoeia*. A list of currently available ICRS can be found on its website (see http://www.edqm.eu).

Orders for ICRS should be sent to:

European Directorate for the Quality of Medicines & HealthCare
7 allée Kastner
CS 30026
F-67081 Strasbourg, France
Fax: +33 (0)3 88 41 27 71 - to the attention of EDQM Sales Section
Email: orders@edqm.eu

The current price for ICRS per package, as well as the cost for the delivery is available on the above-mentioned website.

2. International Infrared Reference Spectra

International infrared reference spectra are provided for use in identification tests as described in monographs of *The International Pharmacopoeia* or other WHO quality assurance documents adopted by the WHO Expert Committee on Specifications for Pharmaceutical Preparations.

The reference spectra are produced from authenticated material using an appropriate sample preparation technique. They are recorded with a Fourier transform infrared spectrophotometer (FTIR). Instructions for the sample preparation are given in *1.7 Spectrophotometry in the infrared region*, *Identification by reference spectrum*.

A spectrum of the test substance is considered to be concordant with a reference spectrum if the transmission minima (absorption maxima) of the principal bands in the test spectrum correspond in position, relative intensities and shape to those in the reference spectrum.

3. Biological reference substances

3.1 Reference substances for the microbiological assay of antibiotics

*[**Note from the Secretariat.** It is intended to add a chapter dealing with the biological, in particular microbiological reference substances referred to in* The International Pharmacopoeia.*]*

4. Use of reference substances of other pharmacopoeias

In order to foster harmonization of pharmacopoeial standards and to exploit possibilities for work sharing between pharmacopoeias the WHO Expert Committee on Specifications for Pharmaceutical Preparations may prescribe reference substances that have been established by other pharmacopoeias for use according to *The International Pharmacopoeia*.

The suitability of these reference substances for the additional intended use is assessed during the elaboration or revision of monographs or other tests in which they are mentioned. With the adoption of these texts the WHO Expert Committee on Specifications on Pharmaceutical Preparations authorizes the use of the respective reference substances for the purposes described in *The International Pharmacopoeia*.

To order these reference substances contact the issuing pharmacopoeia. Any question related to the use of such reference substance according to a monograph of *The International Pharmacopoeia* shall be addressed to the Secretariat of *The International Pharmacopoeia* (empinfo@who.int).

The list of reference substances found suitable for use according to *The International Pharmacopoeia* is published on the website of *The International Pharmacopoeia* (http://www.who.int/medicines/areas/quality_safety/quality_assurance/qas_icrs/en/).

Z - Zinc acetate R.... Zirconyl nitrate TS

Zinc acetate R

$C_4H_6O_4Zn,2H_2O$ (SRIP, 1963, p. 216).

Zinc AsR, granulated

Granulated zinc R that complies with the following tests:

Limit of arsenic. Add 10 mL of stannated hydrochloric acid (~250 g/L) AsTS to 50 mL of water and apply the general test for arsenic; use 10 g of granulated zinc R and allow the reaction to continue for 1 hour; no visible stain is produced.

Test for sensitivity. Repeat the test for arsenic with the addition of 0.1 mL of dilute arsenic AsTS; a faint but distinct yellow-coloured stain is produced.

Zinc bis(dibenzyldithiocarbamate) R

$Zn(C_5H_{10}NS_2)_2$.

Description. A white, crystalline powder.

Solubility. Soluble in chloroform R.

Melting range. 178–180 °C.

Zinc bis(dibenzyldithiocarbamate) TS

Procedure. Dissolve 10.0 mg of zinc bis(dibenzyldithiocarbamate) R in sufficient carbon tetrachloride R to produce 100 mL.

Zinc chloride R

$ZnCl_2$ (SRIP, 1963, p. 217).

Zinc R

Zn (SRIP, 1963, p. 216); granulated, powder or dust.

Zinc standard (20 µg/mL Zn) TS

Procedure. To 4.398 g of zinc sulfate R add 1 mL of acetic acid (~300 g/L) TS and dilute with sufficient water to produce 1000 mL. Dilute 1 mL of this solution to 100 mL with water.

Zinc sulfate R

$ZnSO_4,7H_2O$. Contains not less than 99.0% and not more than 105.0% of $ZnSO_4,7H_2O$.

Description. Colourless crystals or a white, crystalline powder; odourless; efflorescent.

Solubility. Very soluble in water; practically insoluble in ethanol (~750 g/L) TS.

Clarity and colour of solution. A 0.05 g/mL solution is clear and colourless.

Chlorides. Dissolve 0.7 g in a mixture of 2 mL of nitric acid (~130 g/L) TS and 30 mL of water and proceed as described under *2.2.1 Limit test for chlorides*; not more than 0.35 mg/g.

Iron. Use 0.4 g; the solution complies with the *2.2.4 Limit test for iron*; not more than 0.10 mg/g.

pH value. pH of a 0.05 g/mL solution, 4.4–5.6.

Assay. Dissolve about 0.2 g, accurately weighed, in 5 mL of acetic acid (~60 g/L) TS and proceed with the titration as described under *2.5 Complexometric titrations*. Each mL of disodium edetate (0.05 mol/L) VS is equivalent to 14.38 mg of $ZnSO_4,7H_2O$.

Storage. Store at a temperature below 35 °C in a tightly closed container.

Zirconyl nitrate R

Contains not less than 43.5% and not more than 45.5% of ZrO_2.

Description. A white powder.

Solubility. Soluble in water giving a solution that is clear or not more than faintly turbid.

Assay. Dissolve about 0.1 g, accurately weighed, in 5 mL of sulfuric acid (~1760 g/L) TS and add carefully 50 mL of water. Add, with stirring, 5 mL of hydrogen peroxide (~330 g/L) TS and 350 mL of diammonium hydrogen phosphate (100 g/L) TS. Add 40 mL

of sulfuric acid (~1760 g/L) TS and keep the mixture at a temperature of 40–50 °C for 2 hours. Filter and wash with not more than 200 mL of cold ammonium nitrate (50 g/L) TS until the washings no longer give the reaction A for orthophosphates described under *2.1 General identification tests*. Dry and ignite to constant weight. Each g of residue is equivalent to 0.4647 g of ZrO_2.

Zirconyl nitrate TS

Procedure. Dissolve 0.1 g of zirconyl nitrate R in a mixture of 60 mL of hydrochloric acid (~420 g/L) TS and 40 mL of water.

Y - Yeast extract, water-soluble, R.... Yellow stock standard TS

Yeast extract, water-soluble, R.

(SRIP, 1963, p. 215).

Yellow stock standard TS.

Procedure. To 9.5 mL of cobalt colour TS, add 1.9 mL of copper colour TS, 10.7 mL of dichromate colour TS, 4.0 mL of iron colour TS, dilute to 100.0 mL with sulfuric acid (~10 g/l) TS, and mix.

X - Xanthydrol R.... Xylenol orange R

Xanthydrol R.

$C_{13}H_{10}O_2$ (SRIP, 1963, p. 210).

Xanthydrol TS.

Procedure. Dissolve 20 mg of xanthydrol R in 1 mL of hydrochloric acid (~420 g/l) TS and 99 mL of acetic acid (~300 g/l) TS.

m-Xylene

1,3-Dimethylbenzene, C_8H_{10}.

Description. Clear, colourless, flammable liquid, practically insoluble in water, miscible with ethanol (96 per cent).

Relative density d_{20}^{20} = 0.884.

Refractive index n_D^{20} = 1.497.

Boiling point. About 139 °C.

Melting point. About – 47 °C.

Xylene R.

C_8H_{10} (SRIP, 1963, p. 215).

o-Xylene R

1,2-Dimethylbenzene; C_8H_{10}.

Description. Clear, colourless, flammable liquid, practically insoluble in water, miscible with ethanol (~750 g/L) TS

Relative density d_{20}^{20} = 0.881.

Refractive index n_D^{20} = 1.505.

Boiling point. About 144°C.

Xylenol orange indicator mixture R.

Procedure. Mix 0.1 g of xylenol orange R with 10 g of potassium nitrate R.

Xylenol orange R

[3*H*-2,1-Benzoxathiol-3-ylidene bis[(6-hydroxy-5-methyl-*m*-phenylene) methylenenitrilo]] tetraacetic acid, *S, S*-dioxide, $C_{31}H_{32}N_2O_{13}S$.

Description. An orange powder.

Solubility. Soluble in water and ethanol (~750 g/l) TS.

W - Water R.... Water vapour detector tube

Water R

Purified water as defined in the monograph for Purified water.

Note: Unless otherwise specified, all solutions indicated in the tests and assays of *The International Pharmacopoeia* are prepared with water R.

Water, ammonia-free, R

Water that complies with the following additional test: to 50 mL add 2 mL of alkaline potassio-mercuric iodide TS; no colour is produced.

Water, carbon-dioxide-free and ammonia-free, R

Ammonia-free water R that has been treated as described under carbon-dioxide-free water R.

Water, carbon-dioxide-free, R

Water that has been boiled vigorously for a few minutes and protected from the atmosphere during cooling and storage.

Water, distilled R

Water R prepared by distillation.

Water for injections R

Water for injections as described in the monograph for *Water for injections*.

Water BET

Water is suitable if it gives a negative result under the conditions prescribed in *3.4 Test for bacterial endotoxins*. It may be prepared by distilling water three times in an apparatus fitted with an effective device to prevent the entrainment of droplets, or by other means which give water of the requisite quality.

Water, particle-free R

Water R that has been filtered through a membrane with a pore size of 0.22 µm.

Water, sterile, R

Sterile water R that complies with the following additional test:

Pyrogens. Carry out the test as described under *3.5 Test for pyrogens* injecting, per kg of the rabbit's weight, 10 mL of water that has been rendered isotonic by the addition of pyrogen-free sodium chloride R.

Water vapour detector tube

A cylindrical, sealed glass tube containing adsorbent filters and suitable supports for the magnesium perchlorate indicator. The minimum value indicated is 60 µL/L or less with a relative standard deviation of at most ±20%.

V - Vanadium pentoxide R.... Vanillin/sulfuric acid TS2

Vanadium pentoxide R

V_2O_5.

Description. A yellow-brown to rust-brown powder.

Solubility. Slightly soluble in water; soluble in concentrated acids and alkalis; practically insoluble in ethanol (~750 g/L) TS.

Vanadium/sulfuric acid TS.

Procedure. Dissolve 0.20 g of vanadium pentoxide R in 4 mL of sulfuric acid (~1760 g/L) TS and dilute carefully with water to 100 mL.

Vanillin (10 g/L) TS

A solution of vanillin R containing about 10 g of $C_8H_8O_3$ per litre.

Vanillin R

$C_8H_8O_3$ (SRIP, 1963, p. 214).

Vanillin/hydrochloric acid TS

Procedure. Dissolve 1.0 g of vanillin R in sufficient hydrochloric acid (~250 g/L) TS to produce 100 mL.

Note: Vanillin/hydrochloric acid TS must be freshly prepared.

Vanillin/sulfuric acid TS1

Procedure. Dissolve 5 g of vanillin R in 100 mL of sulfuric acid (~1760 g/L) TS.

Note : Vanillin/sulfuric acid TS1 should be freshly prepared.

Vanillin/sulfuric acid TS2

Procedure. Dissolve 1 g of vanillin R in sufficient ethanol (~750 g/L) TS to produce 100 mL. Carefully add, drop by drop, 2 mL of sulfuric acid (~1760 g/L) TS.

Note : Vanillin/sulfuric acid TS2 must be used within 48 hours.

T - Tannic acid (50 g/L) TS.... Tyrosine R

Tannic acid (50 g/L) TS

A solution of tannic acid R containing about 50 g of $C_{76}H_{52}O_{46}$ per litre.

Tannic acid R

$C_{76}H_{52}O_{46}$ (SRIP, 1963, p. 205).

Tartaric acid (10 g/L) TS

A solution of tartaric acid R containing about 10 g of $C_4H_6O_6$ per litre.

Tartaric acid (200 g/L) TS

A solution of tartaric acid R containing about 200 g of $C_4H_6O_6$ per litre.

Tartaric acid (5 g/L) TS

A solution of tartaric acid R containing about 5 g of $C_4H_6O_6$ per litre.

Tartaric acid R

$C_4H_6O_6$ (SRIP, 1963, p. 205).

p-Terphenyl R

1,4-Diphenylbenzene, $C_{18}H_{14}$. Suitable for scintillation counting.

Thioglycolic acid R. See *Mercaptoacetic acid R*.

Testosterone propionate R

$C_{22}H_{32}O_3$. Use testosterone propionate as described in the monograph for *Testosterone propionate*.

Testosterone propionate/ethanol TS

Procedure. Dissolve 10 mg of testosterone propionate R in sufficient ethanol (~750 g/L) TS to produce 10 mLl.

Tetrabromophenolphthalein ethyl ester R

3',3",5',5"-Tetrabromophenolphthalein, ethyl ester; $C_{22}H_{14}Br_4O_4$. Use a suitable reagent grade.

Tetrabromophenolphthalein ethyl ester TS

Procedure. Dissolve 0.10 g of tetrabromophenolphthalein ethyl ester R in sufficient glacial acetic acid R to produce 100 mL.

Note: Tetrabromophenolphthalein ethyl ester TS should be freshly prepared.

Tetrabutylammonium hydrogen sulfate R

TBAHS; $C_{16}H_{37}NO_4S$.

A commercially available reagent of suitable grade.

Description. Colourless crystals or a white, crystalline powder.

Solubility. Freely soluble in water and methanol R; soluble in ethanol (~750 g/L) TS producing a slightly hazy, colourless solution.

Absorbance. A 0.05 g/mL solution of a 1 cm layer measured at a wavelength between 240 nm and 300 nm has an absorbance of not greater than 0.05.

Melting range. 169–173 °C.

Tetrabutylammonium hydroxide (0.1 mol/L) VS

Procedure. Dissolve 40 g of tetrabutylammonium iodide R in 90 mL of dehydrated ethanol R, add 20 g of finely powdered, purified silver oxide R and shake vigorously for 1 hour. Centrifuge a small volume of the mixture and test the supernatant liquid for iodides. If a positive reaction is obtained add an additional 2 g of silver oxide R and shake for a further 30 minutes. Repeat this procedure until the liquid is free from iodides, filter the mixture through a fine sintered glass filter and rinse the reaction vessel and the filter with 3 quantities of dry benzene R, each of 50 mL. Add the washings to the filtrate and dilute to 1000 mL with dry benzene R. Pass dry carbon-dioxide-free nitrogen R through the solution for 5 minutes.

Method of standardization. Titrate 10 mL of dimethylformamide R with the tetrabutylammonium hydroxide solution using 3 drops

of thymol blue/methanol TS as indicator until a pure blue colour is obtained. Immediately add about 0.06 g of benzoic acid R, accurately weighed, stir to effect solution and titrate with the tetrabutylammonium hydroxide solution until the full blue colour of the indicator is again obtained. The solution must be protected from atmospheric carbon dioxide throughout the titration. From the volume of the titrant used in the second titration ascertain the exact concentration of the 0.1 mol/L solution. Each 12.21 mg of benzoic acid is equivalent to 1 mL of tetrabutylammonium hydroxide (0.1 mol/L) VS.

Note: Tetrabutylammonium hydroxide (0.1 mol/L) VS must be standardized immediately before use.

Tetrabutylammonium hydroxide TS

$C_{16}H_{37}NO$. A solution in water containing about 400 g of $C_{16}H_{37}NO$ per litre (~1.5 mol/L).

Tetrabutylammonium hydroxide/methanol TS

Procedure. Dilute a sufficient volume of tetrabutylammonium hydroxide TS with methanol R to obtain a solution containing 0.25 g of $C_{16}H_{37}NO$ per mL.

Tetrabutylammonium iodide R

$C_{16}H_{36}IN$. Contains not less than 98.0% of $C_{16}H_{36}IN$.

Description. White or slightly cream-coloured crystals or a crystalline powder.

Solubility. Soluble in ethanol (~750 g/L) TS.

Sulfated ash. Not more than 0.2 mg/g.

Assay. Dissolve about 1.2 g, accurately weighed, in 30 mL of water. Add 50 mL of silver nitrate (0.1 mol/L) VS and 5 mL of nitric acid (~130 g/L) TS. Titrate the excess of silver nitrate with ammonium thiocyanate (0.1 mol/L) VS using ferric ammonium sulfate (45 g/L) TS as indicator. Each mL of silver nitrate (0.1 mol/L) VS is equivalent to 36.94 mg of $C_{16}H_{36}IN$.

n-Tetradecane R

$C_{14}H_{30}$.

Description. A clear and colourless liquid.

Miscibility. Miscible with ethanol (~750 g/L) TS.

Mass density. ρ_{20} = about 0.76 kg/L.

Refractive index. n_D^{20} = 1.428–1.429.

Tetrachloroethane R

1,1,2,2-Tetrachloroethane, $C_2H_2Cl_4$.

Description. A clear, colourless liquid.

Miscibility. Miscible with 400 parts of water; miscible with ethanol (~750 g/L) TS and ether R.

Boiling range. Not less than 95% distils between 142 and 147 °C.

Refractive index. ρ_{20} = 1.493–1.495.

Mass density. n_D^{20} = 1.590 - 1.595 kg/l.

Tetradecylammonium bromide R

$C_{40}H_{84}BrN$. Chromatographic reagent grade of commerce.

Description. White to almost white crystals, or a crystalline powder.

Melting range. Between 88–89 °C.

Tetraheptylammonium bromide R

$C_{28}H_{60}BrN$. Chromatographic reagent grade of commerce.

Description. White, flaky powder.

Melting range. Between 89-91 °C.

Tetrahydrofuran R

C_4H_8O.

Description. A colourless liquid; odour, characteristic, pungent.

Boiling point. About 66 °C.

Mass density. ρ_{20} = 0.884–0.886 kg/L.

Storage. Store in small, well-filled containers, protected from light.

Labelling. The name and concentration of any suitable preservative, not exceeding 0.1%, should be stated on the label.

Tetramethylammonium hydroxide (~100 g/L) TS

Contains about 100 g/L of $(CH_3)_4NOH$ in water.

Description. A clear and colourless liquid; odour, strong, ammonia-like.

Residue on evaporation. Evaporate 5 mL on a water-bath and dry at 105 °C for 1 hour; it leaves a residue of not more than 1.0 mg (0.2 mg/g).

Ammonia and other amines. Weigh accurately a quantity of the solution, equivalent to about 0.3 g of $(CH_3)_4NOH$, in a low-form weighing bottle tared with 5 mL of water. Add a slight excess of hydrochloric acid (1 mol/L) VS (about 4 mL), evaporate to dryness on a water-bath and dry at 105 °C for 2 hours. The weight of the residue obtained, multiplied by 0.8317, represents the quantity in mg of $(CH_3)_4NOH$ corresponding to within ± 0.2% of that found in the assay.

Assay. Weigh accurately a glass-stoppered flask containing about 15 mL of water. Add a quantity of the solution equivalent to about 0.2 g of $(CH_3)_4NOH$ and weigh again. Add methyl red/ethanol TS and titrate with hydrochloric acid (0.1 mol/L) VS. Each mL of hydrochloric acid (0.1 mol/L) VS is equivalent to 9.115 mg of $(CH_3)_4NOH$.

Storage. Store in a tightly closed container.

Tetramethylammonium hydroxide/ethanol TS

Procedure. Dilute 10 mL of tetramethylammonium hydroxide (~100 g/L) TS with sufficient ethanol (~750 g/L) TS to produce 100 mL.

N,N,N',N'-Tetramethylethane-1,2-diamine R

$C_6H_{16}N_2$

Molecular weight. 116.2.

Other name. N,N,N',N' -Tetramethylethylenediamine.

Description. Colourless liquid, miscible with water and with ethanol (~710 g/L) TS.

Relative density d_{20}^{20}. About 0.78.

Boiling point. About 121 °C.

Thallium standard (2 µg/mL Tl) TS

Procedure. Transfer 235.0 mg of thallous chloride R accurately weighed, to a 1000 mL volumetric flask, dilute to volume with water R and mix. Transfer 1.0 mL of this solution to a 100 mL volumetric flask, dilute to volume with saline TS containing 0.9% of benzyl alcohol R and mix. Each mL of this solution contains 2 µg of thallium.

Thioacetamide R

C_2H_5NS = 75.13 (*62-55-5*).

General reagent grade of commerce.

White crystals or crystalline powder; melting point, about 113 °C.

Thioacetamide reagent TS

Add 1 mL of a mixture of 15 mL of 1m sodium hydroxide, 5 mL of water and 20 mL of glycerol (85%) to 0.2 mL of thioacetamide solution TS, heat in a water bath for 20 seconds, cool and use immediately.

Thioacetamide solution TS

A 4% w/v solution of thioacetamide R.

Thioacetamide, alkaline, TS

Procedure. Dissolve 0.4 g of thioacetamide R in 10 mL of water. Immediately before use add 0.2 mL of this solution to 1 mL of a mixture of 15 mL of sodium hydroxide (1 mol/L) VS, 5 mL of water and 20 mL of glycerol R. Heat on a water-bath for 20 seconds.

Thioglycollic acid R

$C_2H_4O_2S$ 2-Mercaptoacetic acid (MW 92.1) CAS [68-11-1]

A colourless liquid, soluble in alcohol and miscible with water.

Thiourea (0.1 g/L) TS

A solution of thiourea R containing 0.1 g of CH_4N_2S per litre.

Thiourea R

CH_4N_2S (SRIP, 1963, p. 207).

Thorin (2 g/L) TS

Procedure. Dissolve 0.2 g of thorin R in sufficient water to produce 100 mL.

Storage. Store the solution protected from light.

Shelf-life. Use within 1 week of preparation.

Thorin R

2,7-Disodium 4-[(*o*-arsonophenyl)azo]-3-hydroxy-2,7-naphthalenedisulfonate, $C_{16}H_{11}AsN_2Na_2O_{10}S_2$.

Thorium nitrate (0.005 mol/L) VS

Thorium nitrate R dissolved in water to contain 2.401 g of $Th(NO_3)_4$ in 1000 mL.

Method of standardization. Ascertain the exact concentration of the 0.005 mol/L solution in the following manner: transfer 0.050 g, accurately weighed, of sodium fluoride R, previously dried, to a flask and dissolve in sufficient water to produce 250 mL. To 20.0 mL of this solution add 0.6 mL of sodium alizarinsulfonate (1 g/L) TS and then, by drops, sodium hydroxide (0.1 mol/L) VS until the colour changes from pink to yellow. Add 5 mL of acetate buffer, pH 3.0, TS and titrate with the thorium nitrate solution until the yellow colour changes to pinkish yellow. Each 0.8398 mg of sodium fluoride is equivalent to 1 mL of thorium nitrate (0.005 mol/L) VS.

Thorium nitrate R

$Th(NO_3)_4,4H_2O$.

Description. White, slightly deliquescent crystals.

Solubility. Very soluble in water and ethanol (~750 g/L) TS.

Thymine R

5-methylpyrimidine-2,4(1*H*,3*H*)-dione; $C_5H_6N_2O_2$.

A commercially available reagent of suitable grade.

Description. Short needles or plates.

Solubility. Slightly soluble in cold water, soluble in hot water. It dissolves in dilute solution of alkali hydroxyde.

Thymol blue R

Thymolsulfonphthalein, $C_{27}H_{30}O_5S$ (SRIP, 1963, p. 207).

Thymol blue/dimethylformamide TS

Procedure. Dissolve 0.3 g of thymol blue R in sufficient dimethylformamide R to produce 100 mL.

Thymol blue/ethanol TS

Procedure. Dissolve 0.1 g of thymol blue R in sufficient ethanol (~750 g/L) TS to produce 100 mL; filter if necessary.

Thymol blue/methanol TS

Procedure. Dissolve 0.3 g of thymol blue R in sufficient methanol R to produce 100 mL.

Thymol R

$C_{10}H_{14}O$.

Description. Colourless, often large crystals, or a white, crystalline powder; odour, aromatic, resembling that of thyme.

Solubility. Soluble in about 1000 parts of water, in 1 part of ethanol (~750 g/L) TS and in 1.5 parts of ether R.

Melting range. Between 48 and 51 °C; when the melted substance is cooled it remains liquid at a considerably lower temperature.

Residue on volatilization. Volatilize 2 g on a water-bath and dry to constant weight at 105 °C; it leaves a residue of not more than 0.5 mg/g.

Storage. Store in tightly closed containers, protected from light.

Thymol TS1

Procedure. Dissolve 0.225 g of thymol R in sufficient carbon tetrachloride R to produce 100 mL.

Thymol TS2

Procedure. Dilute 10 mL of thymol TS1 to 100 mL with carbon tetrachloride R.

Thymol TS3

Procedure. Dilute 10 mL of thymol TS1 to 150 mL with carbon tetrachloride R.

Thymolphthalein R

$C_{28}H_{30}O_4$ (SRIP, 1963, p. 207).

Thymolphthalein/dimethylformamide TS

Procedure. Dissolve 0.1 g of thymolphthalein R in sufficient dimethylformamide R to produce 100 mL.

Thymolphthalein/ethanol TS

Procedure. Dissolve 0.1 g of thymolphthalein R in 100 mL of ethanol (~750 g/L) TS and filter if necessary.

Titan yellow paper R

Impregnate filter paper with titan yellow TS. Allow to dry at room temperature.

Titan yellow R

$C_{28}H_{19}N_5Na_2O_6S_4$ (SRIP, 1963, p. 208).

Titan yellow TS

Procedure. Dissolve 0.05 g of titan yellow R in sufficient water to produce 100 mL.

Titanium dioxide R

TiO_2.

Description. A white powder; odourless.

Solubility. Practically insoluble in water; slowly soluble, when heated, in sulfuric acid (~1760 g/L) TS.

Titanium dioxide/sulfuric acid TS

Procedure. To 0.1 g of titanium dioxide R add 100 mL of sulfuric acid (~1760 g/L) TS. Heat cautiously with occasional stirring until a clear solution is effected and fumes are evolved; cool.

Storage. Store in glass-stoppered bottles.

Titanium trichloride (0.1 mol/L) VS

Procedure. Dilute 100 mL of titanium trichloride R with 200 mL of hydrochloric acid (~250 g/L) TS and add sufficient carbon-

dioxide-free water R to produce 1000 mL.

Method of standardization. Ascertain the exact concentration immediately before use. With the solution titrate 25 mL of ferric ammonium sulfate (0.1 mol/L) VS acidified with sulfuric acid (~100 g/L) TS in an atmosphere of carbon dioxide R, adding ammonium thiocyanate (75 g/L) TS just before the end-point as indicator. Each mL of ferric ammonium sulfate (0.1 mol/L) VS is equivalent to 15.43 mg of $TiCl_3$.

Titanium trichloride R

A solution of titanium trichloride containing about 15% of $TiCl_3$ (SRIP, 1963, p. 208).

Mass density. ρ_{20} = ~1.2 kg/L.

Toluene R

C_7H_8 (SRIP, 1963, p. 209).

4-Toluenesulfonamide R

$C_7H_9NO_2S$.

Melting range. 135–137 °C.

4-Toluenesulfonic acid R

$C_7H_8O_3S,H_2O$. Contains not less than 98.0% of $C_7H_8O_3S$.

Description. Colourless crystals or a white, crystalline powder.

Solubility. Soluble in water, ethanol (~750 g/L) TS and ether R.

Melting range. 100–105 °C.

Sulfated ash. Not more than 1.0 mg/g.

Assay. Dissolve 0.8 g, accurately weighed, in 50 mL of water and titrate with sodium hydroxide (0.1 mol/L) VS using phenolphthalein/ethanol TS as indicator. Each mL of sodium hydroxide (0.1 mol/L) VS is equivalent to 19.02 mg of $C_7H_8O_3S,H_2O$.

4-Toluenesulfonic acid/ethanol TS

Procedure. Dissolve 20 g of 4-toluenesulfonic acid R in sufficient ethanol (~750 g/L) TS to produce 100 mL.

Tosylchloramide sodium (15 g/L) TS

A solution of tosylchloramide sodium R containing about 16 g of $C_7H_7ClNNaO_2S$ per litre.

Tosylchloramide sodium R

$C_7H_7ClNNaO_2S,3H_2O$. Contains not less than 98.0% of $C_7H_7ClNNaO_2S,3H_2O$.

Description. White crystals or a white, crystalline powder; odour, resembling that of chlorine.

Solubility. Soluble in 7 parts of water and in 2 parts of boiling water; soluble in ethanol (~750 g/L) TS; insoluble in ether R.

Sodium chloride. Treat 1.0 g with 15 mL of dehydrated ethanol R without the aid of heat and filter; it leaves a residue of not more than 15 mg.

Assay. Dissolve 0.4 g, accurately weighed, in 50 mL of water, placed in a glass-stoppered vessel. Add 10 mL of potassium iodide (80 g/L) TS and 5 mL of sulfuric acid (~100 g/L) TS. Allow to stand for 10 minutes and titrate the liberated iodine with sodium thiosulfate (0.1 mol/L) VS. Each mL of sodium thiosulfate (0.1 mol/L) VS is equivalent to 14.08 mg of $C_7H_7ClNNaO_2S,3H_2O$.

Note: Tosylychloramide sodium R is efflorescent.

Tributyl phosphate R

$C_{12}H_{27}O_4P$.

Description. A clear, colourless liquid.

Miscibility. Slightly miscible with water; miscible with most organic solvents.

Mass density. ρ_{20} = about 0.98 kg/L.

Note: Before use, wash the reagent three times as follows: shake 60 mL with 10 mL of a solution containing 1 g of sodium chloride R and 0.1 g of disodium hydrogen phosphate R.

Trichloroacetic acid R

$C_2HCl_3O_2$. (SRIP, 1963, p. 209).

Trichloroethylene R

C_2HCl_3.

Description. A colourless or pale blue, clear, mobile liquid; odour, characteristic, resembling that of chloroform.

Miscibility. Almost immiscible with water; miscible with dehydrated ethanol R and ether R.

Trichlorotrifluoroethane R

1,1,2-Trichloro-1,2,2-trifluoroethane. $C_2Cl_3F_3$.

Description. A colourless, volatile liquid.

Miscibility. Immiscible with water; miscible with acetone R and ether R.

Trichlorotrifluoroethane TS

Procedure. Mix 0.05 µL of trichlorotrifluoroethane R with 1.0 mL of dichloromethane R (other suitable solvents can be used).

Triethylamine R

$C_6H_{15}N$.

Description. A colourless liquid; odour, ammoniacal.

Boiling range. 89–90 °C.

Mass density. ρ_{20} = about 0.73 kg/L.

Refractive index. n_D^{20} = 1.4003.

Triethylenediamine R

1,4-Diazabicyclo[2.2.2]octane; $C_6H_{12}N_2$.

Description. Hygroscopic crystals.

Melting temperature. About 158 °C.

Storage. Store in a tightly closed container.

Trifluoroacetic acid R. $C_2HF_3O_2$

A commercially available reagent of suitable grade.

Triketohydrindene hydrate (1 g/L) TS

A solution of triketohydrindene hydrate R containing about 1 g of $C_9H_4O_3$ per litre.

Triketohydrindene hydrate R

Ninhydrin, $C_9H_4O_3,H_2O$ (SRIP, 1963, p. 210).

Triketohydrindene/butanol TS

Procedure. Dissolve 0.1 g of triketohydrindene hydrate R in sufficient 1-butanol R previously saturated with water to produce 100 mL.

Triketohydrindene/butanol/acetic acid TS

Procedure. Prepare a 20 mg/mL solution of triketohydrindene hydrate R in a mixture of 95 volumes of 1-butanol R and 5 volumes of acetic acid (~120 g/L) TS.

Triketohydrindene/cadmium TS

Procedure. Dissolve 0.050 g of cadmium acetate R in a mixture of 5 mL of water and 1 mL of glacial acetic acid R and add

sufficient ethylmethylketone R to produce 50 mL. Dissolve 20 mg of triketohydrindene hydrate R in 10 mL of this solution.

Note: Prepare immediately before use.

Triketohydrindene/ethanol TS

Procedure. Prepare a saturated solution of triketohydrindene hydrate R in ethanol (~750 g/L) TS.

Triketohydrindene/methanol TS

Procedure. Dissolve 1.0 g of triketohydrindene hydrate R in sufficient methanol R to produce 100 mL.

Note. Triketohydrindene/methanol TS must be freshly prepared.

Triketohydrindene/pyridine/acetone TS

Procedure. Dissolve 0.25 g of triketohydrindene hydrate R in 100 mL of a mixture of equal volumes of pyridine R and acetone R.

Triketohydrindene/pyridine/butanol TS

Procedure. Dissolve 1 g of triketohydrindene hydrate R in 1 mL of pyridine R and dilute with sufficient 1-butanol R to produce 100 mL.

Note: It should be freshly prepared.

Triketohydrindene/sodium metabisulfite TS

Procedure. Dissolve 3 g of triketohydrindene hydrate R in 100 mL of a solution containing 4.55 g of sodium metabisulfite R in 100 mL of water.

Triketohydrindene/stannous chloride TS

Procedure. Dissolve 4 g of triketohydrindene hydrate R in 100 mL of ethylene glycol monomethyl ether R. Shake gently with 1 g of cation exchange resin (300 µm–840 µm) and filter (solution A). Dissolve 0.16 g of stannous chloride R in 100 mL of acetate buffer, pH 5.5, TS (solution B). Immediately before use mix equal volumes of the two solutions.

2,4,4 Trimethylpentan-2-amine R

$C_8H_{19}N$

Molecular weight. 129.3.

Other name. 2-Amino-2,4,4-trimethylpentane; 1,1,3,3-Tetramethylbutylamine

Description. Clear, colourless liquid.

Relative density d_{20}^{20}. About 0.805.

Boiling point. About 140 °C.

2,2,4-Trimethylpentane R

iso -Octane; C_8H_{18} (SRIP, 1963, p. 129).

Trimethylpyridine (50 g/L) TS

A solution of trimethylpyridine R containing about 50 g of $C_8H_{11}N$ per litre.

Trimethylpyridine R

$C_8H_{11}N$. 2,4,6-Trimethylpyridine; sym. collidine.

Description. Liquid; odour, aromatic.

Miscibility. More miscible with cold water than with hot water; miscible with ethanol (~750 g/L) TS; ether R and methanol R.

Relative density. d_4^{20} = 0.914.

Refractive index. n_D^{20} = 1.498.

Trinitrophenol (7 g/L) TS

A solution of trinitrophenol R containing 7 g of $C_6H_3N_3O_7$ per litre.

Trinitrophenol R

$C_6H_3N_3O_7$ (SRIP, 1963, p. 211).

Trinitrophenol, alkaline, TS

Procedure. Mix 20 mL of a 10 mg/mL solution of trinitrophenol R with 10 mL of a 50 mg/mL solution of sodium hydroxide R, dilute with water to 100 mL and mix.

Note: After preparation alkaline trinitrophenol TS should be used for only 48 hours.

Trinitrophenol/ethanol TS

Procedure. Dissolve 33 g of trinitrophenol R in sufficient ethanol (~750 g/L) TS to produce 1000 mL.

Triphenylantimony R

$C_{18}H_{15}Sb$.

Melting temperature. About 55 °C.

Triphenylmethanol R

Triphenylcarbinol; $C_{19}H_{16}O$.

A commercialy available reagent of suitable grade.

Description. Colourless crystals.

Solubility. Practically insoluble in water, freely soluble in ethanol (~750 g/L) TS (ethanol (95%) R).

Trisodium orthophosphate (2 g/L) TS

A solution of trisodium orthophosphate R containing about 2 g of Na_3PO_4 per litre.

Trisodium orthophosphate R

Na_3PO_4,$12H_2O$.

Description. Colourless crystals or a white, crystalline powder.

Solubility. Freely soluble in water; practically insoluble in ethanol (~750 g/L) TS and carbon disulfide R.

Tyrosine R

$C_9H_{11}NO_3$ (SRIP, 1963, p. 212).

U - Uracil R.... Urea R

Uracil R Pyrimidine-2,4(1*H*,3*H*)-dione; $C_4H_4N_2O_2$.

A commercially available reagent of suitable grade.

Uranyl acetate R

$C_4H_6O_6U,2H_2O$ (SRIP, 1963, p. 213).

Uranyl/zinc acetate TS

Procedure. Dissolve 10 g of uranyl acetate R by heating with 50 mL of water and 5 mL of acetic acid (~300 g/L) TS; dissolve 30 g of zinc acetate R by heating with 30 mL of water and 3 mL of acetic acid (~300 g/L) TS. Mix the two solutions, allow to cool to room temperature and remove by filtration any solid material that separates.

Urea R

CH_4N_2O (SRIP, 1963, p. 214).

S - Salicylaldehyde R.... Sulfurous acid TS

Salicylaldehyde R

$C_7H_6O_2$.

Description. A clear, colourless, oily liquid; odour, bitter, almond-like.

Solubility. Slightly soluble in water; soluble in ethanol (~750 g/L) TS and ether R.

Relative density, d_4^{20} = 1.17.

Salicylaldehyde TS

Procedure. Mix 2 g of salicylaldehyde R with 100 mL of methanol R and add 0.1 mL of hydrochloric acid (~420 g/L) TS.

Salicylic acid R

2-hydroxybenzoic acid; $C_7H_6O_3$.

A commercially available reagent of suitable grade.

Storage. Keep protected from light.

Saline TS

A sterile solution of sodium chloride R containing about 9 g/L of NaCl. Sterilization by heating in a steam autoclave at 120 °C for 30 minutes is suitable.

Selenious acid R

H_2SeO_3. Contains not less than 93% of H_2SeO_3.

Description. Colourless or white crystals.

Solubility. Soluble in water and ethanol (~750 g/L) TS.

Assay. Transfer about 0.1 g, accurately weighed, to a glass-stoppered flask and dissolve in 50 mL of water. Add 10 mL of potassium iodide (300 g/L) TS and 5 mL of hydrochloric acid (~420 g/L) TS, mix, insert the stopper into the flask and allow to stand for 10 minutes. Dilute with 50 mL of water, add 3 mL of starch TS and titrate with sodium thiosulfate (0.1 mol/L) VS until the colour is no longer diminished, then titrate with iodine (0.05 mol/L) VS to a blue colour. Subtract the volume of iodine (0.05 mol/L) VS from the volume of sodium thiosulfate (0.1 mol/L) VS equivalent to selenious acid. Each mL of sodium thiosulfate (0.1 mol/L) VS is equivalent to 3.225 mg of H_2SeO_3.

Note: Selenious acid R effloresces in dry air and is hygroscopic in moist air.

Selenious acid/sulfuric acid TS

Procedure. Dissolve 10 mg of selenious acid R in 2 mL of sulfuric acid (~1760 g/L) TS.

Selenium R

Se (SRIP, 1963, p. 172).

Caution. Selenium vapours are toxic.

D-serine R

A commercially available reagent of suitable grade.

Silica gel for chromatography R

A very finely divided (3–10 µm) silica gel. The particle size is indicated after the name of the reagent in the tests where it is used.

Description. A fine, white, homogeneous powder.

Solubility. Practically insoluble in water and ethanol (~750 g/L) TS.

Silica gel R1

Silica gel G.

Description. A white, homogeneous powder.

Composition. A mixture of silica gel (particle size 10–40 µm) and calcium sulfate, hemihydrate (about 130 g/kg).

Silica gel R2

Silica gel HF(UV254).

Description. A white, homogeneous powder.

Composition. Silica gel (particle size 10–40 µm) containing a fluorescent indicator having an optimal intensity at 254 nm (about 15 g/kg).

Silica gel R3

Silica gel H.

Description. A white, homogeneous powder.

Particle size. 10–40 µm.

Silica gel R4

Silica gel GF (UV 254).

Description. A white, homogeneous powder.

Composition. A mixture of silica gel (particle size 10–40 µm) and calcium sulfate, hemihydrate (about 130 g/kg) containing a fluorescent indicator having an optimal intensity at 254 nm (about 15 g/kg).

Silica gel R5

Silica gel 60.

Description. A white, homogeneous powder.

Average pore size. 6 nm.

Silica gel R6

Silica gel 60 (UV 254).

Description. A white, homogeneous powder.

Average pore size. 6 nm.

Composition. Silica gel (average particle size 15 µm) containing a fluorescent indicator having an optimal intensity at 254 nm (about 15 g/kg).

Silica gel R7

Silica gel H, octadecylsilyl (UV 254)

Description. A white, homogeneous powder.

Composition. RP-18 modified silica gel (particle size 9-12 µm) containing a fluorescent indicator having an optimal intensity at 254nm.

Silica gel for chromatography, hexadecylamidylsilyl

Particles of silica gel, the surface of which has been modified with chemically-bonded hexadecylamidylsilyl groups.

Silica gel for chromatography, octadecylsilyl, base-deactivated

A very finely divided silica gel, pretreated before the bonding of octadecylsilyl groups to minimize the interaction with basic compounds.

Silica gel, desiccant, R

Description. An amorphous, partly hydrated SiO_2, occurring in glassy granules of varying sizes. It is frequently coated with a substance that changes colour when the capacity to absorb water is exhausted. Such coloured products may be regenerated (i.e. may regain their capacity to absorb water) by heating at 110 °C until the gel assumes the original colour.

Loss on ignition. Ignite 2 g, accurately weighed, at 950 ± 50 °C to constant weight; the loss is not more than 60 mg/g.

Water absorption. Place about 10 g in a tared weighing-bottle and weigh. Then place the bottle, with the cover removed, for 24

hours in a closed container in which the atmosphere is maintained at 80% relative humidity by being in equilibrium with sulfuric acid having a relative density of 1.19. Weigh again; the increase in weight is not less than 310 mg/g.

Silver nitrate (0.001 mol/L) VS

Silver nitrate R dissolved in water to contain 0.1699 g of $AgNO_3$ in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution following the method described under "Silver nitrate (0.1 mol/L) VS".

Silver nitrate (0.01 mol/L) VS

Silver nitrate R dissolved in water to contain 1.699 g of $AgNO_3$ in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution following the method described under silver nitrate (0.1 mol/L) VS.

Silver nitrate (0.05 mol/L) VS

Silver nitrate R dissolved in water to contain 8.494 g of $AgNO_3$ in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution following the method described under "Silver nitrate (0.1 mol/L) VS".

Silver nitrate (0.1 mol/L) VS

Silver nitrate R dissolved in water to contain 16.99 g of $AgNO_3$ in 1000 mL.

Method of standardization. Ascertain the exact concentration of the 0.1 mol/L solution in the following manner: dilute 40.0 mL of the silver nitrate solution with 100 mL of water. Heat the solution and add slowly, with continuous stirring, hydrochloric acid (~70 g/L) TS until precipitation of the silver is complete. Boil the mixture cautiously for about 5 minutes then allow it to stand in the dark until the precipitate has settled and the supernatant liquid has become clear. Transfer the precipitate completely to a tared filtering crucible and wash it with small portions of water that have been slightly acidified with nitric acid (~1000g/L) TS. Dry the precipitate to constant weight at 110 °C. From the weight of silver chloride calculate the concentration of the silver nitrate solution in mol/L. Protect the silver chloride from light as much as possible during the determination.

Silver nitrate (~0.1 mol/L) TS

A 17 g/L solution of silver nitrate R.

Storage. Protected from light.

Silver nitrate (100 g/L) TS

A solution of silver nitrate R containing 100 g of $AgNO_3$ per litre.

Silver nitrate (40 g/L) TS

A solution of silver nitrate R containing about 42.5 g/L of $AgNO_3$ (approximately 0.25 mol/L).

Silver nitrate R

$AgNO_3$ (SRIP, 1963, p. 173).

Silver nitrate/methanol TS

Procedure. Prepare a saturated solution of silver nitrate R in methanol R.

Silver oxide R

Ag_2O.

Description. A brownish-black, heavy powder; odourless.

Solubility. Practically insoluble in water; freely soluble in nitric acid (~130 g/L) TS and ammonia (~260 g/L) TS.

Substances insoluble in nitric acid. Dissolve 5 g in a mixture of 5 mL of nitric acid (~1000 g/L) TS and 10 mL of water, dilute to about 65 mL with water and filter any undissolved residue on a tared filtering crucible (retain the filtrate for the test for substances not precipitated by hydrochloric acid). Wash the crucible with water until the last washing shows no opalescence with 1 drop of hydrochloric acid (~250 g/L) TS and dry to constant weight at 105 °C; not more than 0.2 mg/g.

Substances not precipitated by hydrochloric acid. Dilute the filtrate obtained in the test for substances insoluble in nitric acid to

250 mL with water, heat to boiling and add drop-wise sufficient hydrochloric acid (~250 g/L) TS to precipitate all of the silver (about 5 mL) avoiding any great excess. Cool, dilute to 300 mL with water and allow to stand overnight. Filter, evaporate 200 mL of the filtrate to dryness in a suitable tared porcelain dish and ignite; not more than 0.5 mg/g.

Alkalinity. Heat 2 g with 40 mL of water on a water-bath for 15 minutes, cool and dilute to 50 mL with water. Filter, discarding the first 10 mL of the filtrate. To 25 mL of the subsequent filtrate add 2 drops of phenolphthalein/ethanol TS and titrate with hydrochloric acid (0.02 mol/L) VS to the disappearance of any pink colour; not more than 0.20 mL is required.

Silver standard (5 µg Ag/mL) TS

Procedure. Dissolve 39.5 mg of silver nitrate R in sufficient water to produce 100 mL. Dilute 1.0 mL of this solution to 100 mL with water.

Soda lime R

(SRIP, 1963, p. 174).

Sodium 1,2-naphthoquinone-4-sulfonate (5 g/L) TS

A solution of sodium 1,2-naphthoquinone-4-sulfonate R containing about 5 g of $C_{10}H_5NaO_5S$ per litre.

Sodium 1,2-naphthoquinone-4-sulfonate R

$C_{10}H_5NaO_5S$.

Description. A yellow or orange, crystalline powder.

Solubility. Soluble in water; insoluble in ethanol (~750 g/L) TS.

Sodium acetate (0.04 mol/L) VS

Sodium acetate R dissolved in water to contain 3.281 g of $C_2H_3NaO_2$ in 1000 mL.

Sodium acetate (150 g/L) TS

A solution of sodium acetate R containing about 150 g/L of $C_2H_3NaO_2$.

Sodium acetate (50 g/L) TS

A solution of sodium acetate R containing about 50 g of $C_2H_3NaO_2$ per litre.

Sodium acetate (60 g/L) TS

A solution of sodium acetate R containing about 60 g of $C_2H_3NaO_2$ per litre.

Sodium acetate R

$C_2H_3NaO_2,3H_2O$. Contains not less than 99.0% of $C_2H_3NaO_2,3H_2O$.

Description. Colourless crystals.

Solubility. Very soluble in water; sparingly soluble in ethanol (~750 g/L) TS.

Clarity and colour of solution. A 0.1 g/mL solution is clear and colourless.

pH value. pH of a 50 mg/mL solution, 7.5–9.2.

Iron. Use 8 g; the solution complies with the *2.2.4 Limit test for iron*; not more than 5.0 µg/g.

Heavy metals. Use 1.0 g for the preparation of the test solution as described in *2.2.3 Limit test for heavy metals*, Procedure 1; determine the heavy metals content according to Method A; not more than 10 µg/g.

Substances reducing permanganate. Dissolve 1 g in 100 mL of boiling water, add 2 mL of sulfuric acid (~100 g/L) TS and 0.05 mL of potassium permanganate (0.02 mol/L) VS and boil for 5 minutes; the pink colour does not entirely disappear.

Assay. Dissolve about 0.4 g, accurately weighed, in 100 mL of glacial acetic acid R and 5 mL of acetic anhydride R. After 5 minutes add 10 drops of 1-naphtholbenzein/acetic acid TS and titrate to a green end-point with perchloric acid (0.1 mol/L) VS as described under *2.6 Non-aqueous titration*, Method A. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 13.61 mg of $C_2H_3NaO_2,3H_2O$.

Sodium acetate/glacial acetic acid (0.1 mol/L) VS

Procedure. Dissolve 5.3 g of anhydrous sodium carbonate R in small portions in 100 mL of glacial acetic acid R1, stirring well

after each addition and add sufficient glacial acetic acid R1 to produce 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution in the following manner: titrate the solution against 15.0 mL of perchloric acid (0.1 mol/L) VS using 2–3 drops of crystal violet/acetic acid TS. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 8.203 mg of $C_2H_3NaO_2$.

Sodium alizarinsulfonate (1 g/L) TS

Procedure. Dissolve 0.11 g of sodium alizarinsulfonate R in sufficient water to produce 100 mL.

Sodium alizarinsulfonate (10 g/L) TS

A solution of sodium alizarinsulfonate R containing about 10 g of $C_{14}H_7NaO_7S$ per litre.

Sodium alizarinsulfonate R

Alizarin Red S, sodium salt of 3,4-dihydroxy-9,10-anthraquinone-2-sulfonic acid; $C_{14}H_7NaO_7S,H_2O$.

Description. A yellow-brown or orange-yellow powder.

Solubility. Freely soluble in water, producing a yellow solution; sparingly soluble in ethanol (~750 g/L) TS.

Sodium arsenite (0.05 mol/l) VS

Procedure. Dissolve 5 g of arsenic trioxide R in a mixture of 20 mL of sodium hydroxide (~80 g/L) TS and 20 mL of water, dilute to 400 mL with water and add hydrochloric acid (~70 g/L) TS until the solution is neutral to litmus paper R. Dissolve 4 g of sodium hydrogen carbonate R in the prepared solution and dilute to 1000 mL with water.

Method of standardization. Ascertain the exact concentration of the 0.05 mol/L solution in the following manner: dilute 25 mL with 50 mL of water, add 5 g of sodium hydrogen carbonate R and titrate with iodine (0.05 mol/L) VS, using starch TS as indicator.

Storage. Add 1 drop of mercury R for the preservation of the solution.

Sodium carbonate (10 g/L) TS

A solution of sodium carbonate R containing about 10.6 g of Na_2CO_3 per litre (approximately 0.1 mol/L).

Sodium carbonate (50 g/L) TS

A solution of sodium carbonate R containing about 50 g/L of Na_2CO_3 (approximately 0.5 mol/L).

Sodium carbonate (75 g/L) TS

A solution of sodium carbonate R containing about 75 g of Na_2CO_3 per litre.

Sodium carbonate (106 g/L) TS

A solution of sodium carbonate R containing about 106 g of Na_2CO_3 per litre (approximately 1 mol/L).

Sodium carbonate (200 g/L) TS

A solution of sodium carbonate R containing 200 g of Na_2CO_3 per litre.

Sodium carbonate R

$Na_2CO_3,10H_2O$ (SRIP, 1963, p. 179).

Sodium carbonate standard TS

Procedure. Dissolve 2.64 g of sodium carbonate R and 2.093 g of sodium hydrogen carbonate R in sufficient carbon-dioxide-free water R to produce 1000 mL.

Sodium carbonate, anhydrous, FeR

Anhydrous sodium carbonate R that complies with the following additional test: Dissolve 4.0 g in 25 mL of water, add 8 mL of hydrochloric acid (~250 g/L) FeTS and proceed with the *2.2.4 Limit test for iron* using 2 mL of iron standard FeTS; not more than 10 µg/g.

Sodium carbonate, anhydrous, R

Na_2CO_3 (SRIP, 1963, p. 179).

Sodium chloride (10 g/L) TS

A solution of sodium chloride R containing about 10 g of NaCl per litre.

Sodium chloride (300g/L) TS

A solution of sodium chloride R containing about 300 g of NaCl per litre.

Sodium chloride (400 g/L) TS

A saturated solution of sodium chloride R containing about 400 g of NaCl per litre.

Sodium chloride (5.84 g/L) TS

A 5.84 g/L solution of sodium chloride R1 in water R.

Sodium chloride (9 g/L) TS

A solution of sodium chloride R containing about 9g of NaCl per litre.

Sodium chloride R

NaCl (SRIP, 1963, p. 181).

Sodium chloride R1

Use sodium chloride as described in the monograph for Sodium chloride.

Sodium chloride, pyrogen-free, R

Sodium chloride R which complies with the following additional test:

Pyrogens. Carry out the test as described under *3.5 Test for pyrogens* injecting, per kg of the rabbit's weight, a solution containing 9 mg in 10 mL of sterile water R.

Sodium citrate (250 g/L) TS

A solution of sodium citrate R containing about 294 g of $C_6H_5Na_3O_7,2H_2O$ in 1000 mL.

Sodium citrate R

$C_6H_5Na_3O_7,2H_2O$.

Contains not less than 99.0% of $C_6H_5Na_3O_7$, calculated with reference to the anhydrous substance.

Description. White, granular crystals or a crystalline powder; odourless. Slightly deliquescent in moist air.

Solubility. Soluble in less than 2 parts of water, practically insoluble in ethanol (~750 g/L) TS.

Appearance of solution. A 100 g/L solution is clear and colourless.

Water. Determined by the Karl Fischer method, keeping the substance in contact with the dehydrated methanol R for 15 minutes; not less than 110 mg/g and not more than 130 mg/g.

Assay. Dissolve about 0.15 g, accurately weighed, in 20 mL of glacial acetic acid R and titrate with perchloric acid (0.1 mol/L) VS as described in *2.6 Non-aqueous titration*, Method A. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 8.603 mg of $C_6H_5Na_3O_7$.

Sodium cobaltinitrite (100 g/L) TS

A solution of sodium cobaltinitrite R containing about 100 g/L of $Na_3Co(NO_2)_6$.

Sodium cobaltinitrite R

$Na_3Co(NO_2)_6$ (SRIP, 1963, p. 182).

Sodium diethyldithiocarbamate (0.8 g/L) TS

A solution of sodium diethyldithiocarbamate R containing about 0.8 g of $C_5H_{10}NNaS_2$ per litre.

Sodium diethyldithiocarbamate R

$C_5H_{10}NNaS_2,3H_2O$ (SRIP, 1963, p. 183).

Sodium dihydrogen phosphate (275 g/L) TS

A solution of sodium dihydrogen phosphate R containing about 275 g of NaH_2PO_4 per litre.

Sodium dihydrogen phosphate (45 g/L) TS

A solution of sodium dihydrogen phosphate R containing about 47 g of NaH_2PO_4 per litre.

Sodium dihydrogen phosphate, anhydrous R

Molecular formula: NaH_2PO_4

Description. White powder, hygroscopic.

Storage. In a tightly closed container.

Sodium dihydrogen phosphate dihydrate R

[sodium biphosphate]; sodium phosphate, monobasic; $NaH_2 PO_4$, 2 H_2O.

Sodium dihydrogen phosphate R

[sodium biphosphate]; sodium phosphate, monobasic; NaH_2PO_4,H_2O (SRIP, 1963, p. 178).

Sodium dithionite R

Sodium hydrosulfite, sodium sulfoxylate; $Na_2O_4S_2$.

Description. A white or greyish white, crystalline powder.

Solubility. Very soluble in water, slightly soluble in ethanol (~750 g/L) TS.

Note : Sodium dithionite R oxidizes in air.

Sodium dithionite (200g/L) TS

A solution of sodium dithionite R containing about 200 g of $Na_2O_4S_2$ per litre.

Sodium dodecyl sulfate R.

Sodium lauryl sulfate; $C_{12}H_{25}NaO_4S$.

Purified grade of commercially available reagent containing not less than 99.0% of $C_{12}H_{25}NaO_4S$.

White, crystalline flakes.

Sodium fluoride R

NaF (SRIP, 1963, p. 183).

Sodium formate R

$CHNaO_2$.

Description. White, deliquescent granules or a crystalline powder; slight odour of formic acid.

Melting temperature. About 253 °C.

Sodium hexanesulfonate R

$C_6H_{13}NaO_3S$.

A commercially available reagent of suitable grade.

Sodium hydrogen carbonate (100 g/L) TS

A solution of sodium hydrogen carbonate R containing about 100 g of $NaHCO_3$ in 1000 mL.

Sodium hydrogen carbonate (40 g/L) TS

A solution of sodium hydrogen carbonate R containing about 42 g of $NaHCO_3$ per litre (approximately 0.5 mol/L).

Sodium hydrogen carbonate R

[sodium bicarbonate]; $NaHCO_3$ (SRIP, 1963, p. 177).

Sodium hydrogen carbonate R

$NaHCO_3$ (SRIP, 1963, p. 177).

Sodium hydroxide (~4 g/L) TS

A solution of sodium hydroxide R containing about 4 g/L of NaOH (approximately 0.1 mol/L).

Sodium hydroxide (~40 g/L) TS

A solution of sodium hydroxide R containing about 40 g/L of NaOH (approximately 1 mol/L).

Sodium hydroxide (~80 g/L) TS

A solution of sodium hydroxide R containing about 80 g/L of NaOH (approximately 2 mol/L).

Sodium hydroxide (~85 g/L) TS

A solution of sodium hydroxide R in water R containing about 85 g/L of NaOH.

Sodium hydroxide (~ 100 g/L) TS

A solution of sodium hydroxide R containing about 100 g/L of NaOH (approximately 2.5 mol/L).

Sodium hydroxide (~150 g/L) TS

A solution of sodium hydroxide R containing about 150 g/L of NaOH.

Sodium hydroxide (~200 g/L) TS

A solution of sodium hydroxide R containing about 200 g/L of NaOH.

Sodium hydroxide (~300 g/L) TS

A solution of sodium hydroxide R containing about 300 g/L of NaOH.

Sodium hydroxide (~400 g/L) TS

A solution of sodium hydroxide R containing about 400 g/L of NaOH.

Sodium hydroxide (~420 g/L) TS

A solution of sodium hydroxide R containing about 420 g/L of NaOH.

Sodium hydroxide (0.01 mol/L) VS

Sodium hydroxide R dissolved in water to contain 0.4001 g of NaOH in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution following the method described under sodium hydroxide (1 mol/L) VS.

Sodium hydroxide (0.01 mol/L), carbonate-free, VS

Sodium hydroxide R dissolved in water to contain 0.4001 g of NaOH in 1000 mL.

Procedure, test for carbonates and method of standardization. Prepare the solution, carry out the test and ascertain the exact concentration following the method described under carbonate-free sodium hydroxide (1 mol/L) VS.

Sodium hydroxide (0.02 mol/L) VS

Sodium hydroxide R dissolved in water to contain 0.8001 g of NaOH in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution following the method described under sodium hydroxide (1 mol/L) VS.

Sodium hydroxide (0.02 mol/L), carbonate-free, VS

Sodium hydroxide R dissolved in water to contain 0.8001 g of NaOH in 1000 mL.

Procedure, test for carbonates and method of standardization. Prepare the solution, carry out the test and ascertain the exact concentration following the method described under carbonate-free sodium hydroxide (1 mol/L) VS.

Sodium hydroxide (0.05 mol/L) VS

Sodium hydroxide R dissolved in water to contain 2.000 g of NaOH in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution following the method described under sodium hydroxide (1 mol/L) VS.

Sodium hydroxide (0.1 mol/L) VS

Sodium hydroxide R, dissolved in water to contain 4.001 g of NaOH in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution following the method described under sodium hydroxide (1 mol/L) VS.

Sodium hydroxide (0.1 mol/L), carbonate-free, VS

Sodium hydroxide R dissolved in water to contain 4.001 g of NaOH in 1000 mL.

Procedure, test for carbonates and method of standardization. Prepare the solution, carry out the test and ascertain the exact concentration following the method described under carbonate-free sodium hydroxide (1 mol/L) VS.

Sodium hydroxide (0.2 mol/L) VS

Sodium hydroxide R, dissolved in water to contain 8.001 g of NaOH in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution following the method described under sodium hydroxide (1 mol/L) VS.

Sodium hydroxide (0.2 mol/L), carbonate-free, VS

Sodium hydroxide R dissolved in water to contain 8.001 g of NaOH in 1000 mL.

Procedure, test for carbonates and method of standardization. Prepare the solution, carry out the test and ascertain the exact concentration following the method described under carbonate-free sodium hydroxide (1 mol/L) VS.

Sodium hydroxide (0.5 mol/L) VS

Sodium hydroxide R dissolved in water to contain 20.00 g of NaOH in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution following the method described under sodium hydroxide (1 mol/L) VS.

Sodium hydroxide (0.5 mol/L), carbonate-free, VS

Sodium hydroxide R dissolved in water to contain 20.00 g of NaOH in 1000 mL.

Procedure, test for carbonates and method of standardization. Prepare the solution, carry out the test and ascertain the exact concentration following the method described under carbonate-free sodium hydroxide (1 mol/L) VS.

Sodium hydroxide (1 mol/L) VS

Sodium hydroxide R dissolved in water to contain 40.01 g of NaOH in 1000 mL.

Method of standardization. Ascertain the exact concentration of the 1 mol/L solution in the following manner: dry about 5 g of potassium hydrogen phthalate R at 105 °C for 3 hours and weigh accurately. If the potassium hydrogen phthalate is in the form of large crystals they should be crushed before drying. Dissolve in 75 mL of carbon-dioxide-free water R and titrate with the sodium hydroxide solution using phenolphthalein/ethanol TS as indicator. Each 0.2042 g of potassium hydrogen phthalate is equivalent to 1 mL of sodium hydroxide (1 mol/L) VS. Standard solutions of sodium hydroxide should be restandardized frequently.

Storage. Solutions of alkali hydroxides absorb carbon dioxide when exposed to air. They should therefore be stored in bottles with suitable non-glass, well-fitting stoppers, provided with a tube filled with soda lime R.

Sodium hydroxide (1 mol/L), carbonate-free, VS

Sodium hydroxide R dissolved in water to contain 40.01 g of NaOH in 1000 mL.

Procedure. Dissolve sodium hydroxide R in water to produce a 400–600 g/L solution and allow to stand. Taking precautions to avoid absorption of carbon dioxide, siphon off the clear supernatant liquid and dilute as required with carbon-dioxide-free water R.

Test for carbonates. Titrate 45 mL of hydrochloric acid (1 mol/L) VS with the carbonate-free sodium hydroxide solution using phenolphthalein/ethanol TS as indicator. At the end-point add just sufficient acid to discharge the pink colour and boil to reduce the volume to 20 mL. Add, whilst boiling, sufficient acid again to discharge the pink colour and prevent its reappearance on continued boiling; not more than 0.1 mL of the acid is required.

Method of standardization. Ascertain the exact concentration of the 1 mol/L solution in the following manner: dry about 5 g of

potassium hydrogen phthalate R at 105 °C for 3 hours and weigh accurately. If the potassium hydrogen phthalate is in the form of large crystals they should be crushed before drying. Dissolve in 75 mL of carbon-dioxide-free water R and titrate with the carbonate-free sodium hydroxide solution using phenolphthalein/ethanol TS as indicator. Each 0.2042 g of potassium hydrogen phthalate is equivalent to 1 mL of carbonate-free sodium hydroxide (1 mol/L) VS. Standard solutions of sodium hydroxide should be restandardized frequently.

Storage. Solutions of alkali hydroxides absorb carbon dioxide when exposed to air. They should therefore be stored in bottles with suitable non-glass, well-fitting stoppers, provided with a tube filled with soda lime R.

Sodium hydroxide (~8.5 g/L) TS

solution of sodium hydroxide R containing about 8.5 g/L of NaOH.

Sodium hydroxide (10 g/L) TS

A solution of sodium hydroxide R containing about 10 g of NaOH per litre (approximately 0.25 mol/L).

Sodium hydroxide (50 g/L) TS

A solution of sodium hydroxide R containing about 50 g of NaOH per litre.

Sodium hydroxide R

NaOH (SRIP, 1963, p. 185).

Sodium hydroxide/ethanol (0.1 mol/L) VS

Sodium hydroxide R dissolved in ethanol (~710 g/L) TS to contain 4.00 g of NaOH in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution in the following manner:

Dissolve 0.10 g of benzoic acid R, accurately weighted, in 10 mL of water R and 40 mL of dehydrated ethanol R. Titrate with the sodium hydroxide/ethanol solution, determining the end-point potentiometrically or using 0.2 mL of thymolphthalein solution R as indicator. Standardize immediately before use.

1 mL of 0.1 M sodium hydroxide/ethanol (0.1 mol/L) VS is equivalent to 12.21 mg of $C_7H_6O_2$.

Sodium hydroxide/ethanol TS

Procedure. Dissolve 50 g of sodium hydroxide R in sufficient ethanol (~750 g/L) TS to produce 1000 mL.

Sodium hydroxide/methanol TS

Procedure. Dissolve 40 g of sodium hydroxide R in sufficient methanol R to produce 1000 mL.

Sodium hypobromite TS

Procedure. Dissolve 2.5 g of sodium hydroxide R in 7.5 mL of water, add 0.5 mL of bromine R and a sufficient quantity of water to produce 10 mL.

Note : Sodium hypobromite TS must be freshly prepared.

Sodium hypochlorite (~40 g/L) TS

Description. A pale, greenish yellow, clear liquid; odour, resembling that of chlorine.

Assay. Introduce 3 mL into a glass-stoppered flask, weigh accurately and add 50 mL of water. Add 2 g of potassium iodide R and 10 mL of acetic acid (~300 g/L) TS and titrate the liberated iodine with sodium thiosulfate (0.1 mol/L) VS, adding 3 mL of starch TS as the end-point is approached. Each mL of sodium thiosulfate (0.1 mol/L) VS is equivalent to 3.723 mg of NaOCl.

Storage. Sodium hypochlorite (~40 g/L) TS must be kept in a light-resistant container at a temperature not exceeding 25 °C.

Sodium hypochlorite TS1

Procedure. Dilute 10 mL of sodium hypochlorite (~40 g/L) TS to 100 mL with water (contains approximately 0.5% of chlorine).

Sodium iodide R

NaI.

Description. White crystalline powder or colourless crystals, hygroscopic.

Solubility. Very soluble in water, freely soluble in alcohol.

Sodium laurilsulfate (10 g/L) TS

A solution of sodium laurilsulfate R containing about 10 g of $C_{12}H_{25}NaO_4S$ per litre.

Sodium laurilsulfate R

Sodium lauryl sulfate; a mixture of sodium alkyl sulfates, consisting mainly of sodium dodecyl sulfates, $C_{12}H_{25}NaO_4S$.

Description. A white or pale yellow powder, crystals, or flakes; odour, faint but characteristic.

Solubility. Very soluble in water giving an opalescent solution; partly soluble in ethanol (~750 g/L) TS.

Sodium mercaptoacetate R

(Sodium thioglycolate R.) $C_2H_3NaO_2S$.

Description. Hygroscopic crystals.

Solubility. Freely soluble in water; slightly soluble in ethanol (~750 g/L) TS.

Sodium metabisulfite (50 g/L) TS

A solution of sodium metabisulfite R containing about 50 g of $Na_2O_5S_2$ per litre.

Sodium metabisulfite R

$Na_2O_5S_2$ (SRIP, 1963, p. 187).

Sodium metaperiodate R

Sodium periodate $NaIO_4$. Contains not less than 98.0% of $NaIO_4$.

Description. White crystals or a white, crystalline powder.

Solubility. Soluble in water.

Assay. Dissolve 0.5 g in 100 mL of water. Add 3 g of sodium hydrogen carbonate R and 3 g of potassium iodide R and titrate the liberated iodine with sodium arsenite (0.05 mol/L) VS. Each mL of sodium arsenite (0.05 mol/L) VS is equivalent to 10.69 mg of $NaIO_4$.

Sodium metaperiodate TS

Procedure. Dissolve 60 g of sodium metaperiodate R in 120 mL of sulfuric acid (0.05 mol/L) VS and dilute to 1000 mL with water. *Do not heat to dissolve the periodate.* If the solution is not clear filter through a sintered-glass filter. Store the solution in a glass-stoppered, light-resistant container.

Suitability test. Pipette 10 mL into a 250 mL volumetric flask, dilute to volume with water and mix. To about 550 mg of glycerol R dissolved in 50 mL of water add, using a pipette, 50 mL of the diluted sodium metaperiodate. For a blank transfer 50 mL of the diluted sodium metaperiodate solution to a flask containing 50 mL of water. Allow the solutions to stand for 30 minutes then to each add 5 mL of hydrochloric acid (~420 g/L) TS and 10 mL of potassium iodide (80 g/L) TS and swirl to mix. Allow to stand for 5 minutes, add 100 mL of water and titrate with sodium thiosulfate (0.1 mol/L) VS, shaking continuously and adding 3 mL of starch TS as the end-point is approached. The ratio of the volume of sodium thiosulfate (0.1 mol/L) VS required for sodium metaperiodate TS to that required for the blank should be between 0.750 and 0.765.

Sodium methoxide (0.1 mol/L) VS

Procedure. Cool in ice-water 150 mL of dehydrated methanol R and add in small portions 2.5 g of freshly cut sodium R. When the metal has dissolved add sufficient toluene R to produce 1000 mL.

Method of standardization. Ascertain the exact concentration of the 0.1 mol/L solution in the following manner: titrate 0.10 g of benzoic acid R, accurately weighed, as described under *2.6 Non-aqueous titration*, Method B. Each 12.21 mg of $C_7H_6O_6$ is equivalent to 1 mL of sodium methoxide (0.1 mol/L) VS.

Note: Sodium methoxide (0.1 mol/L) VS must be standardized immediately before use.

Sodium molybdotungstophosphate TS

Procedure. Boil under a reflux condenser for 2 hours 350 mL of water with 50 g of sodium tungstate R, 12 g of phosphomolybdic acid R and 25 mL of phosphoric acid (~1440 g/L) TS; cool and add sufficient water to produce 500 mL.

Sodium nitrate R

$NaNO_3$.

A commercially available reagent of suitable grade.

Sodium nitrite (0.1 mol/L) VS

Sodium nitrite R, dissolved in water to contain 6.900 g of $NaNO_2$ in 1000 mL.

Method of standardization. Ascertain the exact concentration of the 0.1 mol/L solution in the following manner: place 50.0 mL of potassium permanganate (0.02 mol/L) VS in a glass-stoppered flask, dilute with 300 mL of water, add 25 mL of sulfuric acid (~100 g/L) TS and 20.0 mL of the sodium nitrite solution. Allow the solution to stand for 10 minutes. Then add 2 g of potassium iodide R and titrate with sodium thiosulfate (0.1 mol/L) VS using starch TS as indicator. Perform a blank determination and make any necessary corrections.

Sodium nitrite (1 g/L) TS

A solution of sodium nitrite R containing about 1 g of $NaNO_2$ per litre.

Note: Sodium nitrite (1 g/L) TS must be freshly prepared.

Sodium nitrite (10 g/L) TS

A solution of sodium nitrite R containing about 10 g/L of $NaNO_2$.

Sodium nitrite (100 g/L) TS

A solution of sodium nitrite R containing about 100 g of $NaNO_2$ per litre.

Sodium nitrite (20 g/L) TS

A solution of sodium nitrite R containing about 20 g of $NaNO_2$ per litre.

Sodium nitrite (3 g/L) TS

A solution of sodium nitrite R containing about 3 g of $NaNO_2$ per litre.

Note: Sodium nitrite (3 g/L) TS must be freshly prepared.

Sodium nitrite (35 g/L) TS

A solution of sodium nitrite R containing about 35 g of $NaNO_2$ per litre (approximately 0.5 mol/L).

Sodium nitrite (50 g/L) TS

A solution of sodium nitrite R containing about 50 g of $NaNO_2$ per litre.

Sodium nitrite R

$NaNO_2$ (SRIP, 1963, p. 189).

Sodium nitrite/hydrochloric acid TS

Procedure. Dissolve 0.5 g of sodium nitrite R in sufficient hydrochloric acid (0.1 mol/L) VS to produce 100 mL.

Sodium nitroprusside (8.5 g/L) TS

A solution of sodium nitroprusside R containing about 8.5 g of $Na_2Fe(NO)(CN)_5$ per litre.

Sodium nitroprusside (20 g/L) TS

A solution of sodium nitroprusside R containing about 20 g of $Na_2Fe(NO)(CN)_5$ per litre.

Note: Sodium nitroprusside (20 g/L) TS must be freshly prepared.

Sodium nitroprusside (45 g/L) TS

A solution of sodium nitroprusside R containing about 45 g of $Na_2Fe(NO)(CN)_5$ per litre.

Note: Sodium nitroprusside (45 g/L) TS must be freshly prepared.

Sodium nitroprusside R

$Na_2Fe(NO)(CN)_5,2H_2O$ (SRIP, 1963, p. 190).

Sodium nitroprusside, alkaline, TS

Procedure. Dissolve 1 g of sodium nitroprusside R and 1 g of sodium carbonate R in sufficient water to produce 100 mL.

Sodium octanesulfonate R

$C_8H_{17}NaO_3S$.

Contains not less than 98.0% of $C_8H_{17}NaO_3S$.

A commercially available reagent of suitable grade.

Absorbance. A 0.05 g/mL solution of a 1 cm layer measured at a wavelength of about 250 nm has an absorbance of not greater than 0.01.

Sodium oxalate R

$C_2Na_2O_4$ (SRIP, 1963, p. 190).

Sodium peroxide R.

Na_2O_2 (SRIP, 1963, p. 191).

Sodium phosphate, anhydrous, R

Sodium dihydrogen phosphate anhydrous; NaH_2PO_4.

A commercially available reagent of suitable grade.

Contains not less than 99.0% of NaH_2PO_4.

Sodium R

Na (SRIP, 1963, p. 175).

Sodium rhodizonate dibasic R

3,4,5,6-Tetraoxocyclohexene-1,2-diol; rhodizonic acid disodium salt; $C_6Na_2O_6$.

A commercially available reagent of suitable grade.

Sodium salicyclate R

$C_7H_5NaO_3$. Use sodium salicylate as described in the monograph for *Sodium salicylate*.

Sodium salicylate (11.5 g/L) TS

A solution of sodium salicylate R containing about 11.5 g of $C_7H_5NaO_3$ per litre.

Sodium standard (200 µg Na/mL) TS

Procedure. Dissolve 0.5084 g of sodium chloride R, previously dried at 100–105 °C for 3 hours, in sufficient water to produce 1000 mL.

Sodium sulfate, anhydrous, R

Na_2SO_4 (SRIP, 1963, p. 195).

Sodium sulfide R

$Na_2S,9H_2O$ (SRIP, 1963, p. 195).

Sodium sulfide TS

Procedure. Dissolve 12 g of sodium sulfide R in 25 mL of water and add sufficient glycerol R to produce 100 mL.

Sodium sulfide (100 g/L) TS

Dissolve 1 g of sodium sulfide R in sufficient water R to produce 10 mL. Note: this solution must be freshly prepared.

Sodium sulfite R

$Na_2SO_3,7H_2O$ (SRIP, 1963, p. 196).

Sodium sulfite, anhydrous R

Anhydrous sodium sulfite of a suitable quality should be used.

Sodium tetraborate (10 g/L) TS

A solution of sodium tetraborate R containing about 10 g of $Na_2B_4O_7$ per litre.

Sodium tetraborate R

Borax, $Na_2B_4O_7,10H_2O$.

Description. Transparent, colourless crystals, or a white, crystalline powder; odourless.

Solubility. Soluble in 20 parts of water and in 0.6 part of boiling water; very slightly soluble in ethanol (~750 g/L) TS.

pH value of a 0.01 mol/L solution. Dissolve 0.3814 g in water and dilute to 100 mL using water having a pH of 6.5–7.4. The pH should be from 9.15 to 9.20 at 25 °C.

Chlorides. Dissolve 1.0 g in 20 mL of water, filter if necessary through a chloride-free filter, add 1 mL of nitric acid (~1000 g/L) TS and proceed as described in *2.2.1 Limit test for chlorides*. Sodium tetraborate R contains not more than 250 µg/g.

Sulfates. Dissolve 0.5 g in 20 mL of water, add 2 mL of hydrochloric acid (~70 g/L) TS and filter. Proceed as described in *2.2.2 Limit test for sulfates*. Sodium tetraborate R contains not more than1.0 mg/g.

Sodium tetraborate standard TS

Procedure. Dissolve 3.81 g of sodium tetraborate R in sufficient carbon-dioxide-free water R to produce 1000 mL.

Storage. Store the solution protected from atmospheric carbon dioxide and keep it stoppered at all times except when actually in use.

Sodium tetraphenylborate (30 g/L) TS

A solution of sodium tetraphenylborate R containing about 30 g/L of $C_{24}H_{20}BNa$.

Note: If necessary stir for 5 minutes with 1 g of aluminium hydroxide R or charcoal R and filter to clarify.

Sodium thioglycolate R. *See* Sodium mercaptoacetate R.

Sodium tetraphenylborate R

$C_{24}H_{20}BNa$.

Description. A fluffy, white or almost white powder.

Solubility. Freely soluble in water and acetone R; insoluble in light petroleum R.

pH value. pH of a 20 g/L solution, not less than 7.5.

Sodium thiosulfate (0.002 mol/L) VS

Sodium thiosulfate R, dissolved in water to contain 0.316 g of $Na_2S_2O_3$ in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution following the method described under sodium thiosulfate (0.1 mol/L) VS.

Sodium thiosulfate (0.01 mol/L) VS

Sodium thiosulfate R dissolved in water to contain 1.582 g of $Na_2S_2O_3$ in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution by following the method described under sodium thiosulfate (0.1 mol/L) VS.

Sodium thiosulfate (0.02 mol/L) VS

Sodium thiosulfate R dissolved in water to contain 3.164 g of $Na_2S_2O_3$ in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution following the method described under sodium thiosulfate (0.1 mol/L) VS.

Sodium thiosulfate (0.05 mol/L) VS

Sodium thiosulfate R dissolved in water to contain 7.910 g of $Na_2S_2O_3$ in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution by following the method described under sodium

thiosulfate (0.1 mol/L) VS.

Sodium thiosulfate (0.1 mol/L) VS

Sodium thiosulfate R dissolved in water to contain 15.82 g of $Na_2S_2O_3$ in 1000 mL.

Method of standardization (alternative procedure). Ascertain the exact concentration of the 0.1 mol/L solution in the following manner: to about 40 mL of water in a glass-stoppered conical flask add 10.0 mL of potassium bromate (0.0167 mol/L) VS, 1 g of potassium iodide R and 3 mL of sulfuric acid (~1760 g/L) TS. Allow the solution to stand for 5 minutes and titrate the liberated iodine with the sodium thiosulfate solution, adding 3 mL of starch TS as the end-point is approached. Perform a blank determination on the same quantities of the reagents and make any necessary corrections.

Sodium thiosulfate (320 g/L) TS

A solution of sodium thiosulfate R containing about 320 g of $Na_2S_2O_3$ per litre.

Sodium thiosulfate R

$Na_2S_2O_3,5H_2O$ (SRIP, 1963, p. 197).

Sodium tungstate R

$Na_2O_4W,2H_2O$ (SRIP, 1963, p. 197).

Sorbitol R

$C_6H_{14}O_6$. Contains not less than 97.0% of $C_6H_{14}O_6$.

Description. White granules or powder or a white, crystalline mass.

Solubility. Very soluble in water; sparingly soluble in ethanol (~750 g/L) TS; practically insoluble in ether R.

Assay. Dissolve about 0.2 g, previously dried and accurately weighed, in sufficient water to produce 100 mL. Transfer 10.0 mL to an iodine flask, add 50.0 mL of potassium periodate TS and heat for 15 minutes on a water-bath. Cool, add 2.5 g of potassium iodide R, stopper tightly and shake well. Allow to stand for 5 minutes protected from light and titrate with sodium thiosulfate (0.1 mol/L) VS using 3 mL of starch TS as an indicator. Perform a blank titration and make any necessary corrections. Each mL of sodium thiosulfate (0.1 mol/L) VS is equivalent to 1.822 mg of $C_6H_{14}O_6$.

Storage. Store in a tightly closed container.

Squalane R

2,6,10,15,19,23-Hexamethyltetracosane; $C_{30}H_{62}$.

Description. A colourless, oily liquid.

Solubility. Freely soluble in ether R; slightly soluble in acetone R and ethanol (~750 g/L) TS.

Relative density. d_{20}^{20} = 0.811–0.813.

Refractive index. n_D^{20} = 1.451–1.453.

Stannated hydrochloric acid (~ 250 g/L) AsTS

Refer to Hydrochloric acid (~250 g/L), stannated, AsTS.

Stannous chloride AsTS

Procedure. Prepare from stannous chloride TS by adding an equal volume of hydrochloric acid (~250 g/L) TS, boil down to the original volume and filter through afine-grained filter-paper.

Test for arsenic. To 10 mL add 6 mL of water and 10 mL of hydrochloric acid (~250 g/L) AsTS and distil 16 mL. To the distillate add 50 mL of water and 2 drops of stannous chloride AsTS; then apply the general test for arsenic. The colour of the stain produced is not more intense than that produced from a 1 mL standard stain, showing that the amount of arsenic does not exceed 1 µg/mL.

Stannous chloride R

$SnCl_2,2H_2O$ (SRIP, 1963, p. 198).

Stannous chloride TS

Procedure. Dissolve 330 g of stannous chloride R in 100 mL of hydrochloric acid (~250 g/L) TS and sufficient water to produce 1000 mL.

Stannous chloride/hydrochloric acid TS1

Procedure. Dissolve 10 g of stannous chloride R in sufficient hydrochloric acid (~70 g/L) TS to produce 100 mL.

Starch iodide TS

Procedure. Dissolve 0.75 g of potassium iodide R in 5 mL of water and 2 g of zinc chloride R in 10 mL of water, mix the two solutions and add 100 mL of water. Heat the solution to boiling and add, with constant stirring, a suspension of 5 g of corn or potato starch R in 35 mL of water. Boil for 2 minutes and cool.

Storage. Store in a well-closed container and keep in a cool place.

Starch R

[potato starch R or corn starch R] (SRIP, 1963, p. 199).

Starch TS

Procedure. Mix 0.5 g of starch R or of soluble starch R with 5 mL of water and add this solution, with constant stirring, to sufficient water to produce about 100 mL; boil for a few minutes, cool and filter.

Note: Starch TS must be freshly prepared.

Starch, soluble, R

(SRIP, 1963, p. 199).

Starch/iodide paper R

[starch-iodide paper R] (SRIP, 1963, p. 200).

Starch solution TS

Triturate 1.0 g of soluble starch R with 5 mL of water R and whilst stirring pour the mixture into 100 mL of boiling water R containing 10 mg of mercuric iodide R.

NOTE: commercially available reagents may be used; including mercury-free solutions or those containing alternative preservatives.

Carry out the test for sensitivity each time the reagent is used.

Test for sensitivity. To a mixture of 1 mL of the starch solution and 20 mL of water R, add about 50 mg of potassium iodide R and 0.05 mL of iodine solution TS. The solution is blue.

Strontium chloride hexahydrate R

$SrCl_2,6H_2O$.

A commercially available reagent of suitable grade.

Contains not less than 99.0% and not more than 103.0% of $SrCl_2,6H_2O$.

Strychnine sulfate R

$C_{42}H_{44}N_4O_4,H_2SO_4,5H_2O$ (SRIP, 1963, p. 200).

Sudan red G R

1-(4-Phenylazophenylazo)-2-naphthol; Sudan III; Solvent red 23; C.I. 26100; $C_{22}H_{16}N_4O$.

Description. A reddish brown powder.

Solubility. Practically insoluble in water.

Sudan red TS

Procedure. Dissolve 0.5 g of sudan red G R in 100 mL of glacial acetic acid R1.

Sulfadoxine R

N^1 -(5,6-Dimethoxy-4-pyrimidinyl)sulfanilamide; 4-amino-*N*-(5,6-dimethoxy-4-pyrimidinyl)benzenesulfonamide; $C_{12}H_{14}N_4O_4S$

A commercialy available reagent of suitable grade.

Description. A white or creamy white, crystalline powder.

Solubility. Very slightly soluble in water; slightly soluble in ethanol (~750 g/l) TS and in methanol R; practically insoluble in ether R.

Sulfamethoxazole R

4-Amino-N-(5-methylisoxazol-3-yl)benzenesulfonamide; 4-amino-N-(5-methyl-1,2-oxazol-3-yl)benzenesulfonamide; N1-(5-methyl-3-isoxazolyl)sulfanilamide;$C_{10}H_{11}N_3O_3S$.

A commercially available reagent of suitable grade.

Description. A white or yellowish white, crystalline powder.

Solubility. Very slightly soluble in water; soluble in 50 parts of ethanol (~750 g/L) TS and in 3 parts of acetone R.

Sulfamic acid (5 g/L) TS

A solution of sulfamic acid R containing about 5 g of H_3NO_3S per litre.

Sulfamic acid (50 g/L) TS

A solution of sulfamic acid R containing about 50 g of H_3NO_3S per litre.

Note: Sulfamic acid (50 g/L) TS must be freshly prepared.

Sulfamic acid (80 g/L) TS

A solution of sulfamic acid R containing about 80 g of H_3NO_3S per litre.

Note: Sulfamic acid (80 g/L) TS must be freshly prepared.

Sulfamic acid R

H_3NO_3S.

Description. Colourless or white crystals.

Solubility. Soluble in water; slightly soluble in ethanol (~750 g/L) TS.

4-Sulfamoylbenzoic acid R

p -Sulfamoylbenzoic acid; $C_7H_7NO_4S$.

Melting point. About 291 °C.

Sulfan blue R

Sodium [[[(4-diethylamino)phenyl](2,4-disulfonatophenyl)methylene]cyclohexa-2,5-dien-1-ylidene]diethylammonium. $C_{27}H_{31}N_2NaO_6S_2$. CAS Reg. No. 129-17-9.

Appearance. Violet powder.

Solubility. Soluble in water.

Colour of solution. Dilute solutions are blue and turn yellow on the addition of concentrated hydrochloric acid.

Sulfanilic acid R

$C_6H_7NO_3S$ (SRIP, 1963, p. 201).

Sulfanilic acid, diazotized, TS

Procedure. Dissolve 0.2 g of sulfanilic acid R in 20 mL of hydrochloric acid (1 mol/L) VS with warming, cool in ice, add drop by drop and with continuous stirring 2.5 mL of sodium nitrite (35 g/L) TS, allow to stand in ice for 10 minutes and then add 1 mL of sulfamic acid (50 g/L) TS.

Sulfosalicylic acid (175 g/L) TS

A solution of sulfosalicylic acid R containing about 175 g/L of $C_7H_6O_6S$.

Sulfosalicylic acid R

$C_7H_6O_6S,2H_2O$.

Description. White or slightly pink coloured, needle-like crystals.

Solubility. Soluble in water and ethanol (~750 g/L) TS.

Insoluble matter. Dissolve 5.0 g in 50 mL of water, heat to boiling and digest in a covered beaker on a water-bath for 1 hour. Filter through a tared filtering crucible, wash thoroughly and dry at 105 °C. The weight of the residue does not exceed 1.0 mg.

Sulfated ash. Gently ignite 1.0 g in a tared crucible or dish, other than platinum, until charred. Cool, moisten the residue with 1 mL of sulfuric acid (~1760 g/L) TS and ignite again; not more than 1.0 mg/g.

Sulfur dioxide R

SO_2 (SRIP, 1963, p. 202).

Sulfuric acid (~10 g/L) TS

Procedure. Mix 100 mL of sulfuric acid (~100 g/L) TS with sufficient water to produce 1000 mL.

Sulfuric acid (~50 g/L) TS

Procedure. Mix 500 mL of sulfuric acid (~100 g/L) TS with sufficient water to produce 1000 mL.

Sulfuric acid (~98 g/L) TS

Procedure. Add 55 mL of sulfuric acid (~1760 g/L) TS to sufficient water to produce 1000 mL; d ~ 1.063.

Sulfuric acid (~100 g/L) TS

Procedure. Add 57 mL of sulfuric acid (~1760 g/L) TS to sufficient water to produce 1000 mL (approximately 1 mol/L); *d*~1.065.

Sulfuric acid (~1125 g/L) TS

Sulfuric acid (~1760 g/L) TS, diluted with water to contain about 1125 g of H_2SO_4 per litre; *d*~1.61.

Sulfuric acid (~1760 g/L) TS

[sulfuric acid R] (SRIP, 1963, p. 202); *d* ~ 1.84.

Sulfuric acid (~1760 g/L), nitrogen-free, TS

Sulfuric acid (~1760 g/L) TS containing not less than 1760 g/L of H_2SO_4 and complying with the test for nitrates.

Nitrates. Mix 45 mL with 5 mL of water, cool and add 8 mg of diphenylbenzidine R; the solution is colourless or not more than very pale blue.

Sulfuric acid (~190 g/L) TS

Procedure. Mix 1 volume of sulfuric acid (~1760 g/L) TS with 9 volumes of water and cool. The resulting solution contains about 190 g/L of H_2SO_4 ; *d*~1.12.

Sulfuric acid (~440 g/L) TS

Procedure. Dilute 485 mL of sulfuric acid (~1760 g/L) TS to 1000 mL with water (~4.5 mol/L); *d*~1.25.

Sulfuric acid (~50 g/L) TS.

Procedure. To 50 mL of sulfuric acid (~100 g/L) TS add about 50 mL of water and mix.

Sulfuric acid (~500 g/L) TS

Procedure. Cool separately 10 mL of water R and 5 mL of sulfuric acid (~1760 g/L) TS to about -5 °C. Carefully add the acid to the water keeping the solution as cool as possible and mix gently (approximately 5 mol/L); d~1.29.

Sulfuric acid (~570 g/L) TS

Procedure. Slowly add 3 volumes of sulfuric acid (~1760 g/L) TS to 7 volumes of water while gently stirring and cool; *d*~1.33.

Sulfuric acid (~635 g/L) TS

Sulfuric acid (~1760 g/L) TS diluted with water to contain about 635 g of H_2SO_4 per litre; *d*~1.36.

Sulfuric acid (~700 g/L) TS

Procedure. Slowly add sulfuric acid (~1760 g/L) TS to an equal weight of water while gently stirring and cool; *d*~1.40.

Sulfuric acid (0.005 mol/L) VS

Sulfuric acid (~1760 g/L) TS diluted with water to contain 0.4904 g of H_2SO_4 in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution following the method described under sulfuric acid (0.5 mol/L) VS.

Sulfuric acid (0.01 mol/L) VS

Sulfuric acid (~1760 g/L) TS diluted with water to contain 0.9808 g of H_2SO_4 in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution following the method described under sulfuric acid (0.5 mol/L) VS.

Sulfuric acid (0.05 mol/L) VS

Sulfuric acid (~1760 g/L) TS diluted with water to contain 4.904 g of H_2SO_4 in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution following the method described under sulfuric acid (0.5 mol/L) VS.

Sulfuric acid (0.1 mol/L) VS

Sulfuric acid (~1760 g/L) TS diluted with water to contain 9.808 g of H_2SO_4 in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution following the method described under sulfuric acid (0.5 mol/L) VS.

Sulfuric acid (0.125 mol/L) VS.

Sulfuric acid (~1760 g/L) TS diluted with water to contain 12.52 g of H_2SO_4 in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution following the method described under sulfuric acid (0.5 mol/L) VS.

Sulfuric acid (0.25 mol/L) VS

Sulfuric acid (~1760 g/L) TS diluted with water to contain 24.52 g of H_2SO_4 in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution following the method described under sulfuric acid (0.5 mol/L) VS.

Sulfuric acid (0.5 mol/L) VS

Sulfuric acid (~1760 g/L) TS diluted with water to contain 49.04 g of H_2SO_4 in 1000 mL.

Method of standardization. Ascertain the exact concentration of the 0.5 mol/L solution in the following manner: dissolve about 1.5 g, accurately weighed, of anhydrous sodium carbonate R, previously dried at 270 °C for 1 hour, in 50 mL of water and titrate with the sulfuric acid solution, using methyl orange/ethanol TS as indicator. Each 52.99 mg of anhydrous sodium carbonate is equivalent to 1 mL of sulfuric acid (0.5 mol/L) VS.

Sulfuric acid (90% v/v) TS

Procedure. Cool separately 10 mL of water R and 90 mL of sulfuric acid (~1760 g/L) TS to about -5 °C. Carefully add the acid to the water keeping the solution as cool as possible and mix gently.

Sulfuric acid/ethanol (~0.05 mol/L)

Carefully add 4.9 g of sulfuric acid (~1760 g/L) TS to about 800 mL ethanol (~750 g/L) TS, while mixing gently, and dilute to 1000 mL with ethanol (~750 g/L) TS.

Sulfuric acid/ethanol (10%) TS

Procedure. Cool separately 10 mL of sulfuric acid (~1760 g/L) TS and 90 mL of ethanol (~750 g/L) TS to about -5 °C. Carefully add the acid to the ethanol keeping the solution as cool as possible and mix gently.

Sulfuric acid/ethanol (20%) TS

Cool separately 20 ml of sulfuric acid (~1760 g/L) TS and 60 mL of ethanol (~750 g/L) TS to about -5 °C. Carefully add the acid to

the ethanol, keeping the solution as cool as possible, mix gently and dilute to 100 mL with ethanol.

Note: Sulfuric acid/ethanol (20%) TS must be freshly prepared.

Sulfuric acid/ethanol TS

Procedure. Cool separately 10 mL of ethanol (~750 g/L) TS and 90 mL of sulfuric acid (~1760 g/L) TS to about -5 °C. Carefully add the acid to the ethanol keeping the solution as cool as possible and mix gently.

Sulfuric acid/methanol TS

Procedure. Cool separately 10 mL of sulfuric acid (~1760 g/L) TS and 90 mL of methanol R. Carefully add the acid to the methanol keeping the solution as cool as possible and mix gently.

Sulfurous acid TS

[sulfurous acid R] (SRIP, 1963, p. 204).

R - Red stock standard TS.... Rhodamine B R

Red stock standard TS

Procedure. To 40.5 mL of cobalt colour TS, add 6.1 mL of copper colour TS, 6.3 mL of dichromate colour TS, 12.0 mL of iron colour TS, dilute to 100.0 mL with sulfuric acid (~10 g/L) TS and mix.

Resazurin sodium (1 g/L) TS

A solution of resazurin sodium R containing about 1 g/L of $C_{12}H_6NNaO_4$.

Note: Resazurin sodium (1 g/L) TS must be freshly prepared.

Resazurin sodium R

$C_{12}H_6NNaO_4$ (SRIP, 1963, p. 170).

Resorcinol (20 g/L) TS

A solution of resorcinol R containing 20 g/L of $C_6H_6O_2$.

Resorcinol R

1,3-Dihydroxybenzene, $C_6H_6O_2$ (SRIP, 1963, p. 171).

Resorcinol/toluene TS

Procedure. Shake 0.2 g of resorcinol R with 100 mL of toluene R until saturated and decant.

Note: Resorcinol/toluene TS should be prepared immediately before use.

Rhodamine B R

$C_{28}H_{31}ClN_2O_3$.

Description. Green crystals or reddish-violet powder.

A commercially available reagent of suitable grade.

Solubility. Soluble in water and in alcohol.

Q - Quinaldine red R.... Quinine R

Quinaldine red R.

2-(*p*-Dimethylaminostyryl)quinoline ethiodide; $C_{21}H_{23}IN_2$.

Description. A dark blue-black powder.

Solubility. Sparingly soluble in water; freely soluble in ethanol (~750 g/l) TS.

Melting temperature. About 260°C with decomposition.

Quinaldine red/ethanol TS.

Procedure. Dissolve 0.1 g of quinaldine red R in 100 mL of ethanol (~750 g/l) TS.

Quinaldine red/methanol TS.

Procedure. Dissolve 1.0 g of quinaldine red R in sufficient methanol R to produce 100 mL.

Quinhydrone R.

p-Benzoquinone compound with hydroquinone (1:1); $C_{12}H_{10}O_4$.

Description. Dark green, lustrous crystals or a crystalline powder.

Melting point. About 171 °C.

Quinhydrone/methanol TS

Procedure. Dissolve 2.5 g of quinhydrone R in sufficient methanol R to produce 100 mL.

Quinine R.

$C_{20}H_{24}N_2O_2$.

Description. A white, microcrystalline powder; odourless.

Solubility. Very slightly soluble in water; slightly soluble in boiling water; very soluble in ethanol (~750 g/l) TS; soluble in ether R and benzene R.

Melting temperature. About 175°C.

Identification. Very dilute solutions containing sulfuric acid (~100 g/l) TS show a blue fluorescence. Acid solutions are levorotatory. Dissolve about 5 mg in a mixture of 5 mL of water and 0.3 mL of hydrochloric acid (~70 g/l) TS. Mix the solution with 0.2 mL of bromine TS1 and add 1 mL of ammonia (~35 g/l) TS; an emerald green colour is produced.

Quinine sulfate R

Quinine sulfate of a suitable quality should be used.

P - Pancreatic digest of casein R.... Pyrogallol, alkaline, TS

Pancreatic digest of casein R

(SRIP, 1963, p. 132).

Pancreatin R

Pancreatin is a preparation of mammalian pancreas containing enzymes having protease, lipase and amylase activity. Use a commercially available reagent of suitable grade.

Storage. Pancreatin should be stored in an airtight container, protected from light at a temperature between 2° to 8 °C.

Papaic digest of soybean meal R

(SRIP, 1963, p. 134).

Paracetamol R

Paracetamol as described in the monograph for *Paracetamol*.

Paracetamol, 4-aminophenol-free, R

Paracetamol as described in the monograph for *Paracetamol* or paracetamol recrystallized from water until it complies with the following test:

Dissolve 5 g of the dried material in a mixture of equal volumes of methanol R and water and dilute to 100 mL with this solvent mixture. Add 1.0 mL of alkaline sodium nitroprusside TS, mix and allow to stand for 30 minutes; no blue or green colour is produced.

Paraffin, liquid, R

(SRIP, 1963, p. 135).

Paraformaldehyde R

$(CH_2O)_n$.

Description. A white, crystalline powder; odour, characteristic of formaldehyde.

Solubility. Slowly soluble in cold water, freely soluble in hot water, with evolution of formaldehyde; practically insoluble in ethanol (~750 g/L) TS and ether R.

Solubility in ammonia. Dissolve 1 g in 10 mL of ammonia (~100 g/L) TS; a practically clear and colourless solution is produced.

Sulfated ash. Not more than 1.0 mg/g.

Acidity or alkalinity. Shake 1 g with 20 mL of water for 1 minute and filter; the nitrate is neutral to litmus paper R.

Paraldehyde R

2,4,6-Trimethyl-s-trioxane; $C_6H_{12}O_3$.

Description. Liquid; odour, characteristic, aromatic.

Boiling point. About 124 °C.

Mass density. ρ_{20} = 0.994 kg/L.

Penicillinase R

An enzyme, usually obtained from culture filtrates of a strain of Bacillus cereus, which has the specific property of inactivating penicillin by splitting the bond linking the nitrogen of the thiazolidine to the adjacent carbonyl carbon and thus releasing a carboxyl group. It is precipitated from solutions in water by acetone R, ethanol (~750 g/L) TS and dioxan R but inactivated by several hours' contact with these solvents; it is rapidly inactivated by ethyl acetate R. In place of penicillinase R a sterile filtrate obtained by fermentation of a penicillinase-producing organism in a suitable medium, described below under "Preparation of penicillinase", may also be used directly.

Description. Small, brown, easily pulverizable pieces or granules.

Solubility. Freely soluble in water, forming a slightly opalescent solution.

Preparation of penicillinase. Dissolve 10 g of pancreatic digest of casein R, 2.7 g of potassium dihydrogen phosphate R and 5.9 g

of sodium citrate R in 200 mL of water, adjust the alkalinity to pH 7.2 with sodium hydroxide (~200 g/L) TS and dilute to 1000 mL with water. Dissolve 0.4 g of magnesium sulfate R in 5 mL of water and add 1 mL of ferrous ammonium sulfate (1 g/L) TS and sufficient water to produce 10 mL. Sterilize both solutions by heating in an autoclave, cool, mix, distribute in shallow layers in conical flasks and inoculate with a suitable strain (*Bacillus cereus* NCTC 9946 is suitable). Allow the flasks to stand at 18–37 °C until growth is apparent and then maintain at 35–37 °C for 16 hours, shaking constantly to ensure maximum aeration. Centrifuge and sterilize the supernatant liquid by filtration through a suitable membrane filter.

Penicillinase TS

A sterile aqueous solution of penicillinase R. To test the activity of penicillinase TS carry out the "Penicillinase assay". The time required for iodine decolorization is not more than 36 seconds.

Penicillinase assay. Carry out the assay in test-tubes of borosilicate glass, 15 cm long and about 20 mm in internal diameter, immersed in a water-bath of 30 ± 1 °C. All reagents should have a temperature of 30 °C before use.

To the test-tube add the reagents in the following order: 1.6 mL of gelatin TS, 0.4 mL of penicillinase TS to be tested, 1 drop of starch TS and 1 mL of benzylpenicillin sodium TS, blowing out the last reagent from a 1 mL pipette. Start the stop-watch and after 15 seconds add 2.0 mL of iodine (0.01 mol/L) VS, recording the time of decolorization of iodine from the time of the addition of benzylpenicillin sodium TS. The activity of penicillinase TS is calculated from the results of the assay. The time of decolorization of strictly 36 seconds corresponds to a penicillinase activity equivalent to a rate of decomposition (at 30 °C at pH 7.0) of 220 mg of benzylpenicillin sodium R per hour per mL of penicillinase TS.

Storage. Store between 0 and 2 °C and use within 2–3 days. When dried from the frozen state and kept in sealed ampoules, penicillinase TS may be stored for several months.

n-Pentane R

C_5H_{12}.

A commercially available reagent of suitable grade.

Description. A colourless, volatile liquid.

Boiling point. About 36 °C.

Relative density. d_D^{20} = about 1.359.

Transmittance. Not less than 20% at 200nm, 50% at 210 nm, 85% at 220 nm, 93% at 230 nm and 98% at 240 nm, determined using water in the reference cell.

1-Pentanesulfonic acid sodium salt R

$C_5H_{11}NaO_3S,H_2O$.

Description. A white, crystalline powder.

Solubility. Soluble in water.

Clarity and colour of solution. A solution of 1 g in 25 mL of water is clear and colourless.

Water. Determined by the Karl Fischer method; not more than 20 mg/g.

1-Pentanesulfonic acid TS

Procedure. Dissolve 0.960 g of 1-pentanesulfonic acid sodium salt R in 1000 mL of deaerated acetic acid (5.0 g/L) TS and adjust the pH to 4.3 with ammonia (~260 g/L) TS. Filter and deaerate before use.

Pentoxyverine citrate R

A commercially available reagent of suitable grade.

Pepsin R

Pepsin is prepared from the gastric mucosa of pigs, cattle or sheep. It contains gastric proteinases, active in acid medium (pH of 1 to 5). Use a commercially available reagent of suitable grade.

Storage. Pepsin should be stored in an airtight container protected from light at a temperature between 2° to 8 °C.]

Peptone (1 g/L) TS1

Procedure. Dissolve 1.0 g of peptone R1 (or similar peptic digest of animal tissue) in sufficient water to produce 1000 mL, filter or

centrifuge to clarify, adjust the pH to 7.1 ± 0.2, place 100 mL portions into individual vessels and sterilize by maintaining at 121 °C for 18–20 minutes.

Peptone (1 g/L) TS2

Procedure. Dissolve in water, while heating, 1.0 g of dried peptone R and 9 g of sodium chloride R and dilute with sufficient water to produce 1000 mL. Adjust to pH 8.0–8.4 and boil for 20 minutes. Filter, adjust to pH 7.2–7.4 and sterilize by maintaining at 115 °C for 30 minutes.

Peptone (5 g/L) TS

Procedure. Dissolve in water, while heating, 5.0 g of dried peptone R and 7 g of sodium chloride R and dilute with sufficient water to produce 1000 mL. Adjust to pH 8.0–8.4 and boil for 20 minutes. Filter, adjust to pH 7.2–7.4 and sterilize by maintaining at 115 °C for 30 minutes.

Peptone R1

Dried peptone R that conforms to the following requirement: an autoclaved solution containing 0.02 g/mL is clear and neutral or almost neutral.

Peptone, dried, R

(SRIP, 1963, p. 137.)

Perchloric acid (~1170 g/L) TS

[perchloric acid (70%, w/w) R] (SRIP, 1963, p. 137); d~1.67.

Perchloric acid (~140 g/L) TS

Perchloric acid (~1170 g/L) TS, diluted with water to contain 141 g/L of $HClO_4$; d~1.09.

Perchloric acid (0.02 mol/L) VS

Procedure. Dilute 20 mL of perchloric acid (0.1 mol/L) VS with sufficient glacial acetic acid R1 to produce 100 mL.

Water and method of standardization. Immediately before use determine the content of water and ascertain the exact concentration of the solution following the methods described under perchloric acid (0.1 mol/L) VS.

Perchloric acid (0.05 mol/L) VS

Procedure. To 900 mL of glacial acetic acid R1 at about 25 °C, add 4.2 mL of perchloric acid (~1170 g/L) TS, mix, add 15 mL of acetic anhydride R and mix again. Cool to room temperature, add sufficient glacial acetic acid R1 to produce 1000 mL and allow to stand for 24 hours.

Water and method of standardization. Determine the content of water and ascertain the exact concentration of the solution following the method described under perchloric acid (0.1 mol/L) VS.

Perchloric acid (0.1 mol/L) VS

Procedure. To 900 mL of glacial acetic acid R1, at about 25 °C, add 8.2 mL of perchloric acid (~1170 g/L) TS, mix, add 32 mL of acetic anhydride R and mix again. Cool to room temperature, add sufficient glacial acetic acid R1 to produce 1000 mL and allow to stand for 24 hours.

Water. Determine the content by the Karl Fischer method. If necessary add sufficient water or acetic anhydride R to adjust the content of water to between 0.1 and 2.0 mg/mL and allow to stand for a further 24 hours.

Method of standardization. Ascertain the exact concentration by titrating 0.5 g, accurately weighed, of potassium hydrogen phthalate R, previously dried at 120 °C for 2 hours, using method A, as described under *2.6 Non-aqueous titration*. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 20.42 mg of $C_8H_5KO_4$. Record the temperature at which the standardization is carried out.

Perchloric acid TS

Procedure. Dilute 82 mL of perchloric acid (~1170 g/L) TS with sufficient water to produce 1000 mL (approximately 1 mol/L).

Perchloric acid/dioxan (0.1 mol/L) VS

Procedure. Mix 8.5 mL of perchloric acid (~1170 g/L) TS with sufficient dioxan R, which has been especially purified by adsorption, to produce 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution by titrating 0.7 g of potassium hydrogen phthalate R, accurately weighed and previously dried at 120 °C for 2 hours, using method A, as described under *2.6 Non-aqueous titration*. Each mL of perchloric acid/dioxan (0.1 mol/L) VS is equivalent to 20.42 mg of $C_8H_5KO_4$.

Periodic-acetic acid TS

Procedure. Dissolve 0.446 g of sodium metaperiodate R in 2.5 mL of sulfuric acid (~570 g/L) TS and dilute to 100 mL with glacial acetic acid R.

Petroleum, light, R

[light petroleum R] (SRIP, 1963, p. 108).

Petroleum, light, R1

Description. A colourless, very volatile, highly inflammable liquid.

Boiling range. 40–60 °C.

Mass density. ρ_{20} = 0.630–0.650 kg/L.

Petroleum, light R2

Description. Clear, colourless, flammable liquid without fluorescence.

Solubility. Practically insoluble in water, miscible with ethanol (~750 g/L) TS.

Boiling range. (1.2.4). 50–70 °C.

Mass density. ρ_{20} = 0.661 to 0.664 kg/L.

o-Phenanthroline (1 g/L) TS

Procedure. Dissolve 0.11 g of o-phenanthroline R in sufficient water to produce 100 mL.

o-Phenanthroline R

1,10-Phenanthroline, $C_{12}H_8N_2,H_2O$ (SRIP, 1963, p. 138).

o-Phenanthroline TS

Procedure. Dissolve 0.7 g of ferrous sulfate R in about 70 mL of water, add about 1.5 g of o-phenanthroline R and sufficient water to produce 100 mL.

Phenol (50 g/L) TS

A solution of phenol R containing about 50 g of C_6H_6O per litre.

Phenol R

C_6H_6O.

Description. Colourless, or at most faintly pink, cohering or separate acicular crystals or crystalline masses; odour, characteristic. Corrosive and blanches the skin and mucous membranes.

Solubility. Soluble in about 15 parts of water and in about 100 parts of liquid paraffin R; freely soluble in ethanol (~750 g/L) TS and ether R.

Completeness of solution. 1.0 g dissolves completely in 15 mL of water at 15 °C.

Congealing temperature. Not below 40.5 °C.

Residue on evaporation. Evaporate on a water-bath and dry to constant weight at 105 °C; leaves not more than 0.5 mg/g of residue.

Phenol red R

Phenolsulfonphthalein, $C_{19}H_{14}O_5S$ (SRIP, 1963, p. 139).

Phenol red/ethanol TS

Procedure. Dissolve 0.05 g of phenol red R in a mixture of 2.85 mL of sodium hydroxide (0.05 mol/L) VS and 5 mL of ethanol (~710 g/L) TS. Warm the solution slightly and after cooling dilute with sufficient ethanol (~150 g/L) TS to produce 250 mL.

Phenol red/ethanol TS1

Procedure. Dissolve 0.1 g of phenol red R in a mixture of 2.82 mL of sodium hydroxide (1 mol/L) VS and 20 mL of ethanol (~750 g/L) TS and dilute to 100 mL with water R.

Test for sensitivity. Add 0.1 mL of the phenol red solution to 100 mL of carbon dioxide-free water R; the solution is yellow. Not more than 0.1 mL of sodium hydroxide (0.02 mol/L) VS is required to change the colour to reddish-violet.

Colour change. pH 6.8 (yellow) to pH 8.4 (reddish-violet).

Phenoldisulfonic acid (250 g/L) TS

A commercially available reagent of suitable grade.

Phenoldisulfonic acid TS

Description. A clear liquid which may develop a pale brown colour on storage.

Procedure. Either of the following methods of preparation can be used: (1) to 3 g of phenol R add 20 mL of sulfuric acid (~1760 g/L) TS and heat on a water-bath for 6 hours; store in a stoppered vessel; (2) dilute phenoldisulfonic acid (250 g/L) TS with sulfuric acid (~1760 g/L) TS to contain 150 g of phenol per litre.

Sensitivity to nitrate. Evaporate a solution containing 0.1 mg of potassium nitrate R to dryness in a porcelain dish on a water-bath. Cool, add 1.0 mL of the solution to be examined and allow to stand for 10 minutes. Add 10 mL of water, cool, add 10 mL of ammonia (~100 g/L) TS and dilute to 25 mL with water; a distinct yellow colour is produced when compared with a solution prepared similarly but omitting the potassium nitrate R.

Phenolphthalein R

$C_{20}H_{14}O_4$ (SRIP, 1963, p. 139).

Phenolphthalein/ethanol TS

Procedure. Dissolve 1.0 g of phenolphthalein R in sufficient ethanol (~750 g/L) TS to produce 100 mL.

Phenolphthalein/ethanol TS, diluted

Procedure. Dissolve 0.1 g of phenolphthalein R in sufficient ethanol (~750 g/L) TS to produce 100 mL.

Sensitivity test. To 0.1 mL of the phenolphthalein solution add 100 mL of carbon dioxide-free water R. The solution is colourless. Not more than 0.2 mL of 0.02 M sodium hydroxide is required to change the colour to pink.

Colour change: pH 8.2 (colourless) to pH 10.0 (red).

Phenolphthalein/pyridine TS

Procedure. Dissolve 1.0 g of phenolphthalein R in sufficient pyridine R to produce 100 mL.

Phenoxyacetic acid R

Phenoxyethanoic acid; $C_8H_8O_3$.

Description. White or almost white crystals.

Melting temperature. About 98 °C.

2-Phenoxyethanol R

$C_8H_{10}O_2$.

Description. A clear, colourless, oily liquid; odour, faintly aromatic.

Miscibility. Slightly miscible with water; freely miscible with ethanol (~750 g/L) TS and ether R.

Mass density. ρ_{20} = about 1.1 kg/L.

Refractive index. n_D^{20} = about 1.537.

Freezing point. Not below 12.0 °C.

1,4-Phenylenediamine dihydrochloride R

$C_6H_8N_2$,2HCl.

Description. A white to pale tan, crystalline powder, turning pink on exposure to air.

Solubility. Freely soluble in water; slightly soluble in ethanol (~750 g/L) TS and ether R.

Storage. Keep in a well-closed container, protected from light.

2-Phenylethanol R

$C_8H_{10}O$. A suitable grade to be used in gas-liquid chromatography.

2-Phenylethanol TS

Procedure. Dissolve 1 g of 2-phenylethanol R in sufficient methanol R to produce 50 mL.

Phenylhydrazine hydrochloride (10 g/L) TS

A solution of phenylhydrazine hydrochloride R containing 10 g of $C_6H_8N_2$,HCl in 1000 mL.

Phenylhydrazine hydrochloride R

$C_6H_8N_2$,HCl (SRIP, 1963, p. 140).

Phenylhydrazine R

$C_6H_8N_2$ (SRIP, 1963, p. 140).

Phenylhydrazine/hydrochloric acid TS

Procedure. Dissolve 0.75 g of phenylhydrazine hydrochloride R in 50 mL of water and shake with 2 g of charcoal R. Filter, add 25 mL of hydrochloric acid (~420 g/L) TS and sufficient water to produce 200 mL.

Phenylhydrazine/sulfuric acid TS

Procedure. Dissolve 65 mg of phenylhydrazine hydrochloride R, previously recrystallized from ethanol (~710 g/L) TS, in a sufficient quantity of a mixture of 170 mL of sulfuric acid (~1760 g/L) TS and 80 mL of water to produce 100 mL.

Note: Phenylhydrazine/sulfuric acid TS must be freshly prepared.

Phenyl/methylpolysiloxane R

A suitable grade of a mixture to be used in gas chromatography composed of 5 g of phenylpolysiloxane and 95 g of methylpolysiloxane per 100 g.

pH-Indicator paper R

A paper impregnated with a suitable mixture of colour indicators such that the changes in colour permit estimation of the pH of a solution with an adequate sensitivity (usually 1 pH unit), at least in the pH range 1–10.

Phloroglucinol R

Benzene-1,3,5-triol dihydrate; $C_6H_6O_3$,$2H_2O$.

Description. White or pale cream coloured crystals.

Melting point. About 220 °C.

Phosphate buffer, pH 2.5, TS

Dissolve 3.48 g of dipotassium hydrogen phosphate R in 1000 mL of water R and adjust to pH 2.5 with phosphoric acid R.

Phosphate buffer, pH 3.1, TS

Dissolve 3.48 g of dipotassium hydrogen phosphate R in 1000 mL of water R and adjust to pH 3.1 with phosphoric acid R.

Phosphate buffer, pH 4.0, TS

Procedure. Dissolve 5.04 g of disodium hydrogen phosphate R and 3.01 g of potassium dihydrogen phosphate R in sufficient water to produce 1000 mL and adjust the pH to 4.0 with glacial acetic acid R.

Phosphate buffer, pH 6.4, TS

Procedure. Dissolve 1.36 g of potassium dihydrogen phosphate R in 50 mL of carbon-dioxide-free water R, add 12.60 mL of carbonate-free sodium hydroxide (0.2 mol/L) VS and dilute with sufficient carbon-dioxide-free water R to produce 200 mL.

Phosphate buffer, pH 6.9, TS

Procedure. Dissolve 3.40 g of potassium dihydrogen phosphate R and 3.55 g of disodium hydrogen phosphate R in sufficient carbon-dioxide-free water R to produce 1000 mL.

Phosphate buffer, pH 7.0 (0.01 mol/L) TS

Procedure. Dissolve 0.136 g of potassium dihydrogen phosphate R in sufficient water to produce 100 mL. Separately dissolve 2.681 g of disodium hydrogen phosphate R in sufficient water to produce 100 mL. Mix 38.9 mL of the potassium phosphate solution with 61.1 mL of the sodium phosphate solution.

Phosphate buffer, pH 7.0 (0.067 mol/L), TS

Procedure. Dissolve 0.908 g of potassium dihydrogen phosphate R in sufficient water to produce 100 mL. Separately dissolve 2.38 g of disodium hydrogen phosphate R in sufficient water to produce 100 mL. Mix 38.9 mL of the potassium phosphate solution with 61.1 mL of the sodium phosphate solution.

Phosphate buffer, pH 7.0 (0.1 mol/L) TS

Procedure. Dissolve 1.361 g of potassium dihydrogen phosphate R in 100.0 mL of water. Adjust the pH using a 14,20 g/L solution of anhydrous disodium hydrogen phosphate R.

Phosphate buffer, pH 7.0, TS

Procedure. Dissolve 5.76 g of anhydrous disodium hydrogen phosphate R and 3.55 g of potassium dihydrogen phosphate R in sufficient water to produce 1000 mL.

Phosphate buffer, pH 7.2, TS

Procedure. Dissolve 6.80 g of potassium dihydrogen phosphate R and 1.40 g of sodium hydroxide R in sufficient water to produce 1000 mL.

Phosphate buffer, pH 7.4, TS

Procedure. Dissolve 6.8 g of potassium dihydrogen phosphate R in 250 mL of water and add 393.4 mL of sodium hydroxide (0.1 mol/L) VS.

Phosphate buffer, pH 7.6, TS

Procedure. Place 1.36 g of potassium dihydrogen phosphate R in a 200 mL volumetric flask, dissolve it in water, add 42.4 mL of sodium hydroxide (0.2 mol/L) VS and dilute to volume with water.

Phosphate buffer, pH 8.0 (0.05 mol/L), TS

Procedure. Dissolve 1.361 g of potassium dihydrogen phosphate R in 50 mL of water R and add 46.8 mL of sodium hydroxide (0.2 mol/L) VS. Dilute to 200 mL with water R.

Phosphate buffer, pH 8.0, TS

Procedure. Dissolve 8.95 g of anhydrous disodium hydrogen phosphate R and 0.50 g of potassium dihydrogen phosphate R in sufficient water to produce 1000 mL.

Phosphate buffer, sterile,[1] pH 10.5, TS1

Procedure. Dissolve 35.0 g of dipotassium hydrogen phosphate R in water, add 20 mL of sodium hydroxide (1 mol/L) VS and sufficient water to produce 1000 mL. If necessary adjust the pH to 10.5 with phosphoric acid (~1440 g/L) TS or potassium hydroxide (~110 g/L) TS and then sterilize the solution for 20 minutes in an autoclave at 120 °C.

Phosphate buffer, sterile,[1] pH 4.5, TS

Procedure. Dissolve 13.6 g of potassium dihydrogen phosphate R in sufficient water to produce 1000 mL. If necessary adjust the pH to 4.45–4.55 with phosphoric acid (~1440 g/L) TS or potassium hydroxide (~110 g/L) TS and then sterilize the solution for 20 minutes in an autoclave at 120 °C.

Phosphate buffer, sterile,[1] pH 6.0, TS1

Procedure. Dissolve 2.0 g of dipotassium hydrogen phosphate R and 8.0 g of potassium dihydrogen phosphate R in sufficient water to produce 1000 mL. If necessary adjust the pH to 5.95–6.05 with phosphoric acid (~1440 g/L) TS or potassium hydroxide (~110 g/L) TS and then sterilize the solution for 20 minutes in an autoclave at 120 °C.

Phosphate buffer, sterile,[1] pH 6.0, TS2

Procedure. Dissolve 1.16 g of anhydrous disodium hydrogen phosphate R and 7.96 g of potassium dihydrogen phosphate R in sufficient water to produce 1000 mL. If necessary adjust the pH to 5.95–6.05 with phosphoric acid (~1440 g/L) TS or potassium hydroxide (~110 g/L) TS and then sterilize the solution for 20 minutes in an autoclave at 120 °C.

Phosphate buffer, sterile,[1] pH 6.0, TS3

Procedure. Dissolve 20.0 g of dipotassium hydrogen phosphate R and 80.0 g of potassium dihydrogen phosphate R in sufficient water to produce 1000 mL. If necessary adjust the pH to 5.95–6.05 with phosphoric acid (~1440 g/L) TS or potassium hydroxide (~110 g/L) TS and then sterilize the solution for 20 minutes in an autoclave at 120 °C.

Phosphate buffer, sterile,[1] pH 7.0, TS

Procedure. Dissolve 5.76 g of anhydrous disodium hydrogen phosphate R and 3.55 g of potassium dihydrogen phosphate R in sufficient water to produce 1000 mL. If necessary adjust the pH to 6.95–7.05 with phosphoric acid (~1440 g/L) TS or potassium hydroxide (~110 g/L) TS and then sterilize the solution for 20 minutes in an autoclave at 120 °C.

Phosphate buffer, sterile,[1] pH 7.2, TS

Procedure. Dissolve 6.80 g of potassium dihydrogen phosphate R and 1.4 g of sodium hydroxide R in sufficient water to produce 1000 mL. If necessary adjust the pH to 7.1–7.3 with phosphoric acid (~1440 g/L) TS or potassium hydroxide (~110 g/L) TS and then sterilize the solution for 20 minutes in an autoclave at 120 °C.

Phosphate buffer, sterile,[1] pH 7.8, TS

Procedure. Dissolve 6.8 g of potassium dihydrogen phosphate R in water, add 45.2 mL of sodium hydroxide (1 mol/L) VS and sufficient water to produce 1000 mL. If necessary adjust the pH to 7.8 with phosphoric acid (~1440 g/L) TS or potassium hydroxide (~110 g/L) TS and then sterilize the solution for 20 minutes in an autoclave at 120 °C.

Phosphate buffer, sterile,[1] pH 8.0, TS1

Procedure. Dissolve 16.73 g of dipotassium hydrogen phosphate R and 0.52 g of potassium dihydrogen phosphate R in sufficient water to produce 1000 mL. If necessary adjust the pH to 7.9–8.1 with phosphoric acid (~1440 g/L) TS or potassium hydroxide (~110 g/L) TS and then sterilize the solution for 20 minutes in an autoclave at 120 °C.

Phosphate buffer, sterile,[1] pH 8.0, TS2

Procedure. Dissolve 8.95 g of anhydrous disodium hydrogen phosphate R and 0.50 g of potassium dihydrogen phosphate R in sufficient water to produce 1000 mL. If necessary adjust the pH to 7.9–8.1 with phosphoric acid (~1440 g/L) TS or potassium hydroxide (~110 g/L) TS and then sterilize the solution for 20 minutes in an autoclave at 120 °C.

[1] The adjustment of the pH, if necessary, should be effected before sterilization of the solution.

Phosphate standard (5 µg/mL) TS

Procedure. Dissolve 0.716 g of potassium dihydrogen phosphate R in sufficient water to produce 1000 mL. Immediately before use dilute 1 mL to 100 mL with water.

Phosphate standard buffer, pH 6.8, TS

Procedure. Dissolve 3.40 g of potassium dihydrogen phosphate R and 3.53 g of anhydrous disodium hydrogen phosphate R in sufficient carbon-dioxide-free water R to produce 1000 mL.

Phosphate standard buffer, pH 7.4, TS

Procedure. Dissolve 1.18 g of potassium dihydrogen phosphate R and 4.30 g of anhydrous disodium hydrogen phosphate R in sufficient carbon-dioxide-free water R to produce 1000 mL.

Phosphate/citrate buffer pH 4.5, TS

Procedure. Dissolve 2.15 g of disodium hydrogen phosphate R in 30 mL of water and adjust the pH of the solution to 4.5 with citric acid (20 g/L) TS.

Phosphate/citrate buffer pH 6.0, TS

Procedure. Dissolve 4.52 g of disodium hydrogen phosphate R in 60 mL of water, add 35 mL of citric acid (20 g/L) TS and if necessary adjust the pH of the solution to 6.0.

Phosphomolybdic acid (80 g/L) TS

A solution of phosphomolybdic acid R containing about 100 g of $H_3PO_4,12MoO_3,24H_2O$ per litre.

Phosphomolybdic acid R

$H_3PO_4,12MoO_3,24H_2O$ (SRIP, 1963, p. 141).

Phosphomolybdic acid/ethanol TS

Procedure. Dissolve 5 g of phosphomolybdic acid R in sufficient dehydrated ethanol R to produce 100 mL.

Phosphoric acid (~7.8 g/L) TS

Procedure. Dilute 9.2 g of phosphoric acid (~1440 g/L) TS with sufficient water to produce 1000 mL

Phosphoric acid (~105 g/L) TS

Procedure. Mix about 115 g of phosphoric acid (~1440 g/L) TS with 885 g of water.

Phosphoric acid (~1440 g/L) TS

[phosphoric acid R] (SRIP, 1963, p. 141); d ~ 1.7.

Phosphoric acid (~20g/L) TS

Procedure. To 23 g of phosphoric acid (~1440 g/L) TS add 987 g of water and mix.

Phosphoric acid (~2.8 g/L) TS

Procedure. Dilute 2ml of phosphoric acid (~1440 g/L) TS with sufficient water to produce 1000 mL.

Phosphoric acid (~680 g/L) TS

Procedure: Dilute 680 g of phosphoric acid (~1440 g/L) TS with sufficient water to produce 1000 mL.

Phosphorus pentoxide R

P_2O_5 (SRIP, 1963, p. 142).

Phosphorus, red R

Description. A dark red powder.

Solubility. Insoluble in water and dilute acids.

Soluble matter. Heat 2.0 g with 30 mL of acetic acid (~300 g/L) TS on a water-bath for 15 minutes, cool, dilute to 50 mL, filter, evaporate 25 mL of the filtrate on a water-bath and dry at 110 °C for 2 hours; the residue weighs not more than 50 mg.

Yellow phosphorus. Shake 5.0 g with 20 mL of carbon disulfide R in a glass-stoppered cylinder, filter and immerse in the filtrate a strip of filter-paper, 10 cm by 0.5 cm, previously immersed in copper(II) sulfate (80 g/L) TS, and allow to dry in the air; no stain is produced.

Loss on drying. Dry to constant weight over sulfuric acid (~1760 g/L) TS; it loses not more than 10 mg/g.

Phosphotungstic acid TS

Procedure. Dissolve 25 g of sodium tungstate R in 175 mL of water and add 18.75 mL of phosphoric acid (~1440 g/L) TS. Heat under a reflux condenser for 6 hours, filter and add sufficient water to produce 250 mL.

Storage. Store at a temperature between 2 and 8 °C, protected from light.

Phthalate buffer, pH 3.4, TS

Procedure. Dissolve 2.04 g of potassium hydrogen phthalate R in 50 mL of carbon-dioxide-free water R, add 10.40 mL of hydrochloric acid (0.2 mol/L) VS and dilute with sufficient carbon-dioxide-free water R to produce 200 mL.

Phthalate buffer, pH 3.5, TS

Procedure. Dissolve 2.04 g of potassium hydrogen phthalate R in 50 mL of carbon-dioxide-free water R, add 8.40 mL of hydrochloric acid (0.2 mol/L) VS and dilute with sufficient carbon-dioxide-free water R to produce 200 mL.

Phthalate buffer, pH 4.0, TS

Procedure. Dissolve 2.042 g of potassium hydrogen phthalate R in 50 mL of water, add 0.40 mL of sodium hydroxide (0.2 mol/L) VS and dilute to 200 mL with water.

Phthalein purple R

$C_{32}H_{32}N_2O_{12}$. x H_2O. 2,2',2'',2'''-{3-oxo-2-benzofuran-1(3*H*)-ylidenebis[(6-hydroxyy-5-methyl-3,1-phenylene)methylenenitrilo]}tetraacetic acid.

Description. Yellowish-white to brownish powder.

Solubility. Practically insoluble in water , soluble in ethanol (~750 g/L) TS.

Test for sensitivity. Dissolve 10 mg in 1 mL of ammonia (~260 g/L) TS and dilute to 100 mL with water R. To 5 mL of the solution add 95 mL of water R, 4 mL of ammonia (~260 g/L) TS, 50 mL of ethanol (~750 g/L) TS and 0.1 mL of barium chloride (0.1 mol/L) VS. The solution is blue-violet. Add 0.15 mL of disodium edetate (0.1 mol/L) VS. The solution becomes colourless.

Phthalic anhydride R

$C_8H_4O_3$.

Description. White lustrous needles.

Solubility. Slightly soluble in water, more soluble in hot water; soluble in ethanol (~750 g/L) TS and ether R.

Melting temperature. About 130 °C.

Phthalic anhydride/pyridine TS

Procedure. Add 42 g of phthalic anhydride R, accurately weighed, to 300 mL of freshly distilled pyridine R (refluxed with barium oxide R) containing less than 1 mg/mL of water in a glass-stoppered 1000 mL flask. Use a dark flask or otherwise prevent exposure to light. Shake vigorously until complete solution is effected and allow to stand overnight for completion of the reaction.

Note: Phthalic anhydride/pyridine TS must be freshly prepared.

Piperazine hydrate R

Piperazine hexahydrate; $C_4H_{10}N_2$,$6H_2O$.

Description. Colourless, glossy, deliquescent crystals.

Melting point. 44 °C.

Piperidine R

$C_5H_{11}N$.

Description. A colourless to yellowish liquid; odour, characteristic.

Miscibility. Miscible with water and ethanol (~750 g/L) TS.

Mass density. ρ_{20} = about 0.86 kg/L.

Refractive index. n_D^{20} = about 1.454.

Boiling temperature. About 106 °C.

Congealing temperature. Between 12 and 15 °C.

Plasma substrate R

Note: Use water-repellent equipment (made from materials such as suitable plastics or suitably silicone-treated glass) for taking and handling blood.

Procedure. Collect a sufficient volume of blood from each of at least 5 sheep. A 285 mL volume of blood collected into 15 mL of anticoagulant solution is recommended but smaller volumes may be collected. The blood should be taken either from a live animal or at the time of slaughter using a needle attached to a cannula that is long enough to reach the bottom of the collecting flask. Discard the first few mL and collect only free-flowing blood. Collect the blood in a sufficient quantity of an anticoagulant solution containing 8.7 g of sodium citrate R and 4 mg of aprotinin R in 100 mL of water to give a final ratio of blood to anticoagulant solution of 19 to 1. During and immediately after collection swirl the flask gently to ensure mixing but do not allow frothing to occur. When collection is complete close the flasks and cool to a temperature between 10 and 15 °C. Then pool the contents of all the flasks, with the exception of any that shows obvious haemolysis or clots, and keep the pooled blood at 10–15°C. Within 4 hours of collection centrifuge the pooled and cooled blood at a speed of 1000–2000 g^1 for 30 minutes. Separate the supernatant liquid and centrifuge again at 5000 g for 30 minutes (note: faster centrifugation (20 000 g) may be necessary to clarify the plasma but filtration procedures should not be used). Separate the supernatant liquid and immediately mix thoroughly and distribute the plasma substrate R into small, stoppered containers in portions sufficient for a complete heparin assay (10–30 mL). Without delay, rapidly cool to a temperature below -70 °C by immersing the containers in liquid nitrogen and

store at a temperature below -30 °C. The plasma is suitable for use as plasma substrate R in the assay for heparin if, under the conditions of the assay, it gives a clotting time appropriate to the method of detection used and if it provides reproducible, steep, log dose-response curves. Just before use thaw the quantity of plasma substrate R required in a water-bath at 37 °C and gently swirl until thawing is complete; once thawed it should be kept between 10 and 20 °C and used without delay. The thawed plasma substrate R may be slightly centrifuged if necessary but do not use any filtration procedures.

[1] Acceleration due to gravity = 9.81m/s2.

Platinic chloride (60 g/L) TS

A solution of platinic chloride R containing about 63 g of H_2PtCl_6, per litre.

Platinic chloride R

$H_2PtCl_6,6H_2O$ (SRIP, 1963, p. 144).

Poly(dimethyl)(diphenyl)siloxane R

Stationary phase for gas chromatography. Contains 95% of methyl groups and 5% of phenyl groups.

Poly(dimethyl)siloxane R

Stationary phase for gas chromatography. Contains polysiloxane substituted with 100% of methyl groups

Polydimethylsiloxane R

A commercially available reagent of suitable grade for use in gas chromatography.

Polyethyleneglycol, base-deactivated R

Cross-linked, base-deactivated polyethyleneglycol, specially designed to be used as a stationary phase for gas chromatographic analysis of amine.

Polyoxyethylated castor oil R

Light yellow liquid. It becomes clear above 26 °C.

Polyoxyethylene (23) lauryl ether R.

Synonym: Brij 35, $C_{58}H_{120}O_{24}$;1199.6, CAS 9002-92-0.

A commercially available reagent of suitable grade.

Polysorbate 80 R

The mono ester of oleic acid and tripolyethyleneglycol 300-sorbitan ether.

Description. Lemon to amber coloured, oily liquid.

Miscibility. Miscible with water, producing an odourless and nearly colourless solution. Miscible with ethanol (~750 g/L) TS, ethyl acetate R and vegetable oils; immiscible with mineral oils.

Potassio-cupric tartrate TS

Procedure. Dissolve 7 g of copper(II) sulfate R in sufficient water to produce 100 mL. Separately dissolve 35 g of potassium sodium tartrate R and 10 g of sodium hydroxide R in 100 mL of water. Shortly before use, mix together equal volumes of both solutions.

Potassio-mercuric iodide TS

Procedure. Dissolve 1.355 g of mercuric chloride R in 60 mL of water; separately dissolve 5 g of potassium iodide R in 20 mL of water; mix the two solutions and dilute to 100 mL with water.

Potassio-mercuric iodide, alkaline, TS

Procedure. Dissolve 3.5 g of potassium iodide R and 1.25 g of mercuric chloride R in 80 mL of water, add while stirring a cold, saturated solution of mercuric chloride R in water until a slight red precipitate remains. Then add 12 g of sodium hydroxide R and mix to dissolve, add a little more of the saturated solution of mercuric chloride R and sufficient water to produce 100 mL; allow to stand for 24 hours and decant the clear liquid.

Potassium acetate R

$C_2H_3KO_2$ (SRIP, 1963, p. 144).

Potassium acetate TS

Procedure. Dissolve 100 g of potassium acetate R in sufficient glacial acetic acid R to produce 1000 mL.

Potassium antimonate R

$KSbO_3$ (SRIP, 1963, p. 145).

Potassium antimonate TS

Procedure. Boil 2 g of potassium antimonate R with 95 mL of water until it has dissolved. Cool rapidly and add 50 mL of potassium hydroxide (1 mol/L) VS and 5 mL of sodium hydroxide (1 mol/L) VS. Allow to stand for 24 hours and dilute with sufficient water to produce 150 mL.

Sensitivity to sodium. To 10 mL add 7 mL of sodium hydroxide (0.1 mol/L) VS; a white, crystalline precipitate is formed within 15 minutes.

Note. Potassium antimonate TS must be freshly prepared.

Potassium bicarbonate R

$KHCO_3$ (SRIP, 1963, p. 145).

Potassium bromate (0.00833 mol/L) VS

Potassium bromate R dissolved in water to contain 1.392 g of $KBrO_3$ in 1000 mL.

Potassium bromate (0.0167 mol/L) VS

Potassium bromate R dissolved in water to contain 2.784 g of $KBrO_3$ in 1000 mL.

Potassium bromate (0.0333 mol/L) VS

Potassium bromate R dissolved in water to contain 5.562 g of $KBrO_3$ in 1000 mL.

Potassium bromate (50 g/L) TS

A solution of potassium bromate R containing about 50 g of $KBrO_3$ per litre.

Potassium bromate R

$KBrO_3$ (SRIP, 1963, p. 147).

Potassium bromide (0.119 g/L) TS

A solution of potassium bromide R containing about 0.1190 g of KBr per litre.

Potassium bromide (100 g/L) TS

A solution of potassium bromide R containing about 100 g of KBr per litre.

Potassium bromide (125 g/L) TS

A solution of potassium bromide R containing about 125 g of KBr per litre.

Potassium bromide IR

Potassium bromide R that complies with the following test: the infrared absorption spectrum of a disc prepared as described in Method 3 under *1.7 Spectrophotometry in the infrared region* from potassium bromide R, previously dried at 250 °C for 1 hour, has a substantially flat baseline over the range 4000–670 cm_{-1}; it exhibits no maxima with an absorbance greater than 0.1 above the baseline with the exception of maxima due to water at 3440 and 1630 cm^{-1}.

Potassium bromide R

KBr (SRIP, 1963, p. 148).

Potassium carbonate R

K_2CO_3, $1\frac{1}{2}H_2O$.

Description. Small granular crystals.

Solubility. Very soluble in water; practically insoluble in ethanol (~750 g/L) TS.

Potassium carbonate, anhydrous, R

K_2CO_3.

Description. Granules or a granular powder; hygroscopic.

Solubility. Soluble in 1 part of water; practically insoluble in ethanol (~750 g/L) TS.

Potassium carbonate (150 g/L) TS

Procedure. Dissolve 15 g of anhydrous potassium carbonate R in 100 mL of water R.

Potassium chloride (100 g/L) TS

A solution of potassium chloride R containing about 100 g of KCl per litre.

Potassium chloride (350 g/L) TS

A saturated solution of potassium chloride R containing about 350 g/L of KCl.

Potassium chloride IR

Potassium chloride R that complies with the following test: the infrared absorption spectrum of a disc prepared as described in Method 3 under *1.7 Spectrophotometry in the infrared region*, from potassium chloride R, previously dried at 250 °C for 1 hour, has a substantially flat baseline over the range 4000–670 cm^{-1}; it exhibits no maxima with an absorbance greater than 0.1 above the baseline, with the exception of maxima due to water at 3440 and 1630 cm^{-1}.

Potassium chloride R

KCl (SRIP, 1963, p. 151).

Potassium chromate (100 g/l) TS

A solution of potassium chromate R containing about 97 g of K_2CrO_4 per litre (approximately 0.5 mol/L).

Potassium chromate R

K_2CrO_4 (SRIP, 1963, p. 152).

Potassium cyanide (100 g/L) TS

A solution of potassium cyanide R containing about 100 g of KCN per litre.

Potassium cyanide (50 g/L) TS

A solution of potassium cyanide R containing about 50 g of KCN per litre.

Potassium cyanide PbTS.

Procedure. Dissolve 10 g of potassium cyanide R in 90 mL of water, add 2 mL of hydrogen peroxide (~60 g/L) TS, allow to stand for 24 hours, dilute with water to 100 mL and filter.

Potassium cyanide R

KCN (SRIP, 1963, p. 153).

Potassium dichromate (0.0167 mol/L) VS

Potassium dichromate R dissolved in water to contain 4.904 g of $K_2Cr_2O_7$ in 1000 mL.

Potassium dichromate (100 g/L) TS

A solution of potassium dichromate R containing about 98 g of $K_2Cr_2O_7$ per litre (approximately 0.4 mol/L).

Potassium dichromate R

$K_2Cr_2O_7$ (SRIP, 1963, p. 154).

Potassium dichromate R1

Potassium dichromate R containing not less than 99.9% of $K_2Cr_2O_7$.

Potassium dichromate TS

Procedure. Dissolve about 60 mg, accurately weighed and previously dried at 130 °C, of potassium dichromate R1 in sufficient sulfuric acid (0.005 mol/L) VS to produce 1000.0 mL.

Potassium dichromate TS2

Procedure. Dissolve 1 g of potassium dichromate R in 60 mL of water and cautiously add 7.5 mL of sulfuric acid (~1760 g/L) TS.

Potassium dichromate TS3

Procedure. Dissolve 0.5 g of potassium dichromate R in sufficient sulfuric acid (~100 g/L) TS to produce 100 mL.

Potassium dihydrogen phosphate (6.8 g/L) TS

A solution of potassium dihydrogen phosphate R containing 6.8 g of KH_2PO_4 per litre (0.05 mol/L).

Potassium dihydrogen phosphate (13.6 g/L) TS

A solution of potassium dihydrogen phosphate R containing 13.6 g of KH_2PO_4 per litre (0.1 mol/L).

Potassium dihydrogen phosphate (27.2 g/L) TS

A solution of potassium dihydrogen phosphate R containing 27.2 g of KH_2PO_4 per litre (0.2 mol/L).

Potassium dihydrogen phosphate (70 g/L) TS

A solution of potassium dihydrogen phosphate R containing about 70 g of KH_2PO_4 per litre.

Potassium dihydrogen phosphate (100 g/L) TS.

A solution of potassium dihydrogen phosphate R containing about 100 g of KH_2PO_4 per litre.

Potassium dihydrogen phosphate R

KH_2PO_4 (SPRI, 1963, p. 155).

Potassium ferricyanide (10 g/L) TS

Procedure. Wash about 1 g of crystalline potassium ferricyanide R with a little water and dissolve the washed crystals in sufficient water to produce 100 mL.

Note: Potassium ferricyanide (10 g/L) TS must be freshly prepared.

Potassium ferricyanide (50 g/L) TS

Procedure. Wash about 5 g of crystalline potassium ferricyanide R with a little water and dissolve the washed crystals in sufficient water to produce 100 mL.

Note: Potassium ferricyanide (50 g/L) TS must be freshly prepared.

Potassium ferrocyanide (~53 g/L) TS

A solution of potassium ferrocyanide R containing about 53 g of $K_4Fe(CN)_6$ per litre.

Potassium ferricyanide R

$K_3Fe(CN)_6$ (SRIP, 1963, p. 156).

Potassium ferrocyanide (45 g/L) TS

A solution of potassium ferrocyanide R containing about 50 g of $K_4Fe(CN)_6$ per litre.

Potassium ferrocyanide R

$K_4Fe(CN)_6,3H_2O$ (SRIP, 1963, p. 156).

Potassium gluconate R

$C_6H_{11}KO_7$

Potassium hydrogen phthalate R

$C_8H_5KO_4$ (SRIP, 1963, p. 157).

Potassium hydrogen phthalate standard TS

Procedure. Dissolve 10.21 g of potassium hydrogen phthalate R, previously dried at 120 °C, in sufficient carbon-dioxide-free water R to produce 1000 mL. The pH of this solution is defined as having the value of 4.000 at 15 °C.

Potassium hydrogen sulfate R

$KHSO_4$ (SRIP, 1963, p. 146).

Potassium hydrogen tartrate R

$C_4H_5KO_6$ (SRIP, 1963, p. 158).

Potassium hydrogen tartrate standard TS

Procedure. Add 2 g of potassium hydrogen tartrate R to 100 mL of carbon-dioxide-free water R contained in a glass-stoppered flask and shake the flask vigorously. Let the temperature of the solution reach room temperature, allow the solid to settle and remove it by filtration or decantation.

Note: Potassium hydrogen tartrate standard TS must be freshly prepared.

Potassium hydroxide (~55 g/L) TS

A solution of potassium hydroxide R containing about 55 g/L of KOH (approximately 1 mol/L).

Potassium hydroxide (~110 g/L) TS

A solution of potassium hydroxide R containing about 112 g/L of KOH (approximately 2 mol/L).

Potassium hydroxide (~400 g/L) TS

A solution of potassium hydroxide R containing about 400 g of KOH per litre.

Potassium hydroxide (~450 g/L) TS

A solution of potassium hydroxide R containing about 450 g of KOH per litre.

Potassium hydroxide (~560 g/L) TS

A solution of potassium hydroxide R containing about 560 g of KOH per litre.

Potassium hydroxide (0.01 mol/L) VS

Potassium hydroxide R dissolved in water to contain 0.5610 g of KOH in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution following the method described under potassium hydroxide (1 mol/L) VS.

Potassium hydroxide (0.1 mol/L) VS

Potassium hydroxide R dissolved in water to contain 5.610 g of KOH in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution following the method described under potassium hydroxide (1 mol/L) VS.

Potassium hydroxide/ethanol (0.1 mol/L) VS

Potassium hydroxide R dissolved in ethanol (~710 g/L) TS to contain 5.61 g of KOH in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution following the method described under potassium hydroxide/ethanol (0.5 mol/L) VS

Potassium hydroxide (0.5 mol/L) VS

Potassium hydroxide R dissolved in water to contain 28.05 g of KOH in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution following the method described under potassium hydroxide (1 mol/L) VS.

Potassium hydroxide (1 mol/L) VS

Potassium hydroxide R dissolved in water to contain 56.10 g of KOH in 1000 mL.

Method of standardization. Ascertain the exact concentration of the 1 mol/L solution in the following manner: dry about 5 g of

potassium hydrogen phthalate R at 105 °C for 3 hours and weigh accurately. If the potassium hydrogen phthalate is in the form of large crystals they should be crushed before drying. Dissolve in 75 mL of carbon-dioxide-free water R and titrate with the potassium hydroxide solution using phenolphthalein/ethanol TS as indicator. Each 0.2042 g of potassium hydrogen phthalate is equivalent to 1 mL of potassium hydroxide (1 mol/L) VS. Standard solutions of potassium hydroxide should be restandardized frequently.

Storage. Solutions of alkali hydroxides absorb carbon dioxide when exposed to air. They should therefore be stored in bottles with suitable non-glass, well-fitting stoppers, provided with a tube filled with soda lime R.

Potassium hydroxide R

KOH (SRIP, 1963, p. 159).

Potassium hydroxide/ethanol (0.02 mol/L) VS

Potassium hydroxide R dissolved in ethanol (~710 g/L) TS to contain 1.122 g of KOH in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution following the method described under potassium hydroxide/ethanol (0.5 mol/L) VS.

Potassium hydroxide/ethanol (0.5 mol/L) VS

Potassium hydroxide R dissolved in ethanol (~710 g/L) TS to contain 28.05 g of KOH in 1000 mL.

Method of standardization. Ascertain the exact concentration of the 0.5 mol/L solution in the following manner: dilute 25.0 mL of hydrochloric acid (0.5 mol/L) VS with 50 mL of water and titrate with the potassium hydroxide/ethanol solution using phenolphthalein/ethanol TS as indicator.

Potassium hydroxide/ethanol (1 mol/L) VS

Potassium hydroxide R dissolved in ethanol (~710 g/L) TS to contain 56.10 g of KOH in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution following the method described under potassium hydroxide/ethanol (0.5 mol/L) VS.

Potassium hydroxide/ethanol TS1

Procedure. Dissolve 40 g of potassium hydroxide R in 20 mL of water and add sufficient ethanol (~750 g/L) TS to produce 1000 mL. Allow to stand overnight and pour off the clear liquid.

Potassium hydroxide/ethanol TS2

Procedure. Dissolve 112 g of potassium hydroxide R in sufficient ethanol (~710 g/L) TS to produce 1000 mL (approximately 2 mol/L).

Potassium hydroxide/methanol TS

Procedure. Dissolve 30 g of potassium hydroxide R in sufficient methanol R to produce 1000 mL.

Potassium iodate (0.01 mol/L) VS

Potassium iodate R dissolved in water to contain 2.140 g of KIO_3 in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution following the method described under potassium iodate (0.05 mol/L) VS.

Potassium iodate (0.05 mol/L) VS

Potassium iodate R dissolved in water to contain 10.70 g of KIO_3 in 1000 mL.

Method of standardization. Ascertain the exact concentration of the 0.05 mol/L solution in the following manner. Place 10.0 mL of the potassium iodate solution in a glass-stoppered flask, dilute with 200 mL of water, add 2 g of potassium iodide R and 25 mL of sulfuric acid (~100 g/L) TS. Allow the solution to stand for 10 minutes and titrate the liberated iodine with sodium thiosulfate (0.1 mol/L) VS, adding 3 mL of starch TS as the end-point is approached. Correct for a blank determined on the same quantities of the same reagents.

Potassium iodate (3.6 mg/L) TS

A freshly prepared solution of potassium iodate R containing 3.6 mg of KIO_3 per litre.

Potassium iodate R

KIO_3 (SRIP, 1963, p. 160).

Potassium iodide (100 g/L) TS

A solution of potassium iodide R containing about 100 g of KI per litre.

Potassium iodide (160g/L) TS

A solution of potassium iodide R containing about 160 g of KI per litre.

Potassium iodide (300 g/L) TS

A solution of potassium iodide R containing about 300 g of KI per litre.

Potassium iodide (400 g/L) TS

A solution of potassium iodide R containing about 400 g of KI per litre.

Potassium iodide (60 g/L) TS

A solution of potassium iodide R containing about 60 g of KI per litre.

Potassium iodide (80 g/L) TS

A solution of potassium iodide R containing about 83 g/L of KI (approximately 0.5 mol/L).

Potassium iodide AsR

Potassium iodide R that complies with the following test: Dissolve 10 g of potassium iodide R in 25 mL of hydrochloric acid (~250 g/L) AsTS and 35 mL of water, add 2 drops of stannous chloride AsTS and apply the general test for arsenic; no visible stain is produced.

Potassium iodide R

KI (SRIP, 1963, p. 161).

Potassium iodide, iodinated TS

Procedure. Dissolve 500 mg of iodine R and 1.5 g of potassium iodide R in water R and dilute to 25 mL with the same solvent.

Potassium iodide/starch TS1

Procedure. Dissolve 10 g of potassium iodide R in 95 mL of water and add to it 5 mL of starch TS.

Note: Potassium iodide/starch TS1 must be freshly prepared.

Potassium iodobismuthate TS1

Procedure. Dissolve 100 g of tartaric acid R in 400 mL of water and add 8.5 g of bismuth oxynitrate R. Shake the solution for 1 hour, add 200 mL of potassium iodide (400 g/L) TS and mix. Allow to stand for 24 hours and filter.

Potassium iodobismuthate TS2

Procedure. Dissolve 100 g of tartaric acid R in 500 mL of water and add 50 mL of potassium iodobismuthate TS1.

Potassium iodobismuthate TS3

Stock solution. Suspend 1.7 g of bismuth subnitrate R and 20 g of tartaric acid R in 40 mL of water R. To the suspension add 40 mL of potassium iodide (400 g/L) TS and stir for 1 hour. Filter. The solution may be kept for several days in an amber glass bottle.

Spray solution. Prepare immediately before use. Mix 1 volume of the stock solution with 3 volumes of water R.

Potassium iodobismuthate/acetic acid TS

Procedure. Dissolve 8 g of potassium iodide R in 20 mL of water and add to it a solution composed of 0.85 g of bismuth oxynitrate R dissolved in 40 mL of water and 10 mL of glacial acetic acid R.

Potassium iodoplatinate TS

Procedure. Dissolve 2.5 g of platinic chloride R in 50 mL of water, add 45 mL of a 0.1 g/mL solution of potassium iodide R and dilute to 100 mL with water.

Storage. Store in amber glass containers.

Potassium iodoplatinate TS2

Procedure. Dissolve 0.25 g of platinic chloride R in 2.5 mL of water, add 45 mL of potassium iodide (100 g/L) TS and dilute with sufficient acetone R to produce 100 mL.

Potassium nitrate R

KNO_3 (SRIP, 1963, p. 162).

Potassium nitrite (100 g/L) TS

A solution of potassium nitrite R containing about 100 g/L of KNO_2.

Potassium nitrite R

KNO_2.

Description. White or slightly yellow, deliquescent granules or rods.

Solubility. Soluble in 0.35 part of water; slightly soluble in ethanol (~750 g/L) TS.

Potassium periodate R

KIO_4 (SRIP, 1963, p. 164).

Potassium periodate TS

Procedure. To 2.8 g of potassium periodate R add 200 mL of water followed by 20 mL of sulfuric acid (~1760 g/L) TS added drop by drop while shaking to effect solution; cool and add sufficient water to produce 1000 mL.

Potassium permanganate (0.0002 mol/L) VS

Potassium permanganate R dissolved in water to contain 31.61 mg of $KMnO_4$ in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution following the method described under "Potassium permanganate (0.02 mol/L) VS".

Potassium permanganate (0.002 mol/L) VS

Potassium permanganate R dissolved in water to contain 0.3161 g of $KMnO_4$ in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution following the method described under potassium permanganate (0.02 mol/L) VS.

Potassium permanganate (0.02 mol/L) VS

Potassium permanganate R dissolved in water to contain 3.161 g of $KMnO_4$ in 1000 mL.

Method of standardization. Ascertain the exact concentration of the 0.02 mol/L solution in the following manner: dissolve about 0.2 g, accurately weighed, of sodium oxalate R, previously dried to constant weight at 110 °C, in 250 mL of water. Add 7 mL of sulfuric acid (~1760 g/L) TS, heat to about 70 °C and then slowly add the permanganate solution from a burette, with constant stirring, until a pale pink colour, which persists for 15 seconds, is produced. The temperature at the conclusion of the titration should be not less than 60 °C. Every 6.7 mg of sodium oxalate are equivalent to 1 mL of potassium permanganate (0.02 mol/L) VS. Potassium permanganate solutions should be restandardized frequently.

Storage. Store the solution in tightly closed containers, protected from light.

Potassium permanganate (~ 0.3 g/L) TS

A solution of potassium permanganate R containing about 0.3 g of $KMnO_4$ per litre.

Potassium permanganate (~25 g/L) TS

A solution of potassium permanganate R containing about 25 g of $KMnO_4$ per litre.

Potassium permanganate (~10 g/L) TS

A solution of potassium permanganate R containing about 10 g/L of $KMnO_4$.

Potassium permanganate (~1g/L) TS

A solution of potassium permanganate R containing about 1 g of $KMnO_4$ per litre.

Potassium permanganate R

$KMnO_4$ (SRIP, 1963, p. 165).

Potassium permanganate, basic (~5 g/L) TS

A solution of potassium permanganate R containing about 5 g of $KMnO_4$ per litre of sodium hydroxide (~40 g/L) TS.

Potassium permanganate, basic (~1 g/L) TS

A solution of potassium permanganate R containing about 1 g of $KMnO_4$ per litre of sodium hydroxide (~40 g/L) TS.

Potassium permanganate/phosphoric acid TS

Procedure. Dissolve 3 g of potassium permanganate R in a mixture of 15 mL of phosphoric acid (~1440 g/L) TS and 70 mL of water and dilute to 100 mL with water.

Potassium pyroantimonate R

$KSb(OH)_6$

Description. White or almost white, crystals or crystalline powder.

Solubility. Sparingly soluble in water R.

Potassium pyroantimonate (13 g/L) TS

Dissolve 1.95 g of potassium pyroantimonate R in 95 mL of hot water R. Cool quickly and add a solution containing 2.5 g of potassium hydroxide R in 50 mL of water R and 1 mL of dilute sodium hydroxide (~85 g/L) TS. Allow to stand for 24 hours, filter and dilute to 150 mL with water R.

Potassium sodium tartrate R

[sodium potassium tartrate R] $C_4H_4KNaO_6,4H_2O$ (SRIP, 1963, p. 193).

Potassium sulfate (0.1 g/L) TS

A solution of potassium sulfate R containing about 0.1 g of K_2SO_4 per litre.

Potassium sulfate (174 mg/L) TS

Procedure. Dissolve 174 mg, accurately weighed, of potassium sulfate R in sufficient water to produce 1000 mL.

Potassium sulfate R

K_2SO_4 (SRIP, 1963, p. 165).

Potassium tetraoxalate R

$C_4H_3KO_8,2H_2O$ (SRIP, 1963, p. 166).

Potassium tetraoxalate standard TS

Procedure. Dissolve 25.42 g of potassium tetraoxalate R in sufficient carbon-dioxide-free water R to produce 1000 mL.

Potassium thiocyanate (200 g/L) TS

A solution of potassium thiocyanate R containing 200 g of KCNS per litre.

Potassium thiocyanate R

KCNS. Contains not less than 99.0% of KCNS, calculated with reference to the dried substance.

Description. Colourless crystals.

Solubility. Soluble in 0.5 part of water and in 15 parts of dehydrated ethanol R.

Alkalinity. A 0.1 g/mL solution in carbon-dioxide-free water R is not alkaline to bromothymol blue/ethanol TS.

Ammonia. Boil 1.0 g with 5 mL of sodium hydroxide (~80 g/L) TS; no ammonia is evolved.

Chlorides. Dissolve 1.0 g in a solution of 1 g of ammonium nitrate R in 30 mL of hydrogen peroxide (~60 g/L) TS containing not more than 1 µg/g of chlorides, add 1 g of sodium hydroxide R and warm gently; when the vigorous reaction subsides add a further 30 mL of hydrogen peroxide (~60 g/L) TS and boil for 2 minutes. Cool, add 5 mL of nitric acid (~1000 g/L) TS and 1 mL of

silver nitrate (40 g/L) TS; any opalescence produced is not greater than that produced by treating 1 mL of hydrochloric acid (0.01 mol/L) VS in the same manner.

Sulfates. Dissolve 0.50 g in 20 mL of water and proceed as described under *2.2.2 Limit test for sulfates*; not more than 1.0 mg/g.

Other sulfur compounds. Dissolve 1.0 g in 50 mL of water, add 2 mL of hydrochloric acid (~70 g/L) TS, and titrate with iodine (0.05 mol/L) VS; not more than 0.5 mL of iodine (0.05 mol/L) VS is required.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 20 mg/g.

Assay. Dissolve about 0.4 g, accurately weighed, in 50 mL of water, add 5 mL of nitric acid (~1000 g/L) TS, 50 mL of silver nitrate (0.1 mol/L) VS and 5 mL of ferric ammonium sulfate (45 g/L) TS and titrate the excess of silver nitrate with ammonium thiocyanate (0.1 mol/L) VS. Each mL of silver nitrate (0.1 mol/L) VS is equivalent to 9.718 mg of KCNS.

Note: Potassium thiocyanate R is deliquescent.

2-Propanol R

[*iso*-propanol R]; isopropyl alcohol; C_3H_8O (SRIP, 1963, p. 167).

1-Propanol R

n- Propanol; propan-1-ol, C_3H_8O.

Description. A clear, colourless liquid.

Miscibility. Miscible with water and ethanol (~750 g/L) TS.

Boiling range. Not less than 95% distils between 95 and 98 °C.

Mass density. ρ_{20} = about 0.803 kg/L.

Residue on evaporation. Evaporate on a water-bath and dry to constant weight at 105 °C; it leaves a residue of not more than 0.1 mg/g.

Propylene glycol R.

$C_3H_8O_2$ (SRIP, 1963, p. 168).

Pyridine R

C_5H_5N (SRIP, 1963, p. 169).

Pyridine, anhydrous, R

Pyridine R that has been dried by allowing it to stand over sodium hydroxide R.

Pyridine/acetic anhydride TS

Procedure. Mix 3 volumes of freshly distilled pyridine R with 1 volume of freshly distilled acetic anhydride R.

Note: Pyridine/acetic anhydride TS must be freshly prepared.

Pyrogallol R

Pyrogallic acid; 1,2,3-trihydroxybenzene, $C_6H_6O_3$ (SRIP, 1963, p. 170).

Pyrogallol, alkaline, TS

Procedure. Dissolve 0.5 g of pyrogallol R in 2 mL of water. Dissolve separately 12 g of potassium hydroxide R in 8 mL of water. Immediately before use mix the two solutions.

O - Octanoic acid R.... Oxibendazole R

Octane R

n-Octane; C_8H_{18}; CAS Reg. No. 111-65-9.

Contains not less than 99% of C_8H_{18}.

Octanoic acid R

Caprylic acid, $C_8H_{16}O_2$.

Description. A colourless, oily liquid.

Boiling temperature. About 237 °C.

Mass density. ρ_{20} = about 0.92 kg/L.

Olive oil R

A commercially available reagent of suitable grade.

Opalescence standard TS1

Procedure. Dilute 15 mL of Opalescence stock standard TS with sufficient water to produce 1000 mL.

Shelf-life. Use within 24 hours after preparation.

Opalescence standard TS2

Procedure. Dilute 5.0 mL of Opalescence standard TS1 with sufficient water to produce 100 mL. Mix well and shake before use.

Note: Opalescence standard TS2 must be freshly prepared.

Opalescence standard TS3

Procedure. Dilute 10 mL of opalescence standard TS1 with sufficient water to produce 100 mL. Mix well and shake before use.

Note: Opalescence standard TS3 must be freshly prepared.

Opalescence stock standard TS

Procedure. Dissolve 1.0 g of hydrazine sulfate R in sufficient water to produce 100 mL and allow to stand for 4–6 hours. To 25.0 mL of this solution add a solution of 2.5 g of methenamine R dissolved in 25.0 mL of water, mix well and allow to stand for 24 hours.

Storage. Store in a glass container free from surface defects.

Shelf-life. Use within 2 months after preparation.

Oracet blue B R

Solvent blue 19; a mixture of 1-methylamino-4-anilinoanthraquinone ($C_{21}H_{16}N_2O_2$) and 1-amino-4-anilinoanthraquinine ($C_{20}H_{14}N_2O_2$).

Oracet blue R/acetic acid TS

Procedure. Dissolve 0.5 g of oracet blue B R in sufficient glacial acetic acid R1 to produce 100 mL.

Osmium tetroxide R

OsO_4.

Caution. The fumes are corrosive to the eyes, the mucous membranes and the skin.

Description. Yellow, needle-shaped crystals or a yellow, crystalline mass; hygroscopic; light sensitive; odour, pungent.

Solubility. Soluble in water, ethanol (~750 g/L) TS and ether R.

Ox brain, acetone-dried, R

Procedure. Cut into small pieces a fresh ox brain previously freed from vascular and connective tissue. Place in acetone R for preliminary dehydration. Complete the dehydration by pounding in a mortar 30 g of the material with successive quantities, each of 75 mL of acetone R, until a dry powder is obtained after filtration. Dry at 37 °C for 2 hours or until the odour of acetone is no longer perceptible.

Oxalic acid (0.05 g/L) TS

A solution of oxalic acid R containing 0.05 g of $C_2H_2O_4$ in 1000 mL.

Procedure. Dissolve 0.07 g of oxalic acid R in sufficient water to produce 1000 mL.

Oxalic acid R

$C_2H_2O_4,2H_2O$ (SRIP, 1963, p. 131).

Oxalic acid/sulfuric acid TS

Procedure. Dissolve 5 g of oxalic acid R in a sufficient quantity of a cooled mixture of equal volumes of sulfuric acid (~1760 g/L) TS and water to produce 100 mL.

Oxibendazole R

Oxibendazole of a suitable quality should be used.

Oxytetracycline hydrochloride R

Oxytetracycline hydrochloride of a suitable quality should be used.

N - N-(1-Naphthyl)ethylenediamine hydrochloride (1 g/L) TS... 1-Nitroso-2-naphthol-3,6-disodium disulfonate R

N-(1-Naphthyl)ethylenediamine hydrochloride (1 g/L) TS

A solution of *N*-(1-naphthyl)ethylenediamine hydrochloride R containing about 1 g of $C_{12}H_{14}N_2$,2HCl per litre.

N-(1-Naphthyl)ethylenediamine hydrochloride (5 g/L) TS

A solution of *N*-(1-naphthyl)ethylenediamine hydrochloride R containing about 5 g of $C_{12}H_{14}N_2$,2HCl per litre.

N-(1-Naphthyl)ethylenediamine hydrochloride R

$C_{12}H_{14}N_2$,2HCl (SRIP, 1963, p. 124).

N-(1-Naphthyl)ethylenediamine hydrochloride/1-propanol TS

Procedure. To 7 mL of *N*-(1-naphthyl)ethylenediamine hydrochloride (1 g/l) TS add 3 mL of 1-propanol R.

N-(1-Naphthyl)ethylenediamine hydrochloride/propylene glycol TS

Procedure. Dissolve 0.1 g of *N*-(1-naphthyl)ethylenediamine hydrochloride R in 30 mL of water and dilute to 100 mL with propylene glycol R.

Note. N -(1-Naphthyl)ethylenediamine hydrochloride/propylene glycol TS must be freshly prepared.

N,N'-bis(propan-2-yl)ethane-1,2-diamine R

$C_8H_{20}N_2$

Molecular weight. 144.3.

Other name. N,N'-Bis(1-methylethyl)-1,2-ethanediamine; N,N'-Diisopropylethylenediamine.

Description. Colourless or yellowish, hygroscopic liquid, corrosive, flammable.

Relative density d_{20}^{20}. About 0.798.

Boiling point. About 170 °C.

Naphthalene-1,3-diol R

1,3-Naphthalenediol; $C_{10}H_8O_2$.

Description. Colourless crystals.

Solubility. Freely soluble in water, ethanol (~750 g/L) TS and ether R.

Melting temperature. About 124 °C.

Naphthalene-1,3-diol/ethanol TS

Procedure. Dissolve 0.2 g of naphthalene-1,3-diol R in sufficient ethanol (~750 g/L) TS to produce 100 mL.

2-Naphthol R

[β-naphthol R] $C_{10}H_8O$ (SRIP, 1963, p. 122).

1-Naphthol R

$C_{10}H_8O$.

Description. Colourless crystals or a white, crystalline powder; odour, characteristic.

Solubility. Soluble in 5 parts of ethanol (~750 g/L) TS (may form a slightly opalescent, colourless or almost colourless solution).

Melting range. 93–96 °C.

Sulfated ash. Not more than 0.5 mg/g.

1-Naphthol TS1

Procedure. Dissolve 0.10 g of 1-naphthol R in 3 mL of sodium hydroxide (~150 g/L) TS and dilute with sufficient water to produce 100 mL.

Note: 1-Naphthol TS1 must be prepared immediately before use.

2-Naphthol TS1

Procedure. Dissolve 5 g of 2-naphthol R, freshly recrystallized, in 40 mL of sodium hydroxide (~80 g/L) TS and add sufficient water to produce 100 mL.

Note: 2-Naphthol TS1 must be freshly prepared.

1-Naphtholbenzein R

$C_{27}H_{20}O_3$.

Description. A reddish-brown powder.

Solubility. Practically insoluble in water; soluble in ethanol (~750 g/L) TS, benzene R, ether R and glacial acetic acid R.

1-Naphtholbenzein/acetic acid TS

Procedure. Dissolve 0.2 g of 1-naphtholbenzein R in sufficient glacial acetic acid R to produce 100 mL.

1-Naphthol/ethanol TS

Procedure. Dissolve 0.05 g of 1-naphthol R in 60 mL of ethanol (~750 g/L) TS and add sufficient water to produce 100 mL.

Neutral red R

C.I. 50040; C.I. Basic Red; $C_{15}H_{17}ClN_4$ (SRIP, 1963, p. 124).

Neutral red/ethanol TS

Procedure. Dissolve 0.1 g of neutral red R in sufficient ethanol (~375 g/L) TS to produce 100 mL.

Ninhydrin/2-propanol (5g/L) TS

Procedure. Prepare a 5 g/L solution of ninhydrin R in 2-propanol R.

Ninhydrin R

See under “Triketohydrindene hydrate R”.

Nitric acid (~1000 g/L) TS

[nitric acid (70%) R] (SRIP, 1963, p. 125); *d*~1.41.

Nitric acid (~1000 g/L), cadmium-free and lead-free, TS

[nitric acid, cadmium-free and lead-free (70%) R].

Nitric acid (~200 g/L), cadmium-free and lead-free, TS

Procedure. Dilute 200 mL cadmium-free and lead-free nitric acid (~1000 g/L) TS with water R to produce 1000 mL.

Nitric acid (~130 g/L) TS

Procedure. Dilute 130 mL of nitric acid (~1000 g/L) TS with sufficient water to produce 1000 mL (approximately 2 mol/L); *d*~1.07.

Nitric acid (0.05 mol/L) VS

Nitric acid (~1000 g/L) TS diluted with water to contain 3.151 g of HNO_3 in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution by following the method described under nitric acid (1 mol/L) VS.

Nitric acid (1 mol/L) VS

Nitric acid (~1000 g/L) TS diluted with water to contain 63.10 g of HNO_3 in 1000 mL.

Method of standardization. Ascertain the exact concentration of the 1 mol/L solution in the following manner: Dissolve 2 g of anhydrous sodium carbonate R in 50 mL of water and titrate with the nitric acid solution using 0.1 mL of methyl orange/ethanol TS as indicator until the solution just becomes reddish yellow. Boil for 2 minutes; the solution reverts to yellow. Cool and continue the titration until the reddish yellow colour is restored. Each mL of nitric acid (1 mol/L) VS is equivalent to 0.0530 g of Na_2CO_3.

Nitric acid (15 g/L) TS

Nitric acid (~1000 g/L) TS diluted with water to contain 15.0 g/l of HNO_3.

Nitric acid (3 g/L) TS

Nitric acid (~1000 g/L) TS diluted with water to contain 3.0 g/L of HNO_3.

Nitric acid, fuming, R

HNO_3 (SRIP, 1963, p. 126); *d*~1.5.

4-Nitroaniline R

[p-nitroaniline R] $C_6H_6N_2O_2$ (SRIP, 1963, p. 127).

4-Nitroaniline TS1

Procedure. Dissolve 5 g of 4-nitroaniline R in sufficient hydrochloric acid (1 mol/l) VS to produce 1000 mL.

4-Nitroaniline TS2

Procedure. Dissolve 0.4 g of 4-nitroaniline R in 60 mL of hydrochloric acid (1 mol/L) VS, cool to 15 °C and add sufficient sodium nitrite (100 g/L) TS until 1 drop of the mixture turns starch/iodine paper R blue.

Note: 4-Nitroaniline TS2 must be freshly prepared.

Nitrobenzene R

$C_6H_5NO_2$ (SRIP, 1963, p. 128).

4-Nitrobenzoyl chloride R

[p-nitrobenzoyl chloride R] $C_7H_4ClNO_3$ (SRIP, 1963, p. 128).

Nitrogen monoxide R

NO.

Nitric oxide, washed with water.

A commercially available gas of suitable grade.

Nitrogen monoxide and nitrogen dioxide detector tube

A cylindrical, sealed glass tube containing adsorbent filters and suitable supports for an oxidizing layer Cr(VI) salt and the diphenyl-benzidine indicator. The minimum value indicated is 5 µl/L or less, with a relative standard deviation of at most ±15%. Tubes can be verified with a calibration gas containing the appropriate impurity if a negative result is obtained.

Nitrogen R

N_2 (SRIP, 1963, p. 129).

Nitrogen for chromatography R

Contains not less than 99.95% v/v of N_2.

Nitrogen, oxygen-free, R

Nitrogen R which has been freed from oxygen by passing it through alkaline pyrogallol TS.

Nitromethane R

CH_3NO_2.

Description. A colourless, oily liquid.

Miscibility. Miscible with water, ethanol (~750 g/L) TS, ether R and di-methylformamide R.

Mass density. ρ_{20} = about 1.13 kg/L.

Refractive index. $n_D^{22} = \text{about} 1.380$.

Boiling temperature. About 101 °C.

Nitrophenanthroline R

5-Nitro-1,10-phenanthroline; $C_{12}H_7N_3O_2$.

Description. A white powder; odourless.

Solubility. Soluble in water.

Melting range. 198–200 °C.

Nitrophenanthroline TS

Procedure. Dissolve 0.15 g of nitrophenanthroline R in 15 mL of freshly prepared ferrous sulfate (7 g/L) TS.

1-Nitroso-2-naphthol-3,6-disodium disulfonate (2 g/L) TS

A solution of 1-nitroso-2-naphthol-3,6-disodium disulfonate R containing about 2 g of $C_{10}H_5NNa_2O_8S_2$ per litre.

1-Nitroso-2-naphthol-3,6-disodium disulfonate R

[1-nitroso-2-naphthol-3,6-disodium sulfonate R]. $C_{10}H_5NNa_2O_8S_2$ (SRIP, 1963, p. 129).

N,N,N',N'-tetramethyl-2,2'-oxybis(ethaneamine) R

$C_8H_{20}N_2O$

Molecular weight. 160.3.

Other name. Bis(2-dimethylaminoethyl) ether; 2,2'-Oxybis(N,N-dimethylethylamine).

Description. Colourless, corrosive liquid.

Relative density d_{20}^{20}. About 0.85.

L - Lactobionic acid R.... Litmus TS

Lactobionic acid R

4-O-β-D-Galactopyranosyl-D-gluconic acid; $C_{12}H_{22}O_{12}$.

A commercially available reagent of suitable grade.

Lanthanum nitrate (30 g/L) TS

Procedure. Dissolve 4.3 g of lanthanum nitrate R in 1 mL of nitric acid (~130 g/L) TS and sufficient water to produce 100 mL.

Lanthanum nitrate R

$La(NO_3)_3,6H_2O$. Contains not less than 97.0% of $La(NO_3)_3,6H_2O$.

Description. Colourless crystals; deliquescent.

Solubility. Freely soluble in water.

Assay. Dissolve about 0.75 g, accurately weighed, in 25 mL of water, add 3 mL of nitric acid (~130 g/L) TS, 3 g of methenamine R, about 20 mg of xylenol orange indicator mixture R and titrate with disodium edetate (0.05 mol/L) VS until the solution becomes pure yellow in colour. If fading of the colour of the indicator occurs towards the end of the titration more methenamine R should be added. Each mL of disodium edetate (0.05 mol/L) VS is equivalent to 21.65 mg of $La(NO_3)_3,6H_2O$.

Lead acetate (80 g/L) TS

A solution of lead acetate R in freshly boiled water containing about 80 g/L of $C_4H_6O_4Pb$ (approximately 0.25 mol/L).

Lead acetate paper R

Procedure. Dip white filter-paper into a mixture of 10 volumes of lead acetate (80 g/L) TS and 1 volume of acetic acid (~60 g/L) TS. Allow to dry and cut the paper into strips measuring 15 mm × 40 mm.

Storage. Lead acetate paper R should be kept in a well-closed container.

Lead acetate R

$C_4H_6O_4Pb,3H_2O$ (SRIP, 1963, p. 105).

Lead nitrate (0.05 mol/L) VS

Lead nitrate R, dissolved in water to contain 16.56 g of $Pb(NO_3)_2$ in 1000 mL.

Method of standardization. Ascertain the exact concentration of the 0.05 mol/L solution by diluting 25.0 mL with 200 mL of water, add 10 mL of ammonia buffer TS and about 20 mg of Mordant Black 11 indicator mixture R and titrate with disodium edetate (0.05 mol/L) VS.

Lead nitrate (0.1 mol/L) VS

Lead nitrate R, dissolved in water to contain 33.12 g of $Pb(NO_3)_2$ in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution following the method described under "Lead nitrate (0.05 mol/L) VS".

Lead nitrate (100 g/L) TS

A solution of lead nitrate R containing 100 g of $Pb(NO_3)_2$ per litre.

Lead nitrate paper R

Procedure. Dip strips of suitable filter-paper in a solution of 10 g of lead nitrate R in 100 mL of water and allow to dry.

Lead nitrate R

$Pb(NO_3)_2$ (SRIP, 1963, p. 107).

Lead subacetate TS

Contains not less than 16.7% *m/m* and not more than 17.4% *m/m* of Pb in a form corresponding approximately to the formula $C_8H_{14}O_{10}Pb_3$.

Procedure. Dissolve 40.0 g of lead acetate R in 90 mL of carbon-dioxide-free water R. Adjust the pH to 7.5 with sodium hydroxide (~400 g/L) TS. Centrifuge and use the clear supernatant solution.

Storage. Lead subacetate TS should be stored in a well-closed container.

Lead(IV) oxide R

PbO_2 (SRIP, 1963, p. 105).

Lead, dilute, PbTS.

One millilitre contains 10 µg of lead.

Procedure. Dilute 10 mL of strong lead PbTS with sufficient water to produce 100 mL.

Note: Dilute lead PbTS must be freshly prepared.

Lead, strong, PbTS

One millilitre contains 100 µg of lead.

Procedure. Dissolve 0.1598 g of lead nitrate R in 5 mL of nitric acid (~1000 g/L) TS and sufficient water to produce 1000 mL.

Levarterenol hydrogen tartrate R

$C_8H_{11}NO_3,C_4H_6O_6,H_2O$. Contains not more than 99% of $C_8H_{11}NO_3,C_4H_6O_6$, calculated with reference to the anhydrous substance.

Description. A white, or almost white, crystalline powder; odourless.

Solubility. Soluble in water; slightly soluble in ethanol (~750 g/L) TS; practically insoluble in ether R.

Specific optical rotation. Use a 50 mg/mL solution; $[\alpha]_D^{20\,°C}$ = -10° to -13°.

Water. Determine as described under *2.8 Determination of water by the Karl Fischer method*, Method A, using about 0.5 g of the substance; not less than 45 mg/g and not more than 58 mg/g.

Assay. Dissolve about 0.4 g, accurately weighed, in glacial acetic acid R and titrate with perchloric acid (0.1 mol/L) VS as described under *2.6 Non-aqueous titration*, Method A. Each mL of perchloric acid (0.1 mL/L) VS is equivalent to 31.93 mg of $C_8H_{11}NO_3,C_4H_6O_6$.

Limulus amoebocyte lysate

Reconstitute the lysate as stated on the label. For each batch confirm the stated sensitivity as prescribed under "Sensitivity of the lysate" in *3.4 Test for bacterial endotoxins*. The sensitivity of the lysate is defined as the lowest concentration of endotoxin which yields a firm gel under test conditions and is expressed in endotoxin units per millilitre.

Lincomycin hydrochloride R

Lincomycin hydrochloride of a suitable quality should be used.

Lithium carbonate R

Li_2CO_3.

Description. A white, granular powder; odourless.

Solubility. Sparingly soluble in water; very slightly soluble in ethanol (~750 g/L) TS.

Lithium carbonate/trinitrophenol TS

Procedure. Dissolve 0.25 g of lithium carbonate R and 0.5 g of trinitrophenol R in sufficient water to produce 100 mL.

Lithium chloride (10 g/L) TS

A solution of lithium chloride R containing about 10 g of LiCl per litre.

Lithium chloride R

LiCl.

Description. White, deliquescent crystals or granules.

Solubility. Freely soluble in water; soluble in acetone R, ethanol (~750 g/L) TS and ether R.

Storage. Store in a tightly closed container.

Lithium clavulanate R

Lithium clavulanate of a suitable quality should be used.

Lithium methoxide (0.1 mol/L) VS

Procedure. Dissolve 0.694 g of lithium R in 150 mL of methanol R and add sufficient toluene R to produce 1000 mL.

Method of standardization. Ascertain the exact concentration of the 0.1 mol/L solution in the following manner: dissolve about 0.15 g, accurately weighed, of benzoic acid R in 25 mL of dimethylformamide R and titrate with the lithium methoxide solution to a red end-point using quinaldine red/methanol TS as indicator as described under *2.6 Non-aqueous titration*, Method B. Each 12.21 mg of benzoic acid is equivalent to 1 mL of lithium methoxide (0.1 mol/L) VS. Lithium methoxide solutions must be standardized immediately before use.

Lithium perchlorate R

$LiClO_4$.

Description. Small crystals.

Solubility. Freely soluble in water; sparingly soluble in ethanol (~750 g/L) TS, acetone R, ether R and ethyl acetate R.

Lithium perchlorate/acetic acid TS

Procedure. Dissolve 10.64 g of lithium perchlorate R in sufficient glacial acetic acid R1 to produce 1000 mL.

Lithium R

Li.

Description. A soft metal whose freshly cut surface is silvery-grey, tarnishing rapidly in air.

Solubility. Reacts violently with water, yielding hydrogen and a solution of lithium hydroxide; soluble in methanol R, yielding hydrogen and a solution of lithium methoxide; practically insoluble in ether R.

Litmus paper R

(SRIP, 1963, p. 109).

Litmus R

(SRIP, 1963, p. 108).

Litmus TS

Procedure. Boil 10 g of litmus R with 40 mL of ethanol (~710 g/L) TS for 1 hour and pour away the clear liquid; repeat this operation twice with 30 mL of ethanol (~710 g/L) TS. Digest the washed litmus with 100 mL of water and filter.

M - Macrogol 1000 R.... Morpholine R

Macrogol 1000 R

Description. A white, waxy mass.

Viscosity. At 100 °C, about 17.3 mm^2s^{-1}.

Macrogol 200 R

Description. A clear, colourless or almost colourless viscous liquid.

Solubility. Very soluble in acetone R and in ethanol (~750 g/L) TS; practically insoluble in ether R and in fatty oils.

Macrogol 20000 2-nitroterephthalate R

Macrogol 20000 R modified by treating with 2-nitroterephthalate acid.

Description. A hard, white or almost white, waxy solid.

Solubility. Soluble in acetone

Macrogol 20000 R

Description. White or almost white solid with a waxy or paraffin-like appearance.

Solubility. Very soluble in water, soluble in methylene chloride, practically insoluble in alcohol, in fatty oils and in mineral oils.

Macrogol 200 TS

Procedure. Pour 500 mL of macrogol 200 R into a 1000 mL, round-bottom flask. Evaporate any volatile components using a rotation evaporator. Heat to 60 °C and apply a vacuum with a pressure of 1.5–2.5 kPa for 6 hours.

Macrogol 20M R

Polyethylene glycol 20 000. A suitable grade to be used in gas-liquid chromatography.

Macrogol 400 R

Polyethylene glycol 400. Macrogol 400 R is a polymer of ethylene oxide and water, represented by the formula $H(OCH_2CH_2)_n OH$, in which the average value of *n* lies between 8.2 and 9.1.

Description. Clear colourless (or practically colourless) viscous liquid having a slight characteristic odour; slightly hygroscopic.

Average molecular weight. Transfer to a pressure flask 2.1 g of macrogol 400 R, accurately weighed, and 25.0 mL of phthalic anhydride/pyridine TS. Insert the stopper in the flask, wrap the flask securely with cloth and immerse it in a water-bath maintained at 96–100 °C to the same depth as the mixture in the flask for 1 hour. Remove the flask retaining the cloth wrapping and allow to cool in air to room temperature. To the contents of the flask add 50 mL of carbonate-free sodium hydroxide (0.5 mol/L) VS and 5 drops of phenolphthalein/pyridine TS. Titrate with carbonate-free sodium hydroxide (0.5 mol/L) VS to a pink end-point that remains for not less than 15 seconds. Perform a blank determination in a similar manner. Calculate the average molecular weight by multiplying by 4000 the weight, in g, of the test substance and dividing the result by the difference between the volume, in mL, of carbonate-free sodium hydroxide (0.5 mol/L) VS consumed for the test substance and the blank determination. The average molecular weight is between 380 and 420.

Mass density (ρ_{20}). 1.110–1.140 kg/L.

Congealing point. Between 4 and 8 °C, the congealing point being the average of 4 consecutive temperature readings, the highest and lowest of which differ by not more than 0.4 °C.

pH value. Between 4.5 and 7.5, in a 50 g/L solution.

Acidity or alkalinity. Dissolve 5.0 g in 50 mL of water. Add a few drops of phenol red/ethanol TS. If the solution turns yellow titrate with sodium hydroxide (0.01 mol/L) VS; if the solution turns red titrate with hydrochloric acid (0.01 mol/L) VS. Not more than 2.0 mL of titrant should be required in either case.

Sulfated ash. Not more than 10 mg/g.

Heavy metals. Mix 4 g, accurately weighed, with 1 mL of hydrochloric acid (~70 g/L) TS and dilute with water to 25 mL. The limit is 50 µg/g.

Limit of monoethylene and diethylene glycols. Dissolve 50 g in 75 mL of diphenyl ether R in a 250 mL distillation flask. Slowly distil at a pressure of 100–250 Pa (1–2 mmHg) into a receiver that is graduated to 100 mL in 1 mL subdivisions, until 25 mL of

distillate have been collected. Add 25.0 mL of water to the distillate, shake the receiving flask vigorously and allow the layers to separate. Cool the container in an ice-bath to solidify and facilitate the removal of the layer of diphenyl ether R. Filter the water layer through filter-paper into a glass-stoppered, 50 mL graduated cylinder. To the filtrate add an equal volume of freshly distilled acetonitrile R and shake the cylinder until solution is complete. Pipette 10 mL of the solution into 15 mL of ceric ammonium nitrate TS, mix and within 2–5 minutes determine the absorbance of the resulting solution at about 525 nm. Use a blank consisting of 15 mL of ceric ammonium nitrate TS and 10 mL of acetonitrile (400 g/L) TS. Prepare a standard solution by mixing 10 mL of acetronile (400 g/L) TS, to which 30 mg of diethylene glycol R have been added and 15 mL of ceric ammonium nitrate TS and determine the absorbance within 2–5 minutes at about 525 nm using the same blank as above. The absorbance of the test solution should not exceed that of the standard solution.

Magnesium acetate R

$C_4H_6MgO_4,4H_2O$.

Description. Colourless crystals. Deliquescent.

Solubility. Freely soluble in water and ethanol (~750 g/L) TS.

Magnesium chloride (0.1 mol/L) VS

Procedure. Dissolve 20.5 g of magnesium chloride R in sufficient water to produce 1000 mL.

Method of standardization. Ascertain the exact concentration of the 0.1 mol/L solution, carrying out the complexometric titration of magnesium under *2.5 Complexometric titrations* using 25 mL of magnesium chloride solution. Each mL of disodium edetate (0.1 mol/L) VS is equivalent to 20.33 mg of $MgCl_2,6H_2O$.

Magnesium chloride R

$MgCl_2,6H_2O$ (SRIP, 1963, p. 110).

Magnesium oxide R

MgO.

Description. A white, very fine powder.

Solubility. Very slightly soluble in water; insoluble in ethanol (~750 g/L) TS.

Magnesium oxide R1

Complies with the requirements prescribed for magnesium oxide R with the following modifications:

Arsenic: maximum 2 ppm.

Heavy metals (*2.2.3*): maximum 10 ppm.

Iron: maximum 50 ppm.

Magnesium standard (10 µg/mL Mg) TS

Procedure. Dilute 10 mL of magnesium (0.1 mg/mL) TS with sufficient water to produce 100 mL.

Magnesium sulfate (50 g/L) TS

A solution of magnesium sulfate R containing about 50 g of $MgSO_4$ per litre.

Magnesium sulfate R

$MgSO_4,7H_2O$ (SRIP, 1963, p. 111).

Magnesium sulfate/sulfuric acid TS

Procedure. Dissolve 25 g of magnesium sulfate R in sufficient sulfuric acid (~100 g/L) TS to produce 100 mL.

Maleic acid R

$C_4H_4O_4$.

Description. Colourless crystals.

Melting temperature. About 135 °C.

Manganese dioxide R

MnO_2 (SRIP, 1963, p. 112).

Manganese sulfate (15 g/L) TS

Manganese sulfate R dissolved in water to contain 15.0 g/L of $MnSO_4$.

Manganese sulfate R

$MnSO_4,H_2O$.

Description. Pale-red, slightly efflorescent crystals.

Solubility. Soluble in about 1 part of water and 0.6 part of boiling water; practically insoluble in ethanol (~750 g/L) TS.

Manganese/silver paper R

Procedure. To a mixture of equal volumes of silver nitrate (0.1 mol/L) VS and manganese sulfate (15 g/L) TS, add drop by drop sodium hydroxide (0.1 mol/L) VS until a persistent precipitate is produced and filter. Soak strips of filter-paper (Whatman No. 1 is suitable) for 15 minutes in the solution dry them at ambient temperature, protected from light and acidic or alkaline vapours. The manganese/silver paper R should be colourless.

Test for sensitivity. Place in a cylinder of about 40 mL capacity (height about 80 mm, internal diameter about 30 mm) 1.0 mL of ammonium chloride (10 µg/mL NH_4) TS. Add 9 mL of water and 1 g of magnesium oxide R. Immediately stopper the flask using a polyethylene cap below which a manganese/silver paper R is placed. Swirl the solution carefully so that magnesium particles do not come into contact with the reagent paper. Keep the cylinder at 50–60 °C for 1 hour. A true grey colour is produced on the reagent paper.

Mebendazole R

Mebendazole of a suitable quality should be used.

Meglumine (100 g/L) TS

A solution of meglumine R containing about 100 g of $C_7H_{17}NO_5$ per litre.

Note: Meglumine (100 g/L) TS must be freshly prepared.

Meglumine R

$C_7H_{17}NO_5$. Meglumine as described in the monograph for *Meglumine*.

Menadione R

2-Methyl-1,4-naphthoquinone, $C_{11}H_8O_2$.

Description. Bright yellow crystals.

Melting temperature. About 106 °C.

Mercaptoacetic acid R

(thioglycolic acid R). $C_2H_4O_2S$ (SRIP, 1963, p. 206).

Mercuric acetate R

$C_4H_6HgO_4$ (SRIP, 1963, p. 112).

Mercuric acetate/acetic acid TS

Procedure. Dissolve 50 g of mercuric acetate R in sufficient glacial acetic acid R1 that has been neutralized, if necessary, to crystal violet/acetic acid TS with perchloric acid (0.1 mol/L) VS to produce 1000 mL.

Mercuric bromide AsTS

Procedure. Dissolve 5 g of mercuric bromide R in sufficient ethanol (~750 g/L) TS to produce 100 mL.

Mercuric bromide paper AsR

Procedure. Use smooth, white filter-paper weighing 65–120 g/m^2. The thickness of the paper in mm should be approximately equal numerically to the weight expressed as above, divided by 400. Soak pieces of filter-paper, not less than 25 mm in width, in mercuric bromide AsTS, decant the superfluous liquid, suspend the paper over a non-metallic thread and allow it to dry, protected from light.

Storage. Store the mercuric bromide paper AsR in stoppered bottles in the dark.

Note: Paper that has been exposed to sunlight or to vapours of ammonia must not be used as it produces only a pale stain or no stain at all.

Mercuric bromide R

$HgBr_2$ (SRIP, 1963, p. 113).

Mercuric chloride (2.7 g/L) TS

A solution of mercuric chloride R containing about 2.7 g of $HgCl_2$ per litre.

Mercuric chloride (65 g/L) TS

A solution of mercuric chloride R containing about 65 g of $HgCl_2$ per litre (approximately 0.25 mol/L).

Mercuric chloride R

$HgCl_2$ (SRIP, 1963, p. 113).

Mercuric chloride/ethanol TS

Procedure. Dissolve 2 g of mercuric chloride R in sufficient ethanol (~375 g/L) TS to produce 100 mL.

Mercuric iodide R

Mercury diiodide; HgI_2.

Description. A heavy, crystalline, scarlet-red powder; odourless.

Solubility. Slightly soluble in water; sparingly soluble in ethanol (~750 g/L) TS, acetone R and ether R; soluble in solutions containing an excess of potassium iodide R.

Storage. Mercuric iodide R should be stored protected from light.

Mercuric nitrate (0.01 mol/L) VS

Procedure. Dissolve about 3.5 g, accurately weighed, of mercuric nitrate R in a mixture of 5 mL of nitric acid (~1000 g/L) TS and 500 mL of water and dilute with water to 1000 mL.

Method of standardization. Ascertain the exact concentration of the 0.01 mol/L solution in the following manner: transfer 20.0 mL to a conical flask, add 2 mL of nitric acid (~1000 g/L) TS and 2 mL of ferric ammonium sulfate (45 g/L) TS. Cool to below 20 °C and titrate with ammonium thiocyanate (0.01 mol/L) VS to the first appearance of a permanent brownish colour.

Mercuric nitrate (0.02 mol/L) VS

Procedure. Weigh accurately about 6.85 g of mercuric nitrate R, dissolve in a mixture of 10 mL of nitric acid (~130 g/L) TS and 500 mL of water and dilute with water to 1000 mL.

Method of standardization. Ascertain the exact concentration of the 0.02 mol/L solution following the method described under mercuric nitrate (0.01 mol/L) VS.

Mercuric nitrate R

$Hg(NO_3)_2,H_2O$.

Caution. Mercuric nitrate R is poisonous.

Description. A white or slightly yellow, deliquescent, crystalline powder.

Solubility. Soluble in water in the presence of a small quantity of nitric acid (~1000 g/L) TS.

Mercuric nitrate TS

Procedure. Dissolve 40 g of yellow mercuric oxide R in a mixture of 32 mL of nitric acid (~1000 g/L) TS and 15 mL of water.

Storage. Keep in a container protected from light.

Mercuric oxide, yellow, R

HgO (SRIP, 1963, p. 114).

Mercuric sulfate TS.

Procedure. Mix 5 g of yellow mercuric oxide R with 40 mL of water and, while stirring, add 20 mL of sulfuric acid (~1760 g/L) TS, then add 40 mL of water and stir until completely dissolved.

Mercury R

Hg (SRIP, 1963, p. 115).

Mercury/nitric acid TS

Procedure. Dissolve 3 mL of mercury R in 27 mL of cold fuming nitric acid R and dilute the solution with an equal volume of water.

Storage. The solution should be stored protected from light and for not more than 2 months.

Methanesulfonic acid R

Molecular formula. CH_4O_3S

Description. Colourless and corrosive liquid, strong irritant.

Solubility. Miscible with water.

Density (*d*). ~1.48.

Melting point. About 20 °C.

Methanol for chromatography R

Methanol for chromatography contains not less than 99.8% of CH_3OH and complies with the following tests:

Absorbance (1.6): not more than 0.17 at 225 nm, determined using water R as compensation liquid.

Methanol R

CH_3OH (SRIP, 1963, p. 117).

Methanol, dehydrated, R

Methanol R that complies with the following requirement: water, not more than 1.0 mg/g.

Methenamine R

Hexamethylenetetramine, $C_6H_{12}N_4$. Contains not less than 99.0% of $C_6H_{12}N_4$.

Description. Colourless crystals or a crystalline powder; odourless.

Solubility. Soluble in water and ethanol (~750 g/L) TS.

Acidity and alkalinity. Dissolve 2.5 g in 25 mL of water. To 10 mL add 3 drops of phenolphthalein/ethanol TS; a pink colour is produced which changes to red after the addition of 1 drop of carbonate-free sodium hydroxide (0.1 mol/L) VS. To a further 10 mL aliquot add 3 drops of bromothymol blue/ethanol TS; a blue colour is produced which changes to green-blue after the addition of 3 drops of hydrochloric acid (0.1 mol/L) VS.

Sulfated ash. Not more than 0.5 mg/g.

Assay. Dissolve about 1.5 g, accurately weighed, in 10 mL of water, add 50 mL of sulfuric acid (0.5 mol/L) VS and boil until the odour of formaldehyde is no longer perceptible. Titrate the excess of acid with sodium hydroxide (1 mol/L) VS using methyl red/ethanol TS as indicator. Each mL of sulfuric acid (0.5 mol/L) VS is equivalent to 35.05 mg of $C_6H_{12}N_4$.

Methoxyphenylacetic R

Procedure. Dissolve 2.7 g of methoxyphenylacetic acid R in 6 mL of tetramethylammonium hydroxide (~100 g/L) TS and add 20 mL of dehydrated ethanol R.

Storage. Store in a polyethylene container.

Methoxyphenylacetic acid R

$C_9H_{10}O_3$

Description. White, crystalline powder or white or almost white crystals.

Solubility. Sparingly soluble in water, freely soluble in ethanol (~750 g/L) TS.

Melting point. About 70° C.

Methyl ethyl ketone R

$C_{11}H_{16}N_2$.

Description. A clear liquid.

A commercially available reagent of suitable grade.

Refractive index. n_D^{20} = about 1.565.

Methyl green R

[α-[*p*-Dimethylamino)phenyl]-α-[4-(dimethyliminio)-2,5-cyclohexadien-1-ylidene]-*p*-tolyl]trimethylammonium dichloride; Basic blue 20; C.I. No. 42585; $C_{26}H_{33}Cl_2N_3$.

Description. A green powder.

Solubility. Soluble in water; soluble in sulfuric acid (~1760 g/L) TS giving a yellow solution and turning green on dilution.

Methyl green/iodomercurate paper R

Procedure. Dip strips of suitable filter-paper in a solution of 4 g of methyl green R in 100 mL of water and allow to dry in air. Then immerse the strips for 1 hour in a mixture composed of 14 g of potassium iodide R and 20 g of mercuric iodide R in 100 mL of water. Wash the strips with water until the washings are practically colourless and allow to dry in air.

Storage. Methyl green/iodomercurate paper R should be stored protected from light.

Methyl orange ethanol TS

Procedure. Dissolve 0.04 g of methyl orange R in sufficient ethanol (~150 g/L) TS to produce 100 mL.

Methyl orange R

Sodium salt of 4'-dimethylaminoazobenzene-4-sulfonic acid, $C_{14}H_{14}N_3NaO_3S$ (SRIP, 1963, p. 118).

Methyl orange/acetone TS

A saturated solution of methyl orange R in acetone R.

Methyl orange/ethanol TS1

Procedure . Dissolve 0.1 g of methyl orange R in 80 mL of water R and dilute to 100 mL with ethanol (~750 g/L) TS.

Test for sensitivity. A mixture of 0.1 mL of methyl orange/ethanol TS1 and 100 mL of carbon-dioxide-free water R is yellow. Not more than 0.1 mL of hydrochloric acid (1 mol/L) VS is required to change the colour to red.

Colour change. pH 3.0 (red) to pH 4.4 (yellow).

N-Methyl-N-nitrosotoluene-4-sulfonamide R

$C_8H_{10}N_2O_3S$.

Description. A yellow, crystalline powder.

Solubility. Insoluble in water; soluble in ethanol (~750 g/L) TS and ether R.

Melting temperature. About 60 °C.

3-Methylpentane-2-one R

$C_6H_{12}O$

Molecular weight. 100.2.

Description. Colourless, flammable liquid.

Relative density d_{20}^{20}. About 0.815.

Boiling point. About 118 °C.

N-Methylpiperazine R

$C_5H_{12}N_2$.

Mass density. ρ_{20} = 0.902 kg/L.

Refractive index. n_D^{20} = 1.466.

4(4-Methylpiperidin-1-yl)pyridine R

4(4-Methylpiperidino)pyridine ; $C_{11}H_{16}N_2$.

Description. A clear liquid.

A commercially available reagent of suitable grade.

Refractive index. n_D^{20} = about 1.565.

2-Methylpropanol R

$C_4H_{10}O$

Molecular weight. 74.1.

Other names. Isobutyl alcohol, 2-Methylpropan-1-ol.

Description. Clear colourless liquid.

Solubility. Soluble in water, miscible with ethanol (~710 g/L) TS.

Relative density d_{20}^{20} . About 0.80.

Boing point. About 107 °C.

Methyl red R

4'-Dimethylaminoazobenzene-2-carboxylic acid, $C_{15}H_{15}N_3O_2$ (SRIP, 1963, p. 118).

Methyl red/ethanol TS

Procedure. Dissolve 25 mg of methyl red R in a mixture of 0.95 mL of sodium hydroxide (0.05 mol/L) VS and 5 mL of ethanol (~750 g/L) TS, warm the solution slightly and after cooling dilute with sufficient ethanol (~375 g/L) TS to produce 250 mL.

Methyl red/methylthioninium chloride TS

Procedure. Mix 20 mL of a 0.5 mg/mL solution of methyl red R in ethanol (~150 g/L) TS with 0.4 mL of a 20 mg/mL solution of methylthioninium chloride R in water.

Methyl silicone gum R

A suitable grade to be used in gas-liquid chromatography.

2-Methylpropan-2-amine R

$C_4H_{11}N$

Molecular weight. 73.1.

Other names. 2-Amino-2-methylpropane, *tert*-Butylamine.

Description. liquid,miscible with ethanol (~710 g/L) TS.

Relative density d_{20}^{20}. About 0.694.

*Boiling point.*About 46 °C.

2-Methyl-5-nitroimidazole R

$C_4H_5N_3O_2$.

Melting temperature. About 253 °C.

Methyl violet 2B R

CI 42535; C.I. basic violet.

A commercially available reagent of suitable grade.

Melting point. About 137 °C, with decomposition.

Methylamine hydrochloride (20 g/L) TS

A solution of methylamine hydrochloride R containing about 20 g of CH_5N,HCl per litre.

Methylamine hydrochloride R

CH_5N,HCl.

Description. Deliquescent tetragonal tablets.

Solubility. Soluble in water and dehydrated ethanol R; practically insoluble in acetone R, ether R and ethyl acetate R.

Melting point. About 228 °C.

Methylisobutylketone R

Isopropylacetone, $C_6H_{12}O$. (SRIP, 1963, p. 119).

Methylthioninium chloride (0.2 g/L) TS

Procedure. Dissolve 23 mg of methylthioninium chloride R in sufficient water to produce 100 mL.

Methylthioninium chloride (1 g/L) TS

A solution of methylthioninium chloride R containing about 1 g of $C_{16}H_{18}ClN_3S$ per litre.

Methylthioninium chloride R

[methylene blue]. $C_{16}H_{18}ClN_3S,3H_2O$ (SRIP, 1963, p. 119).

Methylthymol blue mixture R

Procedure. Mix 1 part of methylthymol blue R with 100 parts of potassium nitrate R.

Methylthymol blue R

Tetrasodium [3*H*-2,1-benzoxathiol-3-ylidenebis[(6-hydroxy-5-isopropyl-2-methyl-*m*-phenylene)methylenenitrilo]]tetraacetic acid *S*, *S*-dioxide; $C_{37}H_{44}N_2Na_4O_{13}S$.

Description. A brownish-black powder.

Solubility. Freely soluble in water; very slightly soluble in ethanol (~750 g/L) TS.

Molecular sieve R

CAS Reg. No. 70955-01-0.

Molecular sieve composed of sodium aluminosilicate. It is available as beads or powder with a pore size of 0.4 nm.

When reused, it is recommended that the molecular sieve be regenerated according to the manufacturer's instructions.

Molybdenum trioxide R

MoO_3 (SRIP, 1963, p. 120).

Monoethanolamine (0.1 mol/L) VS

A solution of monoethanolamine R in water to contain 6.108 g of C_2H_7NO in 1000 mL.

Monoethanolamine R

C_2H_7NO.

Description. A clear, colourless to faintly yellow, viscous liquid; odour, ammoniacal.

Miscibility. Miscible with water, methanol R and acetone R.

Boiling temperature. About 170 °C.

Mass density. ρ_{20}= 1.01 kg/L.

Refractive index, n_D^{20} = 1.453 - 1.455.

Mordant Black 11 indicator mixture R

Procedure. Mix 1 g of Mordant Black 11 R with 100 g of sodium chloride R.

Mordant Black 11 R

[eriochrome black R]. C.I. Mordant Black 11, C.I. No. 14645, Eriochrome Black T, Solochrome Black; sodium salt of 2-(2-hydroxy-6-nitro-4-sulfo-1-naphthylazo)-1-naphthol, $C_{20}H_{12}N_3NaO_7S$ (SRIP, 1963, p. 84).

Morpholine R

Tetrahydro-1,4-oxazine; C_4H_9NO (SRIP, 1963, p. 121).

K - Kaolin, light, R.... Kieselguhr R5

Kaolin, light, R

Kaolin as described in the monograph for *Kaolin*.

Kaolin suspension TS

Procedure. Immediately before use mix equal volumes of cephalin TS and a suspension containing 4 g of kaolin R in 1000 mL of sodium chloride (9 g/L) TS.

Karl Fischer reagent TS

A freshly prepared solution contains up to 5.0 mg/mL of water. The solution should not be used if the water equivalent falls below 2.5 mg of water per mL of the reagent.

Procedure. Dissolve 63 g of iodine R in 100 mL of anhydrous pyridine R, cool in ice and pass sulfur dioxide R into the solution until a gain in weight of 32 g has occurred, taking care to avoid absorption of atmospheric moisture. Add sufficient dehydrated methanol R to produce 500 mL and allow to stand for 24 hours. Karl Fischer reagent TS may also be prepared by mixing commercially available solutions of sulfur dioxide in pyridine and of iodine in methanol, which are stable when properly stored, for example, protected from light. The resulting solution should conform to the requirements stated below.

Method of standardization. Ascertain the exact content of water in the following manner: add about 20 mL of dehydrated methanol R to the titration vessel and titrate to the end-point with Karl Fischer reagent TS without recording the volume required. Introduce in an appropriate form a suitable amount of water, accurately weighed, and titrate again to the end-point with Karl Fischer reagent TS, recording the volume. Water might be introduced, for example, as a solution in dry methanol or under the form of a hydrated compound. Calculate the water equivalent of the reagent in mg of water per mL. Karl Fischer reagent TS deteriorates continuously and should be standardized immediately before use or daily as required.

Note: Ethylene glycol monoethyl ether R may be used in the preparation of the reagent instead of dehydrated methanol R.

Kieselguhr R1

Kieselguhr G

Description. A greyish-white powder of an average particle size between 10 and 40 µm, containing per kg about 150 g of calcium sulfate, hemihydrate.

Kieselguhr R2

Kieselguhr GF254

Description. A greyish-white powder of an average particle size between 10 and 40 µm, containing per kg about 150 g of calcium sulfate, hemihydrate and an adequate amount (usually about 15 g/kg) of a fluorescent indicator having a maximum absorption at 254 nm.

Kieselguhr R3

Description. A greyish-white powder of an average particle size between 170 and 200 µm.

Kieselguhr R4

Description. A greyish-white powder of an average particle size between 70 and 150 µm.

Kieselguhr R5

Kieselguhr H

Description. A fine, greyish-white powder; the grey colour becomes more pronounced on triturating the powder with water. The average particle size is between 10 and 40 µm.

I - Imidazole R.... Isopropyl myristate R

Imidazole R

Glyoxaline, $C_3H_4N_2$. Contains not less than 99.0% of $C_3H_4N_2$.

Description. A white, crystalline powder.

Solubility. Soluble in water and ethanol (~750 g/L) TS.

Melting range. 89–93 °C.

Sulfated ash. Not more than 0.5 mg/g.

Assay. Dissolve 0.3 g in 50 mL of water and titrate with sulfuric acid (0.05 mol/L) VS using bromocresol green/ethanol TS as indicator. Each mL of sulfuric acid (0.05 mol/L) VS is equivalent to 6.808 mg of $C_3H_4N_2$.

Imidazole, recrystallized, R

Procedure. Dissolve 25 g of imidazole R in 100 mL of hot toluene R and cool in an ice-bath while stirring. Filter off the crystals with suction using filter-paper Whatman No. 54 or No. 541. Repeat the crystallization and filtration, sucking as dry as possible. Slurry wash the resulting crystals with about 50 mL of ether R and filter. Repeat this process and then wash the crystals on the filter with ether R and suck as dry as possible. Transfer to a shallow dish and dry at room temperature under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) over silica gel, desiccant, R.

Storage. Store in a tightly closed container.

Imidazole/mercuric chloride TS

Procedure. Dissolve 8.25 g of recrystallized imidazole R in 60 mL of water and add 10 mL of hydrochloric acid (5 mol/L) VS. Under continuous stirring add, drop by drop, 10 mL of mercuric chloride (2.7 g/L) TS. If a cloudy solution results discard and prepare a further solution by adding the mercuric chloride solution more slowly. Adjust the pH to 6.80 ± 0.05 with hydrochloric acid (5 mol/L) VS (about 4 mL is required) and add sufficient water to produce 100 mL.

Iminodibenzyl R

10,11-Dihydro-5H-dibenz[b,f]azepine; $C_{14}H_{13}N$.

Description. A pale yellow, crystalline powder.

Melting temperature. About 106 °C.

Iobenguane sulfate R

$C_{16}H_{22}I_2N_6O_4S$.

Description. White crystals.

Melting point. About 160 °C.

Iodide standard (20 µg I/mL) TS

Procedure. Dissolve 26.0 mg of potassium iodide R in sufficient water to produce 100 mL. Dilute 10 mL of this solution to 100 mL with water.

Iodine (0.0001 mol/L) VS

Iodine R and potassium iodide R, dissolved in water to contain 25.38 mg of I_2 and 36 mg of KI in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution following the method described under iodine (0.1 mol/L) VS.

Iodine (0.0005 mol/L) VS

Iodine R and potassium iodide R, dissolved in water to contain 0.127 g of I2 and 0.18 g of KI in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution following the method described under "Iodine (0.1 mol/L) VS".

Iodine (0.005 mol/L) VS

Iodine R and potassium iodide R, dissolved in water to contain 1.269 g of I_2 and 1.80 g of KI in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution following the method described under iodine (0.1 mol/L) VS.

Iodine (0.01 mol/L) VS

Iodine R and potassium iodide R, dissolved in water to contain 2.538 g of I_2 and 3.6 g of KI in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution following the method described under iodine (0.1 mol/L) VS.

Iodine (0.02 mol/L) VS

Iodine R and potassium iodide R, dissolved in water to contain 5.076 g of I_2 and 7.2 g of KI in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution following the method described under iodine (0.1 mol/L) VS.

Iodine (0.05 mol/L) VS

Iodine R and potassium iodide R, dissolved in water to contain 12.69 g of I_2 and 18.0 g of KI in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution following the method described under iodine (0.1 mol/L) VS.

Iodine (0.1 mol/L) VS

Iodine R and potassium iodide R, dissolved in water to contain 25.38 g of I_2 and 36.0 g of KI in 1000 mL.

Method of standardization. Ascertain the exact concentration of the 0.1 mol/L solution by titrating 25.0 mL with sodium thiosulfate (0.1 mol/L) VS, using starch TS as indicator.

Iodine bromide R

IBr.

Description. Blue-black or brownish-black crystals.

Solubility. Freely soluble in water, ethanol (~750 g/L) TS, ether R and glacial acetic acid R.

Melting temperature. About 40 °C.

Storage. Store in a cool place, in a tightly closed container, protected from light.

Iodine bromide TS

Procedure. Dissolve 20 g of iodine bromide R in sufficient glacial acetic acid R to produce 1000 mL.

Storage. Store in a tightly closed container, protected from light.

Iodine pentoxide R

Iodic anhydride; I_2O_5.

A commercially available reagent of suitable grade.

Iodine R

I_2 (SRIP, 1963, p. 101).

Iodine solution TS

To 10.0 mL of M iodine (0.05 mol/L) VS add 0.6 g of potassium iodide R and dilute to 100.0 mL with water R. Prepare immediately before use.

Iodine TS

Procedure. Dissolve 2.6 g of iodine R and 3 g of potassium iodide R in sufficient water to produce 100 mL (approximately 0.1 mol/L).

Iodine/chloroform TS

Procedure. Dissolve 5.0 g of iodine R in sufficient chloroform R to produce 100 mL.

Iodine/ethanol TS

Procedure. Dissolve 10 g of iodine R in sufficient ethanol (~750 g/L) TS to produce 1000 mL.

Iron colour TS

A solution containing 45.0 mg/mL of $FeCl_3,6H_2O$.

Procedure. Prepare a solution containing 4.500 g of $FeCl_3,6H_2O$ in 100 mL, diluting the strong iron colour TS with sulfuric acid (~10 g/L) TS as necessary.

Iron colour, strong, TS

Procedure. Dissolve 6.6 g of ferric chloride R in 120 mL of sulfuric acid (~10 g/L) TS, filter the solution if necessary and determine the concentration of $FeCl_3,6H_2O$.

Assay. Dilute 5.0 mL with sufficient water to produce 25.0 mL. Transfer 10.0 mL of this solution to a flask and add 60 mL of water. Adjust the pH to 2–3 with hydrochloric acid (1 mol/L) VS and ammonia (~100 g/L) TS using congo red paper R. Heat the solution to approximately 45 °C and titrate with disodium edetate (0.05 mol/L) VS using 2 mL of sulfosalicylic acid (175 g/L) TS as indicator until the solution changes from a lilac tint to straw-yellow. Each mL of disodium edetate (0.05 mol/L) VS is equivalent to 13.52 mg of $FeCl_3,6H_2O$.

Iron salicylate TS

Procedure. Dissolve 0.5 g of ferric ammonium sulfate R in 250 mL of water containing 10 mL of sulfuric acid (~100 g/L) TS and dilute with sufficient water to produce 500 mL. To 100 mL of this solution add 50 mL of sodium salicylate (11.5 g/L) TS, 20 mL of acetic acid (~60 g/L) TS and 80 mL of sodium acetate (150 g/L) TS and dilute with water to 500 mL.

Storage. Store in a well-closed container, protected from light.

Note. Iron salicylate must be freshly prepared.

Iron standard FeTS

Procedure. Dissolve 0.173 g of ferric ammonium sulfate R in 100 mL of water, add 5 mL of hydrochloric acid (~70 g/L) TS and sufficient water to produce 1000 mL. Each mL of this solution contains 20 µg of iron.

Iron, reduced, R

Fe (SRIP, 1963, p. 102).

Isobutyl methyl ketone R

4-Methyl-2-pentanone; $C_6H_{12}O$.

Description. A clear, colourless liquid; odour, characteristic.

Boiling point. About 115 °C.

Mass density. ρ_{20} = about 0.80 kg/L.

Isoleucine R

(2*S*,3*S*)-2-Amino-3-methylpentanoic acid; $C_6H_{13}NO_2$

Description. White or almost white, crystalline powder or flakes.

Solubility. Sparingly soluble in water, slightly soluble in ethanol (~750 g/L) TS. It dissolves in dilute mineral acids and in dilute solutions of alkali hydroxides.

L-Isoleucine R

(2S,3S)-2-Amino-3-methylpentanoic acid, $C_6H_{13}NO_2$, content: 98.5% to 101.0% (dried substance).

Isoniazid R

Isoniazid as described in the monograph for *Isoniazid*.

Isopropylamine R

C_3H_9N.

Description. A colourless, volatile liquid with an ammoniacal odour.

Boiling point. About 33 °C.

Mass density. ρ_{20} = about 0.69 kg/L.

Isopropyl iodide R

2-Iodopropane. C_3H_7I. CAS Reg. No. 75-30-9.

Content. Minimum 99%.

Isopropyl myristate R

Propan-2-yl tetradecanoate. $C_{17}H_{34}O_2$.

Description: A clear, colourless, oily liquid.

Miscibility: Immiscible with water, miscible with ethanol, with fatty oils, with liquid paraffin.

H - Helium R.... Hypoxanthine R

Helium R

He. Contains not less than 999.95 mL/L of He.

Heparin RS

World Health Organization International Reference Material. Heparin, porcine, mucosal. 5th International Standard 1998. (Ampoules containing 2031 IU (distributed by the National Institute for Biological Standards and Control (NIBSC), PO Box 1193, Blanche Lane, South Mimms, Potters Bar, Herts EN6 3QH, England.))

Heparinized saline TS

A sterile solution of saline TS containing 50 International Units of heparin in 1 mL.

Heptane R

C_7H_{16} (SRIP, 1963, p. 94).

Hexamethyldisilazane R

$C_6H_{19}NSi_2$.

*Description.*A clear, colourless liquid, having a characteristic odour.

Mass density.ρ_{20} = about 0.77 kg/L.

Hexane R

n-Hexane, C_6H_{14}.

*Description.*A colourless, mobile, highly inflammable liquid.

*Boiling range.*Distils completely over a range of 1 °C between 67.5 and 69.5 °C.

Mass density.ρ_{20} = 0.658-0.659 kg/L

Refractive index. n_D^{20} = 1.374–1.375.

Hexylamine R

Hexaneamine; $C_6H_{15}N$.

A commercially available reagent of suitable grade.

Description. A colourless liquid.

Boiling point. 127–131 °C.

Refractive index. n_D^{20} = about 1.418.

Mass density. ρ_{20} = about 0.766 kg/L.

Histamine dihydrochloride R

$C_5H_9N_3$,2HCl. Contains not less than 98.0% and not more than 101.0% of $C_5H_9N_3$,2HCl, calculated with reference to the dried substance.

*Description.*Colourless crystals or a white crystalline powder; odourless.

*Solubility.*Freely soluble in water and in methanol R; soluble in ethanol (~750 g/L) TS.

*Melting range.*244–246 °C.

*Loss on drying.*Not more than 5.0 mg/g.

*Assay.*Dissolve about 0.15 g, accurately weighed, in 10 mL of water. Add 5 mL of chloroform R and 25 mL of ethanol (~750 g/L) TS. Titrate with carbonate-free sodium hydroxide (0.2 mol/L) VS using 0.5 mL of thymolphthalein/ethanol TS as indicator. Each mL of carbonate-free sodium hydroxide (0.2 mol/L) VS is equivalent to 9.21 mg of $C_5H_9N_3$,2HCl.

Histamine phosphate R

$C_5H_9N_3,2H_3PO_4$. Contains not less than 98.0% and not more than 101.0% of $C_5H_9N_3,2H_3PO_4$, calculated with reference to the anhydrous substance.

*Description.*Colourless, long, prismatic crystals; odourless. Stable in air.

*Solubility.*Soluble in about 5 parts of water; slightly soluble in ethanol (~750 g/L) TS.

*Melting temperature.*About 132 °C.

*Water.*Determined by the Karl Fischer method using about 1.0 g; the water content is 50–60 mg/g.

*Assay.*Dissolve about 0.15 g, accurately weighed, in 10 mL of water. Add 5 mL of chloroform R and 25 mL of ethanol (~750 g/L) TS. Titrate with carbonate-free sodium hydroxide (0.2 mol/L) VS using 0.5 mL of thymolphthalein/ethanol TS as indicator. Each mL of carbonate-free sodium hydroxide (0.2 mol/L) VS is equivalent to 15.36 mg of $C_5H_9N_3,2H_3PO_4$.

Histamine TS

A solution containing 1.0 mg/L of histamine base.

*Procedure.*Prepare histamine TS by diluting strong histamine TS with a sufficient quantity of saline TS.

*Note:*Histamine TS must be freshly prepared.

Histamine, strong, TS

A solution containing 1.00 g/L of histamine base.

*Procedure.*Dissolve 138.1 mg, accurately weighed, of histamine phosphate R or 82.8 mg, accurately weighed, of histamine dihydrochloride R in sufficient water to produce 50.0 mL.

*Storage.*Strong histamine TS should be stored at a temperature not exceeding 4–10 °C, in dark glass bottles with ground-glass stoppers, protected from light.

*Shelf-life.*Do not use longer than 30 days.

Holmium oxide R

Ho_2O_3. Contains not less than 99.9% of Ho_2O_3, the impurities consisting of Er_2O_3 and Dy_2O_3.

*Description.*A tan-coloured powder.

*Solubility.*Insoluble in water.

Holmium perchlorate TS

*Procedure.*Dissolve 40 g of holmium oxide R in sufficient perchloric acid (~140 g/L) TS to produce 1000 mL.

Hydrazine hydrate R

N_2H_4,H_2O. Contains not less than 98.0% of N_2H_4,H_2O.

*Description.*A clear, colourless liquid.

*Miscibility.*Miscible with water.

*Residue on evaporation.*Evaporate to dryness on a water-bath; it leaves a residue of not more than 5.0 mg/g.

*Assay.*Dilute 1 g to 200 mL with water. Neutralize 20 mL of this solution with hydrochloric acid (~420 g/L) TS and add 10 mL in excess. Add 5 mL of potassium cyanide (100 g/L) TS, titrate with potassium iodate (0.05 mol/L) VS until the brown colour which first forms becomes pale, add starch TS and continue the titration until the blue colour disappears. Each mL of potassium iodate (0.05 mol/L) VS is equivalent to 2.503 mg of N_2H_4,H_2O.

Hydrazine sulfate R

$(NH_4)_2,H_2SO_4$.

*Description.*Colourless crystals or a white, crystalline powder.

*Solubility.*Soluble in about 40 parts of water; practically insoluble in ethanol (~750 g/L) TS.

Arsenic. Use a solution of 10 g in 35 mL of boiling water and proceed as described under *2.2.5 Limit test for arsenic*; not more than 1 µg/g.

Sulfated ash.Not more than 1.0 mg/g.

Hydriodic acid R1

HI. CAS Reg. No. 10034-85-2.

Procedure. Prepare by distilling hydriodic acid over red phosphorus, passing carbon dioxide R or nitrogen R through the apparatus during the distillation. Use the colourless or almost colourless, constant-boiling mixture (55 per cent to 58 per cent of HI) distilling between 126 °C and 127 °C.

Storage. Store at a dark place in small, amber glass-stoppered bottles previously flushed with carbon dioxide R or nitrogen R and sealed with paraffin.

Hydriodic acid (~970 g/L) TS

[hydriodic acid R] HI (SRIP, 1963, p. 95).

Hydrochloric acid (~0.365 g/L) TS

Hydrochloric acid (~250 g/L) TS, dilute with water to contain 0.365 g of HCl in 1000 mL.

Hydrochloric acid (~2.19 g/L) TS

Hydrochloric acid (~250 g/L) TS, dilute with water to contain 2.19 g of HCl in 1000 mL.

Hydrochloric acid (~3.65 g/L) TS

Hydrochloric acid (~250 g/L) TS, dilute with water to contain 3.65 g of HCl in 1000 mL.

Hydrochloric acid (~4 g/L) TS

*Procedure.*Dilute 10 mL of hydrochloric acid (~420 g/L) TS with sufficient water to produce 1000 mL (approximately 0.1 mol/L).

Hydrochloric acid (~10 g/L) TS

Hydrochloric acid (~250 g/L) TS, dilute with water to contain 10 g of HCl in 1000 mL.

Hydrochloric acid (~36.5 g/L) TS

Hydrochloric acid (~250 g/L) TS, dilute with water to contain 36.5 g of HCl in 1000 mL.

Hydrochloric acid (~70 g/L) TS

*Procedure.*Dilute 260 mL of hydrochloric acid (~250 g/L) TS with sufficient water to produce 1000 mL (approximately 2 mol/L); *d* ~1.035.

Hydrochloric acid (~103 g/L) TS

Hydrochloric acid (~420 g/L) TS, dilute with water to contain 103 g of HCl in 1000 mL.

Hydrochloric acid (~146 g/L) TS

*Procedure.*Dilute hydrochloric acid (~250 g/L) TS with water to contain approximately 146 g of HCl in 1000 mL (approximately 4 mol/L).

Hydrochloric acid (~200 g/L) TS

Procedure. Dilute hydrochloric acid (~250 g/L) TS with water to contain approximately 200 g of HCl in 1000 mL (approximately 5.5 mol/L).

Hydrochloric acid (~200 g/L) TS

Hydrochloric acid (~420 g/L) TS, dilute with water to contain 200 g of HCl in 1000 mL.

Hydrochloric acid (~206 g/L) TS

Hydrochloric acid (~420 g/L) TS, dilute with water to contain 206 g of HCl in 1000 mL.

Hydrochloric acid (~250 g/L) AsTS

Hydrochloric acid (~250 g/L) TS that complies with the following tests A and B:

A. Dilute 10 mL with sufficient water to produce 50 mL, add 5 mL of ammonium thiocyanate (75 g/L) TS and stir immediately; no colour is produced.

B. To 50 mL add 0.2 mL of bromine AsTS, evaporate in a water-bath until reduced to 16 mL, adding more bromine AsTS if necessary to ensure that an excess, as indicated by the colour, is present throughout the evaporation. Add 50 mL of water and 5 drops of stannous chloride AsTS and apply the general test for arsenic. The colour of the stain produced is not more intense than that produced from a 0.2 mL standard stain showing that the amount of arsenic does not exceed 0.05 µg/mL.

Hydrochloric acid (~250 g/L) FeTS

Hydrochloric acid (~250 g/L) TS that complies with the following additional test: evaporate 5 mL nearly to dryness on a water-bath, add 40 mL of water, 2 mL of citric acid (180 g/L) FeTS and 2 drops of mercaptoacetic acid R; mix, make alkaline with ammonia (~100 g/L) FeTS and dilute to 50 mL with water; no pink colour is produced.

Hydrochloric acid (~250 g/L), stannated, AsTS

*Procedure.*Dilute 1 mL of stannous chloride AsTS with sufficient hydrochloric acid (~250 g/L) AsTS to produce 100 mL.

Hydrochloric acid (~330 g/L) TS

A solution of hydrochloric acid (~420 g/L) TS in water containing approximately 330 g of HCl per litre; *d*~1.15 (about 9 mol/L).

Hydrochloric acid (~420 g/L) TS

[hydrochloric acid, saturated, R] (SRIP, 1963, p. 96); *d*~1.18.

Hydrochloric acid (0.0001 mol/L) VS

Hydrochloric acid (~250 g/L) TS diluted with water to contain 3.647 mg of HCl in 1000 mL.

*Method of standardization.*Ascertain the exact concentration of the solution following the method described under “Hydrochloric acid (1 mol/L) VS”.

Hydrochloric acid (0.005 mol/L) VS

Hydrochloric acid (~250 g/L) TS diluted with water to contain 0.1824 g of HCl in 1000 mL.

*Method of standardization.*Ascertain the exact concentration of the solution following the method described under hydrochloric acid (1 mol/L) VS.

Hydrochloric acid (0.01 mol/L) VS

Hydrochloric acid (~250 g/L) TS diluted with water to contain 0.3647 g of HCl in 1000 mL.

*Method of standardization.*Ascertain the exact concentration of the solution following the method described under hydrochloric acid (1 mol/L) VS.

Hydrochloric acid (0.015 mol/L) VS

Hydrochloric acid (~250 g/L) TS diluted with water to contain 0.5470 g of HCl in 1000 mL.

*Method of standardization.*Ascertain the exact concentration of the solution following the method described under hydrochloric acid (1 mol/L) VS.

Hydrochloric acid (0.02 mol/L) VS

Hydrochloric acid (~250 g/L) TS diluted with water to contain 0.7293 g of HCl in 1000 mL.

*Method of standardization.*Ascertain the exact concentration of the solution following the method described under hydrochloric acid (1 mol/l) VS.

Hydrochloric acid (0.05 mol/L) VS

Hydrochloric acid (~250 g/L) TS diluted with water to contain 1.824 g of HCl in 1000 mL.

*Method of standardization.*Ascertain the exact concentration of the solution following the method described under hydrochloric acid (1 molLl) VS.

Hydrochloric acid (0.1 mol/L) VS

Hydrochloric acid (~250 g/L) TS diluted with water to contain 3.647 g of HCl in 1000 mL.

*Method of standardization.*Ascertain the exact concentration of the solution following the method described under hydrochloric acid (1 mol/L) VS.

Hydrochloric acid (0.2 mol/L) VS

Hydrochloric acid (~250 g/L) TS diluted with water to contain 7.293 g of HCl in 1000 mL.

*Method of standardization.*Ascertain the exact concentration of the solution following the method described under hydrochloric acid (1 mol/L) VS.

Hydrochloric acid (0.5 mol/L) VS

Hydrochloric acid (~250 g/L) TS diluted with water to contain 18.23 g of HCl in 1000 mL.

*Method of standardization.*Ascertain the exact concentration of the solution following the method described under hydrochloric acid (1 mol/L) VS.

Hydrochloric acid (1 mol/L) VS

Hydrochloric acid (~250 g/L) TS diluted with water to contain 36.47 g of HCl in 1000 mL.

*Method of standardization.*Ascertain the exact concentration of the 1 mol/L solution in the following manner: dissolve about 1.5 g, accurately weighed, of anhydrous sodium carbonate R, previously dried at 270 °C for 1 hour, in 50 mL of water and titrate with the hydrochloric acid solution using methyl orange/ethanol TS as indicator. Each 52.99 mg of anhydrous sodium carbonate is equivalent to 1 mL of hydrochloric acid (1 mol/L) VS.

Hydrochloric acid (2 mol/L) VS

Hydrochloric acid (~250 g/L) TS diluted with water to contain 72.93 g of HCl in 1000 mL.

*Method of standardization.*Ascertain the exact concentration of the solution following the method described under hydrochloric acid (1 mol/L) VS.

Hydrochloric acid (5 mol/L) VS

Hydrochloric acid (~250 g/L) TS diluted with water to contain 182.35 g of HCl in 1000 mL.

*Method of standardization.*Ascertain the exact concentration of the solution following the method described under hydrochloric acid (1 mol/L) VS.

Hydrochloric acid (~250 g/L) TS

A solution of hydrochloric acid (~420 g/L) TS in water containing approximately 250 g/L of HCl; *d*~1.12.

Hydrochloric acid ClTS

One millilitre contains 50 µg of Cl.

*Procedure.*Dilute 14.3 mL of hydrochloric acid (0.1 mol/L) VS with sufficient water to produce 1000 mL.

Hydrochloric acid, brominated, AsTS

*Procedure.*To 100 mL of hydrochloric acid (~250 g/L) AsTS add 1 mL of bromine AsTS.

Hydrochloric acid/ethanol (1mol/L) VS

Hydrochloric acid (~250 g/L) TS diluted with dehydrated ethanol R to contain 36.47 g of HCl in 1000 mL of dehydrated ethanol R.

Method of standardization. Ascertain the exact concentration of the solution following the method described under hydrochloric acid (1 mol/L) VS.

Hydrochloric acid/ethanol (0.1 mol/L) VS

Hydrochloric acid (~250 g/L) TS diluted with dehydrated ethanol R to contain 3.647 g of HCl in 1000 mL of dehydrated ethanol R.

Method of standardization. Ascertain the exact concentration of the solution following the method described under hydrochloric acid (1 mol/L) VS.

Hydrochloric acid/methanol (0.01 mol/L) VS

Hydrochloric acid (~250 g/L) TS diluted with methanol R to contain 0.3647 g of HCl in 1000 mL of methanol R.

*Method of standardization.*Ascertain the exact concentration of the solution following the method described under hydrochloric acid (1 mol/L) VS.

Hydrochloric acid/methanol (0.1 mol/L) TS

Hydrochloric acid (~250 g/L) TS diluted with methanol R to contain 3.647 g of HCl in 1000 mL of methanol R.

Hydrocortisone R

$C_{21}H_{30}O_5$. Use Hydrocortisone as described in the monograph for *Hydrocortisone*.

Hydrogen peroxide (~30 g/L) TS

A solution in water containing about 30 g of H_2O_2 per litre.

Hydrogen peroxide (~330 g/L) TS

[hydrogen peroxide (30% R] (SRIP, 1963, p. 97).

Hydrogen peroxide (~60 g/L) TS

A solution in water containing about 60 g of H_2O_2 per litre.

Hydrogen sulfide R

H_2S (SRIP, 1963, p. 98).

Hydrogen sulfide TS

A saturated solution of hydrogen sulfide R in cold water.

Note:Hydrogen sulfide TS must be freshly prepared.

Hydroquinone R

$C_6H_4(OH)_2$.

*Description.*Colourless or almost colourless crystals or a crystalline powder.

*Solubility.*Soluble in water, ethanol (~750 g/L) TS and ether R.

*Melting temperature.*About 173 °C.

*Note.*Hydroquinone R darkens on exposure to air and light.

Hydroxyethylcellulose R

Contains not less than 20% of $C_2H_5O_2$, calculated with reference to the dried substance.

*Description.*A white or yellowish, flaky, heterogeneous mass; odourless.

*Solubility.*Practically insoluble in ethanol (~750 g/L) TS; after soaking for several hours in water, freely soluble in water.

*Colour of solution.*Transfer 2 g to a 200 mL glass-stoppered, conical flask, add 200 mL of carbon-dioxide-free water R, shake and allow to stand for 30 minutes. Repeat this operation until the substance has dissolved and filter through sintered glass. Observe 5 mL of the filtrate; it is colourless (keep the filtrate for the acidity or alkalinity test).

*Loss on drying.*To 1.0 g add 25 mL of water, stir and allow to stand. Repeat this operation until dissolved. Evaporate on a water-bath and dry to constant weight at 110 °C; it loses not more than 0.10 g/g. (Keep the dried substance for the assay.)

*Acidity or alkalinity.*To 10 mL of the filtrate obtained from the test for colour of solution add 2 drops of bromothymol blue/ethanol TS; a yellow colour is produced. Add 0.5 mL of potassium hydroxide (0.01 mol/L) VS; a green or blue solution is produced.

*Assay.*Place into a boiling flask as described under *2.9 Determination of methoxyl*, 0.5 mL of acetic anhydride R, 0.05–0.10 g of phenol R, 0.20 g of red phosphorus R and 5.0 mL of hydriodic acid (~970 g/L) TS; connect the flask to the condenser, pass a slow, uniform stream of carbon dioxide R through the solution and heat for 60 minutes. Cool for 10 minutes and add 0.035 g, accurately weighed, of the dried substance obtained in the test for loss on drying. Proceed with this mixture as described under *2.9 Determination of methoxyl*. For the calculation take an average of 3 determinations. Each mL of sodium thiosulfate (0.1 mol/L) VS is equivalent to 1.018 mg of $C_2H_5O_2$.

Hydroxyethylcellulose TS

*Procedure.*Place 50 mL of water in a 100 mL beaker and add 2.0 g of hydroxyethylcellulose R. After 15 hours stir the solution for 1 minute and centrifuge for 15 minutes. Using a pipette separate 20 mL of the supernatant liquid.

Note:Hydroxyethylcellulose TS must be freshly prepared.

Hydroxylamine hydrochloride (200 g/L) TS

A solution of hydroxylamine hydrochloride R containing about 200 g of NH_2OH,HCl per litre.

Hydroxylamine hydrochloride (70 g/L) TS

*Procedure.*Dissolve 69.5 g of hydroxylamine hydrochloride R in sufficient water to produce 1000 mL (1 mol/L).

Hydroxylamine hydrochloride R

NH_2OH,HCl (SRIP, 1963, p. 99).

Hydroxylamine hydrochloride TS

*Procedure.*Dissolve 1 g of hydroxylamine hydrochloride R in 50 mL of water and add 50 mL of ethanol (~750 g/L) TS and 1 mL of bromophenol blue/ethanol TS; then add sodium hydroxide (0.1 mol/L) VS until the solution becomes green.

Hydroxylamine hydrochloride TS2

Procedure. Dissolve 3.5 g of hydroxylamine hydrochloride R in 95 mL of ethanol (~535 g/L) TS, add 0.5 mL of bromophenol blue (1 g/L) TS and sufficient potassium hydroxide/ethanol (0.5 mol/L) TS until a greenish tint is developed.

Dilute the solution to 100 mL with ethanol (~535 g/L) TS.

8-Hydroxyquinoline R

8-Quinolinol; C_9H_7NO.

*Description.*A white to yellowish white, crystalline powder.

*Solubility.*Practically insoluble in water and ether R; freely soluble in ethanol (~750 g/L) TS and acetone R.

*Melting point.*About 74 °C.

8-Hydroxyquinoline/chloroform TS

*Procedure.*Dissolve 1 g of 8-hydroxyquinoline R in sufficient chloroform R to produce 100 mL.

Hypophosphorous acid R

Phosphinic acid; H_3PO_2 (SRIP, 1963, p. 100).

Hypophosphorous acid, dilute, TS

A solution of hypophosphorous acid R containing about 100 g of H_3PO_2 per 1000 mL.

Hypoxanthine R

1,7-dihydro-6*H*-purin-6-one; $C_5H_4N_4O$.

A commercially available reagent of suitable grade.

Description. A white, crystalline powder.

Solubility. Very slightly soluble in water, sparingly soluble in boiling water, soluble in dilute acids and in dilute alkali hydroxide solutions.

Melting point. Decomposes without melting at about 150 °C.

Thin-Layer Chromatography. Examine as prescribed in the monograph on *Mercaptopurine*; the chromatogram shows only one principal spot.

G - Gelatin R.... Guanine R

Gelatin R

Gelatin of suitable purity.

Gelatin TS

A solution of gelatin R dissolved in phosphate buffer, pH 7.0, TS containing about 10 g/L.

Glucose hydrate R

Monohydrate of α-D-glucopyranose, $C_6H_{12}O_6,H_2O$. Contains not less than 99.0% and not more than 101.5% of $C_6H_{12}O_6$, calculated with reference to the dried substance.

Description. Colourless crystals or a white crystalline or granular powder; odourless.

Solubility. Soluble in about 1 part of water and in about 60 parts of ethanol (~750 g/L) TS; more soluble in boiling water and in boiling ethanol (~750 g/L) TS.

Acidity. Dissolve 5 g in 50 mL of carbon-dioxide-free water R. It requires for neutralization not more than 0.5 mL of carbonate-free sodium hydroxide (0.02 mol/L) VS, phenolphthalein/ethanol TS being used as indicator.

Specific optical rotation. Dissolve 100 mg, previously dried to constant weight, in 1 mL of water, and add a few drops of ammonia (~100 g/L) TS; $[\alpha]_D^{20\,°C}$ = +52° to +53°.

Soluble starch or sulfites. Dissolve 1 g in 10 mL of water and add 1 drop of iodine TS; the liquid is coloured yellow.

Loss on drying. Dry to constant weight at 105 °C; it loses not less than 80 mg/g and not more than 100 mg/g.

Sulfated ash. Not more than 1.0 mg/g.

Assay. Dissolve about 0.1 g, accurately weighed, in 50 mL of water, add 30 mL of iodine (0.1 mol/L) VS, 10 mL of sodium carbonate (50 g/L) TS and allow to stand for 20 minutes. Add 15 mL of hydrochloric acid (~70 g/L) TS and titrate the excess of iodine with sodium thiosulfate (0.1 mol/L) VS using starch TS as indicator. Perform a blank determination and make any necessary corrections. Each mL of iodine (0.1 mol/L) VS is equivalent to 9.008 mg of $C_6H_{12}O_6$.

Glucose, anhydrous, R

$C_6H_{12}O_6$. Use anhydrous glucose as described in the monograph for *Glucose*.

Glycerol R

Propane-1,2,3-triol with small amounts of water, $C_3H_8O_3$. Contains not less than 970 g/kg of $C_3H_8O_3$.

Description. A clear, almost colourless, syrupy and hygroscopic liquid; odourless.

Miscibility. Miscible with water and ethanol (~750 g/L) TS; practically immiscible with ether R.

Mass density (ρ_{20}). Not less than 1.256 kg/L.

Refractive index (n_D^{20}). Not less than 1.469.

Acrolein and other reducing substances. Mix 1 mL with 1 mL of ammonia (~100 g/L) TS and heat in a water-bath at 60 °C for 5 minutes; the liquid is not coloured yellow. Remove from the water-bath and add 3 drops of silver nitrate (40 g/L) TS; the liquid does not become coloured within 5 minutes.

Sulfated ash. Not more than 0.5 mg/mL.

Glycine R

Aminoacetic acid; $C_2H_5NO_2$.

Description. A white, crystalline powder.

Solubility. Very soluble in water; slightly soluble in ethanol (~750 g/L) TS.

Assay. Determine the nitrogen by the Kjeldahl method in the test substance previously dried at 105 °C for 2 hours; between 18.4 and 18.8% of N is found, corresponding to not less than 98.6% and not more than the equivalent of 100.8% of $C_2H_5NO_2$.

Insoluble matter. 10g shows not more than 1.0 mg of insoluble matter (0.1 mg/g).

Sulfated ash. Not more than 0.5 mg/g.

Chlorides. Not more than 0.1 mg of Cl/g.

Sulfates. Not more than 0.05mg of SO_4/g.

Heavy metals. Not more than 0.02 mg/g.

Iron. Not more than 0.01 mg of Fe/g, 3 mL of hydrochloric acid (~420 g/L) TS being used to facilitate solution.

Glycolic acid R

$C_2H_4O_3$. 2-Hydroxyacetic acid. CAS Reg. No. 79-14-1.

Description. Crystals.

Solubility. Soluble in water, in acetone, in ethanol (~750 g/L) TS and in methanol.

Melting point. About 80 °C.

Glyoxal bis(2-hydroxyanil) R

2,2'-(Ethanediylidenedinitrilo)diphenol, $C_{14}H_{12}N_2O_2$.

Description. White crystals.

Solubility. Soluble in hot ethanol (~750 g/L) TS.

Melting temperature. 203–205 °C.

Glyoxal bis(2-hydroxyanil) TS

A solution of glyoxal bis(2-hydroxyanil) R dissolved in ethanol (~750 g/L) TS containing about 10 g/L of $C_{14}H_{12}N_2O_2$.

Green stock standard TS

Procedure. To 3.5 mL of cobalt colour TS add 20.1 mL of copper colour TS, 10.4 mL of dichromate colour TS and 4.0 mL of iron colour TS; dilute to 100.0 mL with sulfuric acid (~10 g/L) TS and mix.

Guanine R

$C_5H_5N_5O$, 2-Amino-1,7-dihydro-6H-purin-6-one.

Amorphous white or almost white powder, practically insoluble in water, slightly soluble in ethanol (96 per cent). It dissolves in ammonia and in dilute solutions of alkali hydroxides.

F - Ferric ammonium sulfate (0.1 mol/L) VS.... Fuchsin/sulfurous acid TS

Ferric ammonium sulfate (0.1 mol/L) VS

Ferric ammonium sulfate R dissolved in a mixture of sulfuric acid (~1760 g/L) TS and water to contain 48.22 g of $FeNH_4(SO_4)_2$,$12H_2O$ in 1000 mL.

Procedure. Dissolve 50 g of ferric ammonium sulfate R in a mixture of 300 mL of water and 6 mL of sulfuric acid (~1760 g/L) TS. Dilute with sufficient water to produce 1000 mL.

Method of standardization. Ascertain the exact concentration of the 0.1 mol/L solution in the following manner: transfer 25 mL to a glass-stoppered flask and add 3 mL of hydrochloric acid (~420 g/L) TS and 2 g of potassium iodide R. Allow the solution to stand for 10 minutes and titrate the liberated iodine with sodium thiosulfate (0.1 mol/L) VS using starch TS as indicator. Perform a blank determination and make any necessary corrections.

Storage. Store this solution in a tightly closed container, protected from light.

Ferric ammonium sulfate (45 g/L) TS

A solution of ferric ammonium sulfate R containing about 45 g/L of $FeNH_4(SO_4)_2$.

Ferric ammonium sulfate R

$FeNH_4(SO_4)_2,12H_2O$ (SRIP, 1963, p. 88).

Ferric ammonium sulfate TS1

Procedure. Dissolve 0.2 g of ferric ammonium sulfate R in 50 mL of water, add 6 mL of nitric acid (~1000 g/L) TS and sufficient water to produce 100 mL.

Ferric ammonium sulfate TS2

Procedure. Dissolve 8.3 g of ferric ammonium sulfate R in sufficient sulfuric acid (0.25 mol/L) VS to produce 1000 mL.

Ferric chloride (25 g/L) TS

A solution of ferric chloride R containing about 27 g/L of $FeCl_3$.

Ferric chloride (50 g/L) TS

A solution of ferric chloride R containing about 50 g of $FeCl_3$ per litre.

Ferric chloride (63 g/L) TS

A solution of ferric chloride R containing about 63 g of FeCl3 per litre.

Ferric chloride (65 g/L) TS

A solution of ferric chloride R containing about 65 g of $FeCl_3$ per litre.

Ferric chloride R

$FeCl_3,6H_2O$ (SRIP, 1963, p. 88).

Ferric chloride/ferricyanide/arsenite TS

Procedure. Prepare 3 separate solutions:

(1) Dissolve 2.7 g of ferric chloride R in 100 mL of hydrochloric acid (~70 g/L) TS.

(2) Dissolve 3.5 g of potassium ferricyanide R in 100 mL of water. This solution should be freshly prepared.

(3) Dissolve 3.8 g of arsenic trioxide R in 25 mL of hot sodium hydroxide (~80 g/L) TS. Allow to cool, add 50 mL of sulfuric acid (~100 g/L) TS and dilute to 100 mL with water.

Immediately before use mix 5 volumes of solution (1), 5 volumes of solution (2) and 1 volume of solution (3).

Ferric chloride/potassium ferricyanide TS

Procedure. Dissolve 2 g of ferric chloride R and 0.10 g of potassium ferricyanide R in sufficient water to produce 20 mL.

Note. Ferric chloride/potassium ferricyanide TS must be freshly prepared.

Ferric chloride/potassium ferricyanide TS1

Procedure. Dissolve 2 g of ferric chloride R and 0.5 g of potassium ferricyanide R in sufficient water to produce 20 mL.

Note. Ferric chloride/potassium ferricyanide TS2 must be freshly prepared.

Ferricyanide standard (50 µg/mL) TS

Procedure. Prepare a solution of potassium ferricyanide R in water to contain 7.8 g of $K_3Fe(CN)_6$ per 100 mL. Dilute 1.0 mL of this solution with sufficient water to produce 1000 mL.

Note. Ferricyanide standard (50 µg/mL) TS must be freshly prepared.

Ferrocyanide standard (100 µg/mL) TS

Procedure. Prepare a solution of potassium ferrocyanide R in water to contain 2.0 g of $K_4Fe(CN)_6,3H_2O$ per 100 mL. Dilute 1.0 mL of this solution with sufficient water to produce 100.0 mL.

Note. Ferrocyanide standard (100 µg/mL) TS must be freshly prepared.

Ferroin TS

Procedure. Dissolve 0.15 g of *o*-phenanthroline R in 10 mL of a solution of ferrous sulfate R prepared by dissolving 0.70 g of clear crystals of ferrous sulfate R in 100 mL of water.

Note: The ferrous sulfate solution must be prepared immediately before dissolving the *o*-phenanthroline.

Storage. Ferroin TS should be stored in well-closed containers.

Ferrous ammonium sulfate (0.1 mol/L) VS

Procedure. Dissolve 40 g of ferrous ammonium sulfate R in 100 mL of sulfuric acid (~190 g/L) TS and dilute to 1000 mL with carbon-dioxide-free water R.

Method of standardization. Ascertain the exact concentration of the 0.1 mol/L solution in the following manner: to 25 mL add 10 mL of sulfuric acid (~100 g/L) TS and 1 mL of phosphoric acid (~1440 g/L) TS and titrate with potassium permanganate (0.02 mol/L) VS. Each mL of potassium permanganate (0.02 mol/L) VS is equivalent to 39.21 mg of $(NH_4)_2Fe(SO_4)_2$.

Ferrous ammonium sulfate (1 g/L) TS

A solution of ferrous ammonium sulfate R containing about 1 g/L of $Fe(NH_4)_2(SO_4)_2$.

Ferrous ammonium sulfate R

$Fe(NH_4)_2(SO_4)_2,6H_2O$ (SRIP, 1963, p. 89).

Ferrous sulfate (0.1 mol/L) VS

Procedure. Dissolve 2.8 g of ferrous sulfate R in 90 mL of freshly boiled and cooled water and add a sufficient quantity of sulfuric acid (~1760 g/L) TS to produce 100 mL.

Method of standardization. Ascertain the exact concentration of the 0.1 mol/L solution in the following manner: to 40.0 mL of the ferrous sulfate solution add 5 mL of phosphoric acid (~1.440 g/L) TS and titrate immediately with potassium permanganate (0.02 mol/L) VS.

Note: Standardize immediately before use.

Ferrous sulfate (15 g/L) TS

A solution of ferrous sulfate R in freshly boiled and cooled water containing about 15 g/L of $FeSO_4$ (approximately 0.1 mol/L).

Note: Ferrous sulfate (15 g/L) TS must be freshly prepared.

Ferrous sulfate (7 g/L) TS

A solution of ferrous sulfate R in freshly boiled and cooled water containing about 7 g of $FeSO_4$ per litre.

Note: Ferrous sulfate (7 g/L) TS must be freshly prepared.

Ferrous sulfate R

$FeSO_4,7H_2O$ (SRIP, 1963, p. 90).

Ferrous sulfate/hydrochloric acid TS

Procedure. Dissolve 0.45 g of ferrous sulfate R in 50 mL of hydrochloric acid (0.1 mol/L) VS and dilute with sufficient carbon-dioxide-free water R to produce 100 mL.

Note : Ferrous sulfate/hydrochloric acid TS should be prepared immediately before use.

Firebrick, pink, R

A suitable grade for use in gas chromatography with an average particle size of about 180–250 µm.

Fluorescein sodium TS

A 2 g/L solution of fluorescein sodium R in water R.

Fluorescein sodium (2 g/L) R

Use fluorescein sodium as described in the monograph for *Fluorescein sodium.*

2-Fluoro-2-deoxy-D-glucose R

$C_6H_{11}FO_5$.

Description. A white crystalline powder.

A commercially available reagent of suitable grade.

Melting point. 174 °C to 176 °C.

Formaldehyde TS

[formaldehyde R]. (SRIP, 1963, p. 91).

Formaldehyde/sulfuric acid TS

Procedure. To 10 mL of sulfuric acid (~1760 g/L) TS add 0.2 mL of formaldehyde TS.

Shelf-life. Use within 1 month after preparation.

Formamide R

CH_3NO (SRIP, 1963, p. 92).

Formic acid (~1080 g/L) TS

[formic acid R]. CH_2O_2 (SRIP, 1963, p. 92) *d* ~ 1.2.

Formic acid, anhydrous, R

CH_2O_2, *d* ~ 1.22. Contains not less than 98.0% of CH_2O_2.

Description. A colourless liquid; odour, pungent.

Miscibility. Miscible with water and ethanol (~750 g/L) TS.

Chlorides. Dilute 1 mL to 15 mL with water and proceed as described under *2.2.1 Limit test for chlorides*. Anhydrous formic acid R contains not more than 0.50 mg/g.

Sulfates. Dilute 0.5 mL to 15 mL with water and proceed as described under *2.2.2 Limit test for sulfates*. Anhydrous formic acid R contains not more than 1.5 mg/g.

Residue on evaporation. Evaporate on a water-bath and dry to constant weight at 105 °C; leaves not more than 0.5 mg/g of residue.

Assay. To a tared flask containing about 10 mL of water, quickly add about 1 mL of the test liquid and weigh. Dilute with 50 mL of water and titrate with carbonate-free sodium hydroxide (1 mol/L) VS using phenolphthalein/ethanol TS as indicator. Each mL of carbonate-free sodium hydroxide (1 mol/L) VS is equivalent to 46.03 mg of CH_2O_2.

Fuchsin TS

Procedure. Pour carefully 40 mL of sulfuric acid (~1760 g/L) TS into 60 mL of water. Allow to cool and add 100 mL of a 1 g/L solution of basic fuchsin R. Dilute with water to 200 mL and allow to stand. An orange-yellow colour develops. Immediately before use dilute the solution with an equal volume of glacial acetic acid R.

Fuchsin, basic, R

[magenta, basic R]. A mixture of rosaniline hydrochloride, $(H_2NC_6H_4)_2C{:}C_6H_3(CH_3){:}NH_2^+Cl^-$ and pararosaniline hydrochloride, $(H_2NC_6H_4)_2C{:}C_6H_4{:}NH_2^+Cl^-$.

Description. Crystals or crystalline fragments, with a glossy, greenish-bronze lustre.

Solubility. Soluble in water, ethanol (~750 g/L) TS and amyl alcohol R.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 0.10 g/g.

Sulfated ash. Ignite 1 g with 0.5 mL of sulfuric acid (~1760 g/L) TS: not more than 3.0 mg/g.

Fuchsin, decolorized, TS

Procedure. Dissolve 1 g of basic fuchsin R in 600 mL of water and cool in an ice-bath; add 20 g of sodium sulfite R dissolved in 100 mL of water; cool in an ice-bath and add slowly, with constant stirring, 10 mL of hydrochloric acid (~250 g/L) TS; dilute with water to 1000 mL. If the resulting solution is turbid it should be filtered and, if brown in colour, it should be shaken with sufficient charcoal R (0.2–0.3 g) to render it colourless and then filtered immediately. Occasionally it is necessary to add 2–3 mL of hydrochloric acid (~250 g/L) TS, followed by shaking, to remove a little residual pink colour. The solution resulting from any of the foregoing modifications should be allowed to stand overnight before use. Decolorized fuchsin TS should be protected from light.

Fuchsin/sulfurous acid TS

Procedure. Dissolve 0.10 g of fuchsin, basic, R in 50 mL of water with gentle heating. To the cooled solution add 20 mL of sodium metabisulfite (50 g/L) TS and 1 mL of hydrochloric acid (~420 g/L) TS. Dilute to 100 mL with water, mix and allow to stand in the dark for 2 hours. Fuchsin/sulfurous acid TS should be colourless and should not be used for a period longer than 24 hours.

E - Endotoxin RS.... 1-Ethylquinaldinium iodide (15 g/L) TS

Endotoxin RS

Second WHO International Standard for Endotoxin as established in 1996, containing 10 000 IU per ampoule, approximately 1 µg of freeze-dried endotoxin from *Escherichia coli*, with 1 mg of PEG and 10 mg of lactose (distributed by the National Institute for Biological Standards and Control (NIBSC), PO Box 1193, Blanche Lane, South Mimms, Potters Bar, Herts EN6 3QH, England) or another suitable preparation, the activity of which has been determined in relation to the WHO International Standard using the gelation test.

Eosin Y (5 g/L) TS

A solution of eosin Y R containing about 5 g of $C_{20}H_6Br_4Na_2O_5$ per litre.

Eosin YR

Sodium tetrabromofluorescein; $C_{20}H_6Br_4N_2O_5$.

Description. Red to brownish lumps or a powder.

Solubility. Freely soluble in water; sparingly soluble in ethanol (~750 g/L) TS.

Epinephrine hydrogen tartrate R

Epinephrine hydrogen tartrate R as described in the monograph for *Epinephrine hydrogen tartrate*, which complies with the following test for the absence of levarterenol:

Levarterenol. Carry out descending *1.14.2 Paper chromatography*. Mix 4 volumes of 1-butanol R, 1 volume of glacial acetic acid R and 5 volumes of water, shake and allow the two layers to separate. Use the lower layer as the stationary phase and the upper layer as the mobile phase. Apply to the paper 20 µL of a solution containing 50 mg/mL of epinephrine hydrogen tartrate R, develop for 5 hours, dry the paper and spray with a freshly prepared 4.4 mg/mL solution of potassium ferricyanide R dissolved in buffer borate, pH 8.0, TS or another buffer having the same pH may be used; only 1 spot appears, which is pink.

Ergosterol R

Provitamin D_2; ergosta-5,7,22-trien-3-ol; $C_{28}H_{44}O$. Contains not less than 95.0% of $C_{28}H_{44}O$.

Description. White or almost white needles or a crystalline powder.

Melting temperature. About 163 °C.

Specific optical rotation. Use a 20 mg/mL solution in chloroform R; $[\alpha]_D^{20\,°C}$ = -133°.

Estradiol hemihydrate R

Estradiol hemihydrate of a suitable quality should be used.

Ethanol (~150 g/L) TS

A solution of about 200 mL of ethanol (~750 g/L) TS diluted with water to 1000 mL.

Ethanol (~375 g/L) TS

A solution of about 500 mL of ethanol (~750 g/L) TS diluted with water to 1000 mL.

Ethanol (~457 g/L) TS

Procedure. Dilute 609 mL of ethanol (~750 g/L) TS with sufficient water to produce 1000 mL.

Ethanol (~535 g/L) TS

Procedure. Dilute 713 mL of ethanol (~750 g/L) TS with sufficient water to produce 1000 mL.

Ethanol (~600 g/L) TS

A solution of about 800 mL of ethanol (~750 g/L) TS diluted with water to 1000 mL.

Ethanol (~675 g/L) TS

A solution of about 900 mL of ethanol (~750 g/L) TS diluted with water to 1000 mL.

Ethanol (~710 g/L) TS.

A solution of about 947 mL of ethanol (~750 g/L) TS diluted with water to 1000 mL.

Ethanol (~750 g/L) TS

[ethanol (95%) R] (SRIP, 1963, p. 84).

Ethanol (~750 g/L), aldehyde-free TS

[ethanol, aldehyde-free, (95%R]. (SRIP, 1963, p. 84).

Ethanol (~750 g/L), sulfate-free, TS

Ethanol (~750 g/L) TS that complies with the following test: evaporate 25 mL of ethanol (~750 g/L) TS to a volume of about 2 mL, add a mixture of 3 mL of hydrochloric acid (~70 g/L) TS and 42 mL of water and 5 mL of barium sulfate suspension TS. Proceed as described in *2.2.2 Limit test for sulfates*. Sulfate-free ethanol (~750 g/L) TS contains not more than 20 µg/mL.

Ethanol (80% v/v) TS

Procedure. Dilute 831 mL of ethanol (~750 g/L) TS to 1000 mL with water R.

Ethanol, dehydrated, R

C_2H_5OH (SRIP, 1963, p. 85).

Ethanol/methanol (95/5) TS

Procedure. To 5 mL of methanol R add 95 mL of dehydrated ethanol R.

Ethanol, neutralized, TS

Procedure. To a suitable quantity of ethanol (~750 g/L) TS add 0.5 mL of phenolphthalein/ethanol TS and just sufficient carbonate-free sodium hydroxide (0.02 mol/L) VS or (0.1 mol/L) VS to produce a faint pink colour.

Note: Prepare neutralized ethanol TS just prior to use.

Ether R

$C_4H_{10}O$ (SRIP, 1963, p. 85).

Ether, peroxide-free, R

Procedure. To 1000 mL of ether R add 20 mL of a solution of 30 g of ferrous sulfate R in 55 mL of water and shake the mixture with 3 mL of sulfuric acid (~1760 g/L) TS. Continue shaking until a small sample no longer produces a blue colour when shaken with an equal volume of a 20 g/L solution of potassium iodide R and 0.1 mL of starch TS.

Ethinylestradiol R

Ethinylestradiol of a suitable quality should be used.

Ethionamide R

Ethionamide of a suitable quality should be used.

Ethyl acetate R

$C_4H_8O_2$ (SRIP, 1963, p. 86).

Ethyl iodide R

C_2H_5I (SRIP, 1963, p. 87).

Ethylene glycol monoethyl ether R

$C_4H_{10}O_2$.

Description. A clear, colourless liquid.

Miscibility. Miscible with water, ethanol (~750 g/L) TS, ether R and acetone R.

Boiling range. Not less than 95% distils at between 133 and 135 °C.

Mass density (ρ_{20}). About 0.93 kg/L.

Ethylene glycol monomethyl ether R

2-Methoxyethanol; $C_3H_8O_2$.

Description. A colourless liquid.

Boiling temperature. About 125 °C.

Mass density. ρ_{20} = about 0.96 kg/L.

Ethylene oxide R

C_2H_4O.

A commercially available gas of suitable grade.

Ethylene oxide TS

Procedure. Weigh 1.0 g of cold ethylene oxide stock solution R (equivalent to 2.5 mg of ethylene oxide) into a cold flask containing 40 g of cold macrogol 200 TS. Mix and determine the exact mass and dilute to a calculated mass to obtain a solution containing 50 µg of ethylene oxide per 1.0 g of solution. Weigh 10.0 g into a flask and dilute with sufficient water to produce 50 mL (10 µg/mL of ethylene oxide). Dilute 10 mL of this solution to 50 mL with water (2 µg/mL of ethylene oxide).

Note : Ethylene oxide TS should be prepared immediately before use.

Ethylene oxide stock solution R

Note : *All operations should be carried out in a fume-hood. The operator must protect both hands and face by wearing polyethylene protective gloves and an appropriate face mask*.

Procedure. Into a dry, clean test-tube, cooled in a mixture of 1 part of sodium chloride R and 3 parts of crushed ice, introduce a slow current of ethylene oxide R gas, allowing condensation onto the inner wall of the test-tube. Using a glass syringe, previously cooled to -10 °C, inject about 300 µL (corresponding to about 0.25 g) of liquid ethylene oxide R into 50 mL of macrogol 200 TS. Determine the absorbed quantity of ethylene oxide by weighing before and after absorption. Dilute to 100 mL with macrogol 200 TS. Mix well before use.

Assay. To 10 mL of a 500 g/L suspension of magnesium chloride R in dehydrated ethanol R add 20 mL of hydrochloric acid/ethanol (0.1 mol/L) VS, stopper the flask, shake to obtain a saturated solution and allow to stand overnight to equilibrate. Weigh 5 g of the prepared ethylene oxide stock solution R (containing about 2.5 g/L) into the flask and allow to stand for 30 minutes. Titrate with potassium hydroxide/ethanol (0.1 mol/L) VS, determining the end-point potentiometrically. Carry out a blank titration, replacing the substance to be examined with the same quantity of macrogol 200 TS. Calculate the content of ethylene oxide in mg/g.

Storage. Keep in a tightly closed container in a refrigerator at 4 °C.

Ethylenediamine R

$C_2H_8N_2$.

Description. A colourless to pale yellow, clear liquid; odour, ammonia-like.

Miscibility. Miscible with water and ethanol (~750 g/L) TS; slightly miscible with ether R.

Boiling temperature. About 116 °C.

Mass density. ρ_{20} = about 0.898 kg/L.

Storage. Store in a tightly closed container, protected from air and acidic vapours.

2-Ethylhexanoic acid R

$C_8H_{16}O_2$

Molecular weight. 144.2.

*Description.*Colourless liquid.

Relative density d_{20}^{20}. About 0.91.

Related substances. Carry out the test as described under *1.14.1 Chromatography*, Gas chromatography using the conditions given in the test for 2-ethylhexanoic acid in the monograph on Potassium clavulanate. Prepare the following solution: suspend 0.2 g of 2-ethylhexanoic acid in 5 mL of water R, add 3 mL of 33% (V/V) solution of hydrochloric acid R and 5 mL of hexane R, shake for 1 minute, allow the layers to separate and use the upper layer. Inject 1 µL of this solution. The sum of the area of any peaks,

other than the principal peak and the peak due to the solvent, is not greater than 2.5% of the area of the principal peak

Ethylmethylketone R

C_4H_8O.

Description. A clear, colourless, mobile liquid; odour, characteristic.

Miscibility. Miscible with water, ethanol (~750 g/L) TS and ether R.

Boiling range. 79–80 °C.

Mass density. ρ_{20} = about 0.805 kg/L.

1-Ethylquinaldinium iodide R

1-Ethyl-2-methylquinolinium iodide; $C_{12}H_{14}IN$.

A commercially available reagent of suitable grade.

Description. A yellow-green solid.

Solubility. Sparingly soluble in water.

1-Ethylquinaldinium iodide (15 g/L) TS

A solution containing about 15 g of 1-ethylquinaldinium iodide R per litre.

C - Cadmium R.... Cytosine R

Cadmium R

Cd

A commercially available reagent of suitable grade.

Cadmium standard (1000 µg Cd/mL) TS

Procedure. Dissolve 0.100 g of cadmium R in sufficient amount of equal volumes of hydrochloric acid (~330 g/L) TS and water R and dilute to 100 mL with a 1% V/V solution of hydrochloric acid (~330 g/L) TS.

Note. For the preparation of this test solution commercially available cadmium standard solution 1000 µg Cd/mL can also be used.

Cadmium acetate R

$(CH_3CO_2)_2Cd,2H_2O$. Contains not less than 98.0% of $(CH_3CO_2)_2Cd,2H_2O$.

Description. Colourless crystals.

Solubility. Soluble in water.

Assay. Dissolve 1 g, accurately weighed, in 50 mL of water, add 25 mL of ammonia (~260 g/L) TS and titrate with disodium edetate (0.1 mol/L) VS, using methylthymol blue mixture R as indicator, until the blue solution becomes colourless or grey. Each mL of disodium edetate (0.1 mol/L) VS is equivalent to 26.65 mg of $(CH_3CO_2)_2Cd,2H_2O$.

Caesium chloride R

CsCl.

A commercially available reagent of suitable grade.

Calcium acetate (0.25 mol/L) VS

Calcium acetate R, dissolved in water to contain 44.04 g of $Ca(C_2H_3O_2)_2,H_2O$ in 1000 mL.

Calcium acetate R

$Ca(C_2H_3O_2)_2,H_2O$ (SRIP, 1963, p. 56).

Calcium carbonate R1

$CaCO_3$ (SRIP, 1963, p. 56).

Calcium carbonate R2

$CaCO_3$. Calcium carbonate R1 of suitable quality to serve as a primary standard for the standardization of disodium edetate.

Calcium chloride (3.7g/L) TS

A solution of anhydrous calcum chloride R containing about 9 g of $CaCl_2$ per litre.

Calcium chloride (55 g/L) TS

A solution of hydrated calcium chloride R containing about 55 g/L of $CaCl_2$ (approximately 0.5 mol/L).

Calcium chloride, anhydrous, R

[calcium chloride R] $CaCl_2$ (SRIP, 1963, p. 58).

Calcium chloride, hydrated, R

$CaCl_2,6H_2O$ (SRIP, 1963, p. 58).

Calcium fluoride R

CaF_2.

Description. A white powder.

Solubility. Practically insoluble in water; slightly soluble in dilute acids.

Calcium gluconate R

$C_{12}H_{22}CaO_{14},H_2O$.

A commercially available reagent of suitable grade.

Calcium hydroxide R

$Ca(OH)_2$ (SRIP, 1963, p. 59).

Calcium hydroxide TS

Procedure. Prepare a saturated solution of calcium hydroxide R.

Note: Calcium hydroxide TS must be freshly prepared.

Calcium standard (10 µg/mL Ca) TS

Procedure. Dissolve 2.50 g of dried calcium carbonate R2 in 15 mL of acetic acid (~300 g/L) TS and dilute with water to 1000 mL (solution A). Dilute 10.0 mL of this solution with water to produce 1000 mL.

Calcium standard (100 µg/mL Ca), ethanolic, TS

Procedure. Dilute 100.0 mL of solution A, described under calcium standard (10 µg/mL Ca) TS, with sufficient ethanol (~750 g/L) TS to produce 1000 mL.

Calcium sulfate R

$CaSO_4,2H_2O$ (SRIP, 1963, p. 62).

Calcium sulfate TS

Procedure. Shake 5 g of calcium sulfate hemihydrate R for 1 hour with 100 mL of water and filter.

Calcium sulfate, hemihydrate R

Plaster of Paris, $CaSO_4, \frac{1}{2} H_2O$.

Description. A white powder which, when mixed with half its weight of water, rapidly solidifies to a hard and porous mass.

Calcon carboxylic acid indicator mixture R

Procedure. Mix 0.1 g of calcon carboxylic acid R with 10 g of anhydrous sodium sulfate R.

Calcon carboxylic acid R

2-Hydroxy-1-(2-hydroxy-4-sulfo-1-naphthyl-azo)-3-naphthoic acid; $C_{21}H_{14}N_2O_7S,3H_2O$.

Description. A dark-brown powder with a violet tint.

Solubility. Practically insoluble in water; slightly soluble in methanol R and in ethanol (~750 g/L) TS; freely soluble in solutions of alkali hydroxides.

Calcon indicator mixture R

Procedure. Mix 0.1 g of calcon R with 10 g of anhydrous sodium sulfate R.

Calcon R

Monosodium salt of 2-hydroxy-1-[(2-hydroxy-1-naphthyl)-azo]naphthalene-4-sulfonic acid; C.I. Mordant Black 17, C.I. No. 15705, Eriochrome Blue Black R, Solochrome Dark Blue; $C_{20}H_{13}N_2NaO_5S$.

Carbazole R

Dibenzopyrrole; $C_{12}H_9N$.

A commercially available reagent of suitable grade.

Melting point. about 245 °C.

Carbomer R

Carbomer suitable for thin-layer chromatography. A high relative molecular mass cross-linked polymer of acrylic acid; it contains a large proportion (56-68%) of carboxylic acid (-COOH) groups after drying at 80 °C for 1 hour.

pH value. The pH of a 10 g/L suspension is about 3.

Viscosity. Whilst stirring continuously prepare a suspension containing 2.5 g in 500 mL of water. Maintain at 25 ± 0.2 °C for 30 minutes then add 0.2 mL of phenolphthalein/ethanol TS, 1 mL of bromothymol blue/ethanol TS and, whilst stirring, neutralize using a mixture of equal volumes of sodium hydroxide (~400 g/L) TS and water until a uniform blue colour is obtained (check the pH which must be 7.3–7.8). The dynamic viscosity of the neutralized preparation is 30–40 Pa s (300–400 poise).

Carbon dioxide detector tube

A cylindrical, sealed glass tube containing adsorbent filters and suitable supports for hydrazine and crystal violet indicators. The minimum value indicated is 100 µl/L or less, with a relative standard deviation of at most ±15%. Tubes can be verified with a calibration gas containing the appropriate impurity if a negative result is obtained.

Carbon dioxide R

CO_2.

Description. A colourless gas; odourless.

Solubility. Soluble in about 1.3 parts by volume of water.

Carbon disulfide IR

Carbon disulfide R that complies with the following test: The infrared absorption spectrum of a 1.0 mm layer, as described in method 4 under *1.7 Spectrophotometry in the infrared region* and examined over the range 4000–670 cm^{-1} shows an absorbance of less than 0.1 in the regions 4000–3030 cm^{-1}, 2635–2440 cm^{-1}, 2000–1755 cm^{-1}, and 1265–935 cm^{-1}, and an absorbance of less than 0.17 in the region 800–715 cm^{-1}.

Carbon disulfide R

CS_2 (SRIP, 1963, p. 62).

Carbon monoxide detector tube

A cylindrical, sealed glass tube containing adsorbent filters and suitable supports for di-iodine pentoxide, selenium dioxide and fuming sulfuric acid indicators. The minimum value indicated is 5 µl/L or less with a relative standard deviation of at most ±15%. Tubes can be verified with a calibration gas containing the appropriate impurity if a negative result is obtained.

Carbon monoxide R

CO.

A commercially available gas of suitable grade.

Carbon tetrachloride R

CCl_4 (SRIP, 1963, p. 63).

Carboxymethylcellulose R

A suitable grade for column chromatography.

Cefadroxil R

Cefadroxil of a suitable quality should be used.

Cellulose R1

Microcrystalline cellulose suitable for thin-layer chromatography.

Description. A fine, white homogeneous powder.

Particle size. Less than 30 µm.

Note: A suspension of about 25 g of cellulose R1 in 90 mL of water is used in the preparation of the coating for thin-layer chromatographic plates.

Cellulose R2

Cellulose suitable for thin-layer chromatography.

Description. A fine, white homogeneous powder.

Particle size. Less than 30 µm.

Note: A suspension of about 15 of cellulose R2 in 100 mL of water is used in the preparation of the coating for thin-layer chromatographic plates.

Cellulose R3

Cellulose suitable for thin-layer chromatography.

Description. A fine, white homogeneous powder.

Composition. Cellulose (particle size less than 30 µm) containing a fluorescent indicator having an optimal intensity at 254 nm.

Note: A suspension of about 25 g of cellulose R3 in 100 mL of water is used in the preparation of the coating for thin-layer chromatographic plates.

Cefadroxil R

(6R,7R)-7-{[(2R)-2-amino-2-(4-hydroxyphenyl)acetyl]amino}-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid. $C_{16}H_{17}N_3O_5S$.

A commercially available reagent of suitable grade.

Cephaëline hydrochloride R

$C_{28}H_{38}N_2O_4,2HCl,7H_2O$.

Description. A white, crystalline powder.

Specific optical rotation. Use a 20 mg/mL solution; $[\alpha]_D^{20\,°C}$ = +25°.

Cephalin TS

Procedure. Place a quantity between 0.5 and 1.0 g of acetone-dried ox brain R into a centrifuge tube, add 20 mL of acetone R and allow to stand for 2 hours. Centrifuge for 2 minutes and decant the supernatant liquid. Dry the residue under reduced pressure, add to it 20 mL of chloroform R and allow to stand for 2 hours, shaking frequently. Separate the solid material by filtration or centrifugation and evaporate the chloroform under reduced pressure. Suspend the residue in 5–10 mL of sodium chloride (9 g/L) TS. Solvents used to prepare cephalin TS should contain a suitable antioxidant, for example, a solution of 0.02 g/L of butylated hydroxyanisole R.

Storage. Store in a freezer or keep in a freeze-dried state.

Note : The reagent must be used within 3 months.

Ceric ammonium nitrate (0.01 mol/L) VS

Procedure. Dissolve 5.482 g of ceric ammonium nitrate R in sufficient nitric acid (1 mol/L) VS to produce 1000 mL and filter.

Method of standardization. Ascertain the exact concentration of the 0.01 mol/L solution in the following manner: measure accurately 2.0 mL of freshly standardized ferrous ammonium sulfate (0.1 mol/L) VS into a flask and dilute with water to about 100 mL. Add 1 drop of nitrophenanthroline TS and titrate with the ceric ammonium nitrate solution to a colourless end-point. From the volume of ferrous ammonium sulfate (0.1 mol/L) VS taken and the volume of ceric ammonium nitrate solution consumed, calculate the molarity.

Ceric ammonium nitrate R

$Ce(NO_3)_4,2NH_4NO_3$.

Description. Small orange-red monoclinic crystals.

Solubility. Very soluble in water.

Insoluble matter. To 5 g, accurately weighed, add 10 mL of sulfuric acid (~1760 g/L) TS, stir and cautiously add 90 mL of water to dissolve. Heat to boiling and digest in a covered beaker on a water-bath for 1 hour. Filter through a tared filtering crucible, wash thoroughly and dry at 105 °C. The weight of the residue does not exceed 2.5 mg.

Assay. Dissolve 2.5 g, accurately weighed and previously dried at 85 °C for 24 hours, in 10 mL of sulfuric acid (~190 g/L) TS and add 40 mL of water. Add a few drops of *o*-phenanthroline TS and titrate with ferrous sulfate (0.1 mol/L) VS. Each mL of ferrous sulfate (0.1 mol/L) VS is equivalent to 54.8 mg of $Ce(NO_3)_4,2NH_4NO_3$.

Ceric ammonium sulfate (0.1 mol/L) VS

Procedure. Dissolve 65.0 g of ceric ammonium sulfate R in a mixture of 500 mL of water and 30 mL of sulfuric acid (~1760 g/L) TS. Allow to cool and dilute to 1000 mL with water.

Method of standardization. Ascertain the exact concentration of the 0.1 mol/L solution in the following manner: accurately weigh about 0.2 g of arsenic trioxide R1 and dissolve by gently heating in 15 mL of sodium hydroxide (0.2 mol/L) VS. Add to the clear solution 50 mL of sulfuric acid (~100 g/L) TS, 0.15 mL of a 2.5 mg/mL solution of osmium tetroxide R in sulfuric acid (~100 g/L) TS and 0.1 mL of *o*-phenanthroline TS. Titrate the solution with the ceric ammonium sulfate solution until the red colour disappears. Titrate slowly as the end-point is approached.

Ceric ammonium sulfate R

Ammonium cerium (IV) sulfate dihydrate, $Ce(SO_4)_2,2(NH_4)_2SO_4,2H_2O$. Contains not less than 95.0% of $Ce(SO_4)_2,2(NH_4)_2SO_4,2H_2O$.

Description. Yellow crystals or an orange-yellow, crystalline powder.

Solubility. Slowly soluble in water; insoluble in ethanol (~750 g/L) TS.

Assay. Dissolve about 1 g, accurately weighed, in 50 mL of sulfuric acid (~100 g/L) TS, add 0.1 mL of a 10 mg/mL solution of osmium tetroxide R and titrate with sodium arsenite (0.05 mol/L) VS using *o*-phenanthroline TS as indicator. Each mL of sodium arsenite (0.05 mol/L) VS is equivalent to 63.26 mg of $Ce(SO_4)_2,2(NH_4)_2SO_4,2H_2O$.

Ceric ammonium sulfate/nitric acid TS

Procedure. Dissolve 5 g of ceric ammonium sulfate R in sufficient nitric acid (~130 g/L) TS to produce 100 mL.

Ceric amonium nitrate TS

Procedure. Dissolve 6.25 g of ceric ammonium nitrate R in 10 mL of nitric acid (15 g/L) TS.

Shelf-life. Use within 3 days of preparation.

Ceric sulfate (0.1 mol/L) VS

Procedure. Dissolve ceric sulfate R, equivalent to 33.23 g of $Ce(SO_4)$, in a mixture of 28 mL of sulfuric acid (~1760 g/L) TS and 500 mL of water, dilute to 1000 mL and mix. Allow the solution to stand for 48 hours and filter through a sintered glass filter.

Method of standardization. Ascertain the exact concentration of the 0.1 mol/L solution in the following manner: place about 25 mL, accurately measured, in a glass-stoppered flask, dilute with 80 mL of water, add 10 mL of phosphoric acid (~105 g/L) TS and 2.5 g of potassium iodide R and allow the solution to stand for 15 minutes. Add 1 g of sodium carbonate R and titrate with sodium thiosulfate (0.1 mol/L) VS, using starch TS as indicator.

Ceric sulfate (35 g/L) TS

A solution of ceric sulfate R containing about 33 g/L of $Ce(SO_4)_2$.

Ceric sulfate R

Usually $Ce(SO_4)_2,4H_2O$ (SRIP, 1963, p. 63).

Charcoal R

(SRIP, 1963, p. 64).

Chloralose R

$C_8H_{11}Cl_3O_6$.

Description. A colourless, crystalline powder.

Melting temperature. About 187 °C.

Specific optical rotation. Use a 50 mg/mL solution in ethanol (~750 g/L) TS; $[\alpha]_D^{20}$ = +19°.

Chloraniline R

4-Chloroaniline, C_6H_6ClN.

Description. White or faintly coloured crystals.

Melting temperature. About 70 °C.

Chlorbutol R

A commercially available reagent of suitable grade

Chloride standard (5 µg/L) TS

Procedure. Weigh accurately 82.4 mg of sodium chloride R and dissolve in sufficient water to produce 100 mL. Dilute 1.0 mL of this solution with water to 100 mL.

Chlorine R

Cl_2 (SRIP, 1963, p. 65).

Chlorine TS

A saturated solution of chlorine R in water.

Note: Chlorine TS must be freshly prepared.

7-Chloro-1-cyclopropyl-1,4-dihydro-4-oxo-6-(piperazin-1-yl)quinoline-3-carboxylic acid RS

(ciprofloxacin by-compound A). International Chemical Reference Substance.

2-Chloro-2-deoxy-D-glucose R

$C_6H_{11}ClO_5$.

Description. A white crystalline, hygroscopic powder, soluble in water and in dimethyl sulphoxide and insoluble in alcohol.

A commercially available reagent of suitable grade.

1-Chloro-2,4 dinitrobenzene R

$C_6H_3ClN_2O_4$.

A commercially available reagent of suitable grade.

Melting point. About 144 °C.

1-Chloro-2,4 dinitrobenzene/ethanol TS

Procedure. Weigh 5 g of 1-Chloro-2,4 dinitrobenzene R and dissolve in sufficient ethanol (~750 g/L) TS to produce 100 mL.

2-Chloro-4-nitroaniline R

$C_6H_5ClN_2O_2$.

Description. A yellow to brown, crystalline powder.

Solubility. Slightly soluble in water; soluble in ethanol (~750 g/L) TS.

Melting range. 106–108 °C.

Sulfated ash. Not more than 0.5 mg/g.

4-Chloroacetanilide R

C_8H_8ClNO.

Description. Colourless, needle-shaped crystals or a white to pale yellow, crystalline powder.

Solubility. Practically insoluble in water; soluble in ethanol (~750 g/L) TS and ether R.

Melting temperature. About 180 °C.

Chloroform R

$CHCl_3$ (SRIP, 1963, p. 66).

Chloroform, ethanol-free, R

Procedure. Shake 20 mL of chloroform R gently but thoroughly with 20 mL of water for 3 minutes, draw off the chloroform layer and wash twice more with 20 mL quantities of water. Finally filter the chloroform through a dry filter-paper, shake it well with 5 g of powdered anhydrous sodium sulfate R for 5 minutes, allow the mixture to stand for 2 hours and decant or filter the clear

chloroform.

Chromazurol S R

$C_{23}H_{13}Cl_2NaO_9S$.

Description. A brownish-black powder.

A commercially available reagent of suitable grade.

Solubility. Soluble in water; slightly soluble in alcohol.

Chromic acid TS

Procedure. Dissolve 84 g of chromium trioxide R in 700 mL of water and add slowly while stirring 400 mL of sulfuric acid (~1760 g/L) TS.

Chromium trioxide R

CrO_3 (SRIP, 1963, p. 68).

Cinchonidine R

$C_{19}H_{22}N_2O$.

Description. A white, crystalline powder.

Solubility. Soluble in ethanol (~750 g/L) TS.

Melting temperature. About 207 °C.

Specific optical rotation. Use a 50 mg/mL solution in ethanol (~750 g/L) TS; $[\alpha]_D^{20\,°C}$ = -105° to 110°.

Cinchonine R

$C_{19}H_{22}N_2O$ (SRIP, 1963, p. 69).

Citrate buffer, pH 4.0, TS

Procedure. Dissolve 10.5 g of citric acid R in about 100 m of water, add 100 m of sodium hydroxide (1 mol/L) VS and dilute to 500 mL with water. Dilute 100 mL of hydrochloric acid (0.1 mol/L) VS with the solution prepared above to produce 250 mL.

Citrate buffer, pH 5.4, TS

Procedure. Dissolve 2.101 g of citric acid R in water, add 20 mL of sodium hydroxide (1 mol/L) VS and dilute with sufficient water to produce 100 mL. Mix 76.5 mL of this solution with 23.5 mL of sodium hydroxide (0.1 mol/L) VS.

Citrate buffer, pH 5.0, TS

Procedure. Dissolve 20.17 g of citric acid R in 800 mL of water R, adjust to pH 5.0 with sodium hydroxide (~400 g/L) TS and dilute to 1000 mL with water R.

Citrate buffer, pH 5.4, TS

Procedure. Dissolve 2.101 g of citric acid R in water, add 20 mL of sodium hydroxide (1 mol/L) VS and dilute with sufficient water to produce 100 mL. Mix 76.5 mL of this solution with 23.5 mL of sodium hydroxide (0.1 mol/L) VS.

Citric acid (180 g/L) FeTS

A solution of citric acid FeR containing about 183 g/L of $C_6H_8O_7$.

Citric acid (20 g/L) TS

A solution of citric acid R containing about 20 g of $C_6H_8O_7$ per litre.

Citric acid FeR

Citric acid R that complies with the following test: Dissolve 0.5 g of citric acid R in 40 mL of water, add 2 drops of mercaptoacetic acid R, mix, make alkaline with ammonia (~100 g/L) FeTS and dilute to 50 mL with water; no pink colour is produced.

Citric acid PbR

Citric acid R free of lead.

Citric acid R

$C_6H_8O_7,H_2O$ (SRIP, 1963, p. 69).

Citric acid, copper-free, R

Citric acid R, that complies with the following additional test: Dissolve 0.50 g in 20 mL of water, make alkaline with ammonia (~100 g/L) TS, dilute to 50 mL with water and add 1 mL of sodium diethyldithiocarbamate (0.8 g/L) TS; no yellow colour is produced.

Cobalt colour TS

A solution containing 60.0 g/l of $CoCl_2,6H_2O$.

Procedure. Prepare a solution containing 6.000 g of $CoCl_2,6H_2O$ in 100 mL by diluting the strong cobalt colour TS with sulfuric acid (~10 g/L) TS, as necessary.

Cobalt colour, strong, TS

Procedure. Dissolve 8.0 g of cobaltous chloride R in 120 mL of sulfuric acid (~10 g/L) TS, filter the solution if necessary and determine the concentration of $CoCl_2,6H_2O$.

Assay. Dilute 5.0 mL with sufficient water to produce 100 mL. Transfer 10.0 mL of this solution to a glass-stoppered flask, add 10 mL of water, 0.5 mL of hydrogen peroxide (~60 g/L) TS and 10 mL of sodium hydroxide (~80 g/L) TS. Add a few boiling chips to the flask and boil the contents of the flask until the excess of hydrogen peroxide is completely decomposed (approximately 10 minutes). Cool the flask, add 20 mL of water, 1 g of potassium iodide R and 25 mL of hydrochloric acid (2 mol/L) VS. Close the flask with the stopper and allow to stand until the precipitate dissolves. Titrate the liberated iodine with sodium thiosulfate (0.01 mol/L) VS using starch TS as indicator. Each mL of sodium thiosulfate (0.01 mol/L) VS is equivalent to 2.380 mg of $CoCl_2,6H_2O$.

Cobalt(II) chloride (30 g/L) TS

A solution of cobalt(II) chloride R containing about 30 g of $CoCl_2$ per litre.

Cobalt(II) chloride (5 g/L) TS

A solution of cobalt(II) chloride R containing about 5 g of $CoCl_2$ per litre.

Cobalt(II) chloride R

Cobaltous chloride; $CoCl_2,6H_2O$ (SRIP, 1963, p. 70).

Cobalt(II) nitrate (10 g/L) TS

Procedure. Dissolve about 1.6 g of cobalt(II) nitrate R in sufficient water to produce 100 mL.

Cobalt(II) nitrate (100 g/L) TS

A solution of cobalt(II) nitrate R containing about 100 g of $Co(NO_3)_2$ per litre.

Cobalt(II) nitrate R

$Co(NO_3)_2,6H_2O$.

Description. Small red crystals.

Solubility. Very soluble in water.

Cobaltous chloride R

$CoCl_2,6H_2O$ (SRIP, 1963, p. 70).

Cobaltous chloride TS

Procedure. Dissolve 6.5 g of cobaltous chloride R in a sufficient quantity of a mixture of 2.5 mL of hydrochloric acid (~250 g/L) TS and 97.5 mL of water to produce 100 mL.

Codeine R

$C_{18}H_{21}NO_3,H_2O$.

Description. Colourless crystals or a white, crystalline powder; odourless.

Solubility. Slightly soluble in water; freely soluble in ethanol (~750 g/L) TS and ether R.

Melting temperature. About 156 °C.

Specific optical rotation. Use a 20 mg/mL solution in ethanol (~750 g/L) TS; $[\alpha]_D^{20\,°C}$ = -142° to -146°.

Colbatous thiocyanate TS

Procedure. Dissolve 6.8 g of cobaltous chloride R and 4.3 g of ammonium thiocyanate R in sufficient water to produce 100 mL.

Congo red paper R

(SRIP, 1963, p. 72).

Copper colour TS

A solution containing 60.0 g/L of $CuSO_4,5H_2O$.

Procedure. Prepare a solution containing 6.000 g of $CuSO_4,5H_2O$ in 100 mL by diluting the strong copper colour TS with sulfuric acid (~10 g/L) TS as necessary.

Copper colour, strong, TS

Procedure. Dissolve 8.0 g of copper(II) sulfate R in 120 mL of sulfuric acid (~10 g/L) TS, filter the solution if necessary and determine the concentration of $CuSO_4,5H_2O$.

Assay. Dilute 5.0 mL with sufficient water to produce 100 mL. Transfer 10.0 mL of this solution to a glass-stoppered flask, add 20 mL of water, 1 g of potassium iodide R and 5 mL of glacial acetic acid R. After 10 minutes titrate the liberated iodine with sodium thiosulfate (0.01 mol/L) VS using starch TS as indicator. Each mL of sodium thiosulfate (0.01 mol/L) VS is equivalent to 2.497 mg of $CuSO_4,5H_2O$.

Copper edetate TS

Procedure. To 2 mL of a 20 mg/mL solution of copper(II) acetate R add 2 mL of disodium edetate (0.1 mol/lLVS and dilute to 50 mL with water.

Copper standard (10 µg/mL Cu) T.

Procedure. Dissolve 0.393 g of copper(II) sulfate R in sufficient water to produce 100 mL and dilute 10.0 mL of this solution to produce 1000 mL.

Copper standard (5 µg/mL Cu) TS

Procedure. Dissolve 0.982 g of copper(II) sulfate R in 1000 mL of hydrochloric acid (0.1 mol/L) VS. Transfer 2.0 mL of this solution to a 100 mL volumetric flask, dilute to volume with hydrochloric acid (0.1 mol/L) VS and mix. Each mL of this solution contains 5 µg of copper.

Copper standard TS1

Procedure. Dissolve 1.965 g of copper(II) sulfate R, accurately weighed, in sufficient hydrochloric acid (0.1 mol/L) VS to produce 1000 mL.

Copper standard TS2

Procedure. Transfer 3.0 mL of copper standard TS1 to a 1000 mL flask and dilute with hydrochloric acid (0.1 mol/L) VS to produce 1000 mL. This solution contains 1.5 µg of Cu per mL.

Copper tetramine hydroxide TS

Procedure. Dissolve 34.5 g of copper(II) sulfate R in 100 mL of water. Stir and add, drop by drop, ammonia (~260 g/L) TS until the precipitate formed has completely dissolved. Keep the temperature below 20 °C and add slowly, while stirring, 30 mL of sodium hydroxide (~400 g/L) TS. Filter the precipitate through a sintered glass filter (porosity 16–40 µm) and wash with water until the filtrate is clear. Add 200 mL of ammonia (~260 g/L) TS to the precipitate, stir and filter.

Copper(II) acetate (45 g/L) TS

A solution of copper(II) acetate R containing about 50 g of $C_4H_6CuO_4,H_2O$ per litre.

Copper(II) acetate R

$C_4H_6CuO_4,H_2O$. Contains not less than 98.0% of $C_4H_6CuO_4,H_2O$.

Description. Blue-green crystals or powder; odour, resembling that of acetic acid.

Solubility. Soluble in water.

Assay. Dissolve 0.8 g, accurately weighed, in 50 mL of water, add 2 mL of acetic acid (~300 g/L) TS and 3 g of potassium iodide R and titrate the liberated iodine with sodium thiosulfate (0.1 mol/L) VS using starch TS as indicator, until only a faint blue colour remains; add 2 g of potassium thiocyanate R and continue the titration until the blue colour disappears. Each mL of sodium thiosulfate (0.1 mol/L) VS is equivalent to 19.97 mg of $C_4H_6CuO_4,H_2O$.

Copper (I) bromide R

CuBr; 143.45; *[7787-70-4]*

Description. Pale green powder.

Use a suitable grade.

Copper(II) chloride R

$CuCl_2,2H_2O$.

Description. Bluish green, deliquescent crystals.

Solubility. Freely soluble in water; soluble in ethanol (~750 g/L) TS; slightly soluble in ether R.

Copper(II) chloride/ammonia TS

Procedure. Dissolve 22.5 g of copper(II) chloride R in 200 mL of water and add 100 mL of ammonia (~260 g/L) TS.

Copper(II) sulfate (1 g/L) TS

A solution of copper(II) sulfate R containing 1 g of $CuSO_4$ per litre.

Copper(II) Sulfate (10 g/L) TS

A solution of Copper(II) sulfate R containing 10 g of CuSO4 per litre.

Copper(II) sulfate (160 g/L) TS

A solution of copper(II) sulfate R containing about 160 g/L of $CuSO_4$.

Copper(II) sulfate (80 g/L) TS

A solution of copper(II) sulfate R containing about 80 g of $CuSO_4$ per litre (approximately 0.5 mol/L).

Copper(II) sulfate R

$CuSO_4,5H_2O$ (SRIP, 1963, p. 72).

Copper (II) sulfate R, anhydrous

CuSO4.

Description. Greenish-grey powder, hygroscopic.

Solubility. Freely soluble in water, slightly soluble in methanol and practically insoluble in ethanol (~750 g/L) TS.

Copper (II) sulfate pentahydrate R

$CuSO_4,5H_2O$, [7758-99-8]; blue, crystalline powder or transparent, blue crystals, content: 99.0% to 101.0%.

Copper(II) sulfate/ammonia TS

Procedure. Dissolve 50 g of copper(II) sulfate R in 1000 mL of ammonia (~35 g/L) TS.

Copper(II) sulfate/pyridine TS

Procedure. Dissolve 4 g of copper(II) sulfate R in 90 mL of water and add 30 mL of pyridine R.

Note: Copper(II) sulfate/pyridine TS must be freshly prepared.

o-Cresol R

2-Methylphenol; C_7H_8O.

Description. Colourless to pale brownish-yellow crystals or liquid; odour, resembling that of phenol.

Miscibility. Miscible with ethanol (~750 g/L) TS and ether R; miscible with about 50 parts of water.

Mass density. ρ_{20} = about 1.05 kg/L.

Refractive index. n_D^{20} = 1.540–1.550.

Boiling temperature. About 190 °C.

Freezing temperature. Not below 30.5 °C.

Residue on evaporation. Evaporate on a water-bath and dry to constant weight at 105 °C; it leaves a residue of not more than 1.0 mg/mL.

Storage. Store in a tightly closed container, protected from light and oxygen.

Note: On exposure to light and air *o*-cresol R darkens in colour.

Cresol red R

$C_{21}H_{18}O_5S$.

Description. A red-brown powder.

Solubility. Slightly soluble in water; soluble in ethanol (~750 g/L) TS and in dilute solutions of alkali hydroxides.

Cresol red/ethanol TS

Procedure. Warm 0.05 g of cresol red R with 2.65 mL of sodium hydroxide (0.05 mol/L) VS and 5 mL of ethanol (~710 g/L) TS; after solution has been effected add sufficient ethanol (~150 g/L) TS to produce 250 mL.

Crystal violet R

$C_{25}H_{30}ClN_3$ (SRIP, 1963, p. 73).

Crystal violet/acetic acid TS

A solution of crystal violet R dissolved in glacial acetic acid R1 containing about 5 g/L.

Crystal violet/acetic acid TS1

A solution of crystal violet R dissolved in anhydrous acetic acid R containing about 5 g/L.

Test for sensitivity. To 50 mL of anhydrous acetic acid R, add 0.1 mL of the crystal violet solution. On addition of 0.1 mL of perchloric acid (0.1 mol/L) VS, the bluish-purple solution turns bluish-green.

Culture medium Cm1

Procedure. Dissolve 6.0 g of dried peptone R, 4.0 g of pancreatic digest of casein R, 3.0 g of water-soluble yeast extract R, 1.5 g of beef extract R, 1.0 g of glucose hydrate R and 10–20 g of agar R in sufficient water to produce 1000 mL.

Note: The quantity of agar R used should permit the culture medium to be of adequate firmness to support cylinders or to permit holes to be cut without tearing the gel layer.

Culture medium Cm2

Procedure. Dissolve 17.0 g of pancreatic digest of casein R, 3.0 g of papaic digest of soybean meal R, 5.0 g of sodium chloride R, 2.5 g of dipotassium hydrogen phosphate R, 2.5 g of glucose hydrate R and 10–20 g of agar R in about 500 mL of water. Heat the solution, add 10.0 g of polysorbate 80 R and dilute immediately with a sufficient amount of water to produce 1000 mL.

Note: The quantity of agar R used should permit the culture medium to be of adequate firmness to support cylinders or to permit holes to be cut without tearing the gel layer.

Culture medium Cm3

Procedure. Dissolve 9.4 g of dried peptone R, 4.7 g of water-soluble yeast extract R, 2.4 g of beef extract R, 10.0 g of sodium chloride R, 10.0 g of glucose hydrate R and 15–25 g of agar R in sufficient water to produce 1000 mL.

Note: The quantity of agar R used should permit the culture medium to be of adequate firmness to support cylinders or to permit holes to be cut without tearing the gel layer.

Culture medium Cm8

Procedure. Dissolve 10 g of dried peptone R, 10 g of beef extract R, 10 g of glycerol R, 3.0 g of sodium chloride R and 17 g of agar R in sufficient water to produce 1000 mL. Adjust the pH with sodium hydroxide (0.05 mol/L) VS to 6.9–7.1, and sterilize in an autoclave at 121 °C for 18–20 minutes.

Culture medium Cm9

Procedure. Dissolve 10 g of dried peptone R, 10 g of beef extract R, 10 g of glycerol R and 3.0 g of sodium chloride R in sufficient water to produce 1000 mL. Adjust the pH with sodium hydroxide (0.05 mol/L) VS to 6.9–7.1, and sterilize in an autoclave at 121 °C for 18–20 minutes.

Cupri-tartaric TS

Procedure. Dissolve 34.6 g copper (II) sulfate R in sufficient water to produce
100 mL. Separately dissolve 173 g of potassium sodium tartrate R and 50 g sodium hydroxide in 400 mL water R; heat to boiling, allow to cool and dilute to 500 mL with water R. Shortly before use mix together equal volumes of both solutions.

Cyanide/oxalate/thiosulfate TS

Procedure. To 2.0 mL of ammonia (~100 g/L) TS, add in the following order: 1.5 mL of ammonium oxalate (50 g/L) TS, 15 mL of potassium cyanide (50 g/L) TS, 45 mL of sodium acetate (60 g/L) TS, 120 mL of sodium thiosulfate (320 g/L) TS, 75 mL of sodium acetate (60 g/L) TS and 35 mL of hydrochloric acid (1 mol/L) VS.

Note: Cyanide/oxalate/thiosulfate TS must be prepared immediately before use.

Cyanoethylmethyl silicone gum R

A suitable grade to be used in gas-liquid chromatography.

Cyanogen bromide TS

Caution. Very toxic; avoid inhalation of vapours.

Procedure. Add drop by drop, while cooling, potassium cyanide (100 g/L) TS to bromine TS1 until the colour disappears.

Note: Cyanogen bromide TS must be prepared immediately before use.

Cyclohexane R

C_6H_{12} (SRIP, 1963, p. 74).

Cyclohexane R1

Cyclohexane R showing a fluorescence that is not more intense than that of a solution of 2 µg/mL of quinine R in sulfuric acid (0.05 mol/L) VS when measured at 460 nm using a 1 cm layer and an excitation beam at 365 nm.

Cyclohexylenedinitrilotetra-acetic acid R

trans-Cyclohexylene-1,2-dinitrilo-N,N,N',N'-tetra-acetic acid, $C_{14}H_{22}N_2O_8,H_2O$.

Description. A white or almost white, crystalline powder.

Melting point. About 204 °C.

3-Cyclohexylpropionic acid R

$C_9H_{16}O_2$

*Molecular weight.*156.2.

Description. Clear liquid.

Relative density d_{20}^{20}. About 0.998.

*Boiling point.*About 130 °C.

L-Cystine R

$C_6H_{12}N_2O_4S_2$ (SRIP, 1963, p. 75).

Cytosine R

4-Aminopyrimidin-2(1*H*)-one; $C_4H_5N_3O$.

A commercially available reagent of suitable grade.

D - Dantron R.... Dragendorff reagent, modified, TS

Dantron R

1,8-dihydroxyanthraquinone; $C_{14}H_8O_4$.

A commercially available reagent of suitable grade.

Description. An orange, microcrystalline powder.

Solubility. Practically insoluble in water; slightly soluble in ethanol (~750 g/L) TS and ether R.

Melting point. About 193 °C.

Decaethylene glycol monodocecyl ether R.

Polyoxyethylene 10 lauryl ether; $C_{32}H_{66}O_{11}$.

A commercially available reagent of suitable grade.

Demeclocycline hydrochloride R

Demeclocycline hydrochloride of a suitable quality should be used.

1,4-Di[2-(4-methyl-5-phenyloxazole)]benzene R

3,3'-Diaminobenzidine tetrahydrochloride R

$C_{12}H_{14}N_4,4HCl,2H_2O$.

A commercially available reagent of suitable grade.

Description. An almost white or slightly pink powder.

Dimethyl-POPOP $C_{26}H_{20}N_2O_2$. Suitable for scintillation counting.

3,3'-Diaminobenzidine tetrahydrochloride (5g/L) TS

A solution of 3,3'-diaminobenzidine tetrahydrochloride R containing 5 g of $C_{12}H_{14}N_4,4HCl$ per litre.

Diammonium hydrogen phosphate (100 g/L) TS

A solution of diammonium hydrogen phosphate R containing about 100 g of $(NH_4)_2HPO_4$ per litre.

Diammonium hydrogen phosphate R

[ammonium phosphate R]. $(NH_4)_2HPO_4$ (SRIP, 1963, p.38).

Diatomaceous support R.

Description. White granules of silica consisting chiefly of the skeletons of diatoms. The material is flux-calcined to sequester coloured metallic oxides in a colourless form and is supplied commercially in various forms, such as: acid-washed, silanized and alkali-washed.

Diazepam R

Diazepam of a suitable quality should be used.

Diazobenzenesulfonic acid TS

Procedure. To 0.9 g of sulfanilic acid R add 10 mL of hydrochloric acid (~250 g/L) TS and sufficient water to produce 100 mL. To 3 mL of this solution add 5 mL of sodium nitrite (3 g/L) TS, cool in ice for 5 minutes, add a further 20 mL of sodium nitrite (3 g/L) TS and again cool in ice; dilute with water to 100 mL, keeping the solution cold.

Note: Diazobenzenesulfonic acid TS must be freshly prepared and should not be used until at least 15 minutes after its preparation.

Diazomethane TS

Caution. Diazomethane is explosive in the gaseous state and its explosive decomposition is easily initiated by rough surfaces. Do not use apparatus with ground-glass joints or boiling stones of any kind. Diazomethane is highly toxic and all operations should be conducted under a well-ventilated hood.

Procedure. Prepare a solution of 0.4 g of potassium hydroxide R in 10 mL of ethanol (~750 g/L) TS and add it to a solution of

2.14 g of *N*-methyl-*N* -nitrosotoluene-4-sulfonamide R in 30 mL of ether R while cooling in ice. If a precipitate forms add just sufficient ethanol (~750 g/L) TS to dissolve it. After 5 minutes gently distil the ethereal diazomethane solution from a water-bath. Diazomethane TS contains about 10 g of CH_2N_2 per litre.

Alternative procedures. Other procedures for the evolution of diazomethane, using other starting materials, may also be employed, provided the resulting solution has the required concentration of CH_2N_2.

Dibromomethane R

Methylene bromide, CH_2Br_2.

Description. Colourless to yellowish liquid.

Miscibility. Miscible with ethanol (~750 g/L) TS, ether R and acetone R.

Dibutyl ether R

Di-*n*-butyl ether, $C_8H_{18}O$.

Caution. Dibutyl ether R tends to form explosive peroxides especially when anhydrous.

Description. A colourless liquid.

Miscibility. Practically immiscible with water; miscible with ethanol (~750 g/L) TS and ether R.

Boiling range. 140–143 °C.

Mass density. ρ_{20} = 0.769 kg/L.

Refractive index. n_D^{20} = 1.344.

Dibutyl phthalate R

Di-*n*-butyl phthalate, $C_{16}H_{22}O_4$.

Description. A clear, colourless or faintly coloured liquid.

Miscibility. Very slightly miscible with water; miscible with ethanol (~750 g/L) TS and ether R.

Mass density. ρ_{20} = 1.043-1.048 kg/L.

Refractive index. n_D^{20} = 1.492–1.495.

Sulfated ash. Not more than 0.2 mg/mL.

Dichloroethane R

1,2-Dichloroethane, $C_2H_4Cl_2$ (SRIP, 1963, p. 76).

Dichlorofluorescein R

$C_{20}H_{10}Cl_2O_5$.

Description. A light orange-coloured, crystalline powder.

Solubility. Sparingly soluble in water; soluble in ethanol (~750 g/L) TS.

Dichlorofluorescein TS

Procedure. Dissolve 0.2 g of dichlorofluorescein R in 100 mL of methanol R.

Dichloromethane R

Methylene chloride, CH_2Cl_2.

Description. A clear colourless, mobile liquid.

Miscibility. Freely miscible with ethanol (~750 g/L) TS and ether R.

Boiling range. Not less than 95% distils between 39 and 41 °C.

Residue on evaporation. Leaves, after evaporation on a water-bath and drying at 105 °C, not more than 0.5 mg/mL.

2,6-Dichloroquinone chlorimide R

$C_6H_2Cl_3NO$ (SRIP, 1963, p. 77).

2,6-Dichloroquinone chlorimide/ethanol TS

Procedure. Dissolve 0.5 g of 2,6-dichloroquinone chlorimide R in sufficient ethanol (~750 g/L) TS to produce 100 mL.

Dichromate colour TS

A solution containing 4.904 g/L of $K_2Cr_2O_7$.

Procedure. Prepare a solution containing 490.35 mg of $K_2Cr_2O_7$ in 100 mL by diluting the strong dichromate colour TS with sulfuric acid (~10 g/L) TS as necessary.

Dichromate colour, strong, TS

Procedure. Dissolve 6.0 g of potassium dichromate R in 120 mL of sulfuric acid (~10 g/L) TS, filter the solution if necessary and determine the concentration of $K_2Cr_2O_7$.

Assay. Dilute 5.0 mL with sufficient water to produce 50 mL. Transfer 10.0 mL of this solution to a glass-stoppered flask, add 10 mL of water, 2 g of potassium bicarbonate R and 20 mL of sulfuric acid (~100 g/L) TS. Loosely close the flask with its stopper. When the gas evolution has ceased add 1 g of potassium iodide R, keep the flask for 5 minutes in a dark place and titrate the liberated iodine with sodium thiosulfate (0.1 mol/L) VS using starch TS as indicator. Each mL of sodium thiosulfate (0.1 mol/L) VS is equivalent to 4.904 mg of $K_2Cr_2O_7$.

Diethoxytetrahydrofuran R

$C_8H_{16}O_3$. Mixture of the cis and trans isomers.

Description. Colourless or slightly yellow, clear liquid.

Miscibility. Practically immiscible with water; miscible with ethanol (~750 g/L) TS and ether R.

Mass density. ρ_{20} = about 0.975 kg/L.

Refractive index. n_D^{20} = 1.418.

Diethoxytetrahydrofuran/acetic acid TS

Procedure. Mix 1 mL of diethoxytetrahydrofuran R with sufficient glacial acetic acid R to produce 100 mL.

Diethyl phthalate R

$C_{12}H_{14}O_4$.

Mass density. ρ_{20} = about 1.117 kg/L.

Refractive index. n_D^{20} = 1.500–1.505.

Diethylamine R

$C_4H_{11}N$. Contains not less than 99.5% of $C_4H_{11}N$.

Description. A clear, colourless liquid.

Mass density. ρ_{20} = 0.702-0.704 kg/L.

Refractive index. n_D^{20} = 1. –1.386.

Assay. Add about 3 g, accurately weighed, to 50 mL of sulfuric acid (0.5 mol/L) VS and titrate the excess acid with sodium hydroxide (1 mol/L) VS, using methyl red/ethanol TS as indicator. Each mL of sulfuric acid (0.5 mol/L) VS is equivalent to 73.14 mg of $C_4H_{11}N$.

Diethylaminoethylcellulose R

A suitable grade for column chromatography.

Diethylene glycol R

$C_4H_{10}O_3$.

Description. A colourless to faintly yellow liquid having a mild odour.

Miscibility. Freely miscible with water, ethanol (~750 g/L) TS, ether R and acetone R.

Boiling range. Between 240 and 250 °C.

Mass density (ρ_{20}). 1.117–1.120 kg/L.

Acidity. Transfer 60 g to a 250 mL conical flask, add phenolphthalein/ethanol TS and titrate with potassium hydroxide/ethanol (0.02 mol/L) VS to a pink colour that remains stable for at least 15 seconds. Not more than 2.5 mL should be consumed.

Diethylene glycol succinate R

A suitable grade to be used in gas-liquid chromatography.

Diethylphenylenediamine sulfate TS

Procedure. To 250 mL of water add about 2 mL of sulfuric acid (~1760 g/L) TS and 50 mL of disodium edetate (0.01 mol/L) VS. Dissolve 1.1 g of *N,N* -diethyl-*p*-phenylenediamine sulfate R into this solution and dilute with sufficient water to produce 1000 mL.

N,N-Diethyl-p-phenylenediamine sulfate R

N ,*N*-Diethyl-1,4-phenylenediamine sulfate; $C_{10}H_{16}N_2,H_2SO_4$.

A commercially available reagent of suitable grade.

Description. A white or slightly coloured powder.

Melting point. About 185 °C with decomposition.

Storage. *N,N*-Diethyl-*p*-phenylenediamine sulfate R should be kept protected from light.

2,7-Dihydroxynaphthalene R

$C_{10}H_8O_2$. Naphthalene-2,7-diol. CAS Reg. No. 582-17-2

Description. Needles.

Solubility. Soluble in water and in ethanol (~750 g/L) TS.

Melting point. About 190 °C.

2,7-Dihydroxynaphthalene TS

Dissolve 10 mg of 2,7-dihydroxynaphthalene R in 100 mL of sulfuric acid (~1760 g/L) TS and allow to stand until decolorised.

Storage. Use within 2 days.

Digitonin R

$C_{55}H_{90}O_{29}$ (SRIP, 1963, p. 78).

Digitonin TS

Procedure. Dissolve 0.10 g of digitonin R in sufficient ethanol (~710 g/L) TS to produce 10 mL.

Note: Digitonin TS must be freshly prepared.

Diisopropyl ether R

Isopropyl ether; $C_6H_{14}O$.

Description. A colourless liquid; odour, characteristic.

Boiling point. About 68 °C.

Note. Diisopropyl ether is highly flammable.

Dimethyl sulfoxide R

C_2H_6OS.

Description. A colourless liquid; odourless or with a slight but unpleasant odour.

Mass density. ρ_{20} = 1.10 kg/L.

Dimethylacetamide R

C_4H_9NO.

Description. A colourless liquid.

Boiling temperature. About 165 °C.

Mass density. ρ_{20} = 0.94 kg/L.

Dimethylamine R

C_2H_7N.

Description. A low-boiling liquid, 7 °C; odour, characteristic.

Solubility. Soluble in water, ethanol (~750 g/L) TS and ether R.

Dimethylamine/ethanol TS

A solution of dimethylamine R in ethanol (~750 g/L) TS containing about 330 g/L of C_2H_7N.

Assay. Dilute 2 mL to 10 mL with ethanol (~750 g/L) TS. Transfer 2 mL to a flask containing 50.0 mL of sulfuric acid (0.05 mol/L) VS and mix. Titrate the excess acid with sodium hydroxide (0.1 mol/L) VS using methyl red/ethanol TS as indicator. Each mL of sulfuric acid (0.05 mol/L) VS is equivalent to 4.508 mg of C_2H_7N.

4-Dimethylaminobenzaldehyde R

[dimethylaminobenzaldehyde R]. $C_9H_{11}NO$ (SRIP, 1963, p.78).

4-Dimethylaminobenzaldehyde TS1

Procedure. Dissolve 0.125 g of 4-dimethylaminobenzaldehyde R in a cooled mixture of 65 mL of sulfuric acid (~1760 g/L) TS and 35 mL of water and add 0.2 mL of ferric chloride (25 g/L) TS.

Note: 4-Dimethylaminobenzaldehyde TS1 must be freshly prepared.

4-Dimethylaminobenzaldehyde TS2

Procedure. Dissolve 0.80 g of 4-dimethylaminobenzaldehyde R in a cooled mixture of 80 g of ethanol (~750 g/L) TS and 20 g of sulfuric acid (~1760 g/L) TS.

4-Dimethylaminobenzaldehyde TS3

Procedure. Dissolve 0.5 g of 4-dimethylaminobenzaldehyde R in 50 mL of ethanol (~750 g/L) TS, add 1 mL of hydrochloric acid (~420 g/L) TS and dilute with sufficient ethanol (~750 g/L) TS to produce 100 mL.

4-Dimethylaminobenzaldehyde TS4

Procedure. Dissolve 2 g of 4-dimethylaminobenzaldehyde R in a mixture of 5 mL of hydrochloric acid (~420 g/L) TS and 95 mL of glacial acetic acid R.

4-Dimethylaminobenzaldehyde TS5

Procedure. Dissolve without heating 2 g of 4-dimethylaminobenzaldehyde R in a mixture of 45 mL of water and 55 mL of hydrochloric acid (~420 g/L) TS.

4-Dimethylaminobenzaldehyde TS6

Procedure. Dissolve 0.2 g of 4-dimethylaminobenzaldehyde R in 20 mL of ethanol (~750 g/L) TS and add 0.5 mL of hydrochloric acid (~420 g/L) TS. Shake the solution with charcoal R and filter. The colour of this test solution is less intense than that of iodine (0.0001 mol/L) VS.

Note: 4-Dimethylaminobenzaldehyde TS6 must be freshly prepared.

4-Dimethylaminobenzaldehyde TS7

Procedure. Dissolve 0.1 g of 4-dimethylaminobenzaldehyde R in 1 mL of hydrochloric acid (~420 g/L) TS, dilute with ethanol (~750 g/L) to produce 100 mL.

4-Dimethylaminocinnamaldehyde R

$C_{11}H_{13}NO$.

Description. Orange crystals, or a crystalline powder; odour, characteristic.

Solubility. Practically insoluble in water; freely soluble in hydrochloric acid (~70 g/L) TS; sparingly soluble in ethanol (~750 g/L) TS and ether R.

4-Dimethylaminocinnamaldehyde TS1

Procedure. Dissolve 2 g of 4-dimethylaminocinnamaldehyde R in a mixture of 100 mL of hydrochloric acid (5 mol/L) VS and 100 mL of ethanol (~750 g/L) TS.

Storage. Store the solution at a temperature of about 0 °C.

4-Dimethylaminocinnamaldehyde TS2

Procedure. Dilute 20 mL of 4-dimethylaminocinnamaldehyde TS1 with sufficient ethanol (~750 g/L) TS to produce 100 mL.

Note: 4-Dimethylaminocinnamaldehyde TS2 must be freshly prepared.

N,N-Dimethylaniline R

$C_8H_{11}N$.

Description. A colourless liquid darkening on storage.

Miscibility. Practically immiscible with water, miscible with ethanol (~750 g/L) TS and ether R.

Boiling point. About 193 °C.

Mass density. ρ_{20} = 0.96 kg/L.

Dimethylformamide R

C_3H_7NO.

Description. A clear and colourless liquid, having a characteristic odour.

Miscibility. Miscible with water and ethanol (~750 g/L) TS.

Boiling range. Not less than 25% distils at between 152 and 156 °C.

Mass density (ρ_{20}). 0.945–0.947 kg/L.

Acidity and alkalinity. Dissolve 1 g in 10 mL of water, add 2 drops of phenolphthalein/ethanol TS; not more than 0.2 mL of carbonate-free sodium hydroxide (0.01 mol/L) VS is required to produce a red colour. Add 0.3 mL of hydrochloric acid (0.01 mol/L) VS and 5 drops of methyl, red/ethanol TS; an orange colour is produced.

N,N-Dimethyloctylamine R

Octyldimethylamine; $C_{10}H_{23}N$.

A commercially available reagent of suitable grade.

Description. A colourless liquid.

Boiling point. About 195 °C.

Dimidium bromide R

$C_{20}H_{18}BrN_3$. 3,8-Diamino-5-methyl-6-phenylphenanthridinium bromide. CAS Reg. No. 518-67-2.

Description. Dark-red crystals.

Solubility. Slightly soluble in water at 20 °C, sparingly soluble in water at 60 °C and in ethanol (~750 g/L) TS.

Storage. Store protected from light.

Dimidium bromide/sulfan blue TS

Procedure. Dissolve separately 0.5 g of dimidium bromide R and 0.25 g of sulfan blue R in 30 mL of a hot mixture of 1 volume of anhydrous ethanol R and 9 volumes of water R, stir, mix the two solutions, and dilute to 250 mL with the same mixture of solvents. Mix 20 mL of this solution with 20 mL of a 14.0 per cent V/V solution of sulfuric acid R previously diluted with about 250 mL of water R and dilute to 500 mL with water R.

Storage. Store protected from light.

Dinitrobenzene R

$C_6H_4N_2O_4$ (SRIP, 1963, p. 79).

Dinitrobenzene/ethanol TS

Procedure. Dissolve 1 g of dinitrobenzene R in sufficient ethanol (~750 g/L) TS to produce 100 mL.

2,4-Dinitrochlorobenzene R

$C_6H_4ClN_2O_4$ (SRIP, 1963, p. 80).

Dinitrogen oxide R

N_2O.

A commercially available gas of suitable grade.

Dinonyl phthalate R

$C_{26}H_{42}O_4$.

Description. A colourless to pale yellow, viscous liquid.

Mass density. ρ_{20} = 0.97–0.98 kg/L.

Refractive index. n_D^{20} = 1.482–1.489

Water. Determine as described under *2.8 Determination of water by the Karl Fischer method* using about 2 mL of the liquid; not more than 1.0 mg/mL.

Acidity. Shake 5.0 g with 25 mL of water for 1 minute. Allow to stand, filter the aqueous layer and add to it 5 drops of phenolphthalein/ethanol TS; not more than 0.3 mL of carbonate-free sodium hydroxide (0.1 mol/L) VS is required for the neutralization (0.5 mg/g expressed as phthalic acid).

Dioxan R

1,4-Dioxane, $C_4H_8O_2$.

Caution. It is dangerous to determine the boiling range or the residue on evaporation before complying with the test for peroxides.

Description. A clear, colourless liquid.

Miscibility. Miscible with water, ethanol (~750 g/L) TS and ether R.

Boiling range. Not less than 95% distils at between 101 and 105 °C.

Melting temperature. Solidifies when cooled in ice and does not completely remelt at temperatures below 10 °C.

Residue on evaporation. Evaporate on a water-bath and dry to constant weight at 105 °C; it leaves a residue of not more than 0.1 mg/mL.

Mass density (ρ_{20}). About 1.031 kg/L.

Water. Determined by the Karl Fischer method, not more than 5.0 mg/mL.

Peroxide. Add 5 mL to a mixture of 1 g of potassium iodide R dissolved in 10 mL of water, 5 mL of hydrochloric acid (~70 g/L) TS, 2 mL of starch TS and mix; not more than a faint blue or brown colour is produced.

Diphenyl ether R

Phenyl ether, $C_{12}H_{10}O$.

Description. A colourless liquid.

Miscibility. Immiscible with water; freely miscible with glacial acetic acid R and with most organic solvents.

Boiling temperature. About 259 °C.

Melting range. 26–28 °C.

Diphenylamine R

$C_{12}H_{11}N$ (SRIP, 1963, p. 81).

Diphenylamine/sulfuric acid TS

Procedure. Dissolve 1.0 g of diphenylamine R in 100 mL of sulfuric acid (~1760 g/L) TS.

Storage. Diphenylamine/sulfuric acid TS must be colourless and should be kept protected from light.

Diphenylbenzidine R

$C_{24}H_{20}N_2$.

Description. A white, or faintly grey-coloured, crystalline powder.

Solubility. Insoluble in water; slightly soluble in ethanol (~750 g/L) TS and acetone R.

Melting range. 246–250 °C.

Sulfated ash. Not more than 1.0 mg/g.

Nitrates. Dissolve 8 mg in a cooled mixture of nitrogen-free sulfuric acid (~1760 g/L) TS and 5 mL of water; the solution is colourless or not more than very pale blue.

1,5-Diphenylcarbazide R

$C_{13}H_{14}N_4O$.

Description. A white, crystalline powder, gradually turning pink on exposure to air.

Melting point. About 174 °C.

Diphenylcarbazide TS

Procedure. Dissolve 0.2 g of 1,5-diphenylcarbazide R in a mixture of 10 mL of glacial acetic acid R and 90 mL of ethanol (~710 g/L) TS.

Sensitivity test to chromate. Dilute 0.5 mL of potassium dichromate (0.0167 mol/L) VS with water to 1000 mL. Dilute 5 mL of this solution to 50 mL with water, add 0.2 mL of hydrochloric acid (2 mol/L) VS and 0.5 mL of diphenylcarbazide TS; a reddish violet colour is produced.

Diphenylcarbazone R

$C_{13}H_{12}N_4O$ (SRIP, 1963, p. 81).

Diphenylcarbazone/ethanol TS

A solution of diphenylcarbazone R dissolved in ethanol (~750 g/L) TS containing about 1 g/L of $C_{13}H_{12}N_4O$.

2,5-Diphenyloxazole R

PPO, $C_{15}H_{11}NO$. Suitable for scintillation counting.

Dipotassium hydrogen phosphate R

K_2HPO_4 (SRIP, 1963, p. 81).

2,2'-Dipyridyl R

2,2’-Bipyridyl. $C_{10}H_8N_2$. Contains not less than 99% of $C_{10}H_8N_2$.

A commercially available reagent of suitable grade.

Disodium chromotropate (10 g/L) TS

A solution of disodium chromotropate R containing about 9.5 g of $C_{10}H_6Na_2O_8S_2$ per litre.

Disodium chromotropate R

[chromotropic acid sodium salt R]. $C_{10}H_6Na_2O_8S_2,2H_2O$.

Description. A yellow to light-brown powder.

Solubility. Freely soluble in water; insoluble in ethanol (~750 g/L) TS.

Identification. To 0.5 mL of a 2 mg/mL solution add 10 mL of water and 1 drop of ferric chloride (25 g/L) TS; a green colour is produced.

Sensitivity. Dissolve 5 mg in 10 mL of a mixture of 9 mL of sulfuric acid (~1760 g/L) TS and 4 mL of water. Separately dilute 0.5 mL of formaldehyde TS with water to make 1000 mL. Transfer to each of two separate test-tubes 5 mL of the disodium chromotropate solution, to one add 0.2 mL of the formaldehyde solution and heat both tubes in a water-bath for 30 minutes; a violet colour is produced in the tube containing the formaldehyde solution.

Disodium chromotropate TS

Procedure. Dissolve 5 mg of disodium chromotropate R in 10 mL of a mixture of 9 mL of sulfuric acid (~1760 g/L) TS and 4 mL of water.

Disodium edetate (0.01 mol/L) VS

Disodium edetate R, dissolved in water to contain 3.342 g of $C_{10}H_{14}N_2Na_2O_8$ in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution following an appropriate method, e.g. as described under disodium edetate (0.05 mol/L) VS.

Disodium edetate (0.05 mol/L) VS

Disodium edetate R, dissolved in water to contain 16.71 g of $C_{10}H_{14}N_2Na_2O_8$ in 1000 mL.

Method of standardization. Ascertain the exact concentration by an appropriate method. The following method is suitable: Transfer about 200 mg of calcium carbonate R2, accurately weighed, to a 400 mL beaker, add 10 mL of water and swirl to form a slurry. Cover the beaker with a watch glass and introduce 2 mL of hydrochloric acid (~70 g/L) TS from a pipette inserted between the lip of the beaker and the edge of the watch glass. Swirl the contents of the beaker to dissolve the calcium carbonate. Wash down the sides of the beaker, the outer surface of the pipette and the watch glass with water and dilute with water to about 100 mL. While stirring the solution, preferably with a magnetic stirrer, add about 30 mL of the disodium edetate solution from a 50 mL burette. Add 10 mL of sodium hydroxide (~80 g/L) TS and 0.3 g of calcon indicator mixture R or of calcon carboxylic acid indicator mixture R and continue the titration with the disodium edetate solution to a blue end-point. Each 5.005 mg of calcium carbonate is equivalent to 1 mL of disodium edetate (0.05 mol/L) VS.

Disodium edetate (0.1 mol/L) VS

Disodium edetate R, dissolved in water to contain 33.42 g of $C_{10}H_{14}N_2Na_2O_8$ in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution following an appropriate method, e.g. as described under disodium edetate (0.05 mol/L) VS.

Disodium edetate (10 g/L) TS

A solution of disodium edetate R containing about 10 g of $C_{10}H_{14}N_2Na_2O_8$ per litre.

Disodium edetate (20 g/L) TS

A solution of disodium edetate R containing about 20 g of $C_{10}H_{14}N_2Na_2O_8$ per litre.

Disodium edetate R

$C_{10}H_{14}N_2Na_2O_8,2H_2O$ (SRIP, 1963, p. 82).

Disodium hydrogen phosphate (100 g/L) TS

A solution of disodium hydrogen phosphate R containing about 100 g of Na_2HPO_4 per litre.

Disodium hydrogen phosphate (28.4 g/L) TS

A solution of anhydrous disodium hydrogen phosphate R containing 28.4 g of Na_2HPO_4 per litre.

Disodium hydrogen phosphate (40 g/L) TS

A solution of disodium hydrogen phosphate R containing about 40 g of Na_2HPO_4 per litre.

Disodium hydrogen phosphate R

[sodium phosphate R]. $Na_2HPO_4,12H_2O$ (SRIP, 1963, p.192).

Disodium hydrogen phosphate, anhydrous, R

[sodium phosphate, anhydrous, R]. Na_2HPO_4 (SRIP, 1963, p. 193).

5,5'-Dithiobis(2-nitrobenzoic acid) R

3-Carboxy-4-nitrophenyl disulfide; $C_{14}H_8N_2O_8S_2$.

A commercially available reagent of suitable grade.

5,5'-Dithiobis-2-nitrobenzoic acid/methanol TS

Procedure. Dissolve 0.198 g of 5,5'-Dithiobis(2-nitrobenzoic acid) R in sufficient methanol R to produce 500 mL.

Storage. Keep under refrigeration, and warm to room temperature before use.

Dithiol R

$C_7H_8S_2$ CAS[496-74-2]

Toluene-3, 4-dithiol. 4-methylbenzene-1,2-dithiol.

Hygroscopic white crystals soluble in methanol and in alkali hydroxides solutions.

Dithiol reagent R

Prepare immediately before use. Dissolve 1 g of dithiol R by adding 2 mL thioglycollic acid R and dilute to 250 mL with 20 g/L solution of sodium hydroxide R.

Dithizone R

$C_{13}H_{12}N_4S$ (SRIP, 1963, p. 83).

Dithizone standard TS

Procedure. Dissolve 10 mg of dithizone R in 1000 mL of chloroform R.

Storage. Store the solution in a glass-stoppered, lead-free bottle, protected from light and kept at a temperature not exceeding 4 °C.

Dithizone TS

Procedure. Dissolve 0.10 g of dithizone R in sufficient ethanol (~750 g/L) TS to produce 100 mL.

Docusate sodium R

Sodium 1,4-bis[(2-ethylhexyl)oxy]-1,4-dioxobutane-2-sulfonate.

A commercially available reagent of suitable grade.

Domiphen bromide (10 g/L) TS

A solution of domiphen bromide R containing about 10 g of $C_{22}H_{40}BrNO$ per litre.

Domiphen bromide R

Dodecyldimethyl(2-phenoxyethyl)ammonium bromide; $C_{22}H_{40}BrNO$.

Description. Colourless or faintly yellow, crystalline flakes.

Solubility. Freely soluble in water and ethanol (~750 g/L) TS; soluble in acetone R.

Dopamine hydrochloride RS.

International Chemical Reference Substance.

Dragendorff reagent TS

Procedure. Shake vigorously to dissolve 0.85 g of bismuth subnitrate R in 10 mL of glacial acetic acid R and 40 mL of water (*solution A*). Dissolve 8 g of potassium iodide R in 20 mL of water (*solution B*). Immediately before use mix together equal volumes of *solutions A* and *B* and glacial acetic acid R.

Storage. Solutions A and *B* must be protected from light.

Dragendorff reagent, modified, TS

Procedure. Add 20 mL of acetic acid (~60 g/L) TS to 4 mL of a mixture of equal volumes of *solutions A* and *B* of Dragendorff reagent TS.

Note: Prepare this solution immediately before use.

B - Barium chloride (0.1 mol/L) VS.... Butylated hydroxytoluene R

Barium chloride (0.1 mol/L) VS

Barium chloride R, dissolved in water R to contain 20.8 g of $BaCl_2$ in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution by following the method described under barium chloride (0.5 mol/L) VS.

Barium chloride (0.5 mol/L) VS

Barium chloride R, dissolved in water to contain 104.2 g of $BaCl_2$ in 1000 mL.

Method of standardization. Ascertain the exact concentration of the 0.5 mol/L solution in the following manner: Place 10.0 mL of sulfuric acid (0.5 mol/L) VS in a flask, dilute with 40 mL of water, add 2 drops of thorin (2 g/L) TS and titrate slowly with the barium chloride solution to a reddish colour.

Barium chloride (50 g/L) TS

A solution of barium chloride R containing about 52 g/L of $BaCl_2$ (approximately 0.25 mol/L).

Barium chloride (120 g/L) TS

A solution of barium chloride R containing about 120 g of barium chloride per litre.

Barium chloride R

$BaCl_2,2H_2O$ (SRIP, 1963, p. 45).

Barium hydroxide (0.15mol/L) VS

Barium hydroxide R dissolved in carbon-dioxide-free water R to contain 25.7 g of $Ba(OH)_2$ in 1000 mL.

Barium hydroxide (15 g/L)TS

A solution of barium hydroxide R in carbon-dioxide-free water R containing about 15 g of $Ba(OH)_2$ per litre.

Note: Barium hydroxide (15 g/L) TS must be freshly prepared.

Barium hydroxide R

$Ba(OH)_2,8H_2O$ (SRIP, 1963, p. 46).

Barium nitrate (0.01 mol/L) VS

Barium nitrate R, dissolved in water to contain 2.614 g of $Ba(NO_3)_2$ in 1000 mL.

Method of standardization. Ascertain the exact concentration of the 0.01 mol/L solution in the following manner: Place 10.0 mL of sulfuric acid (0.01 mol/L) VS in a flask, dilute with 40 mL of water, add 2 drops of thorin (2 g/L) TS and 2 drops of methylthioninium chloride (0.2 g/L) TS and titrate slowly with the barium nitrate solution from yellow to a pink colour.

Barium nitrate R

$Ba(NO_3)_2$ (SRIP, 1963, p. 47).

Barium oxide R

BaO.

Description. White to yellowish-white lumps or powder. Absorbs moisture and carbon dioxide on exposure to air.

Storage. Store in tightly closed containers.

Barium sulfate suspension TS

Procedure. Mix 15 mL of barium chloride (0.5 mol/L) VS with 55 mL of water and 20 mL of sulfate-free ethanol (~750 g/L) TS, add 5 mL of potassium sulfate (174 mg/L) TS and dilute with sufficient water to produce 100 mL.

Note. Barium sulfate suspension TS must be freshly prepared.

Beef extract R

A residue from beef broth obtained by extracting fresh, sound, lean beef by cooking with water and evaporating the resulting broth at a low temperature, usually under reduced pressure, until a thick pasty residue is obtained.

Benzalkonium chloride TS

A mixture of alkylbenzyldimethylammonium chlorides. It contains in 1 litre not less than 470 g and not more than 530 g of alkylbenzyldimethylammonium chlorides, calculated as $C_{22}H_{40}ClN$.

Description. A clear, colourless to pale yellow, syrupy liquid; odour, aromatic.

Miscibility. Miscible with water and ethanol (~750 g/L) TS.

Assay. Dissolve 4 g, accurately weighed, in sufficient water to produce 100 mL. Transfer 25 mL to a separator, add 25 mL of chloroform R, 10 mL of sodium hydroxide (0.1 mol/L) VS, and 10 mL of freshly prepared potassium iodide (50 g/L) TS. Shake well, allow to separate and run off the chloroform layer. Shake the aqueous solution with 3 further quantities of chloroform R, each of 10 mL, and discard the chloroform solutions. Add 40 mL of hydrochloric acid (~420 g/L) TS, cool and titrate with potassium iodate (0.05 mol/L) VS until the solution becomes pale brown in colour. Add 2 mL of chloroform R and continue the titration until the chloroform becomes colourless. Titrate a mixture of 20 mL of water, 6 mL of potassium iodide (80 g/L) TS and 40 mL of hydrochloric acid (~420 g/L) TS with potassium iodate (0.05 mol/L) VS in a similar manner; the difference between the titrations represents the amount of potassium iodate (0.05 mol/L) VS required. Each mL of potassium iodate (0.05 mol/L) VS is equivalent to 35.40 mg of $C_{22}H_{40}ClN$. Determine the mass density using a pycnometer as described in *1.3 Determination of mass density*, relative density and weight per millilitre and calculate in g/L the proportion of $C_{22}H_{40}ClN$.

Note: A solution in water foams strongly when shaken.

Benzalkonium chloride TS1

Procedure. Dilute 2 mL of benzalkonium chloride TS with sufficient water to produce 100 mL.

Benzene R

C_6H_6 (SRIP, 1963, p. 48).

Benzethonium chloride R

Benzyldimethyl[2-[2-[4-(1,1,3,3-tetramethylbutyl)phenoxy]ethoxy]ethyl]ammonium chloride. $C_{27}H_{42}ClNO_2$. CAS Reg. No. 121-54-0.

Description . White or yellowish-white powder.

Solubility . Very soluble in water and in ethanol (~750 g/L) TS, freely soluble in dichrloromethan R.

Melting point. About 158- 165 °C, after drying at 100- 105 to constant mass

Storage. Store protected from light.

Benzethonium chloride (0.004 mol/L) VS

Procedure. Dissolve 1.792 g of benzethonium chloride R, previously dried to constant mass at 100 °C to 105 °C, in water R and dilute to 1000.0 mL with the same solvent.

Method of standardization. Dissolve 0.350 g of the dried substance in 35 mL of a mixture of 30 volumes of anhydrous acetic acid R and 70 volumes of acetic anhydride R. Titrate with perchloric acid (0.1 mol/L) VS, using 0.05 mL of crystal violet /acetic acid TS1 as indicator. Carry out a blank titration. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 44.81 mg of $C_{27}H_{42}ClNO_2$.

Benzoic acid R

$C_7H_6O_2$. Contains not less than 99.8% of $C_7H_6O_2$.

Description. Colourless, light, feathery crystals or a white, microcrystalline powder; odour, characteristic, faint.

Solubility. Slightly soluble in water; freely soluble in ethanol (~750 g/L) TS and ether R.

Methanol-insoluble substances. Dissolve 20 g in 200 mL of methanol R and digest under complete reflux for 30 minutes. Filter through a tared filtering crucible, wash thoroughly with methanol R and dry at 105 °C; it leaves a residue of not more than 1.0 mg.

Assay. Dissolve about 0.5 g, accurately weighed, in 15 mL of ethanol (~750 g/L) TS, previously neutralized to phenol red/ethanol TS, add 20 mL of water and titrate with sodium hydroxide (0.1 mol/L) VS, using phenol red/ethanol TS as indicator. Repeat the operation without the substance being examined and make any necessary correction. Each mL of sodium hydroxide (0.1 mol/L) VS is equivalent to 12.21 mg of $C_7H_6O_2$.

Benzophenone R

Diphenyl ketone; $C_{13}H_{10}O$.

A commercially available reagent of suitable grade.

Melting point. About 49 °C.

Benzoyl chloride R

C_7H_5ClO (SRIP, 1963, p. 50).

Benzoyl peroxide, hydrous R

$C_{14}H_{10}O_4,\times H_2O$.

A commercially available reagent of suitable grade.

Description. A white, amorphous or granular powder.

Note : For safety reasons it should be kept moistened with about 23% w/w water.

Benzyl alcohol R

C_7H_8O.

Description. A colourless liquid; almost odourless.

Miscibility. Miscible with 25 parts of water; miscible with ethanol (~750 g/L) TS and ether R.

Boiling temperature. About 204 °C.

Mass density. ρ_{20} = about 1.05 kg/L.

Benzyl benzoate R

$C_{14}H_{12}O_2$.

Benzyl benzoate as described in the monograph for *Benzyl benzoate*.

A commercially available reagent of suitable grade.

Description. A clear, colourless, oily liquid.

Benzylpenicillin sodium R

$C_{16}H_{17}N_2NaO_4S$. Contains not less than 96.0% and not more than 102.0% of $C_{16}H_{17}N_2NaO_4S$, calculated with reference to the dried substance.

Description. A white or almost white, crystalline powder; odourless or with a faint characteristic odour.

Solubility. Soluble in about 0.5 part of water; practically insoluble in ether R.

Benzylpenicillin sodium TS

Procedure. Dissolve 0.03 g of benzylpenicillin sodium R in sufficient phosphate buffer, pH 7.0, TS, to produce 10 mL. This solution contains not less than 3 mg/mL of benzylpenicillin sodium R.

4,4'-Bis(dimethylamino)benzophenone R

Tetramethyldiaminobenzophenone; $C_{17}H_{20}N_2O$.

Other name. Michler's ketone.

Melting point. About 176 °C.

Bismuth oxynitrate R

Approximately $4BiNO_3(OH)_2,BiO(OH)$ (SRIP, 1963, p. 50).

Bismuth subnitrate R

Bismuth hydroxide nitrate oxide; $Bi_5O(OH)_9(NO_3)_4$. Bismuth subnitrate is a basic salt, the composition of which varies with the conditions under which it is prepared. It contains not less than 71.5% and not more than 74.5% of Bi, calculated with reference to the dried substance.

Description. A white powder.

Solubility. Practically insoluble in water and ethanol (~750 g/L) TS; soluble in hydrochloric acid (~250 g/L) TS and nitric acid (~1000 g/L) TS.

Blue tetrazolium R

$C_{40}H_{32}Cl_2N_8O_2$. 3,3'-Dianisole-bis-[4,4'-(3,5-diphenyl) tetrazolium chloride].

Description. Lemon-yellow crystals.

Solubility. Slightly soluble in water; freely soluble in ethanol (~750 g/L) TS and methanol R; practically insoluble in acetone R and ether R.

Molar absorptivity. Its molar absorptivity in methanol R at 252 nm, is not less than 60 000.

Suitability test. Prepare the following standard solution: Dissolve in ethanol (~750 g/L) TS a suitable quantity of hydrocortisone R, previously dried at 105 °C for 3 hours and accurately weighed, and prepare by a step-by-step dilution a solution containing about 30 µg/mL. Transfer 10, 15 and 20 mL quantities of the standard solution to separate glass-stoppered 50ml conical flasks. Add 10 mL and 5 mL, respectively, of ethanol (~750 g/L) TS to the flasks containing the 10 and 15 mL quantities of the standard solution and swirl to mix. To each of the flasks, and to a fourth flask, representing the blank, containing 20 mL of ethanol (~750 g/L) TS, add 2.0 mL of a solution prepared by dissolving 0.05 g of the blue tetrazolium R in 10 mL of ethanol (~750 g/L) TS, mix and then add 2.0 mL of tetramethylammonium hydroxide/ethanol TS. Mix, allow the flasks to stand in the dark for 90 minutes and determine the absorbances at 525 nm against the blank. Plot the absorbances on the abscissa and the amount of hydrocortisone on the ordinate scale of arithmetic coordinate paper and draw the curve of best fit; the absorbance of each solution is proportional to the concentration and the absorbance of the solution containing 200 µg of hydrocortisone is not less than 0.50.

Blue tetrazolium/ethanol TS

Procedure. Dissolve 0.5 g of blue tetrazolium R in sufficient aldehyde-free ethanol (~750 g/L) TS, warming slightly if necessary, to produce 100 mL.

Blue tetrazolium/sodium hydroxide TS

Procedure. Immediately before use mix 1 volume of a 2 mg/mL solution of blue tetrazolium R in water with 3 volumes of a 0.12 g/mL solution of sodium hydroxide R in methanol TS.

Borate buffer, pH 8.0, TS

Procedure. Dissolve 0.25 g of boric acid R and 0.30 g of potassium chloride R in 50 mL of carbon-dioxide-free water R, add 3.97 mL of carbonate-free sodium hydroxide (0.2 mol/L) VS, and dilute with sufficient carbon-dioxide-free water R to produce 200 mL.

Borate buffer, pH 9.0, TS

Procedure. Dissolve 1.24 g of boric acid R in about 100 mL of water, add 8.3 mL of sodium hydroxide (1 mol/L) VS and add sufficient water to produce 200 mL.

Borate buffer, pH 9.6, TS

Procedure. Dissolve 0.25 g of boric acid R and 0.30 g of potassium chloride R in 50 mL of carbon-dioxide-free water R, add 36.85 mL of carbonate-free sodium hydroxide (0.2 mol/L) VS and dilute with sufficient carbon-dioxide-free water R to produce 200 mL.

Boric acid (50 g/L) TS

A solution of boric acid R containing about 50 g/L of H_3BO_3.

Boric acid R

H_3BO_3. Contains not less than 99.0% of H_3BO_3.

Description. White crystals or scales of a somewhat pearly lustre or a white, crystalline powder.

Solubility. Soluble in 20 parts of water, in 3 parts of boiling water and in 16 parts of ethanol (~750 g/L) TS.

Water-insoluble substances. 1.0 g dissolves in 30 parts of water; the solution is clear and colourless.

Ethanol-insoluble substances. 1.0 g dissolves in 10 mL of boiling ethanol (~750 g/L) TS; the solution is not more than faintly turbid.

Assay. Dissolve about 1 g, accurately weighed, in 30 mL of water; add 50 mL of glycerol R, previously neutralized to phenolphthalein/ethanol TS and titrate with carbonate-free sodium hydroxide (1 mol/L) VS, using phenolphthalein/ethanol TS as indicator. Each mL of carbonate-free sodium hydroxide (1 mol/L) VS is equivalent to 61.83 mg of H_3BO_3.

Brilliant green R

[4-[*p*-(Diethylamino)-α-phenylbenzylidene]-2,5-cyclohexadien-1-ylidene]diethylammonium hydrogen sulfate; $C_{27}H_{34}N_2O_4S$; C.I. 42040; Malachite green G; C.I. Basic green 1.

Description. Small, glistening, golden crystals.

Brilliant green/acetic acid TS

Procedure. Dissolve 0.5 g of brilliant green R in sufficient glacial acetic acid R1 to produce 100 mL.

Bromine AsTS

Procedure. Dissolve 30 g of potassium bromide R in 40 mL of water, add 30 g of bromine R and dilute with sufficient water to produce 100 mL. The solution complies with the following test: evaporate 10 mL nearly to dryness on a water-bath, add 50 mL of water, 10 mL of hydrochloric acid (~250 g/L) AsTS, sufficient stannous chloride AsTS to reduce the remaining bromine and apply the general test for arsenic. The colour of the stain produced is not more intense than that produced from a 1 mL standard stain, showing that the amount of arsenic does not exceed 1 µg/mL.

Bromine R

Br_2 (SRIP, 1963, p. 51).

Bromine TS1

A saturated solution of bromine R.

Bromocresol green R

$C_{21}H_{14}Br_4O_5S$ (SRIP, 1963, p. 52).

Bromocresol green TS1

Procedure. Dissolve 0.05 g of bromocresol green R and 1.021 g of potassium hydrogen phthalate R in 6 mL of sodium hydroxide (0.2 mol/L) VS and dilute to 100 mL with water. Filter if necessary.

Bromocresol green/ethanol TS

Procedure. Warm 0.1 g of bromocresol green R with 2.9 mL of sodium hydroxide (0.05 mol/L) VS and 5 mL of ethanol (~710 g/L) TS; after solution has been effected add a sufficient quantity of ethanol (~150 g/L) TS to produce 250 mL.

Bromocresol purple R

$C_{21}H_{16}Br_2O_5S$ (SRIP, 1963, p. 52).

Bromocresol purple/ethanol TS.

Procedure. Dissolve 0.05 g of bromocresol purple R in 100 mL of ethanol (~750 g/L) TS and filter if necessary.

Bromophenol blue (1g/L) TS.

A solution of bromophenol blue R containing about 1.0 g of $C_{19}H_{10}Br_4O_5S$ per litre.

Bromophenol blue R

$C_{19}H_{10}Br_4O_5S$ (SRIP, 1963, p. 52).

Bromophenol blue TS

Procedure. Dissolve 0.05 g of bromophenol blue R with gentle heating in 3.73 mL of sodium hydroxide (0.02 mol/L) VS and dilute to 100 mL with water.

Bromophenol blue/ethanol TS.

Procedure. Warm 0.1 g of bromophenol blue R with 3.2 mL of sodium hydroxide (0.05 mol/L) VS and 5 mL of ethanol (~710 g/L) TS; after solution has been effected add a sufficient quantity of ethanol (~150 g/L) TS to produce 250 mL.

Bromothymol blue R

$C_{27}H_{28}Br_2O_5S$ (SRIP, 1963, p. 53).

Bromothymol blue/dimethylformamide TS

Procedure. Dissolve 1.0 g of bromothymol blue R in sufficient dimethylformamide R to produce 100 mL.

Bromothymol blue/ethanol TS

Procedure. Warm 0.1 g of bromothymol blue R with 3.2 mL of sodium hydroxide (0.05 mol/L) VS and 5 mL of ethanol (~710 g/L) TS; after solution has been effected add a sufficient quantity of ethanol (~150 g/L) TS to produce 250 mL.

Brown stock standard TS

Procedure. To 35.0 mL of cobalt colour TS add 17.0 mL of copper colour TS, 8.0 mL of dichromate colour TS, dilute to 100.0 mL with iron colour TS and mix.

2-Butanol R

$C_4H_{10}O$ (SRIP, 1963, p. 53).

1-Butanol R

[*n*-Butanol R]. $C_4H_{10}O$ (SRIP, 1963, p. 54).

***tert* -Butanol R**

2-Methylpropan-2-ol; $(CH_3)_3COH$.

Description. A colourless liquid or solid.

Miscibility. Miscible with water, ethanol (~750 g/L) TS and ether R.

Boiling range. Not less than 95% distils between 81 and 83 °C.

Melting range. 24–26 °C.

Mass density. ρ_{20}= 0.778–0.782 kg/L.

Residue on evaporation. Evaporate on a water-bath and dry to constant weight at 105 °C; it leaves a residue of not more than 0.05 mg/mL.

Butyl acetate R

$C_6H_{12}O_2$.

Description. A clear, colourless, flammable liquid; odour, characteristic.

Miscibility. Slightly miscible with water; miscible with ethanol (~750 g/L) TS.

Mass density. ρ_{20} = about 0.88 kg/L.

3-tert-Butyl-4-hydroxyanisole R

$C_{11}H_{16}O_2$; 180.24; *[25013-16-5]*

Description. White to light yellow powder.

Use a suitable grade.

tert-Butyl methyl ether R

1,1-Dimethylethyl methyl ether; $C_5H_{12}O$.

A commercially available reagent of suitable grade.

Description. A clear, colourless liquid; inflammable.

Refractive index. n_D^{20} = 1.3756.

Relative density. d_4^{20} = 0.740–0.742.

1-Butylamine R

1-Aminobutane; $C_4H_{11}N$.

Description. A colourless to pale yellow inflammable liquid.

Miscibility. Miscible with water, ethanol (~750 g/L) TS and ether R.

Boiling range. Not less than 95% distils between 76 and 78 °C.

Mass density, ρ_{20} = about 0.740 kg/L.

Water. Determine as described under *2.8 Determination of water by the Karl Fischer method* using about 5 mL of the liquid; not more than 10 mg/mL.

Acid impurities. To 50 mL add 5 drops of azo violet TS and titrate quickly with sodium methoxide (0.1 mol/L) VS to a deep blue end-point taking precautions to prevent absorption of atmospheric carbon dioxide, e.g. by use of an atmosphere of nitrogen; not more than 1.0 mL of sodium methoxide (0.1 mol/L) VS is required for neutralization.

Butylated hydroxyanisole R

Use $C_{11}H_{16}O_2$.

Butylated hydroxyanisole as described in the monograph in *Butylated hydroxyanisole*.

Butylated hydroxytoluene R.

2,6-Di-*tert*-butyl-4-methylphenol, $C_{15}H_{24}O$.

Description. Colourless crystals, or a white, crystalline powder.

Solubility. Practically insoluble in water; freely soluble in ethanol (~750 g/L) TS; very soluble in ether R.

Melting temperature. About 70 °C.

Sulfated ash. Not more than 1.0 mg/g.

A - Acacia R.... Azo violet TS

Acacia R

The dried gummy exudate from the stems and branches of *Acacia senegal* (L.) Willd. and of other species of *Acacia* of African origin.

Description. Rounded or ovoid tears of varying diameters from about 1 cm to 3 cm; yellowish white or pale amber; odourless.

Solubility. Very slowly soluble in twice its weight of water, leaving only a very small residue of vegetable particles; practically insoluble in ethanol (~750 g/L) TS and ether R.

Ash. Not more than 50 mg/g.

Acid-insoluble ash. Not more than 5.0 mg/g.

Insoluble matter. Mix 5 g of powdered or finely ground material with 100 mL of water and 10 mL of hydrochloric acid (~70 g/L) TS and boil gently for 15 minutes, stirring frequently. Filter while hot through a sintered glass crucible, wash the residue with hot water and dry to constant weight at 105 °C; not more than 5 mg/g.

Tannin. Dissolve 1 g in 10 mL of water and add 0.1 mL of ferric chloride (25 g/L) TS; no bluish-black colour or blackish precipitate is produced.

Acetaldehyde R

Ethanal; C_2H_4O.

Description. A clear, colourless, flammable liquid.

Miscibility. Miscible with water and ethanol (~750 g/L) TS.

Refractive index. n_D^{20} = 1.332

Relative density. d_{20}^{20} = 0.788

Boiling point. About 21°C.

Acetate buffer, pH 3.0, TS

A buffer mixture of pH 3.0.

Procedure. Dissolve 12 g of sodium acetate R in water, add 6 mL of glacial acetic acid R and dilute with sufficient water to produce 100 mL.

Acetate buffer, pH 3.5, TS

Procedure. Dissolve 25.0 g of ammonium acetate R in 25 mL of water R and add 38.0 mL of hydrochloric acid (~250 g/l) TS. Adjust the pH, if necessary, with hydrochloric acid (~70 g/L) TS or ammonia (~100 g/L) TS. Dilute with water R to 100.0 mL.

Acetate buffer, pH 4.5, TS

Procedure. Dissolve 10.9 g of sodium acetate R in 100 mL of water, add 8 mL of glacial acetic acid R, mix and dilute to 1000 m with water.

Acetate buffer, pH 4.6, TS

Procedure. Dissolve 5.4 g of sodium acetate R in 50 mL of water, adjust to pH 4.6 with glacial acetic acid R and dilute to 100 mL with water.

Acetate buffer, pH 4.7, TS

Procedure. Dissolve 8.4 g of sodium acetate R in 100 mL of water, add 3.35 mL of glacial acetic acid R, mix and dilute to 1000 mL with water.

Acetate buffer, pH 5.0, TS

Procedure. Dissolve 13.6 g of sodium acetate R in 100 mL of water, add 6 mL of glacial acetic acid R, mix and dilute to 1000 mL with water.

Acetate buffer, pH 5.5, TS

Procedure. Dissolve 54.4 g of sodium acetate R in 50 mL of water, heating to 35 °C, if necessary. After cooling, slowly add 10 mL of glacial acetic acid R. Shake and dilute to 1000 mL with water.

Acetate buffer, pH 6.0, TS

Procedure. Dissolve 100 g of ammonium acetate R in 300 mL of water. Add 4.1 mL of glacial acetic acid R, adjust the pH to 6.0 using either ammonia (~100 g/L) TS or acetic acid (~300 g/L) TS and dilute to 500 mL with water.

Acetate standard buffer TS

Procedure. To 10 mL of acetic acid (~60 g/L) TS add 10 mL of sodium hydroxide (1 mol/L) VS and dilute with sufficient carbon-dioxide-free water R to produce 1000 mL.

Acetic acid (~10 g/L) TS

Acetic acid (~300 g/L) TS, diluted with water to contain 10 g of $C_2H_4O_2$ per litre (approximately 0.17 mol/L); *d*~1.0016

Acetic acid (~90 g/L) TS

Acetic acid (~300 g/L) TS, diluted with water to contain about 90 g of $C_2H_4O_2$ per litre (approximately 1.5 mol/L).

Acetic acid (~120 g/L) TS

Acetic acid (~300 g/L) TS, diluted with water to contain 120 g of $C_2H_4O_2$ per litre (approximately 2 mol/L); *d*~1.016.

Acetic acid (~300 g/L) TS

A solution of glacial acetic acid R containing about 300 g/L of $C_2H_4O_2$ (approximately 5 mol/L); *d*~1.037.

Acetic acid (~60 g/L) PbTS

Acetic acid (~60 g/l) TS that complies with the following test: Evaporate 20 mL of acetic acid (~60 g/L) TS almost to dryness on a water-bath, add 25 mL of water and carry out the test for heavy metals. The heavy metals limit is 3 µg/mL.

Acetic acid (~60 g/L) TS

Acetic acid (~300 g/L) TS, diluted to contain about 60 g/L of $C_2H_4O_2$ (approximately 1 mol/L); *d*~1.008.

Acetic acid (0.07 mol/L) VS

A solution prepared by diluting 4.2 mL of glacial acetic acid R to 1000 mL with water.

Acetic acid (5.0 g/L) TS

Acetic acid (~300 g/L) TS, diluted with water to contain about 5.0 g of $C_2H_4O_2$ per litre; *d*~1.0007.

Acetic acid (~10 g/L) TS

Acetic acid (~300 g/L) TS, diluted with water to contain about 10 g of $C_2H_4O_2$ per litre.

Acetic acid, anhydrous, R

Glacial acetic acid R for use in non-aqueous titrations containing not less than 99.6% w/w of $C_2H_4O_2$ and that complies with the following test:

Water. Not more than 4 mg/g, determined as described under *2.8 Determination of water by the Karl Fischer method*, Method A. If the water content is greater than 4 mg/g it may be adjusted by adding the calculated amount of acetic anhydride R.

Acetic acid, glacial, R

$C_2H_4O_2$ (SRIP, 1963, p. 25); *d*~1.048.

Acetic acid, glacial, R1

Glacial acetic acid R, that complies with the following tests:

Substances reducing dichromate. To 10 mL add 1.0 mL of potassium dichromate (0.0167 mol/L) VS and cautiously add 10 mL of sulfuric acid (~1760 g/l) TS. Cool the solution to room temperature and allow to stand for 30 minutes. While swirling the solution dilute slowly and cautiously with 50 mL of water, cool and add 1.5 mL of freshly-prepared potassium iodide (80 g/L) TS. Titrate the liberated iodine with sodium thiosulfate (0.1 mol/L) VS, adding 3 mL of starch TS as the end-point is approached. Perform a blank titration and make any necessary corrections. Not less than 0.60 mL of sodium thiosulfate (0.1 mol/L) VS is consumed.

Substances reducing permanganate. Add 40 mL to 10 mL of water. Cool to 15 °C, add 0.30 mL of potassium permanganate (0.02 mol/L) VS and allow to stand at 15 °C for 10 minutes; the pink colour is not entirely discharged.

Acetic anhydride R

$C_4H_6O_3$ (SRIP, 1963, p. 26).

Acetic anhydride/dioxan TS

Procedure. To 50 mL of dioxan R add 1 mL of acetic anhydride R (approximately 0.2 mol/L).

Acetone R

C_3H_6O (SRIP, 1963, p. 27).

Acetonitrile (400 g/L) TS

Procedure. Mix 1 volume of acetonitrile R with 1 volume of water. The resulting solution contains about 400 g/L of C_2H_3N.

Acetonitrile for chromatography R

Acetonitrile for chromatography contains not less than 99.9% of C_2H_3N and complies with the following tests.

Absorbance (1.6): not more than 0.10 at 200 nm, determined using water R as compensation liquid.

Acetonitrile R

Methyl cyanide, C_2H_3N.

Description. A clear, colourless liquid.

Miscibility. Freely miscible with water.

Acetyl chloride R

C_2H_3ClO. Contains not less than 98.0% of C_2H_3ClO in both assay A and assay B (see below).

Description. A clear, colourless or very slightly yellow liquid.

Phosphorus compounds. Carefully treat 1 mL with 1 mL of water, add 1 mL of nitric acid (~1000 g/L) TS, boil, cool, dilute with 20 mL of water, add 10 mL of ammonium molybdate/nitric acid TS and allow to stand at about 40 °C for 2 hours; no yellow precipitate is produced.

Assay. (A) Dissolve about 1 g, accurately weighed, in 50 mL of carbonate-free sodium hydroxide (1 mol/L) VS and titrate with sulfuric acid (0.5 mol/L) VS, using phenolphthalein/ethanol TS as indicator. Each mL of carbonate-free sodium hydroxide (1 mol/L) VS is equivalent to 7.850 mg of C_2H_3ClO. (B) Dilute the neutralized liquid from A to 250 mL with water, mix and titrate 50 mL with silver nitrate (0.1 mol/L) VS, using potassium chromate (100 g/L) TS as indicator. Each mL of silver nitrate (0.1 mol/L) VS is equivalent to 7.850 g of C_2H_3ClO.

Aciclovir R

Aciclovir of a suitable quality should be used.

Adipic acid R

$C_6H_{10}O_4$. CAS Reg. No. 124-04-9.

Description. Prisms.

Solubility. Freely soluble in methanol, soluble in acetone, practically insoluble in light petroleum.

Melting point. About 152 °C.

Agar R

(SRIP, 1963, p. 27).

Alum R

Aluminium potassium sulfate dodecahydrate; $KAl(SO_4)_2,12H_2O$ (SRIP, 1963, p. 29).

Ammonia/ethanol/methanol (1/95/5) TS

Procedure. To 1 mL of ammonia (~206 g/L) TS add 99 mL of Ethanol / methanol (95/5) TS.

Ammonia/methanol (10/90) TS

Procedure. To 10 mL of ammonia (~206 g/L) TS add 90 mL of methanol R.

Ammonium acetate (~0.40 g/L) TS

A solution of ammonium acetate R containing about 0.385 g of $C_2H_7NO_2$ per litre (approximately 0.005 mol/L).

Amoxicillin trihydrate R

Amoxicillin trihydrate of a suitable quality should be used.

Aluminium chloride R

$AlCl_3,6H_2O$ (SRIP, 1963, p. 30).

Aluminium chloride TS

Procedure. Dissolve 65.0 g of aluminium chloride R in sufficient water to produce 100 mL, add 0.5 g of charcoal R, stir for 10 minutes and filter. While stirring add to the filtrate sufficient sodium hydroxide (0.5 mol/L) VS to adjust the pH to 1.5.

Aluminium hydroxide R

Hydrated $Al(OH)_3$.

Description. A white, odourless powder.

Solubility. Practically insoluble in water and in ethanol (~750 g/L) TS.

Aluminium oxide R

Al_2O_3.

A suitable grade for use in thin-layer chromatography.

Aluminium R

Al (SRIP, 1963, p. 29); wire, granules or sheets.

Aluminium standard (2 ppm Al) TS

Procedure. Immediately before use dilute with water R to 100 times its volume a solution containing aluminium potassium sulphate R equivalent to 0.352 g of $AlK(SO_4)_2$, $12H_2O$ and 10 mL of diluted sulphuric acid R in 100 mL.

Aluminium standard (10 µg Al/mL) TS

Procedure. Dissolve 17.6 mg of alum R in 5 mL of sulfuric acid (0.05 mol/L) VS and dilute to 100 mL with water.

Note. For the preparation of this test solution commercially available aluminium nitrate standard solution 1000 µg Al^{3+}/mL or aluminium nitrate nonahydrate can also be used.

4-Amino-6-chloro-1,3-benzenedisulfonamide R

$C_6H_8ClN_3O_4S_2$.

Description. A white, odourless powder.

Solubility. Soluble in ammonia (~100 g/L) TS; practically insoluble in water.

Identification. The absorption spectrum of a 5 µg/mL solution in methanol R exhibits maxima at about 223 nm, 265 nm and 312 nm. The absorptivity at 265 nm is about 64.0 ($A^{1\%}_{1cm}$ = 640).

Sulfated ash. Ignite 2 g; not more than 1.0 mg/g.

4-Aminoantipyrine R

$C_{11}H_{13}N_3O$. 4-Amino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one; ampyrone; aminopyrazolone.

Description. Pale yellow crystals or powder.

Solubility. Sparingly soluble in water; freely soluble in ethanol (~750 g/L) TS; very slightly soluble in ether R.

Melting temperature. About 108 °C.

4-Aminoantipyrine TS1

Procedure. Dissolve 0.125 g of 4-aminoantipyrine R in 25 mL of methanol R containing 0.25 mL of hydrochloric acid (~420 g/L) TS.

4-Aminoantipyrine TS2

Procedure. Dissolve about 0.1 g of 4-aminoantipyrine R in 30 mL of water and add a mixture of 10 mL of sodium carbonate (200 g/L) TS and 2 mL of sodium hydroxide (1 mol/L) VS; dilute with sufficient water to produce 100 mL.

Note: 4-Aminoantipyrine TS2 must be freshly prepared.

4-Aminobenzoic acid R

$C_7H_7NO_2$. Contains not less than 98.5% of $C_7H_7NO_2$.

Description. White or slightly yellow crystals or a crystalline powder; odourless.

Solubility. Soluble in 170 parts of water, in 9 parts of boiling water, in 8 parts of ethanol (~750 g/L) TS and in 50 parts of ether R.

Melting range. 186–189 °C.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry at 105 °C for 2 hours; it loses not more than 2.0 mg/g.

Assay. Transfer to a beaker about 0.3 g, accurately weighed and previously dried at 105 °C for 2 hours, add 5 mL of hydrochloric acid (~420 g/L) TS, 50 mL of water and stir until dissolved. Cool to about 15 °C, add about 25 g of crushed ice and slowly titrate with sodium nitrite (0.1 mol/L) VS until a glass rod dipped into the titrated solution produces an immediate blue ring when touched to starch/iodide paper R. When the titration is complete the end-point is reproducible after the mixture has been allowed to stand for 1 minute. Each mL of sodium nitrite (0.1 mol/L) VS is equivalent to 13.71 mg of C7H7NO2.

Storage. Store in a tightly closed container, protected from light.

2-Aminobutanol R

$C_4H_{11}NO$.

Description. A colourless or light yellow, clear liquid.

Miscibility. Miscible with water and methanol R.

Mass density. ρ_{20} = 0.944–0.950 kg/L.

Refractive index. n_D^{20} = 1.450–1.455.

Identification. Dissolve 0.05 g in 4 mL of ethanol (~750 g/L) TS, add 0.5 mL of a 2.5 mg/mL solution of triketohydrindene hydrate R and warm on a water-bath; a violet colour is produced.

4-Aminophenol R

C_6H_7NO.

Description. A white or almost white, crystalline powder.

Melting temperature. About 184 °C with decomposition.

Aminopolyether R

4,7,13,16,21,24-Hexaoxa-1,10-diazabicyclo[8,8,8]hexacosane ; $C_{18}H_{36}N_2O_6$.

A commercially available reagent of suitable grade.

Melting point. 70 °C to 73 °C.

Ammonia (~100 g/L) FeTS

Ammonia (~100 g/l) TS that complies with the following test: Evaporate 5 mL of ammonia (~100 g/L) TS nearly to dryness on a water-bath, add 40 mL of water, 2 mL of citric acid (180 g/L) FeTS, and 2 drops of mercaptoacetic acid R; mix, make alkaline with ammonia (~100 g/L) FeTS and dilute to 50 mL with water; no pink colour is produced.

Ammonia (~100 g/L) PbTS

Ammonia (~100 g/l) TS that complies with the following test: Evaporate 5 mL of ammonia (~100 g/L) TS to dryness on a water-bath, add to the residue 1 mL of hydrochloric acid (~70 g/L) TS and evaporate to dryness. Dissolve the residue in 2 mL of acetic acid (~60 g/L) PbTS, dilute with water to 25 mL and carry out the test for heavy metals. Prepare the blank in a similar way. The heavy metals limit is 2 µg/mL.

Ammonia (~100 g/L) TS

Ammonia (~260 g/L) TS, diluted to contain about 100 g/L of NH_3 (approximately 6 mol/L); *d*~0.956.

Ammonia (~17 g/L) TS

Ammonia (~100 g/L) TS, diluted to contain about 17 g of NH_3 per litre (approximately 1 mol/L); *d*~0.992.

Ammonia (~260 g/L) TS

[ammonia, strong, R] (SRIP, 1963, p. 31); *d*~0.894.

Ammonia (~35 g/L) TS

Ammonia (~100 g/L) TS, diluted to contain about 35 g of NH_3 per litre (approximately 2 mol/L); *d*~0.985.

Ammonia (~50 g/L) TS

Ammonia (~260 g/L) TS, diluted with water to contain about 50 g of NH_3 per litre (approximately 3 mol/L); *d*~0.977.

Ammonia (~10 g/L) TS

Ammonia (~100 g/L) TS, diluted to contain about 10 g of NH_3 per litre (approximately 1% (w/v)).

Ammonia buffer TS

Procedure. Dissolve 67.5 g of ammonium chloride R in 570 mL of ammonia (~260 g/L) TS and dilute with water to 1000 mL.

Ammonia buffer TS2

Procedure. Dissolve 67.5 g of ammonium chloride R in 650 mL of ammonia (~260 g/L) TS and dilute with water to produce 1000 mL.

Ammonium acetate (100 g/L) TS

A solution of ammonium acetate R containing 100 g of $C_2H_7NO_2$ per litre.

Ammonium acetate (2 g/L) TS

A solution of ammonium acetate R containing about 2 g of $C_2H_7NO_2$ per litre.

Note: Ammonium acetate (2 g/L) TS must be freshly prepared.

Ammonium acetate (40 g/L) TS

A solution of ammonium acetate R containing about 38.5 g of $C_2H_7NO_2$ per litre (approximately 0.5 mol/L).

Note: Ammonium acetate (40 g/L) TS must be freshly prepared.

Ammonium acetate (50g/L) TS

A solution of ammonium acetate R containing about 50 g of $C_2H_7NO_2$ per litre.

Ammonium acetate (80 g/L) TS

A solution of ammonium acetate R containing about 77 g/L of $C_2H_7NO_2$ (approximately 1 mol/l).

Ammonium acetate TS

Procedure. Dissolve 150 g of ammonium acetate R in water, add 3 mL of glacial acetic acid R and dilute with sufficient water to produce 1000 mL.

Note: Ammonium acetate TS must be used within 1 week of preparation.

Ammonium acetate buffer, pH 4.62, TS

Procedure. Adjust the pH of ammonium acetate (100 g/L) TS to 4.62 using acetic acid (~60 g/L) TS.

Ammonium acetate R

$C_2H_7NO_2$ (SRIP, 1963, p. 32).

Ammonium bicarbonate R.

Analytical reagent grade of commerce containing not less than 99% of NH_4HCO_3.

Ammonium carbonate R

$(NH_4)_2CO_3$ (SRIP, 1963, p. 33).

Ammonium carbonate R1

A mixture of varying proportions of ammonium hydrogen carbonate (NH_4HCO_3) and ammonium carbamate (NH_2COONH_4). It liberates not less than 30 per cent m/m of NH_3.

Description. White or almost white translucent mass.

Solubility. Slowly soluble in about 4 parts of water. It is decomposed by boiling water.

Assay. Dissolve 2.00 g in 25 mL of water R. Slowly add 50.0 mL of hydrochloric acid (1 mol/L) VS, titrate with sodium hydroxide (1 mol/L) VS, using 0.1 mL of methyl orange/ethanol TS1 as indicator. Each mL of hydrochloric acid (1 mol/L) VS is equivalent to 17.03 mg of NH_3.

Storage. Store at a temperature below 20 °C.

Ammonium carbonate (158 g/L) TS

A 158 g/L solution of ammonium carbonate R1.

Ammonium chloride (10 µg/mL NH4) TS

Procedure. Dissolve 0.296 g, accurately weighed, of ammonium chloride R in sufficient water to produce 1000 mL. Dilute 10 mL of this solution to 100 mL.

Shelf-life. Use the solution within 2 weeks of its preparation.

Ammonium chloride (100 g/L) TS

A solution of ammonium chloride R containing about 100 g of NH_4Cl per litre.

Ammonium chloride (20g/L) TS

A solution of ammonium chloride R containing about 20g of NH_4Cl per litre.

Ammonium chloride buffer, pH 10.0, TS

A buffer mixture of pH 10.0.

Procedure. Dissolve 7.0 g of ammonium chloride R in 57 mL of ammonia (~260 g/L) TS and dilute with sufficient water to produce 100 mL.

Ammonium chloride buffer, pH 10.5, TS

A buffer mixture of pH 10.5.

Procedure. Dissolve 6.95 g of ammonium chloride R in 75 mL of ammonia (~260 g/L) TS and dilute to 100 mL with water.

Ammonium chloride R

NH_4Cl (SRIP, 1963, p. 33).

Ammonium chloride TS (Nessler's reagent)

Procedure. Dissolve 3.15 g of ammonium chloride R in a sufficient quantity of ammonia-free water R to produce 1000 mL.

Ammonium chloride, dilute, TS

Procedure. To 10 mL of ammonium chloride TS add a sufficient quantity of ammonia-free water R to produce 1000 mL.

Ammonium dihydrogen phosphate R

$(NH_4)H_2PO_4$

Ammonium formate R

CH_5NO_2. Deliquescent crystals or granules, very soluble in water R, soluble in dehydrated ethanol R. Melting range: 119 °C to 121 °C. Storage in an airtight container.

Ammonium mercurithiocyanate TS

Procedure. Dissolve 30 g of ammonium thiocyanate R and 27 g of mercuric chloride R in sufficient water to produce 1000 mL.

Ammonium molybdate (45 g/L) TS

A solution of ammonium molybdate R containing about 47 g of $(NH_4)6Mo7O24$ per litre.

Ammonium molybdate (95 g/L) TS

A solution of ammonium molybdate R containing about 95 g/L of $(NH4)_6Mo_7O_{24}$.

Ammonium molybdate R

$(NH_4)_6Mo_7O_{24},4H_2O$ (SRIP, 1963, p. 34).

Ammonium molybdate/ceric sulfate/sulfuric acid TS

Procedure. Dissolve 2.5 g ammonium molybdate R and 1.0 g ceric sulfate R in sulfuric acid (~100 g/L) to produce 100 mL.

Ammonium molybdate/nitric acid TS

Procedure. Dissolve 50 g of ammonium sulfate R in 500 mL of nitric acid (~1000 g/l) TS using a 2000 mL conical flask or beaker. Dissolve separately in a beaker 150 g of ammonium molybdate R in 400 mL of boiling water. After cooling pour this solution slowly, while stirring, into the acid solution and dilute with water to 1000 mL. Allow to stand for 2–3 days and filter.

Storage. Store in well-closed, brown glass bottles and keep in a cool place.

Ammonium molybdate/sulfuric acid TS

Procedure. Dissolve 0.5 g of ammonium molybdate R in sufficient sulfuric acid (~1760 g/L) TS to produce 10 mL.

Ammonium molybdate/vanadate TS

Procedure. Shake 4 g of finely powdered ammonium molybdate R and 0.1 g of finely-powdered ammonium vanadate R with 70 mL of water. Add 20 mL of nitric acid (~1000 g/L) TS and dilute to 100 mL with water.

Ammonium nitrate (50 g/L) TS

A solution of ammonium nitrate R containing about 50 g of NH_4NO_3 per litre.

Ammonium nitrate R

NH_4NO_3 (SRIP, 1963, p. 35).

Ammonium nitrate TS

Procedure. Dissolve 1.6 g of ammonium nitrate R in 30 mL of water, add 3.0 mL of ammonia (~260g/L) TS and dilute with sufficient water to produce 100 mL.

Ammonium oxalate (25 g/L) TS

A solution of ammonium oxalate R containing about 27 g/L of $C_2H_8N_2O_4$.

Ammonium oxalate (50 g/L) TS

A solution of ammonium oxalate R containing about 50 g of $C_2H_8N_2O_4$ per litre.

Ammonium oxalate R

$C_2H_8N_2O_4,H_2O$ (SRIP, 1963, p. 36).

Ammonium persulfate R

$(NH_4)_2S_2O_8$ (SRIP, 1963, p. 37).

Ammonium persulfate/phosphate buffer TS

Procedure. Dissolve 10 g of ammonium persulfate R in sufficient phosphate buffer, pH 7.4, TS to produce 100 mL.

Ammonium pyrrolidinedithiocarbamate (10 g/L) TS

Procedure. Immediately before use wash 10 g of ammonium pyrrolidinedithiocarbamate R three times, each with 25 mL of isobutyl methyl ketone R, filter and dry the substance. Then dissolve 1.0 g in sufficient water to produce 100 mL.

Ammonium pyrrolidinedithiocarbamate R

Ammonium 1-pyrrolidinecarbodithioate; $C_5H_{12}N_2S_2$. Reagent grade quality.

Ammonium reineckate (10 g/L) TS

A solution of ammonium reineckate R containing about 10 g of $NH_4[Cr(NH_3)_2(SCN)_4]$ per litre.

Ammonium reineckate R

$NH_4[Cr(NH_3)_2(SCN)_4],H_2O$ (SRIP, 1963, p. 39).

Ammonium sulfamate (25 g/L) TS

A solution of ammonium sulfamate R containing about 25 g of $NH_4OSO_2NH_2$ per litre.

Ammonium sulfamate (5 g/L) TS

A solution of ammonium sulfamate R containing about 5 g of $NH_4OSO_2NH_2$ per litre.

Ammonium sulfamate (50 g/L) TS

A solution of ammonium sulfamate R containing 50 g of $NH_4OSO_2NH_2$ per litre.

Ammonium sulfamate R

$NH_4OSO_2NH_2$ (SRIP, 1963, p. 39).

Ammonium sulfate R

$(NH_4)_2SO_4$ (SRIP, 1963, p. 40).

Ammonium sulfate (50 g/L) TS

Transfer 50 g ammonium sulfate R in a 1000 mL volumetric flask and make up to volume with water R.

Ammonium sulfide TS

Procedure. Prepare a saturated solution of hydrogen sulfide R in ammonia (~100 g/L) TS. To 25 mL of this solution add 50 mL of ammonia (~100 g/L) TS.

Ammonium thiocyanate (0.01 mol/L) VS

Ammonium thiocyanate R, dissolved in water to contain 0.7612 g of NH_4SCN in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution following the method described under ammonium thiocyanate (0.1 mol/L) VS.

Ammonium thiocyanate (0.05 mol/L) VS

Ammonium thiocyanate R, dissolved in water to contain 3.806 g of NH_4SCN in 1000 mL.

Method of standardization. Ascertain the exact concentration of the solution following the method described for ammonium thiocyanate (0.1 mol/L) VS.

Ammonium thiocyanate (0.1 mol/L) VS

Ammonium thiocyanate R, dissolved in water to contain 7.612 g of NH_4SCN in 1000 mL.

Method of standardization. Ascertain the exact concentration of the 0.1 mol/L solution in the following manner: Place 30.0 mL of silver nitrate (0.1 mol/L) VS in a glass-stoppered flask. Dilute with 50 mL of water, add 2 mL of nitric acid (~1000 g/L) TS and then titrate with the ammonium thiocyanate solution to the first appearance of a red-brown colour, using 2 mL of ferric ammonium sulfate (45 g/L) TS as indicator.

Ammonium thiocyanate (10g/L) TS

A solution of ammonium thiocyanate R containing 10g of NH_4SCN per litre.

Ammonium thiocyanate (75 g/L) TS

A solution of ammonium thiocyanate R containing about 75 g/l of NH_4SCN (approximately 1 mol/L).

Ammonium thiocyanate R

NH_4SCN (SRIP, 1963, p. 40).

Ammonium thiocyanate/cobalt(II) nitrate TS

Procedure. Dissolve 20 g of ammonium thiocyanate R and 5 g of cobalt(II) nitrate R in 100 mL of water. Add sufficient sodium chloride R to saturate the solution.

Ammonium vanadate R

NH_4VO_3.

Description. A white to slightly yellowish, crystalline powder.

Solubility. Slightly soluble in water; soluble in ammonia (~100 g/L) TS.

Ammonium vanadate TS

Dissolve 0.5 g of Ammonium vanadate in 1.5 mL water and dilute to 100 mL with sulfuric acid.

Ampicillin trihydrate R

(2*S*,5*R*,6*R*)-6-[(R)-2-Amino-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate; [2*S*-[2*α*,5*α*,6*β* (*S* *)]]-6-[(aminophenylacetyl)amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate. $C_{16}H_{19}N_3O_4S,3H_2O$.

A commercially available reagent of suitable grade.

Amyl alcohol R

$C_5H_{12}O$ (SRIP, 1963, p. 42).

Aniline (25 g/L) TS

A solution of aniline R containing about 25 g of C_6H_7N per litre.

Aniline R

C_6H_7N (SRIP, 1963, p. 43).

Anisaldehyde R

4-Methoxybenzaldehyde; $C_8H_8O_2$.

Description. A colourless to pale yellow, oily liquid.

Solubility. Very slightly soluble in water, miscible with ethanol (~750 g/L) TS and ether R.

Mass density. ρ_{20} = about 1.125 kg/L.

Boiling point. About 248 °C.

Anisaldehyde TS

Procedure. Mix in the following order 0.5 mL of anisaldehyde R, 10 mL of glacial acetic acid R, 85 mL of methanol R and 5 mL of sulfuric acid (~1760 g/L) TS.

Anisaldehyde/methanol TS

Procedure. Slowly add 10 mL of glacial acetic acid R and 5 mL of sulfuric acid (~1760 g/L) TS to 55 mL of methanol R and cool to room temperature. Separately add 0.5 mL of anisaldehyde R to 30 mL of methanol R. Mix the two solutions thoroughly.

Storage. Keep anisaldehyde/methanol TS protected from light.

Note: Anisaldehyde/methanol TS should be freshly prepared.

Anisaldehyde/sulfuric acid TS

Procedure. Add 5 mL of anisaldehyde R to 10 mL of sulfuric acid (~1760 g/L) TS.

Anthrone R

$C_{14}H_{10}O$.

Description. A pale yellow, crystalline powder.

Solubility. Practically insoluble in water; slightly soluble in ethanol (~750 g/L) TS and in sulfuric acid (~100 g/L) TS.

Solubility in carbon tetrachloride R. Add 0.5 g to 10 mL of carbon tetrachloride R; a clear, non-fluorescent solution is produced.

Melting range. 154–156 °C.

Anthrone TS

Procedure. Dissolve 35 mg of anthrone R in 100 mL of sulfuric acid (~1760 g/L) TS.

Anthrone TS2

Procedure. Dissolve 200 mg of anthrone R in 100 mL of sulfuric acid (~1760 g/L) TS.

Antimony sodium tartrate (50 g/L) TS

A solution of antimony sodium tartrate R containing about 50 g/L of $C_4H_4NaO_7Sb$.

Antimony sodium tartrate R

$C_4H_4NaO_7Sb$.

Description. Hygroscopic, transparent or whitish scales or powder.

Solubility. Soluble in 1.5 parts of water; practically insoluble in ethanol (~710 g/L) TS.

Antimony trichloride R

$SbCl_3$. Contains not less than 97.0% of $SbCl_3$.

Description. Colourless crystals.

Solubility. Very soluble in dehydrated ethanol R and in chloroform R (may form a slightly turbid solution).

Chloroform-insoluble substances. Dissolve 5.0 g in 25 mL of chloroform R, filter through a tared filtering crucible, wash the crucible with several portions of chloroform R and dry at 105 °C; it leaves a residue of not more than 1.0 mg.

Assay. Dissolve 0.5 g, accurately weighed, in 30 mL of water containing 4.0 g of potassium sodium tartrate R, add 2 g of sodium hydrogen carbonate R and titrate with iodine (0.1 mol/L) VS. Each mL of iodine (0.1 mol/L) VS is equivalent to 11.41 mg of SbCl3.

Note: In moist air fumes may be evolved.

Antimony trichloride TS

Procedure. Dissolve 22 g of antimony trichloride R in 100 mL of ethanol-free chloroform R, add 2.5 mL of acetyl chloride R and allow to stand for 30 minutes.

Aprotinin R

A polypeptide consisting of a chain of 58 amino acids. A commercially available reagent of suitable grade.

Arachis oil R

Use arachis oil as described in the monograph for *Arachis oil*.

Argon R

Ar. Contains not less than 99.995% of Ar.

Carbon monoxide. When used as described in the test for carbon monoxide in medicinal gases, after passage of 10 litres of argon at a flow rate of 4 litres per hour, not more than 0.05 mL of sodium thiosulfate (0.002 mol/L) VS is required for the titration (0.6 µL/L).

Arsenic trioxide R

As_2O_3 (SRIP, 1963, p. 44).

Arsenic trioxide R1

Arsenic trioxide R that has been prepared according to either of the following methods:

1. Recrystallize arsenic trioxide R from a boiling mixture of 20 parts of hydrochloric acid (~420 g/L) TS and 5 parts of water. After cooling collect the crystals and recrystallize them from boiling water until the mother liquor has a pH greater than 4.0. Dry the crystals to constant weight over silica gel, desiccant, R.

2. Sublime arsenic trioxide R in an appropriate apparatus.

pH value. Heat to boiling for a few minutes 1.0 g in 20 mL of water, filter and cool; the filtrate has a pH greater than 4.0.

Chlorides. Dissolve 10 mg in sufficient water to produce 10 mL. Acidify with 1 drop of nitric acid (~1000 g/L) TS and add 2 drops of silver nitrate (0.1 mol/L) VS; the solution remains clear and colourless for not less than 2 minutes.

Sulfides. To a solution of 5.0 g in a mixture of 10 mL of sodium hydroxide (~80 g/L) TS and 15 mL of water add 2 drops of lead acetate (80 g/L) TS; the solution remains colourless.

Loss on drying. Dry to constant weight over silica gel, desiccant, R; it loses not more than 0.1 mg/g.

Sulfated ash. Not more than 0.1 mg/g.

Arsenic, dilute, AsTS

One millilitre contains 10 µg of arsenic.

Procedure. Dilute 1 mL of strong arsenic AsTS with sufficient water to produce 100 mL.

Note: Dilute arsenic AsTS must be freshly prepared.

Arsenic, strong, AsTS

Procedure. Dissolve 0.132 g of arsenic trioxide R in 6 mL of sodium hydroxide (~80 g/L) TS, by gentle heating. Dilute the cooled solution with 20 mL of water and add 50 mL of hydrochloric acid (~250 g/L) TS and sufficient water to produce 100 mL.

Azo violet R

4-(4-Nitrophenylazo)resorcinol; Magneson I: $C_{12}H_9N_3O_4$.

Description. A red powder.

Melting temperature. About 193 °C with decomposition.

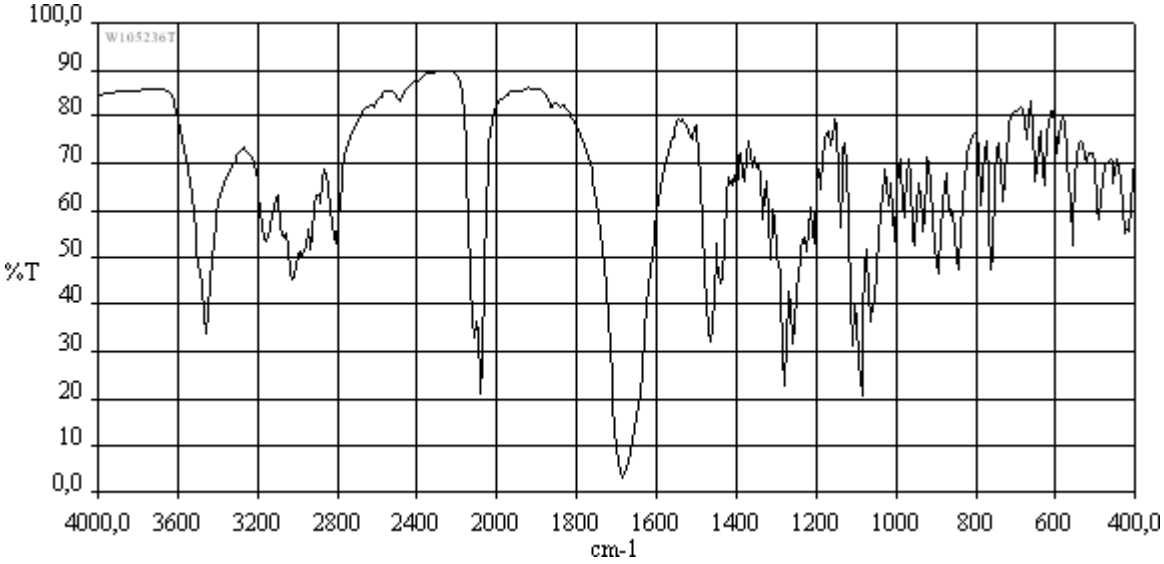
Azo violet TS

Procedure. Dissolve 0.2 g of azo violet R in a mixture of 1 volume of toluene R and 2 volumes of cyclohexane R.

Zidovudine (RS00154)

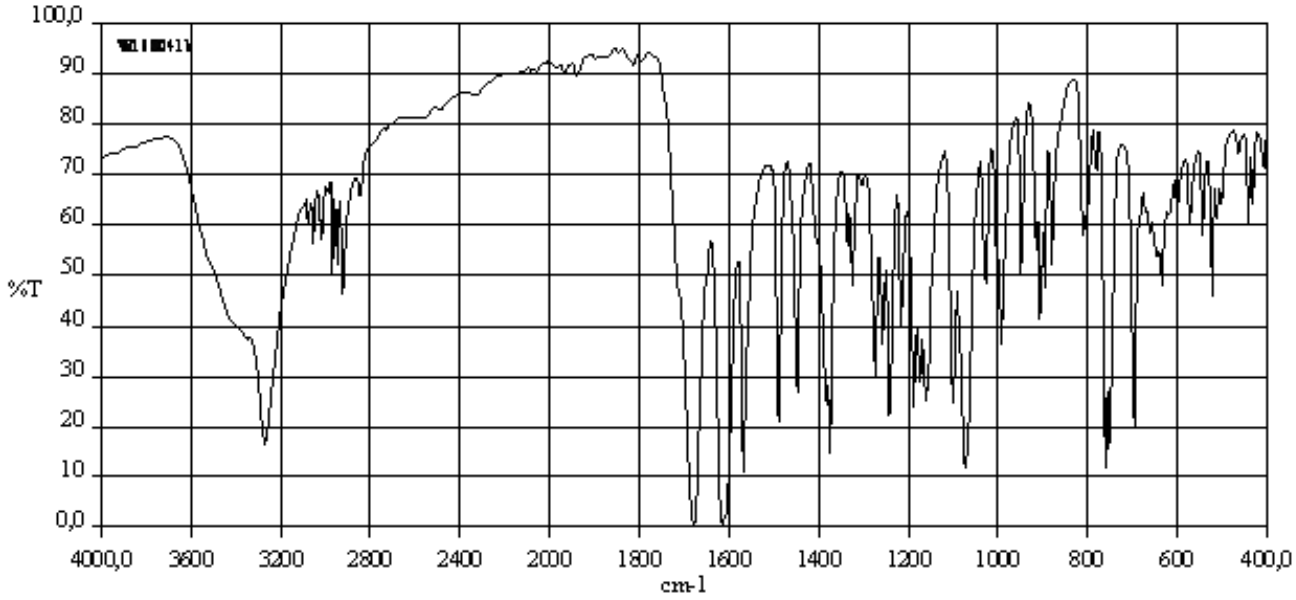
Instrument: Fourier Transform

Phase: Potassium bromide disc



Warfarin (RS00125)

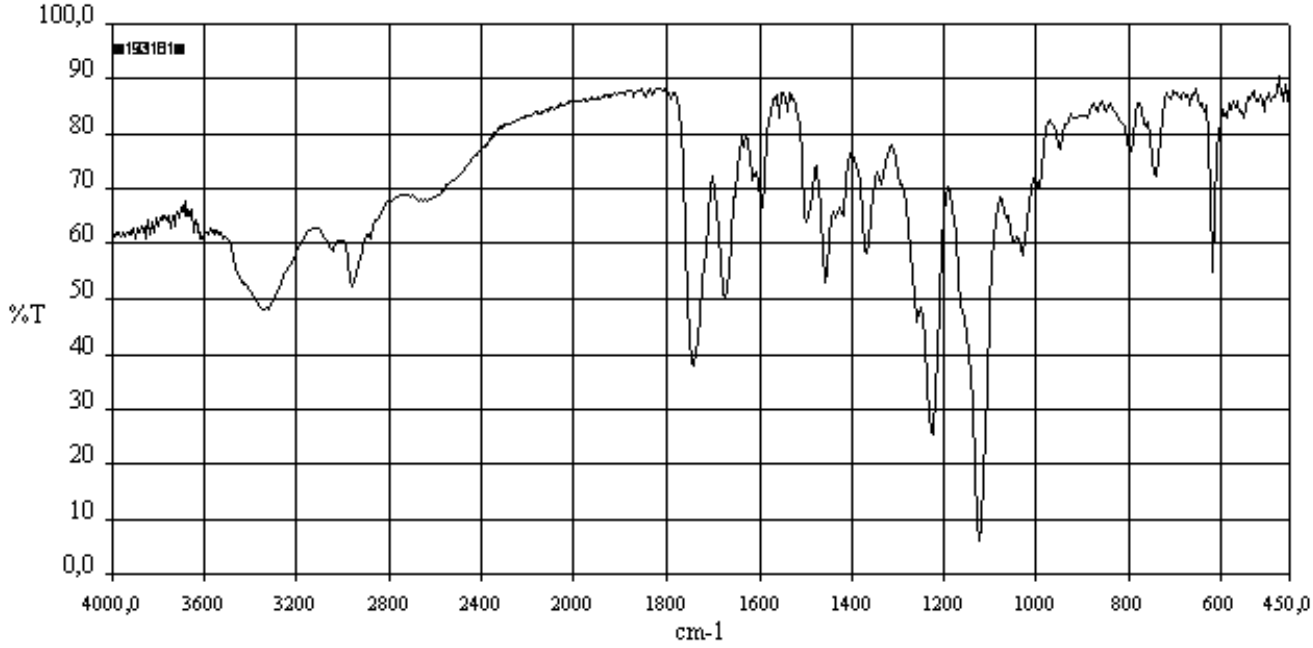
Instrument: Fourier Transform
Phase: Potassium bromide disc



Vincristine sulfate (RS00124)

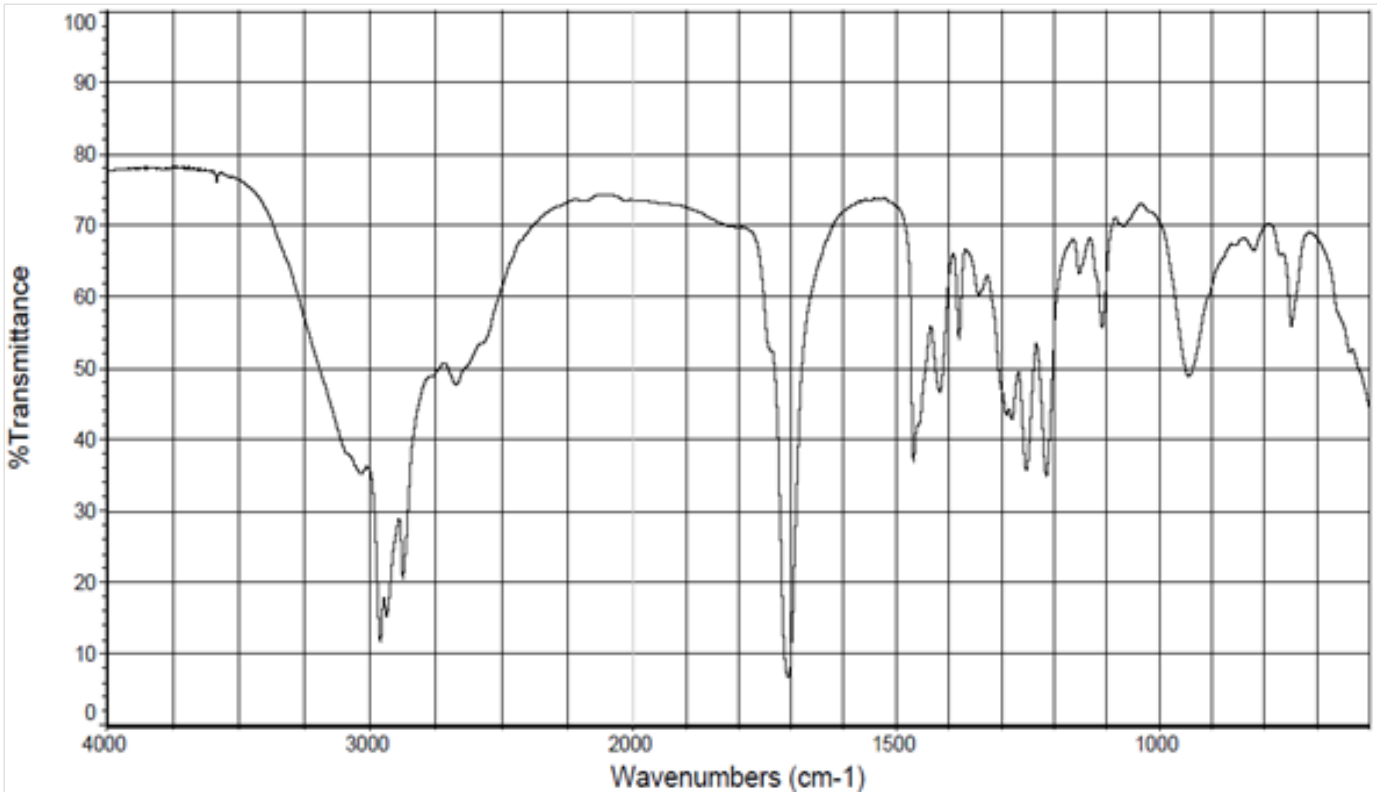
Instrument: Fourier Transform

Phase: Potassium bromide disc



Valproic acid (RS00170)

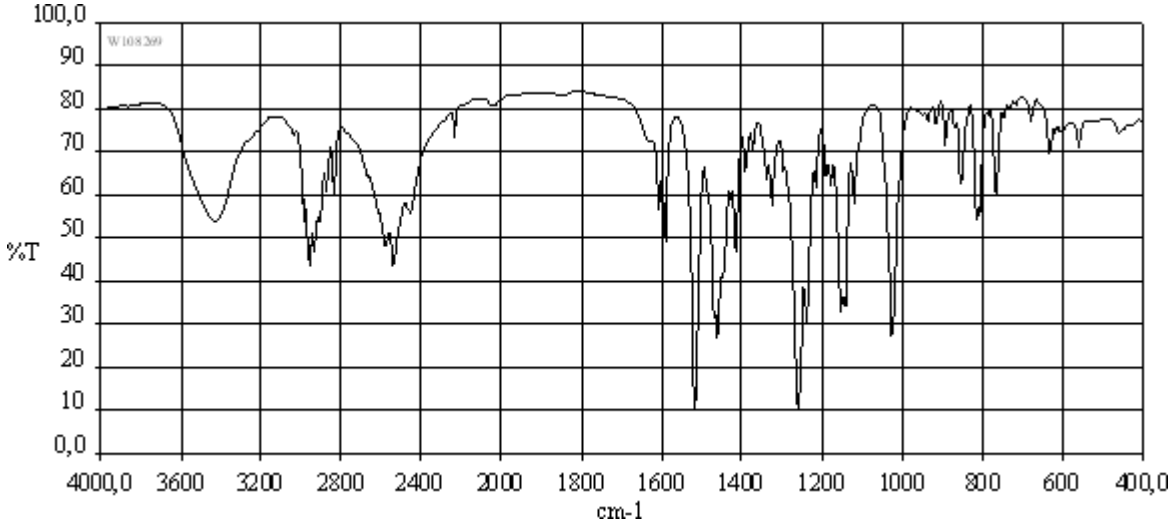
Instrument: Fourier Transform
Phase: Potassium bromide disc



Verapamil hydrochloride (RS00153)

Instrument: Fourier Transform

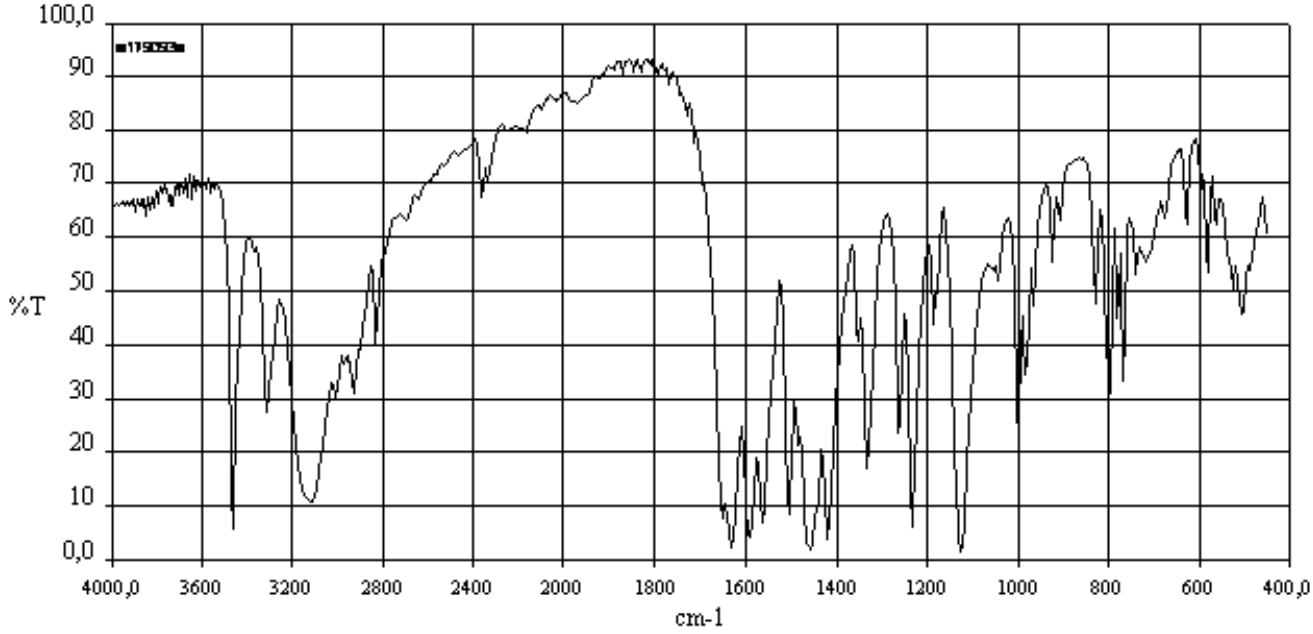
Phase: Potassium bromide disc



Trimethoprim (RS00123)

Instrument: Fourier Transform

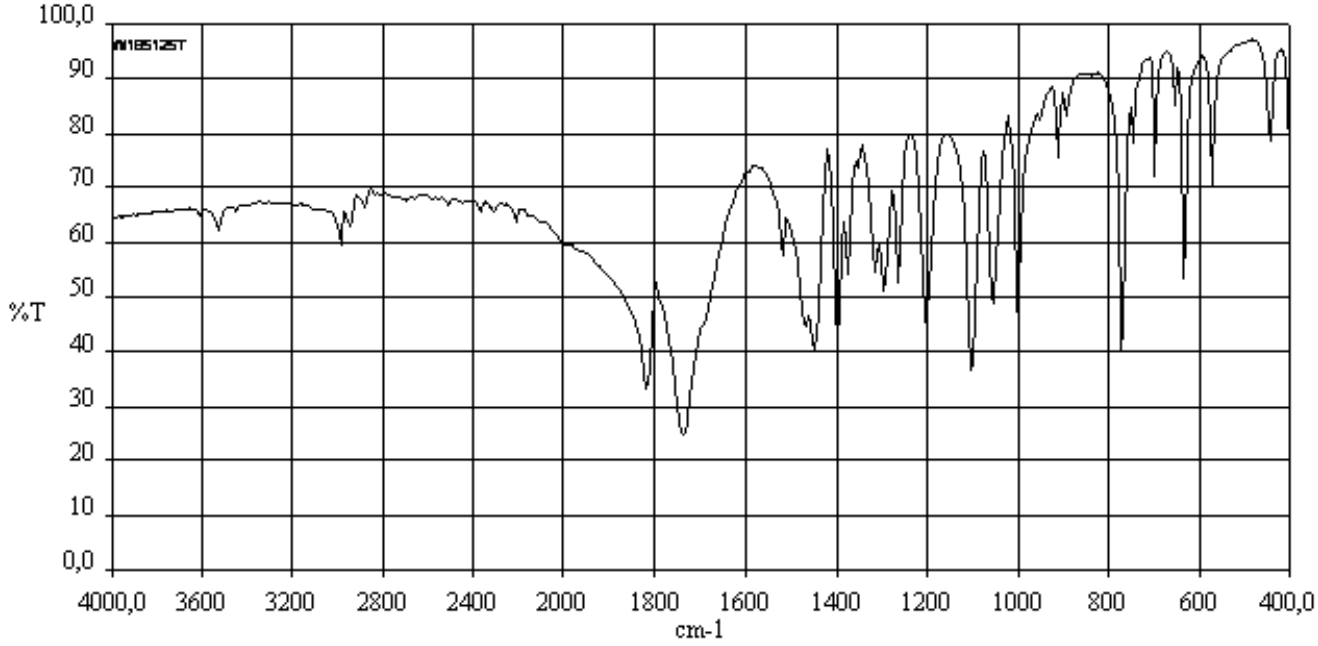
Phase: Potassium bromide disc



Trimethadione (RS00122)

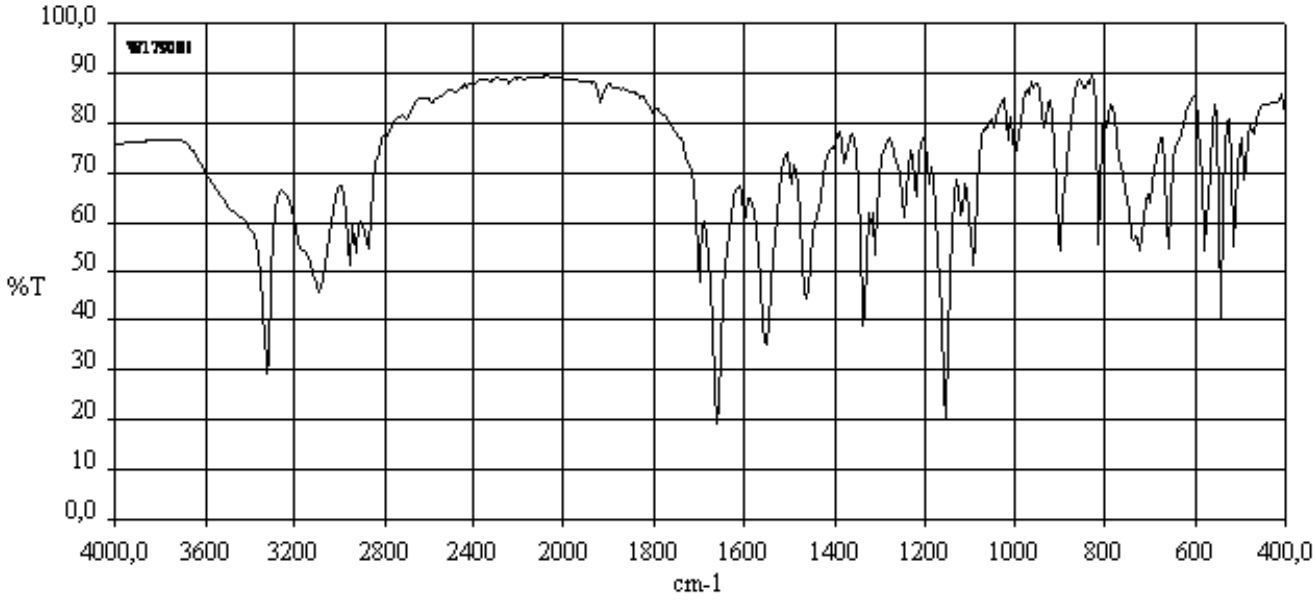
Instrument: Fourier Transform

Phase: Potassium bromide disc



Tolbutamide (RS00121)

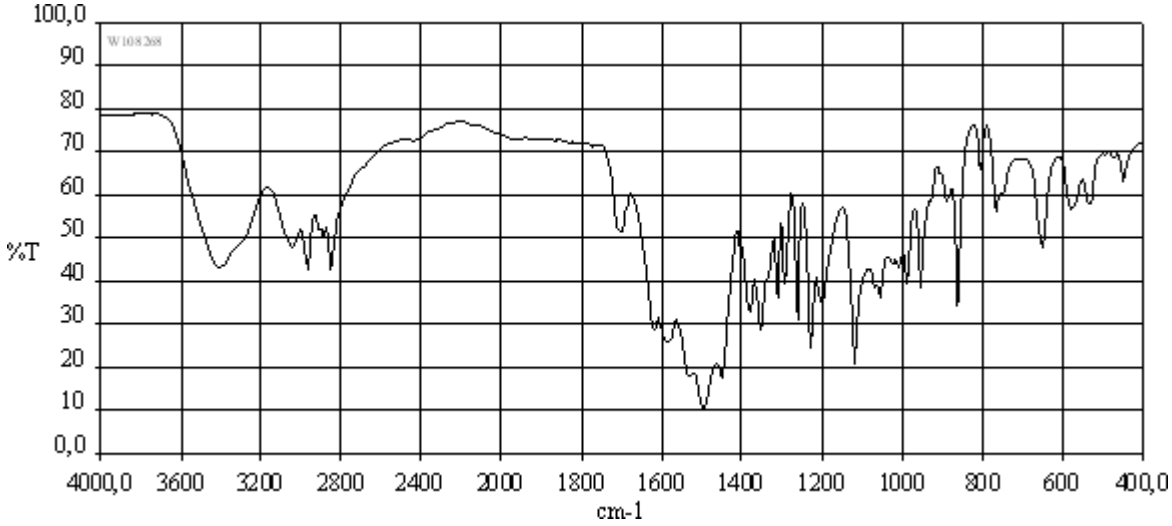
Instrument: Fourier Transform
Phase: Potassium bromide disc



Timolol maleate (RS00152)

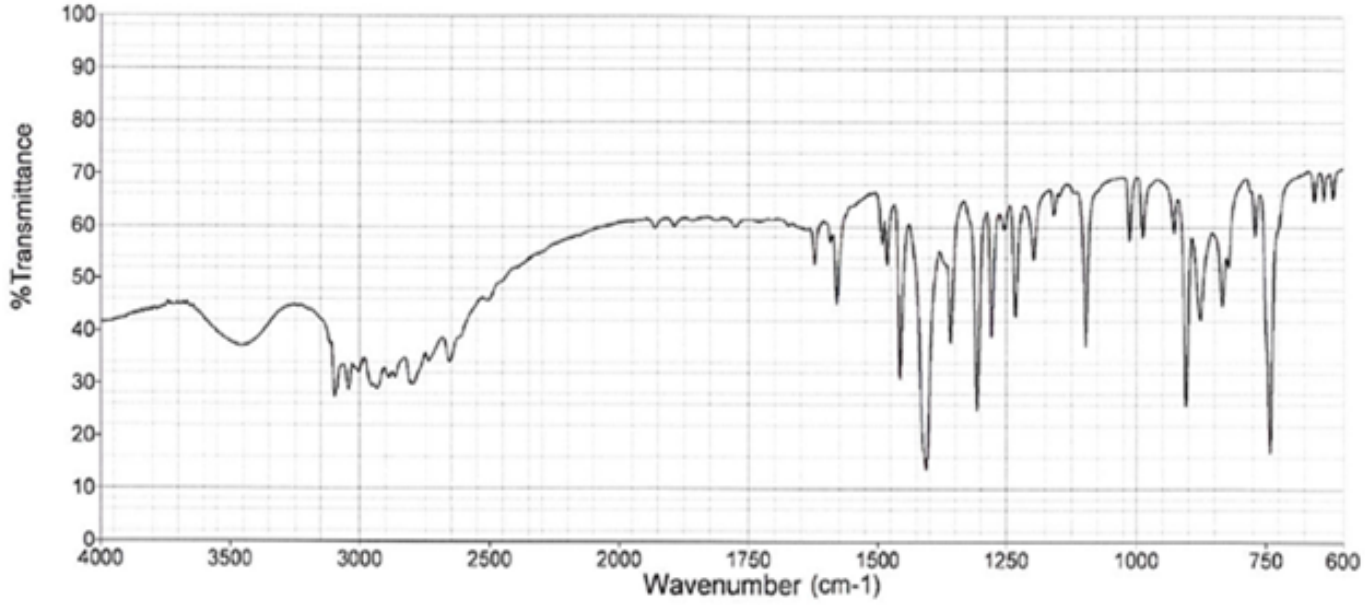
Instrument: Fourier Transform

Phase: Potassium bromide disc



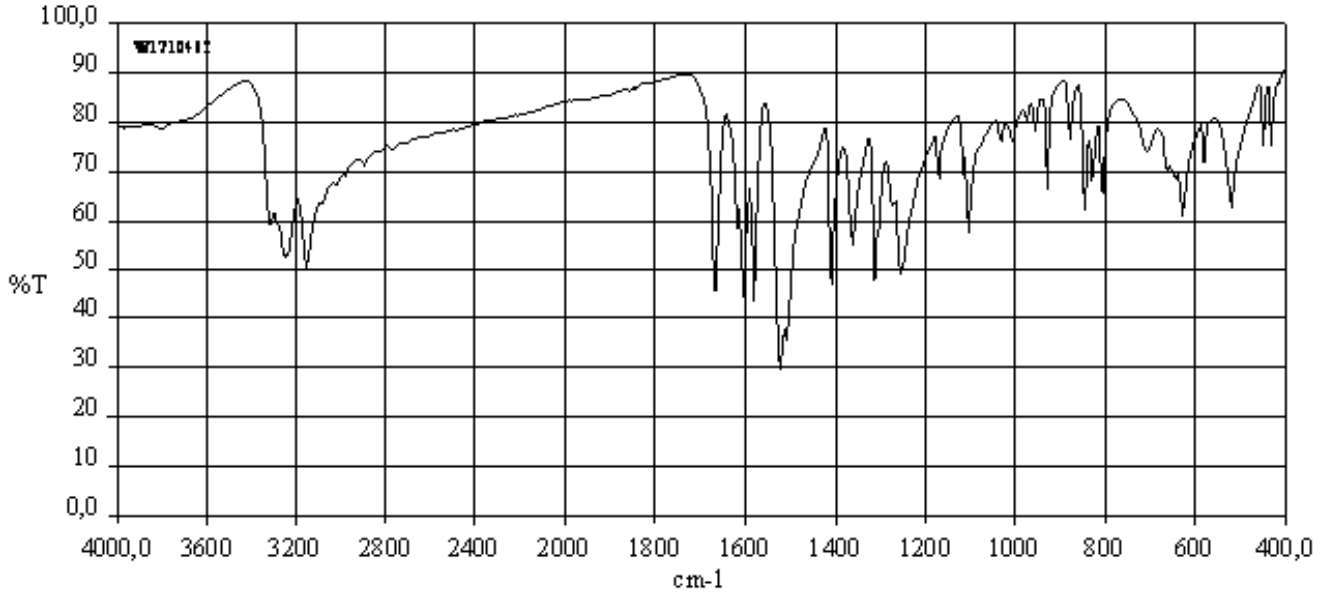
Tiabendazole (RS00169)

Instrument: Fourier Transform
Phase: Potassium bromide disc



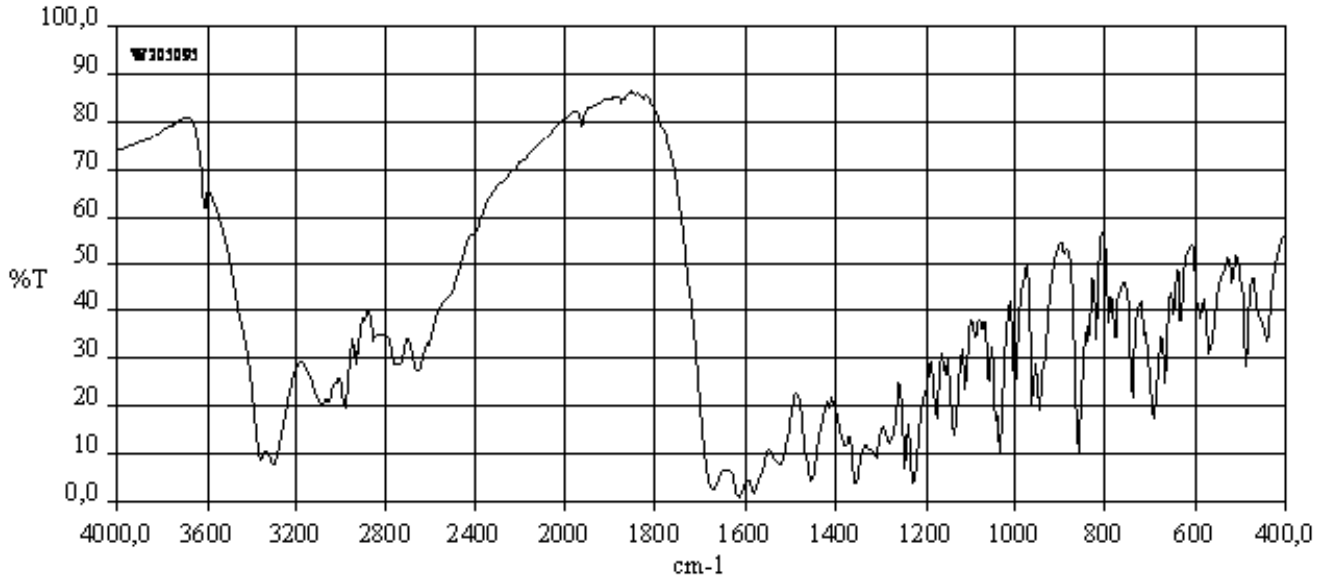
Thioacetazone (RS00120)

Instrument: Fourier Transform
Phase: Potassium bromide disc



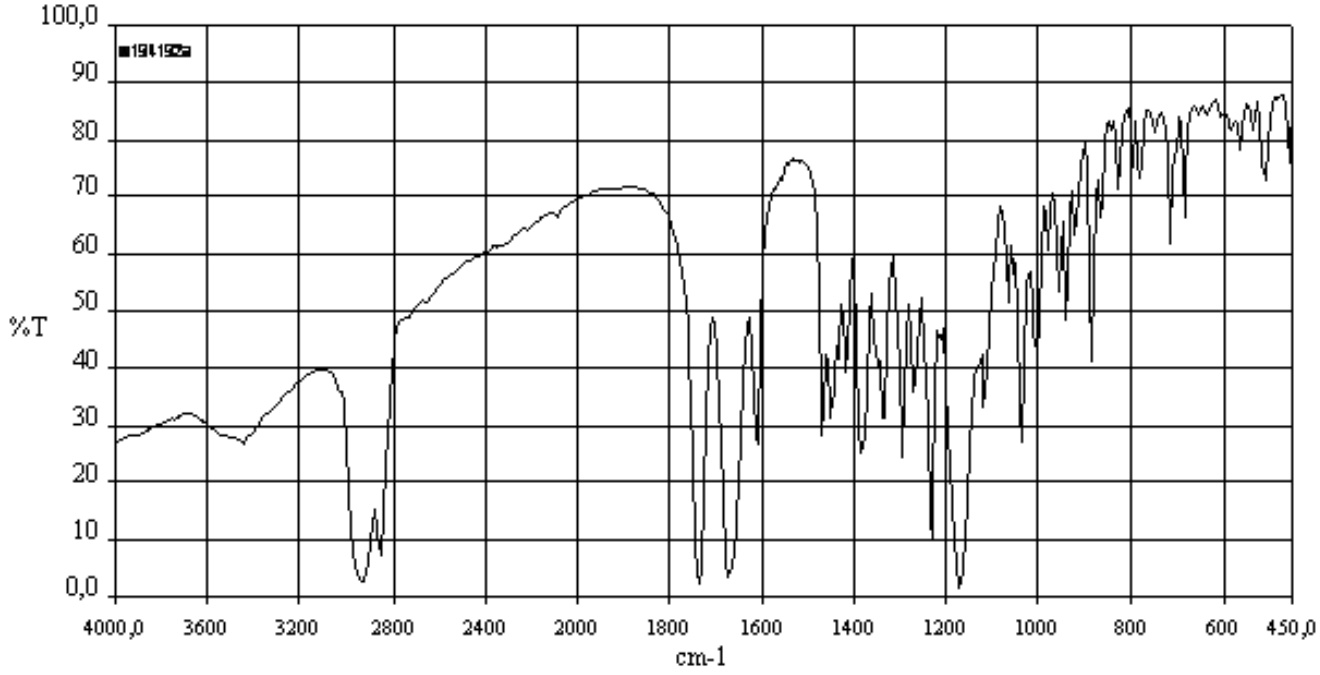
Tetracycline hydrochloride (RS00119)

Instrument: Fourier Transform
Phase: Potassium bromide disc



Testosterone enantate (RS00117)

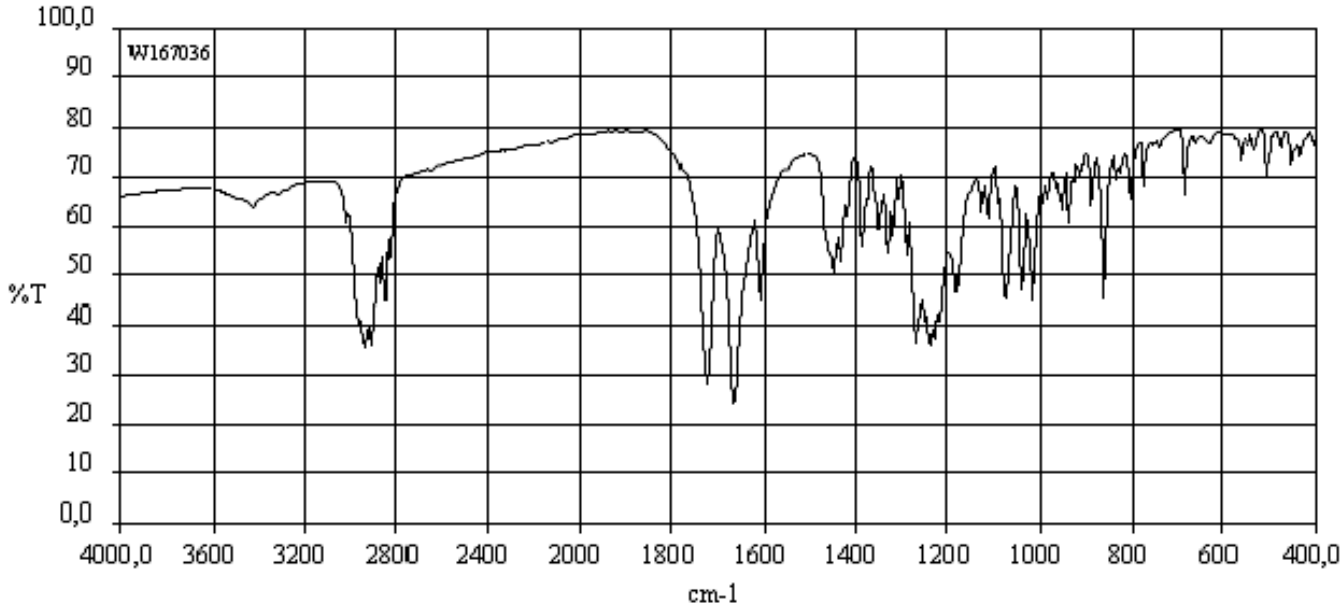
Instrument: Fourier Transform
Phase: Potassium bromide disc



Testosterone propionate (RS00118)

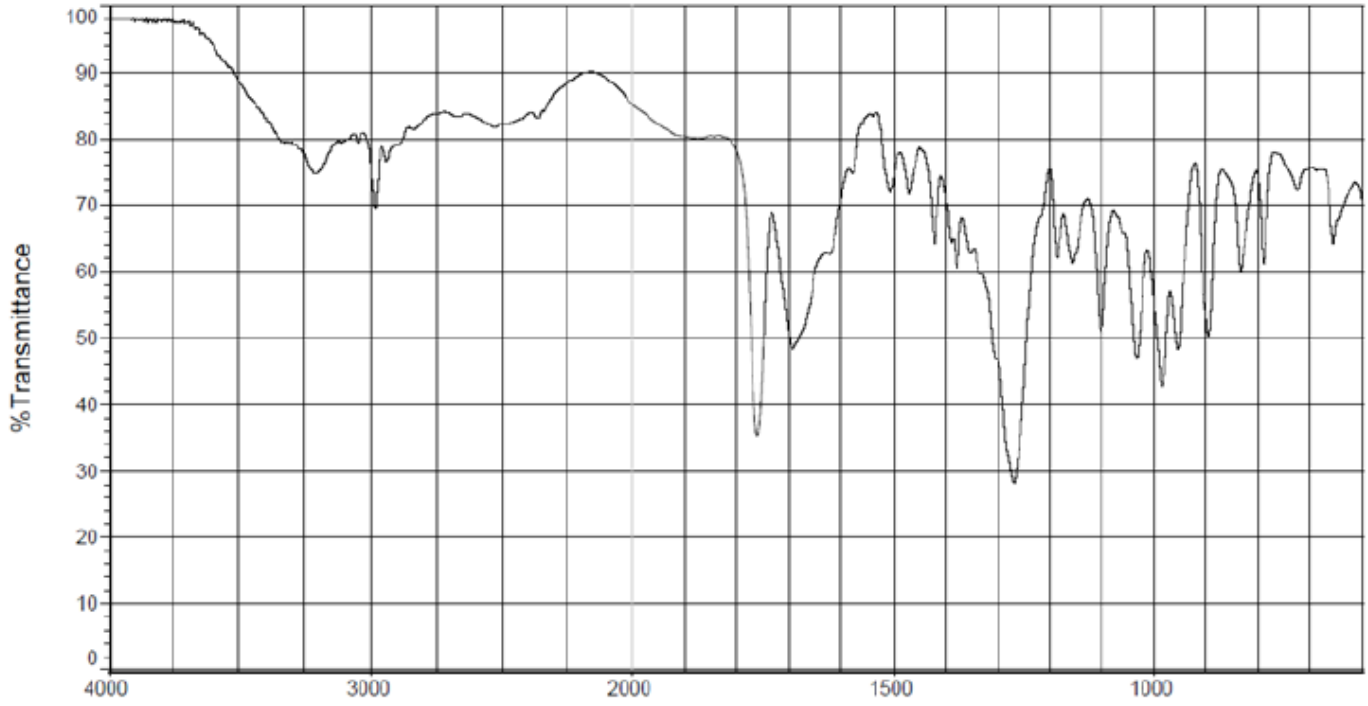
Instrument: Fourier Transform

Phase: Potassium bromide disc



Tenofovir disoproxil fumarate

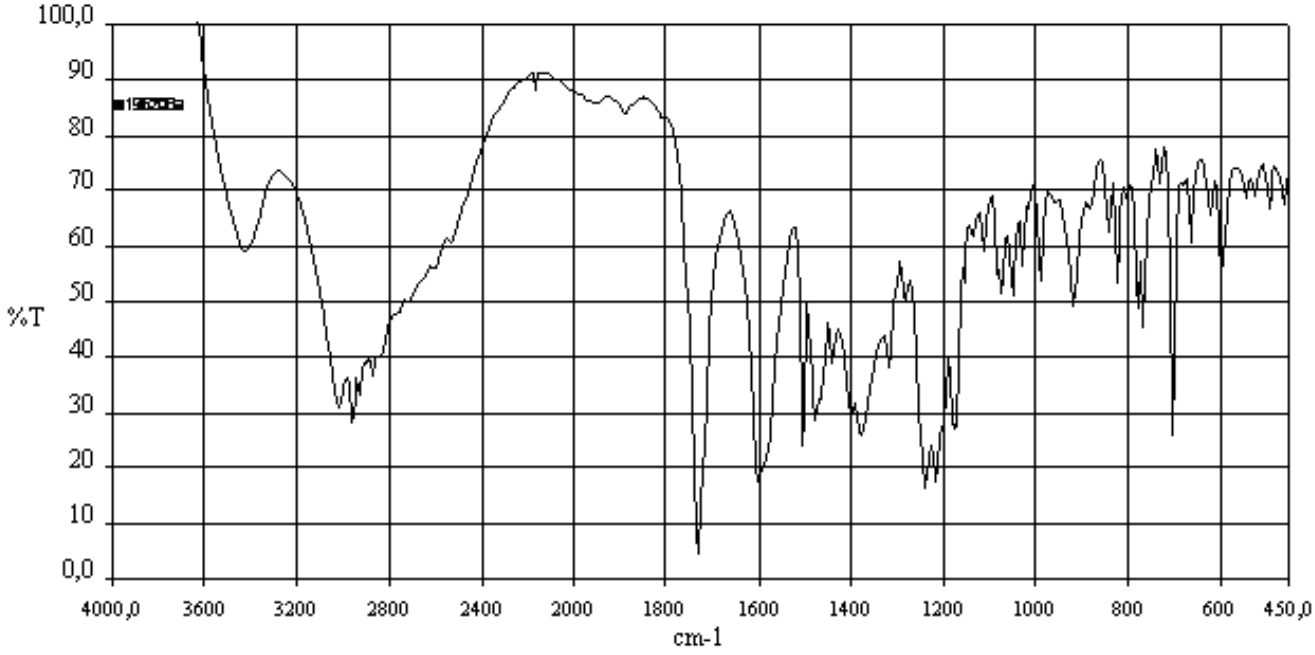
Instrument: Fourier Transform
Phase: Potassium bromide disc



IR File Name : 48744REC*1

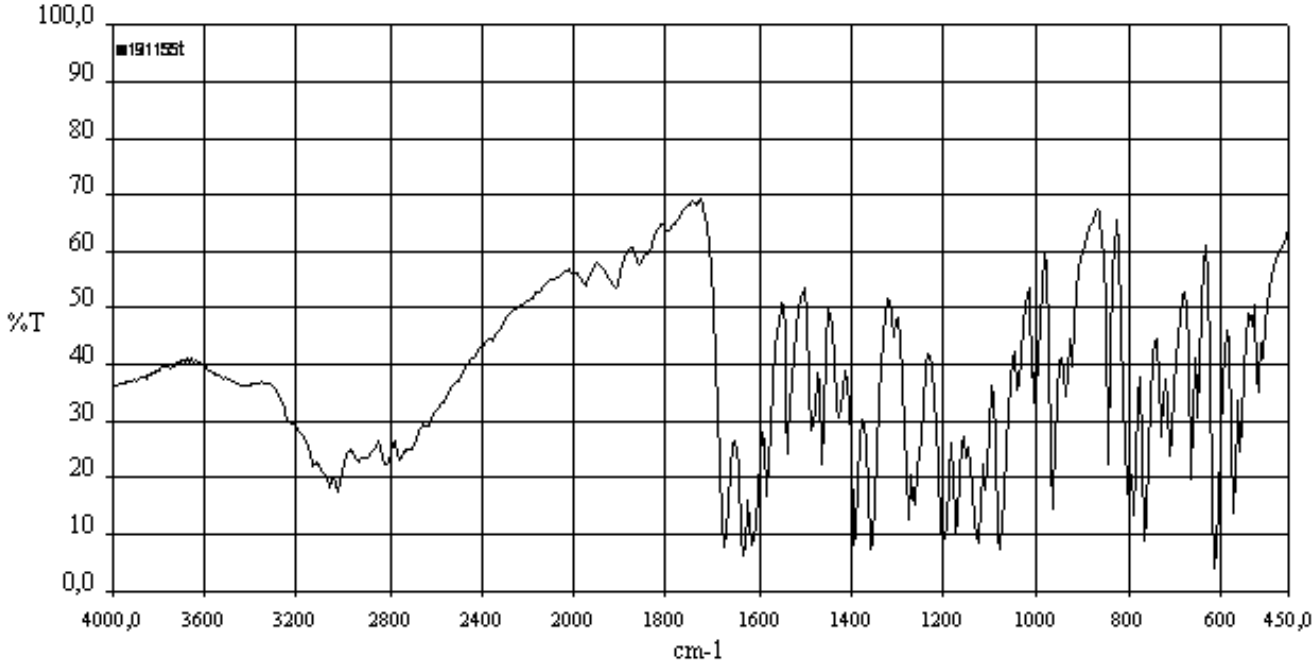
Tamoxifen citrate (RS00116)

Instrument: Fourier Transform
Phase: Potassium bromide disc



Sulfasalazine (RS00115)

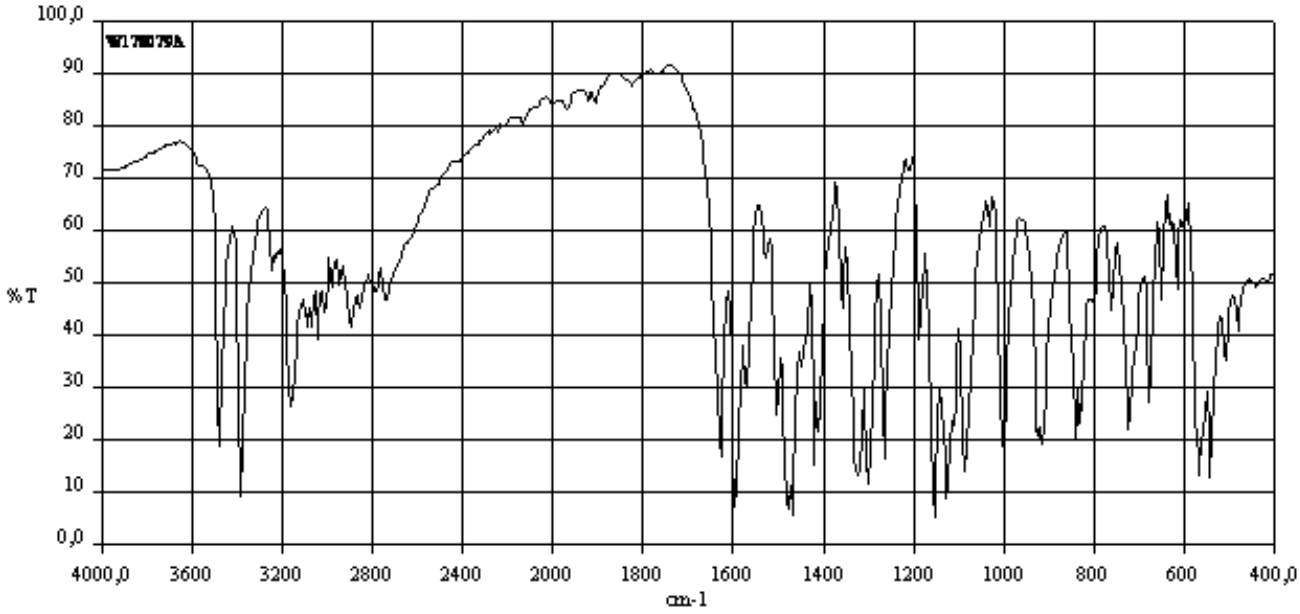
Instrument: Fourier Transform
Phase: Potassium bromide disc



Sulfamethoxypyridazine (RS00114)

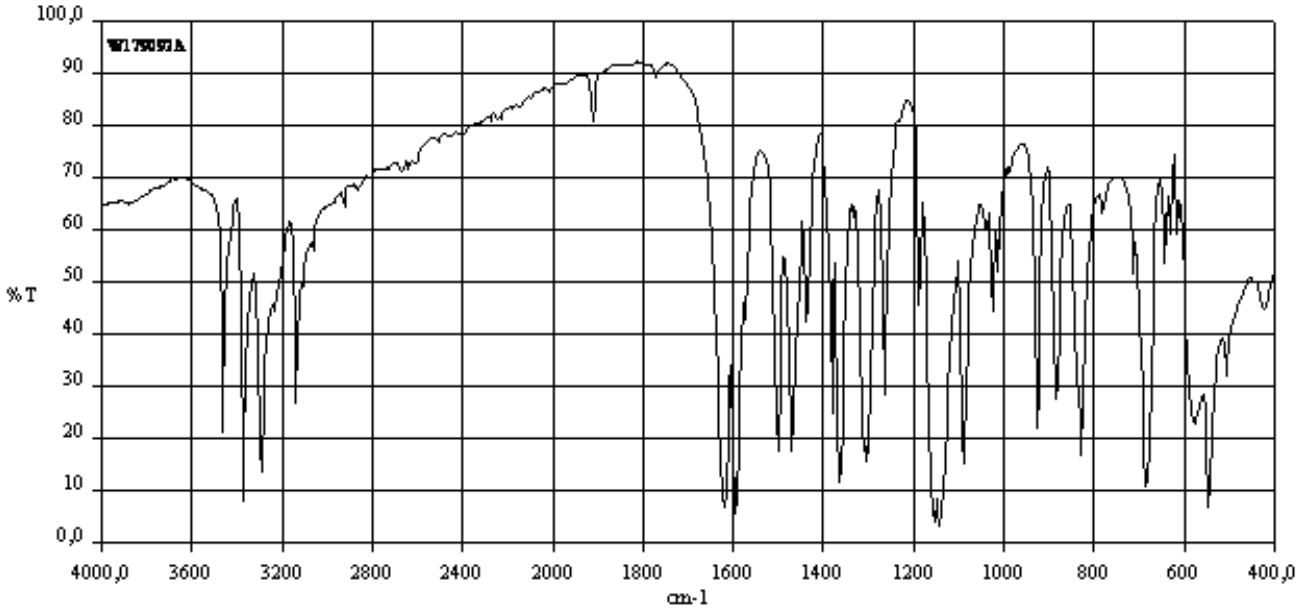
Instrument: Fourier Transform

Phase: Potassium bromide disc



Sulfamethoxazole (RS00113)

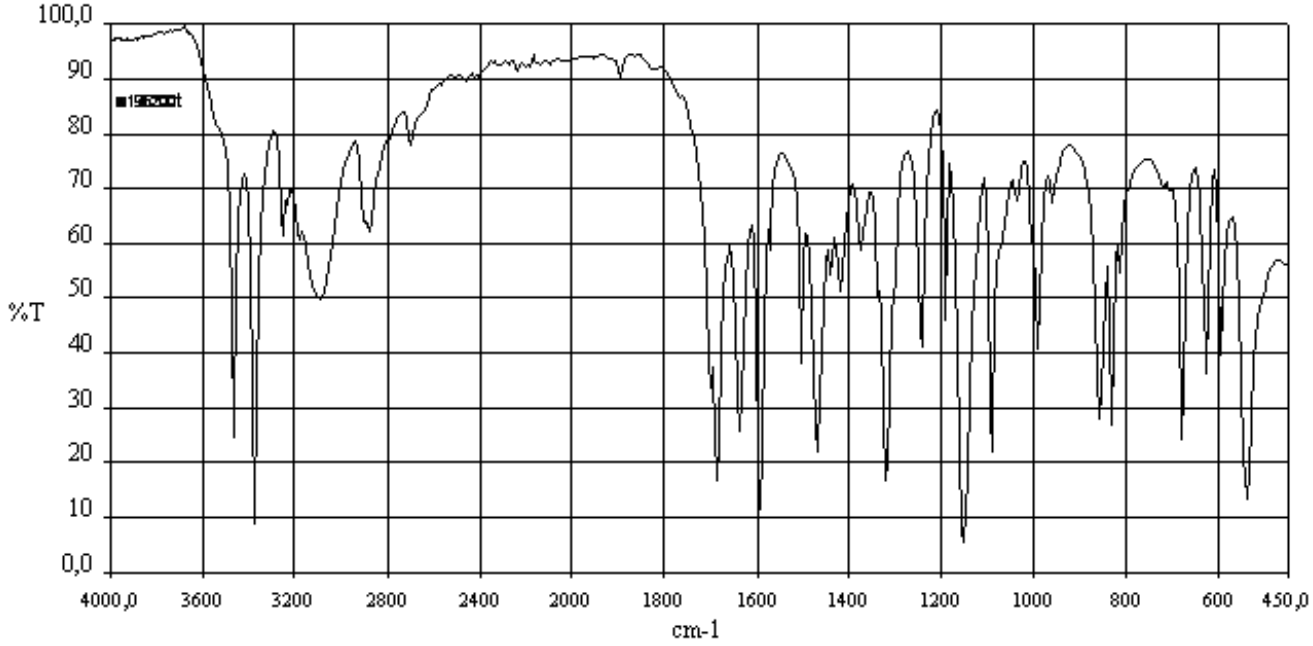
Instrument: Fourier Transform
Phase: Potassium bromide disc



Sulfacetamide (RS00112)

Instrument: Fourier Transform

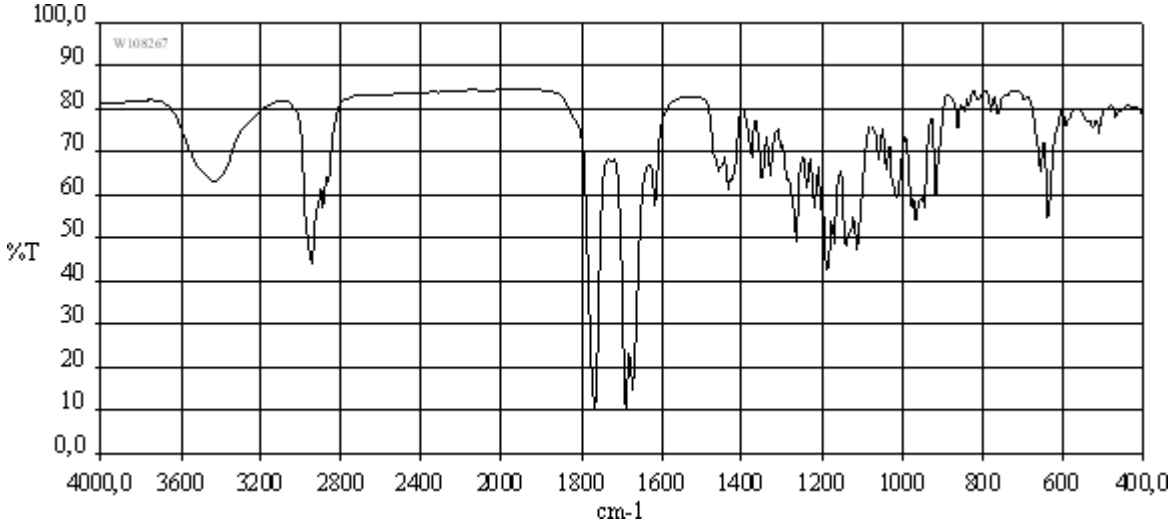
Phase: Potassium bromide disc



Spironolactone (RS00150)

Instrument: Fourier Transform

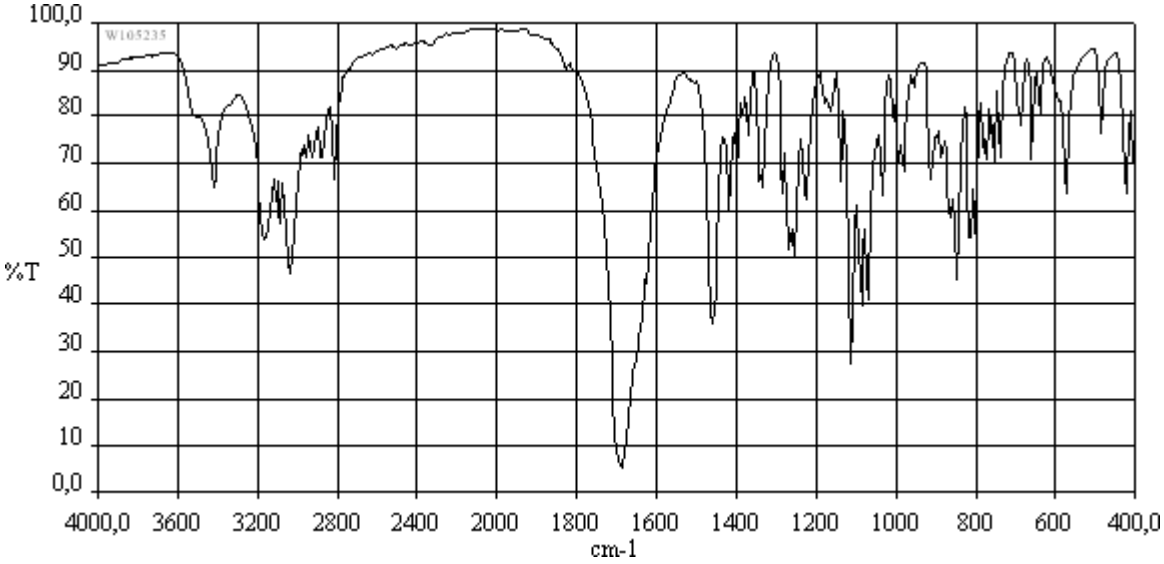
Phase: Potassium bromide disc



Stavudine (RS00151)

Instrument: Fourier Transform

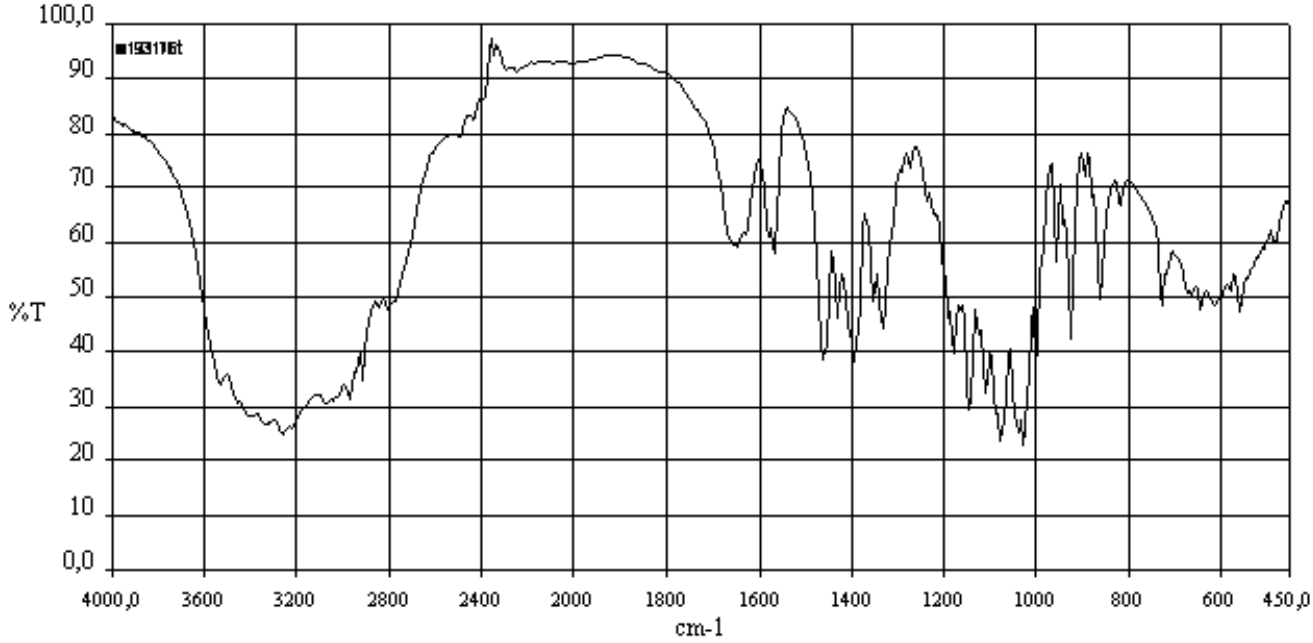
Phase: Potassium bromide disc



Spectinomycin hydrochloride (RS00111)

Instrument: Fourier Transform

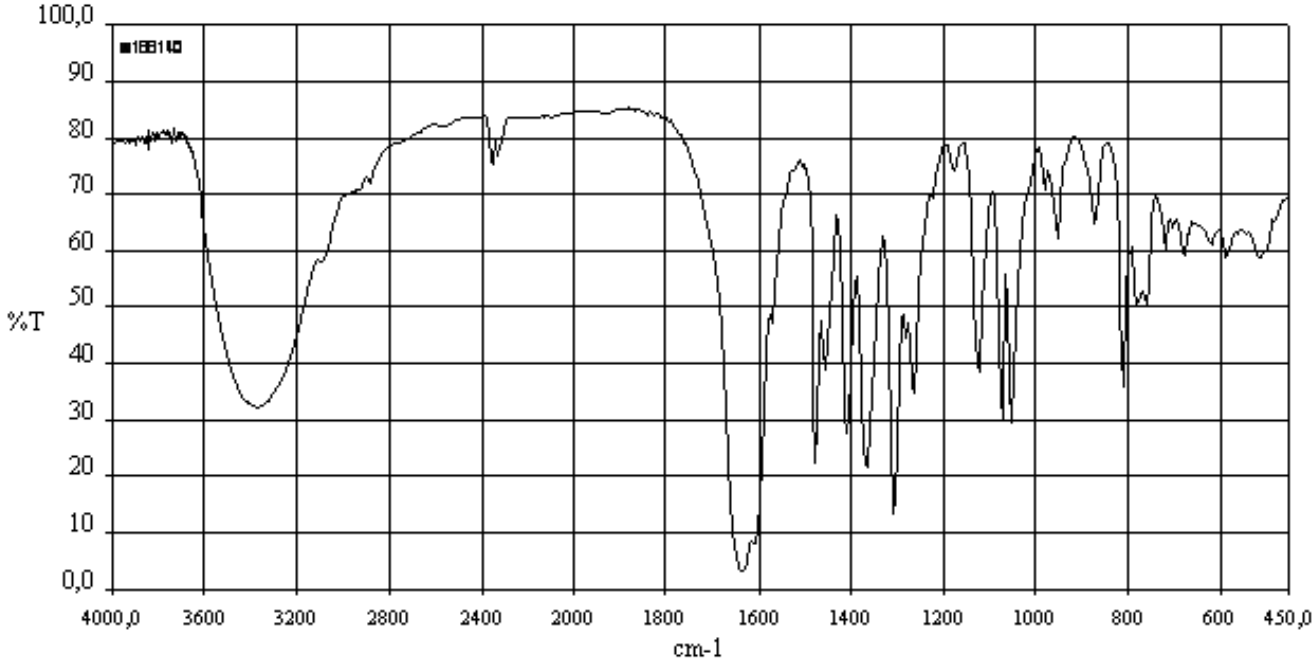
Phase: Potassium bromide disc



Sodium cromoglicate (RS00110)

Instrument: Fourier Transform

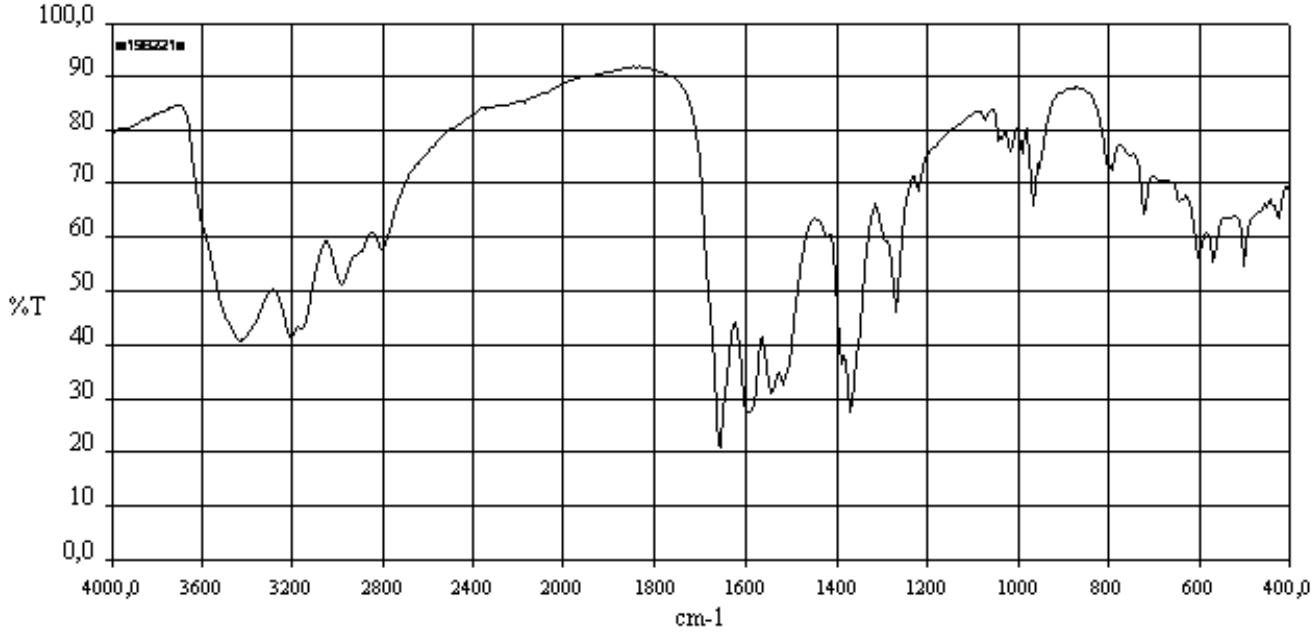
Phase: Potassium bromide disc



Sodium amidotrizoate (RS00109)

Instrument: Fourier Transform

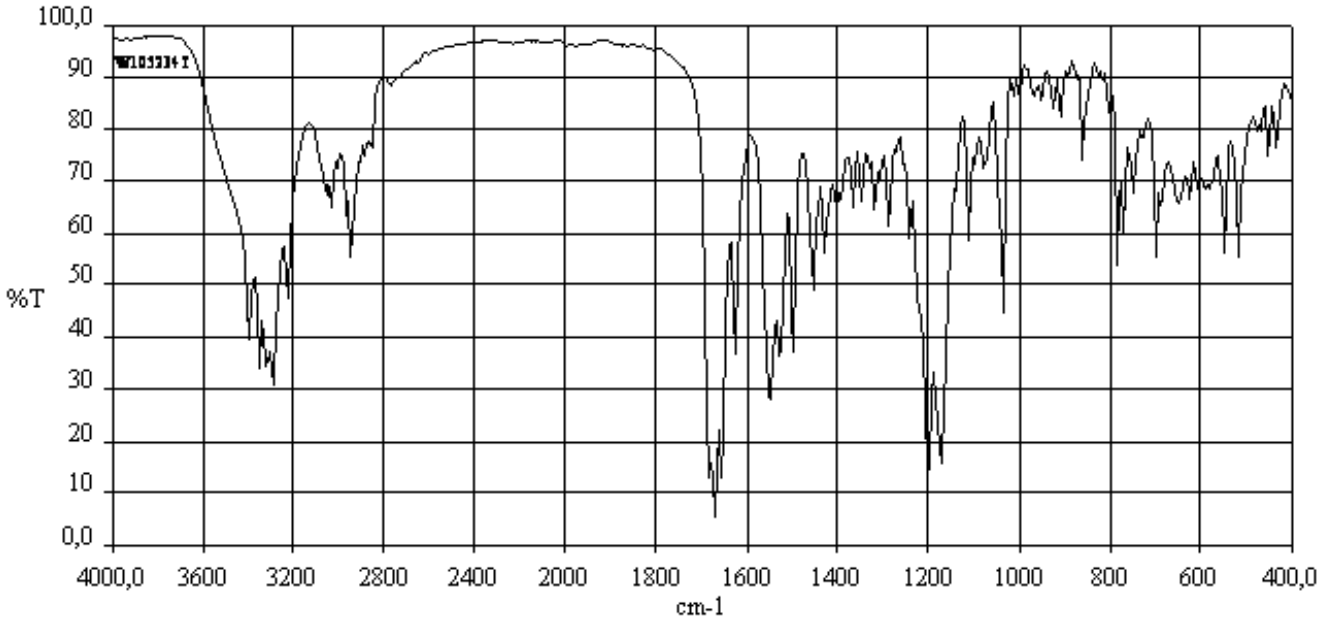
Phase: Potassium bromide disc



Saquinavir mesilate (RS00108)

Instrument: Fourier Transform

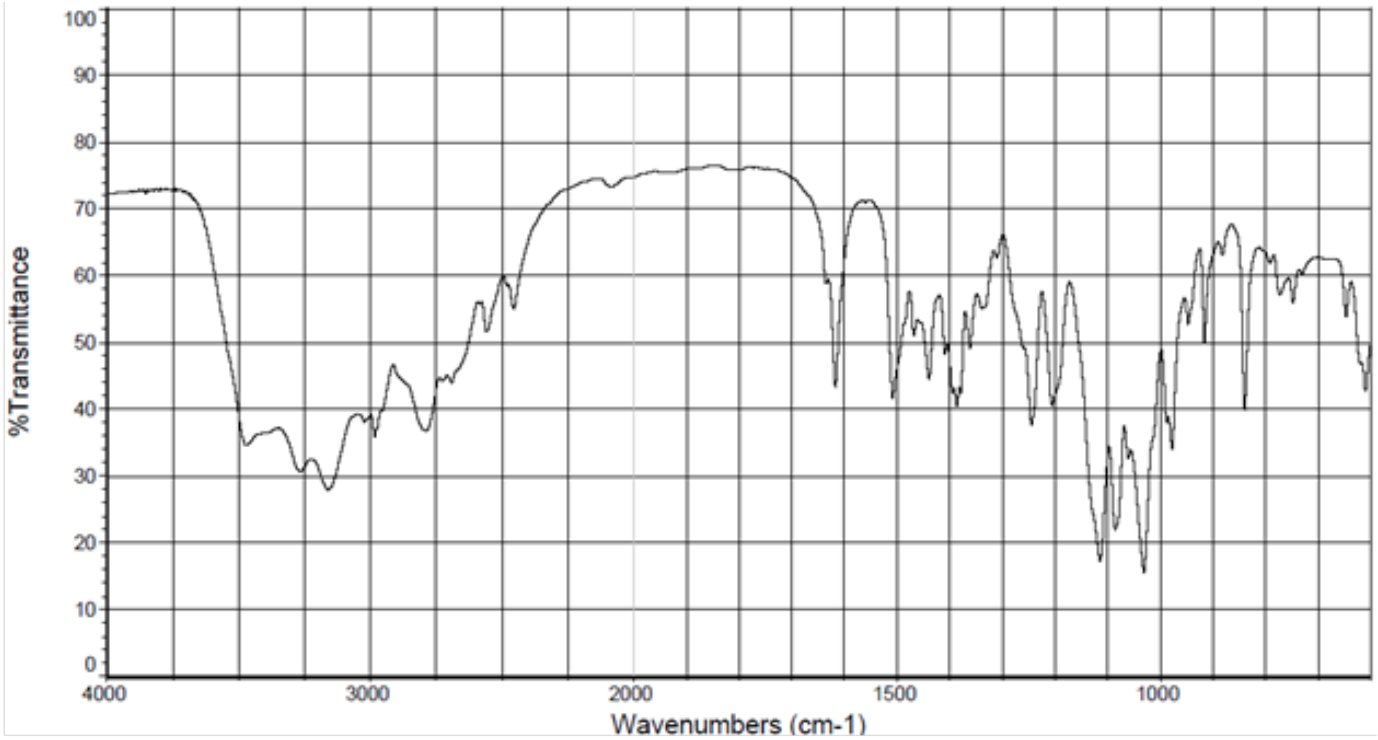
Phase: Potassium bromide disc



Salbutamol sulfate (RS00168)

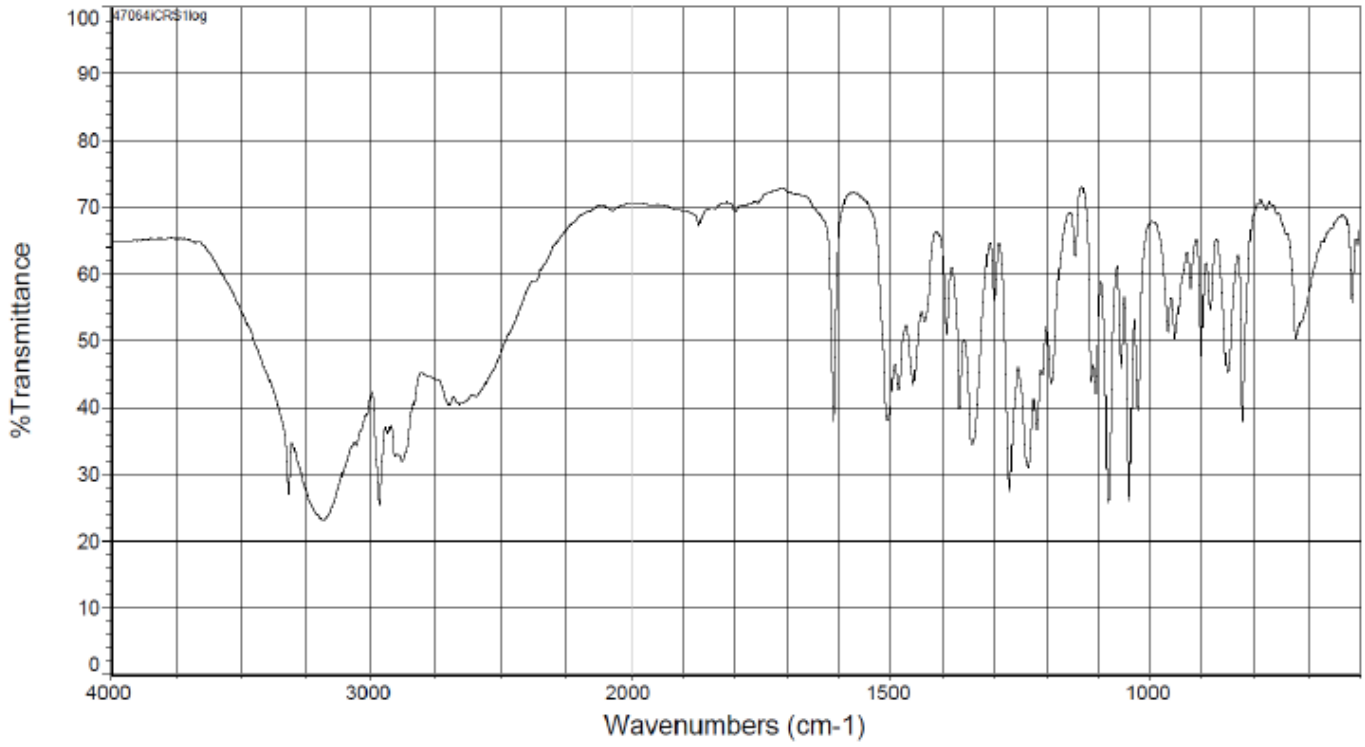
Instrument: Fourier Transform

Phase: Potassium bromide disc



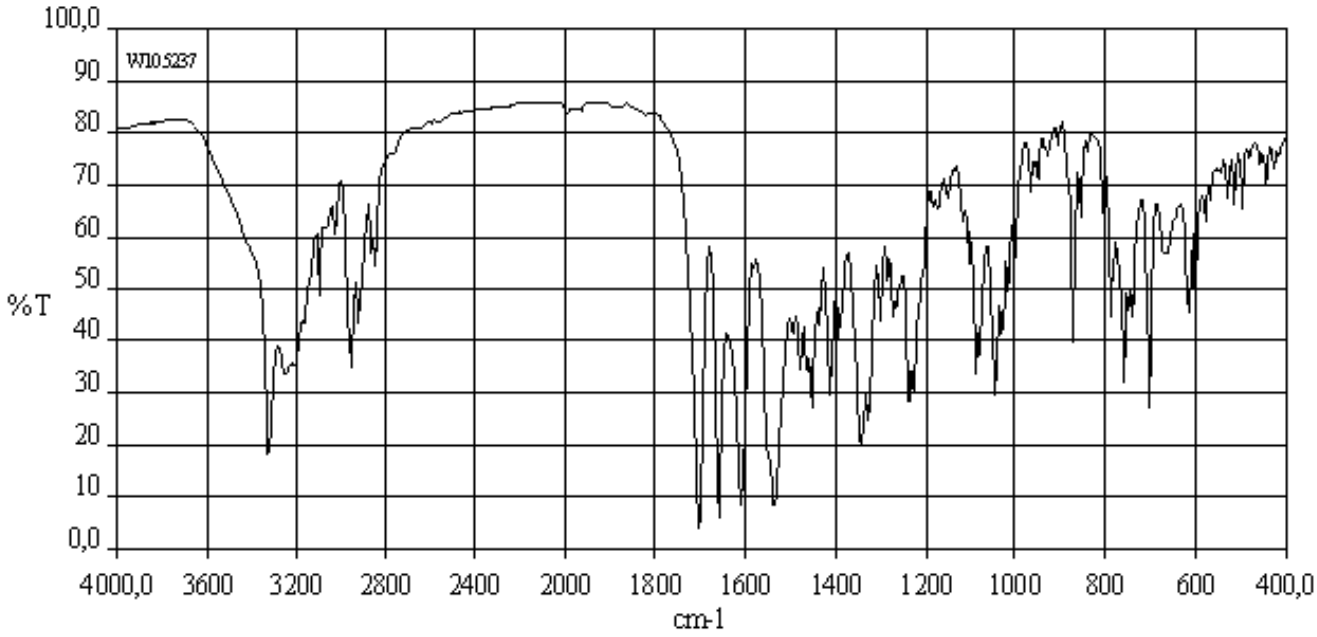
Salbutamol

Instrument: Fourier Transform
Phase: Potassium bromide disc



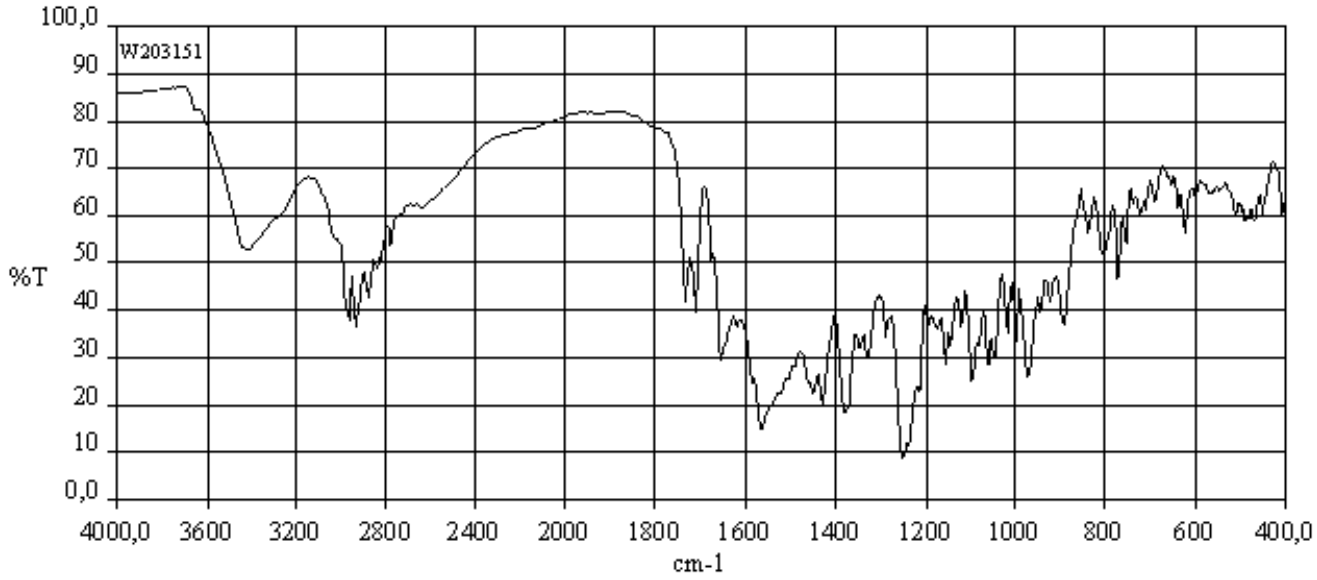
Ritonavir (RS00107)

Instrument: Fourier Transform
Phase: Potassium bromide disc



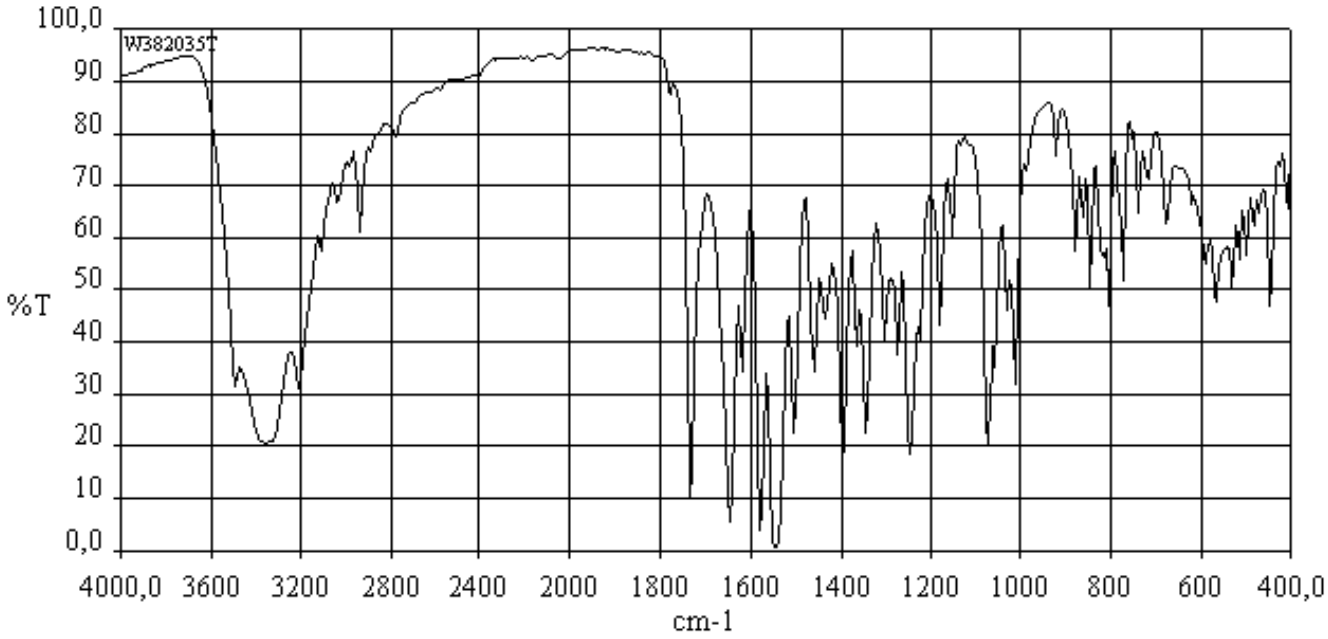
Rifampicin (RS00106)

Instrument: Fourier Transform
Phase: Potassium bromide disc



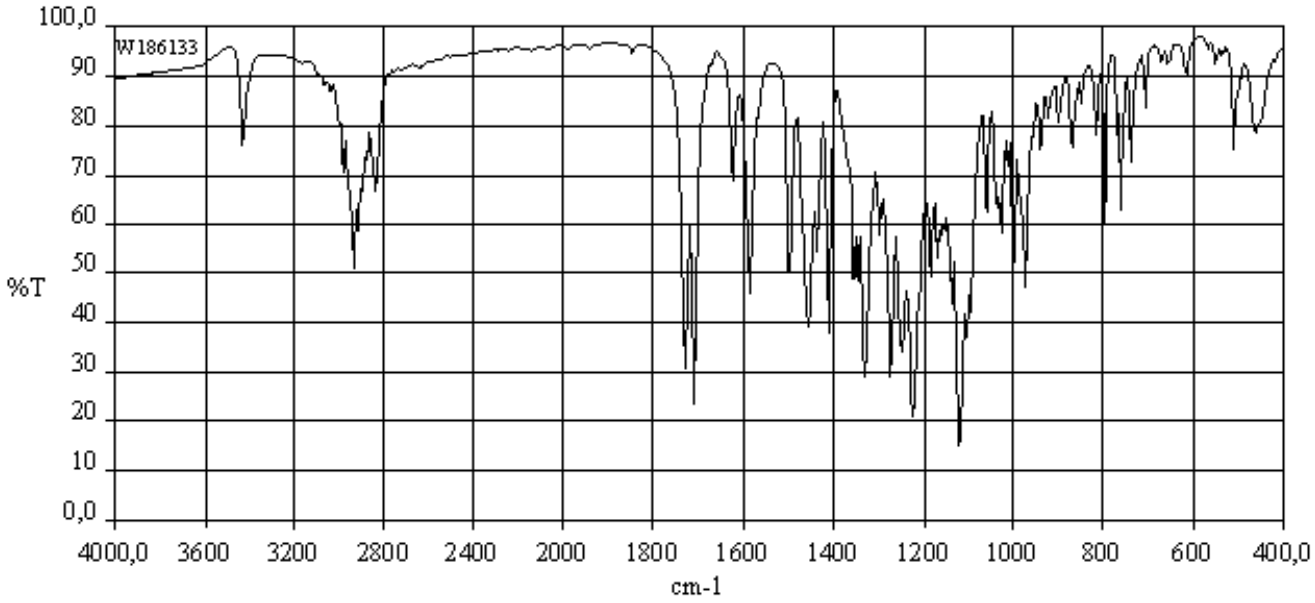
Riboflavin (RS00105)

Instrument: Fourier Transform
Phase: Potassium bromide disc



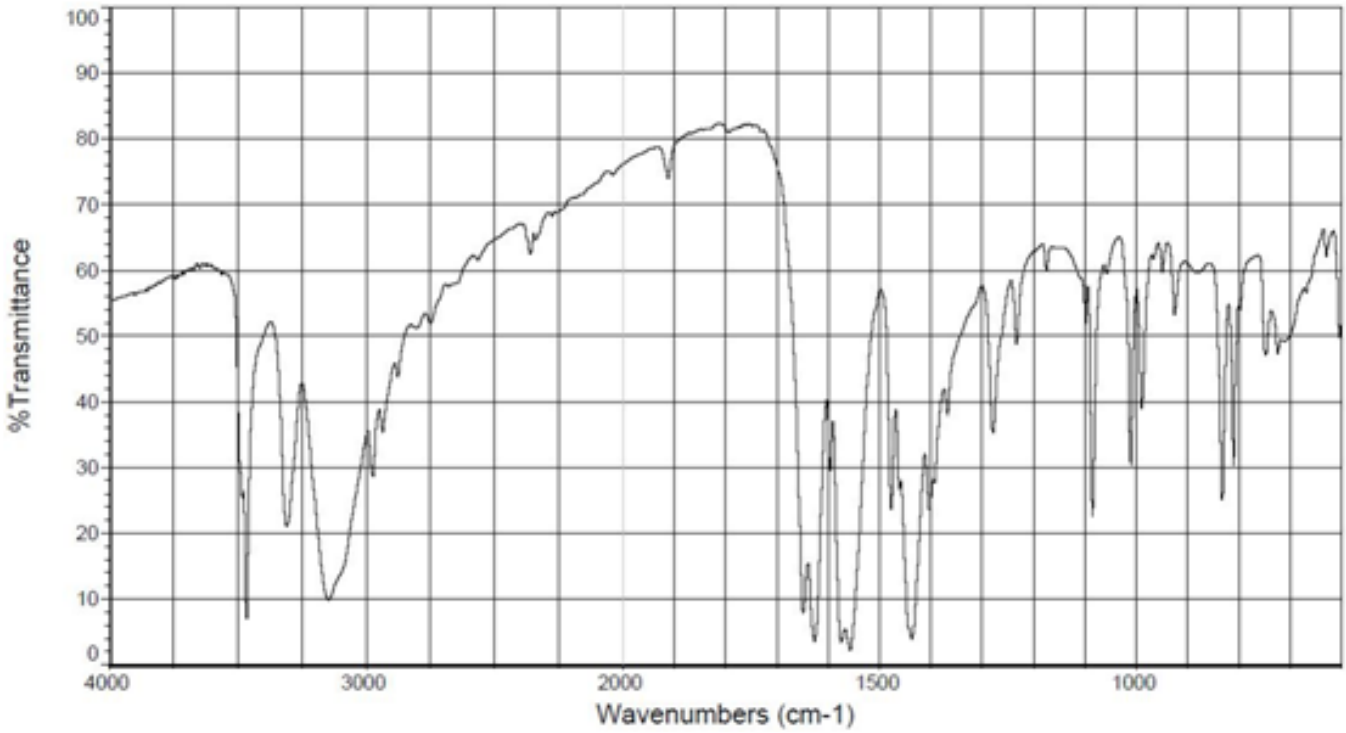
Reserpine (RS00104)

Instrument: Fourier Transform
Phase: Potassium bromide disc



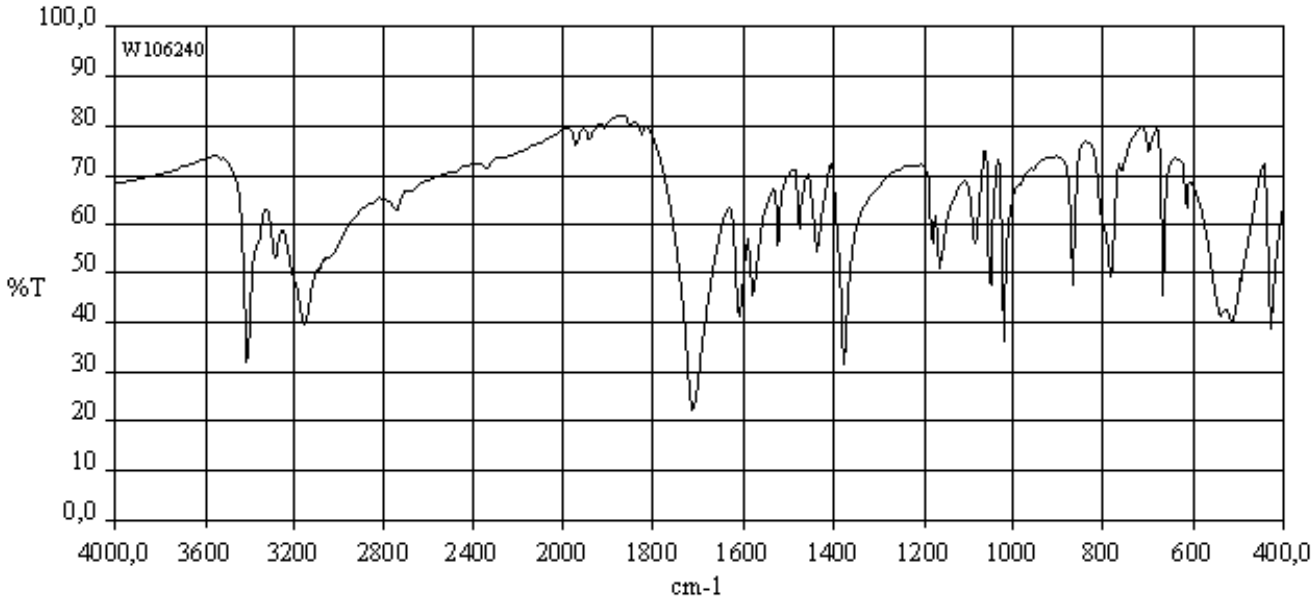
Pyrimethamine (RS00163)

Instrument: Fourier Transform
Phase: Potassium bromide disc



Pyrazinamide (RS00103)

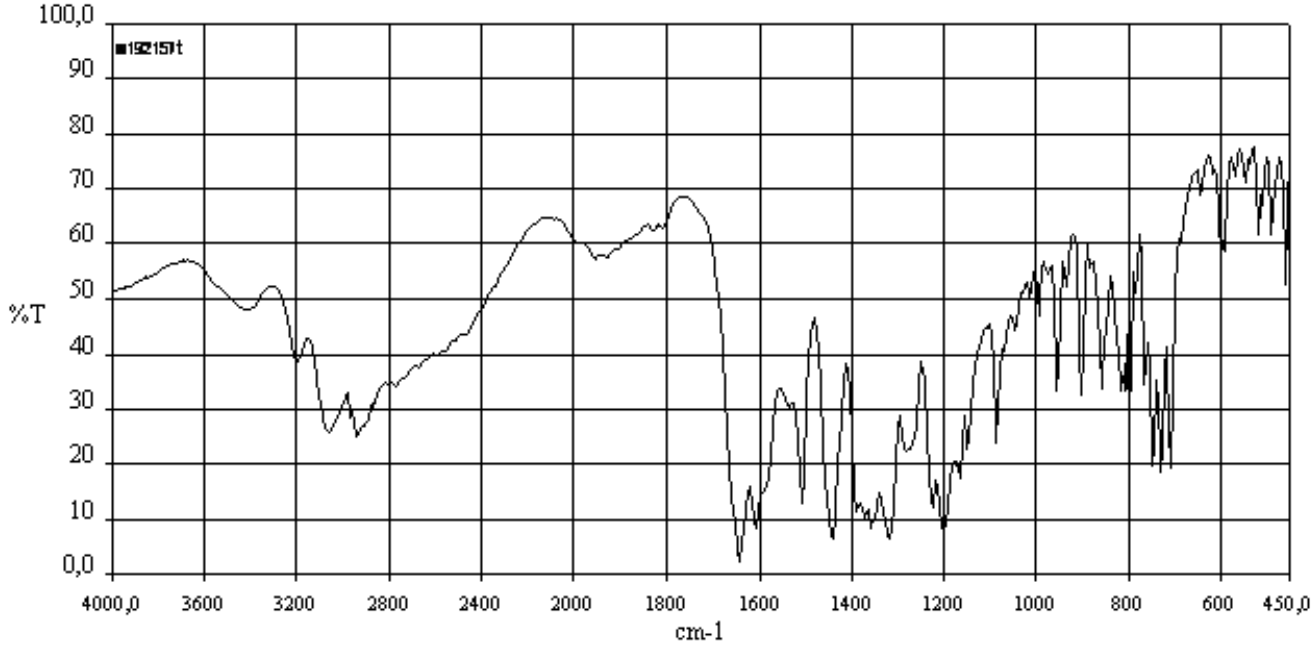
Instrument: Fourier Transform
Phase: Potassium bromide disc



Pyrantel embonate (pyrantel pamoate) (RS00102)

Instrument: Fourier Transform

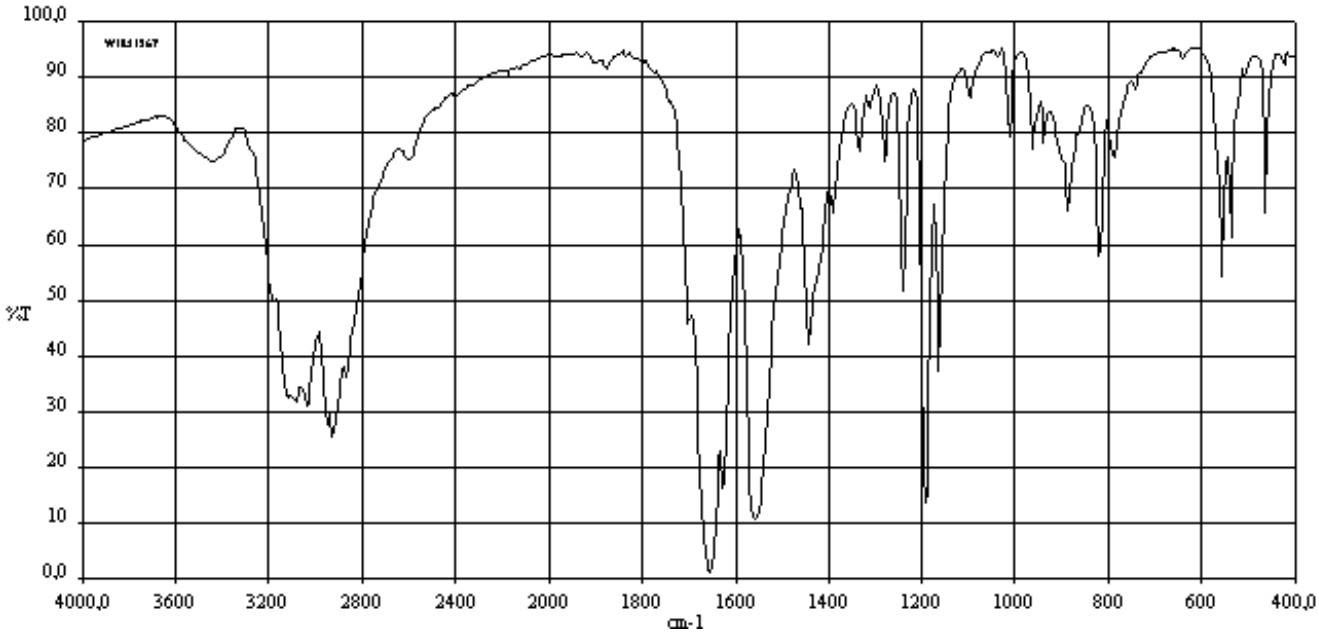
Phase: Potassium bromide disc



Propylthiouracil (RS00101)

Instrument: Fourier Transform

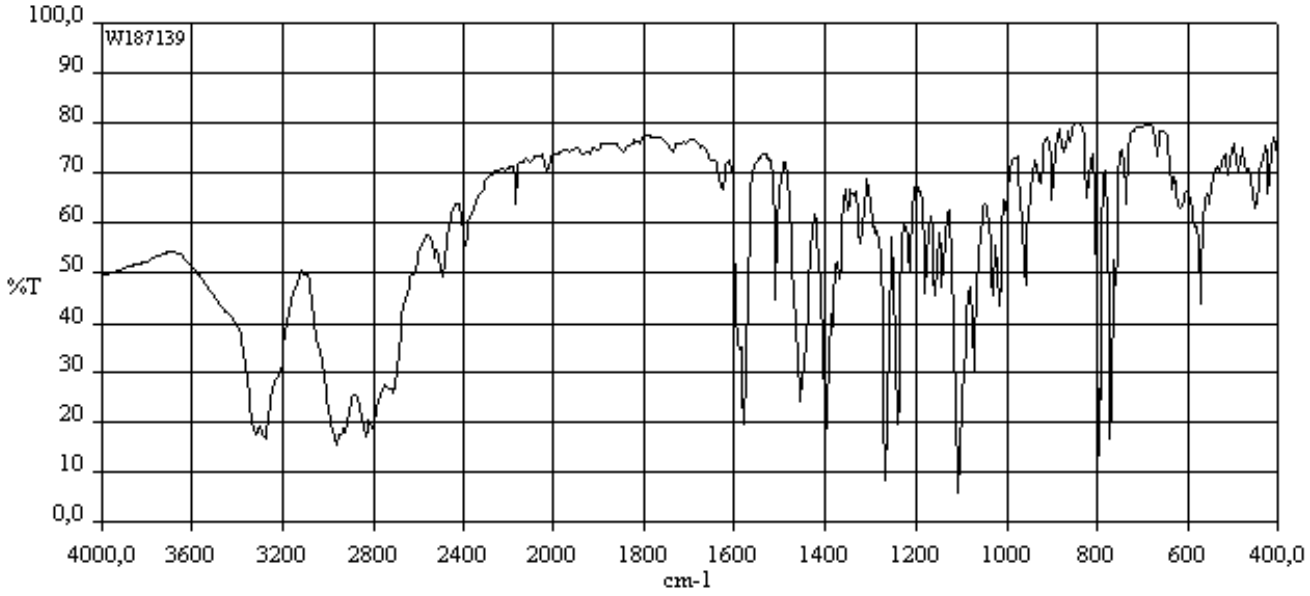
Phase: Potassium bromide disc



Propranolol hydrochloride (RS00100)

Instrument: Fourier Transform

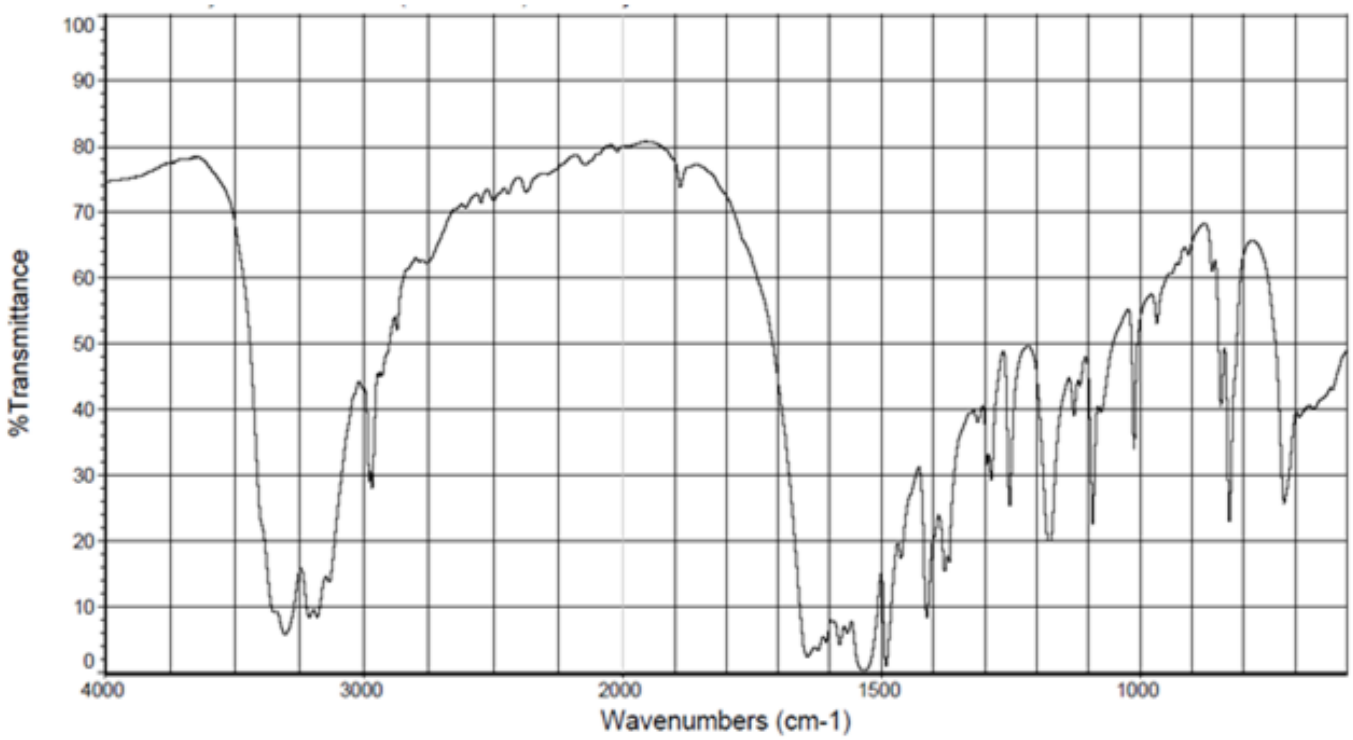
Phase: Potassium bromide disc



Proguanil hydrochloride (RS00162)

Instrument: Fourier Transform

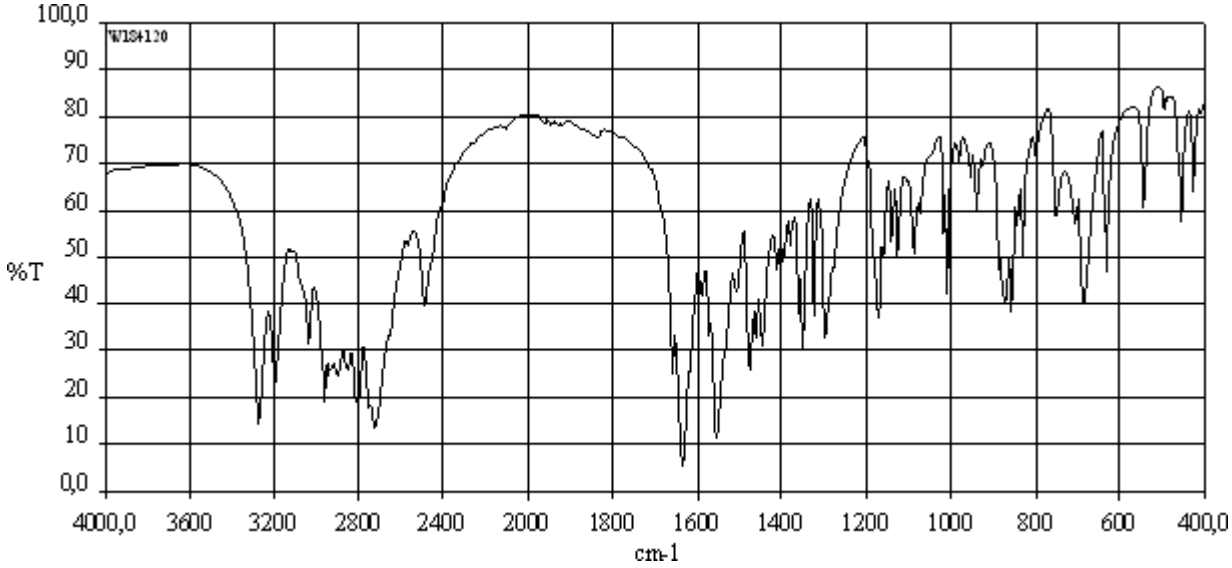
Phase: Potassium bromide disc



Procarbazine hydrochloride (RS00149)

Instrument: Fourier Transform

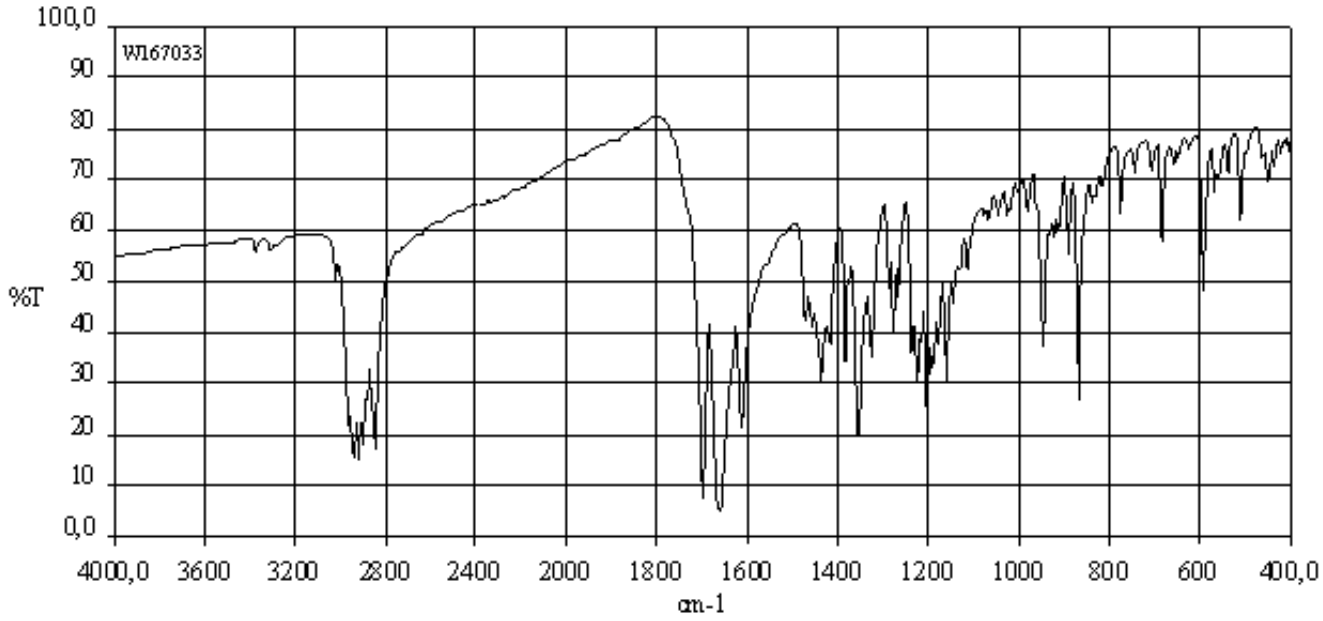
Phase: Potassium bromide disc



Progesterone (RS0099)

Instrument: Fourier Transform

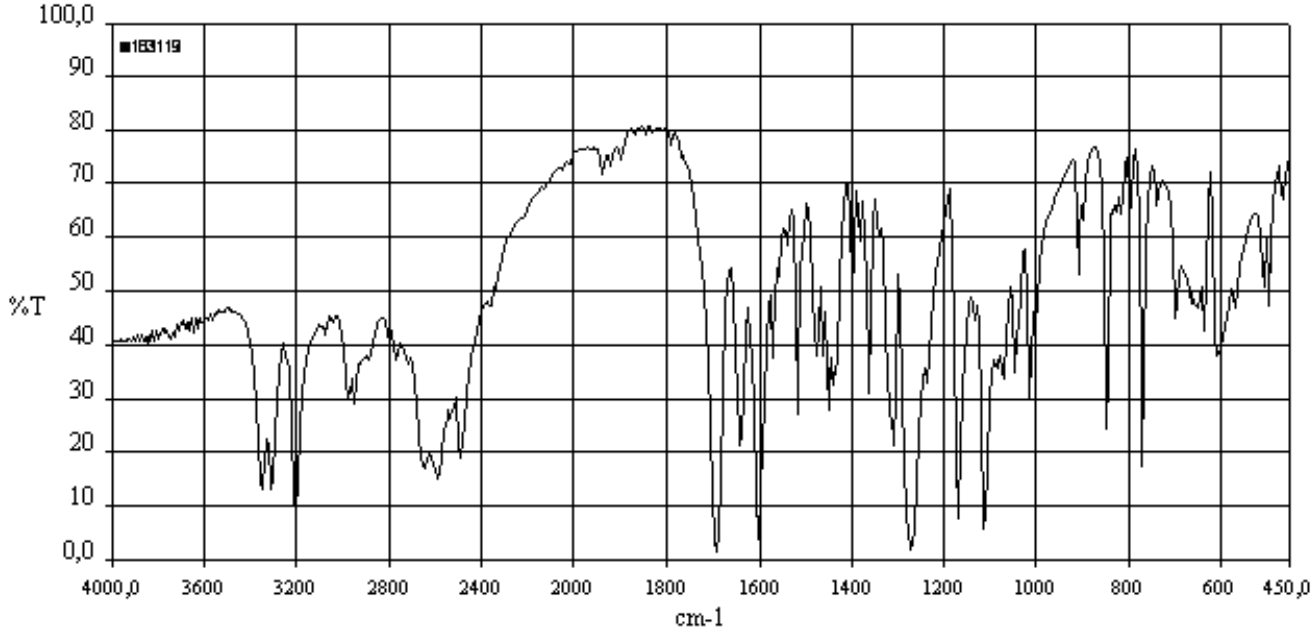
Phase: Potassium bromide disc



Procaine hydrochloride (RS0098)

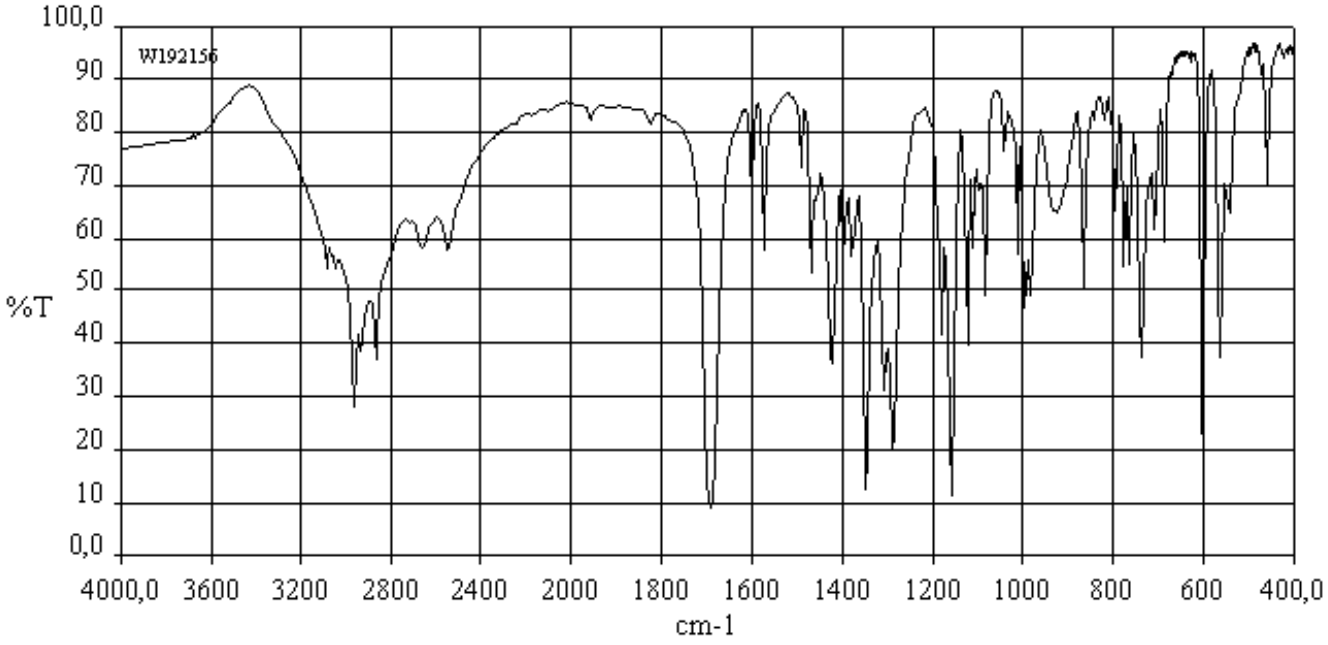
Instrument: Fourier Transform

Phase: Potassium bromide disc



Probenecid (RS0097)

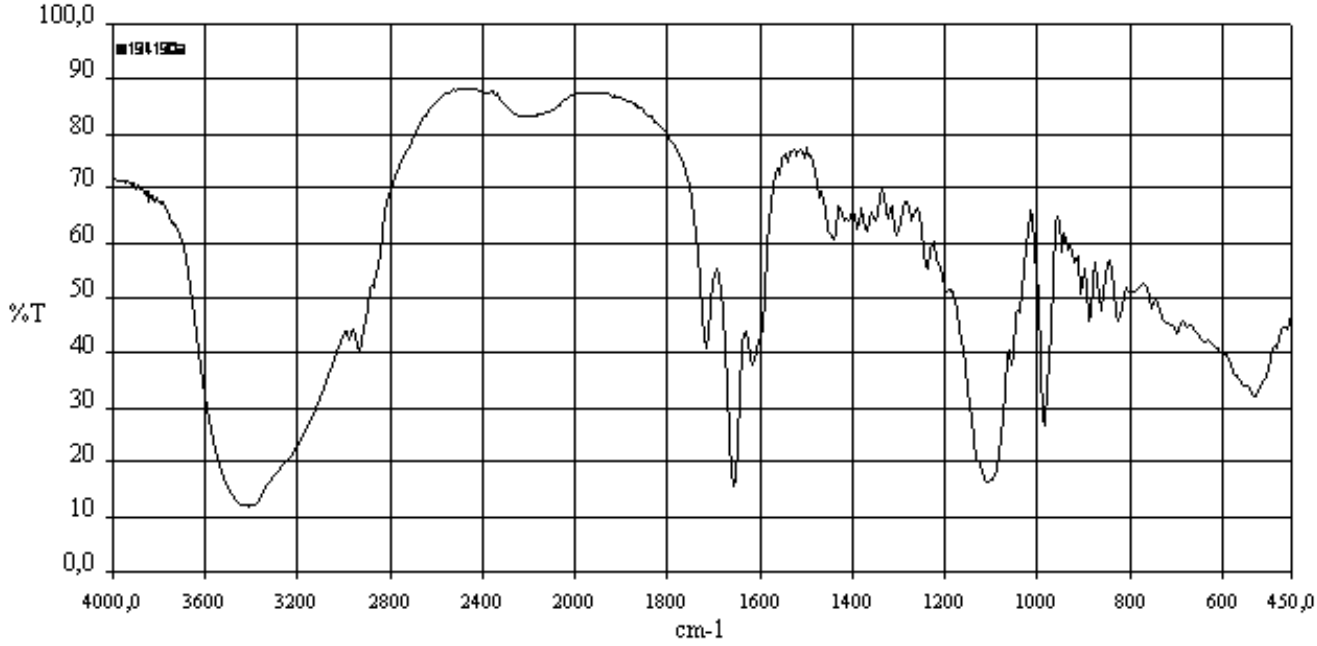
Instrument: Fourier Transform
Phase: Potassium bromide disc



Prednisolone sodium phosphate (RS0096)

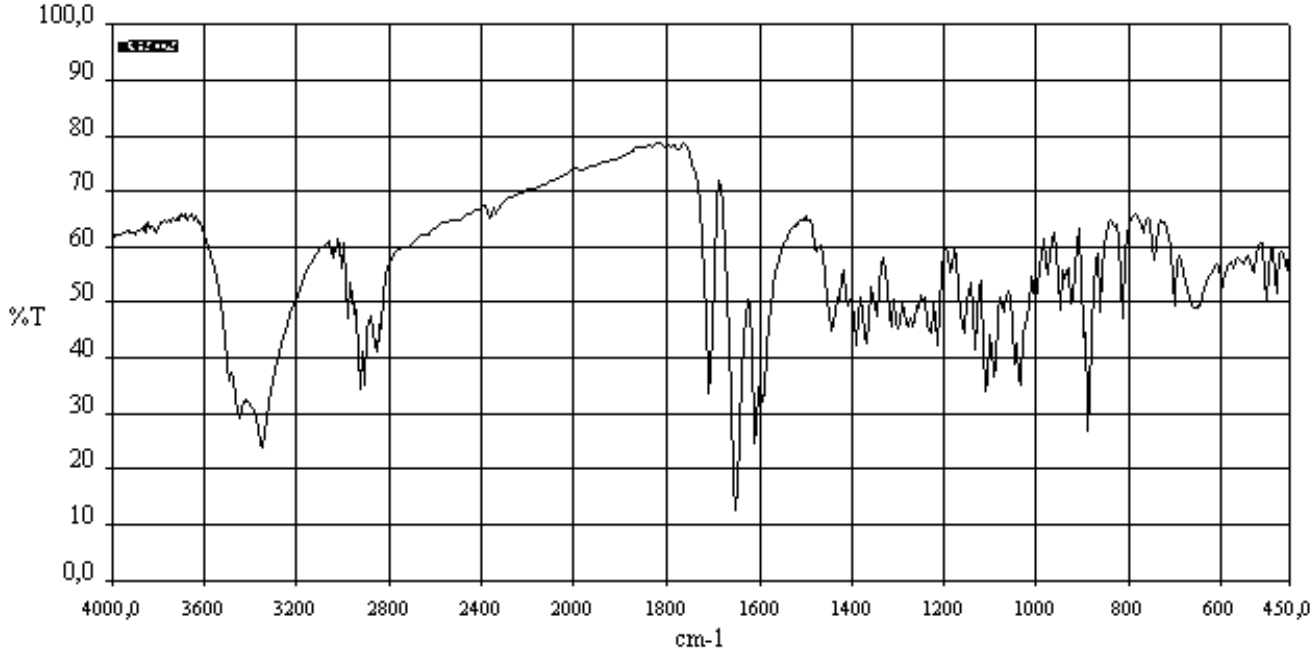
Instrument: Fourier Transform

Phase: Potassium bromide disc



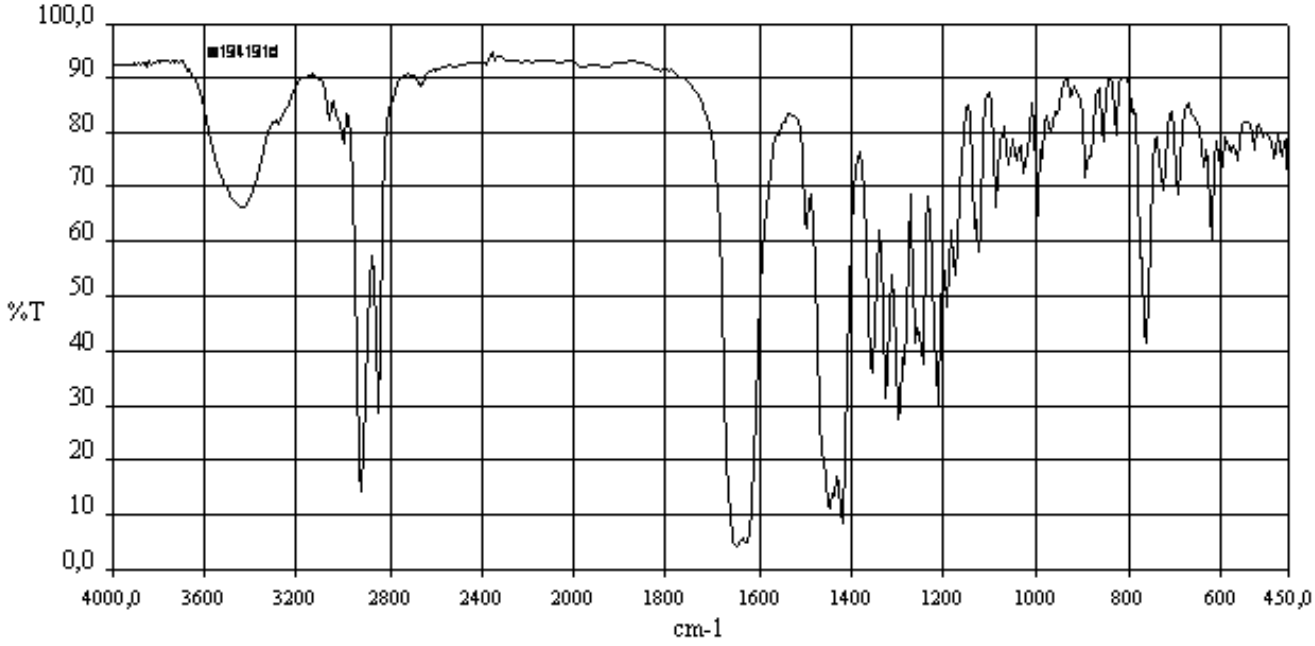
Prednisolone (RS0095)

Instrument: Fourier Transform
Phase: Potassium bromide disc



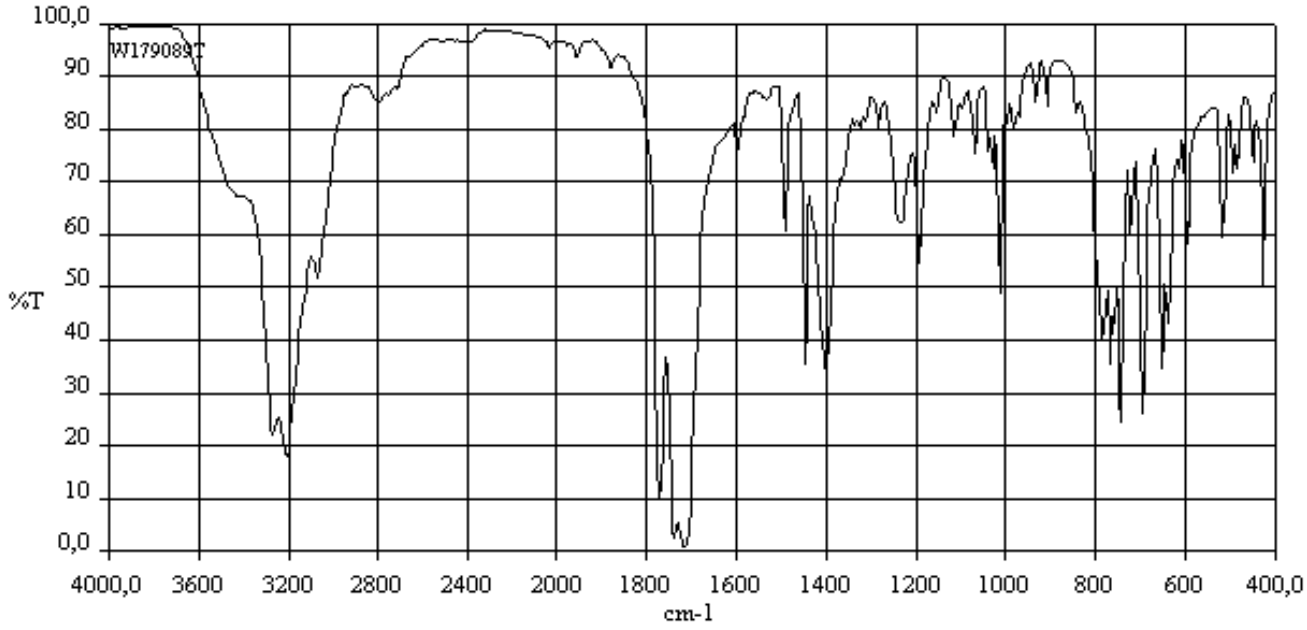
Praziquantel (RS0094)

Instrument: Fourier Transform
Phase: Potassium bromide disc



Phenytoin (RS0093)

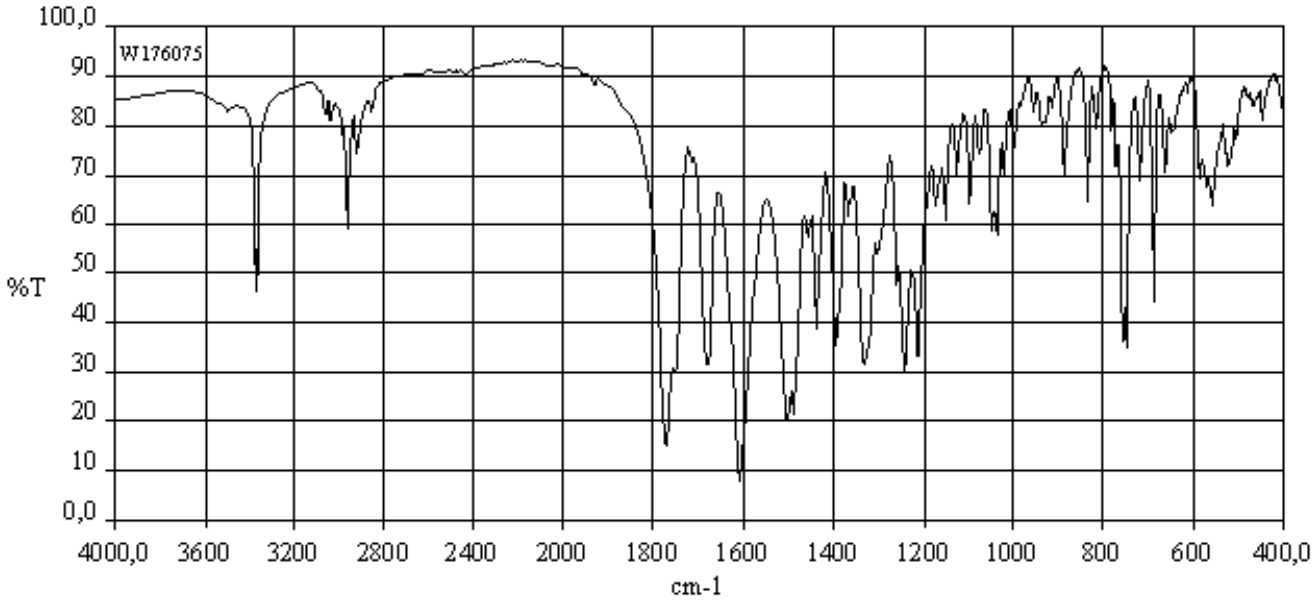
Instrument: Fourier Transform
Phase: Potassium bromide disc



Phenoxymethylpenicillin potassium (RS0092)

Instrument: Fourier Transform

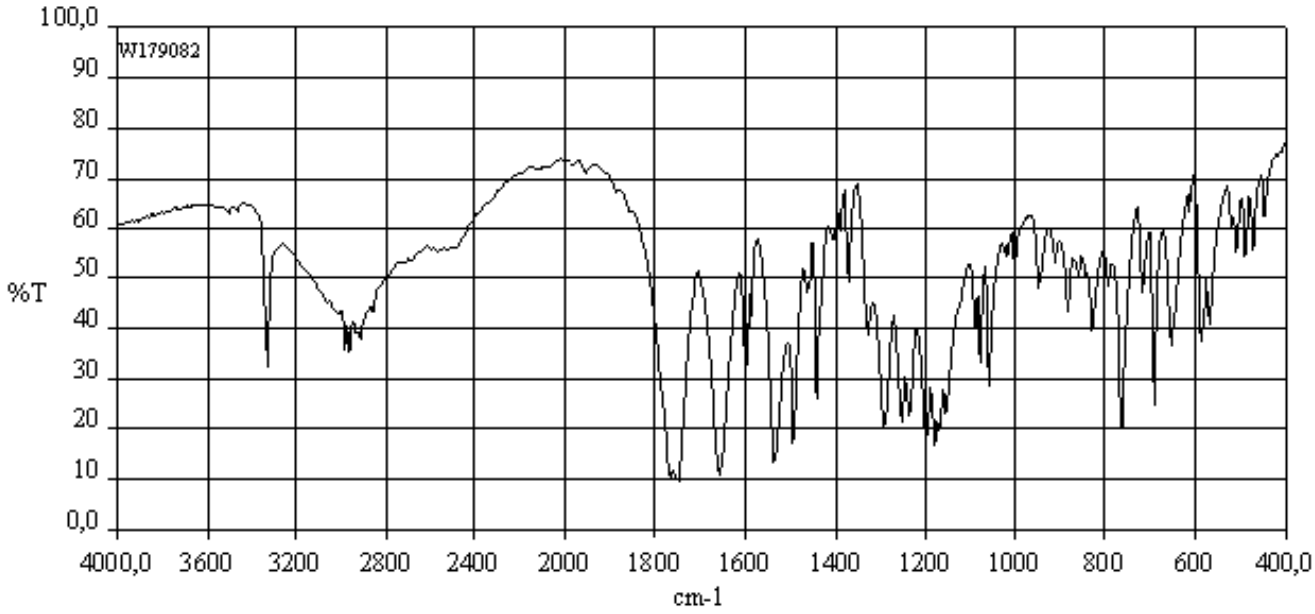
Phase: Potassium bromide disc



Phenoxymethylpenicillin (RS0090)

Instrument: Fourier Transform

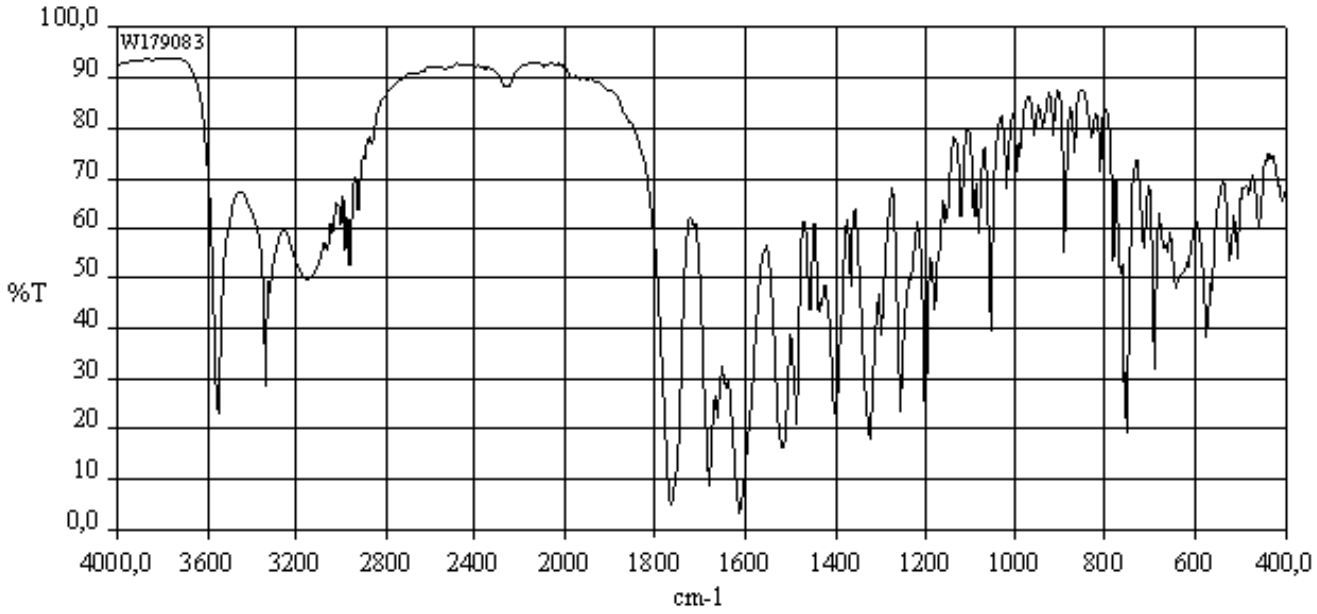
Phase: Potassium bromide disc



Phenoxymethylpenicillin calcium (RS0091)

Instrument: Fourier Transform

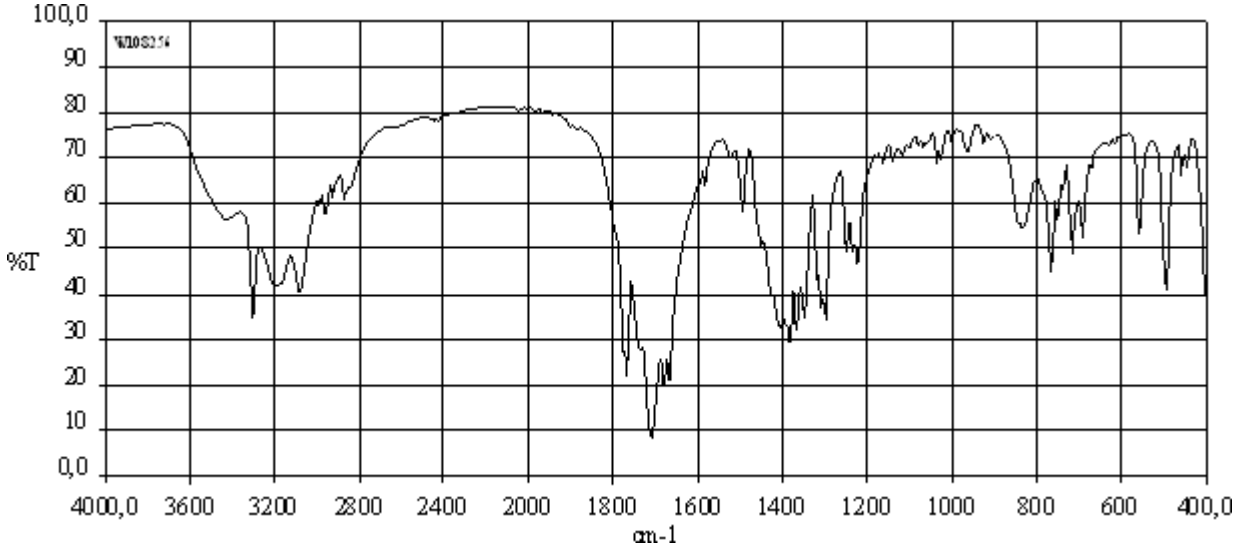
Phase: Potassium bromide disc



Phenobarbital (RS00148)

Instrument: Fourier Transform

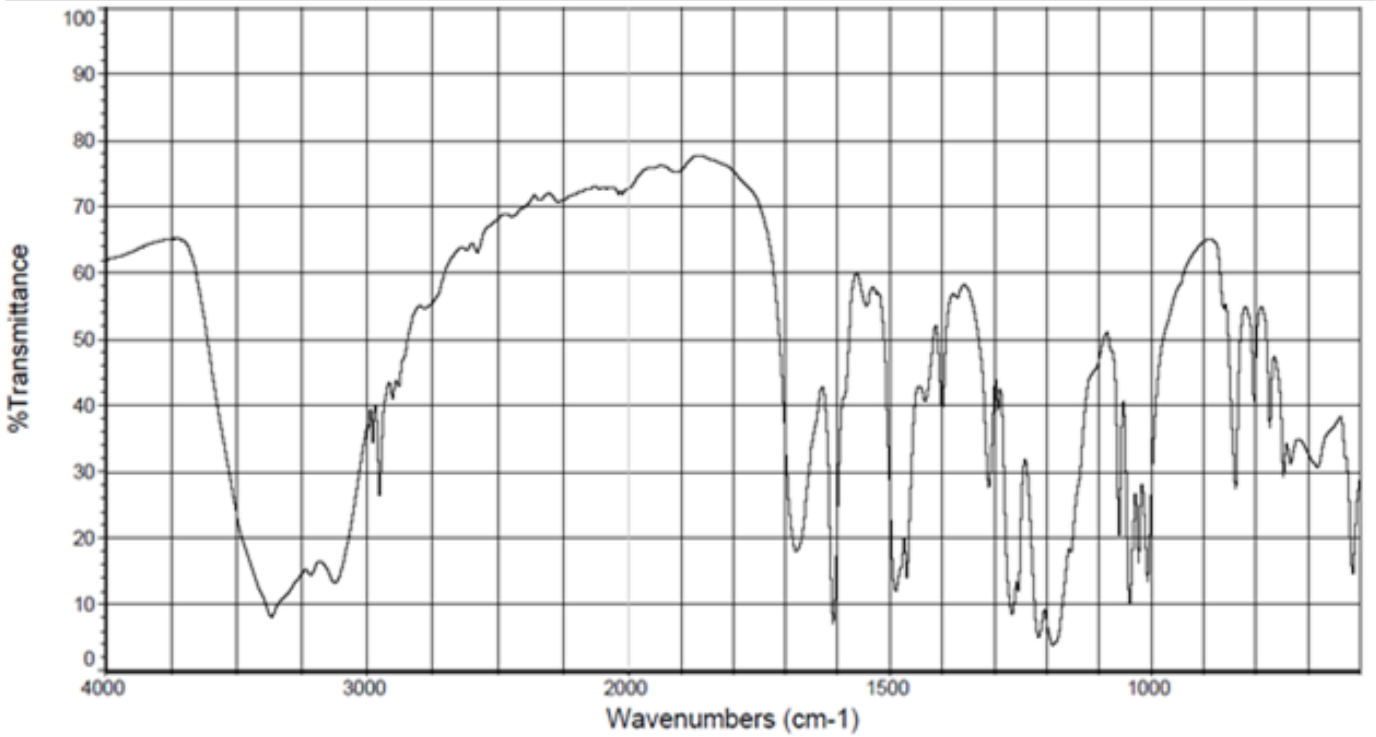
Phase: Potassium bromide disc



Pentamidine isetionate (RS00167)

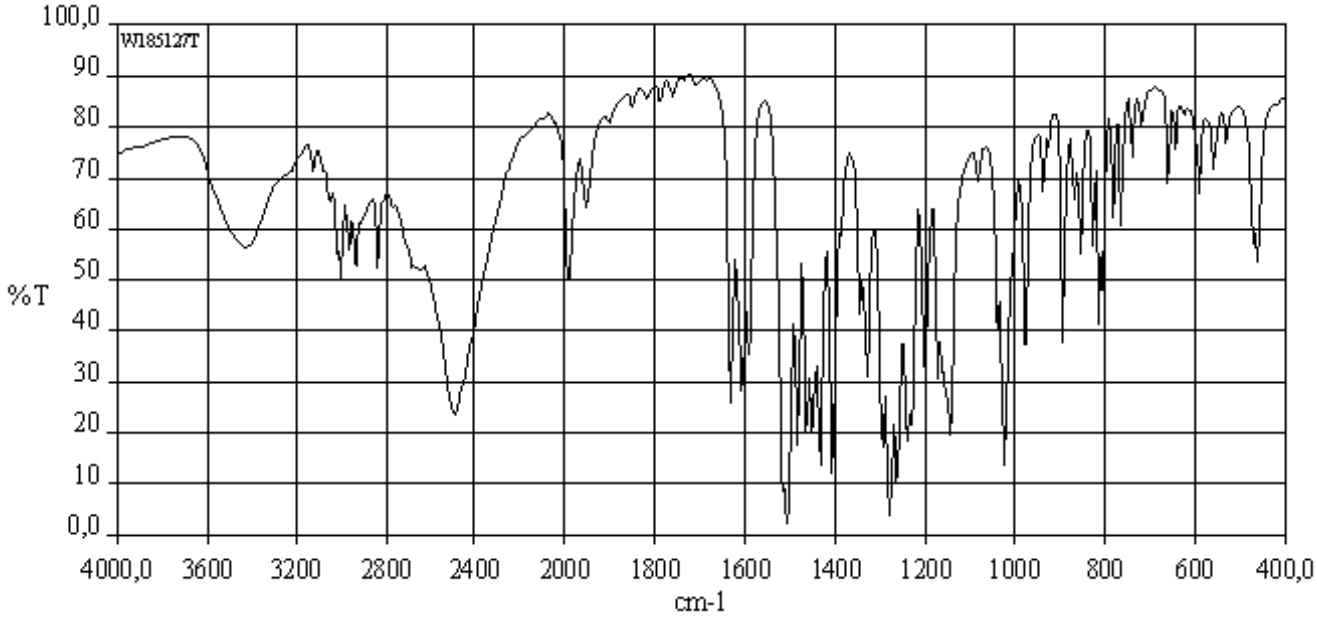
Instrument: Fourier Transform

Phase: Potassium bromide disc



Papaverine hydrochloride (RS0089)

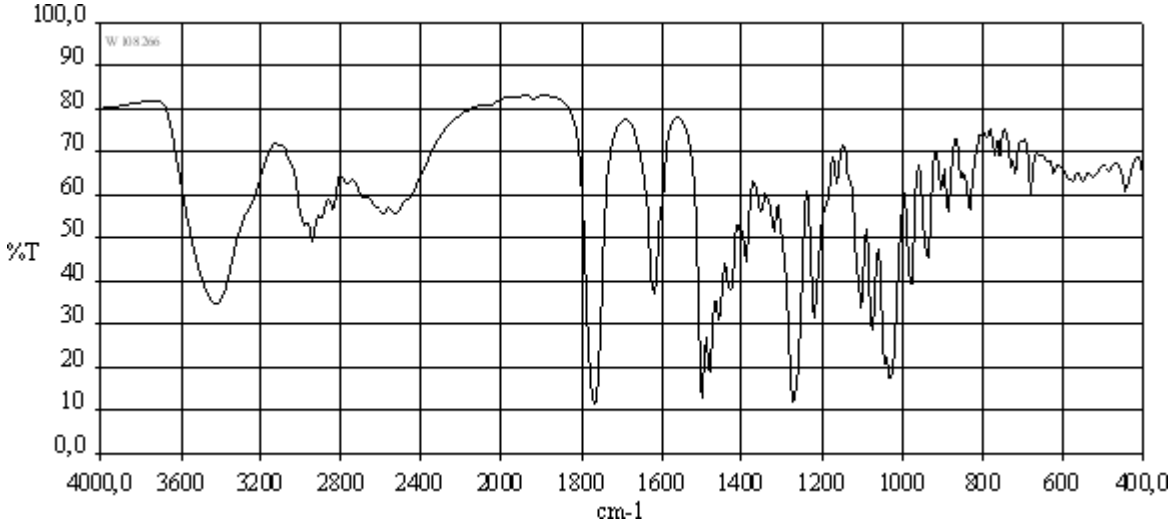
Instrument: Fourier Transform
Phase: Potassium bromide disc



Noscapine hydrochloride (RS00147)

Instrument: Fourier Transform

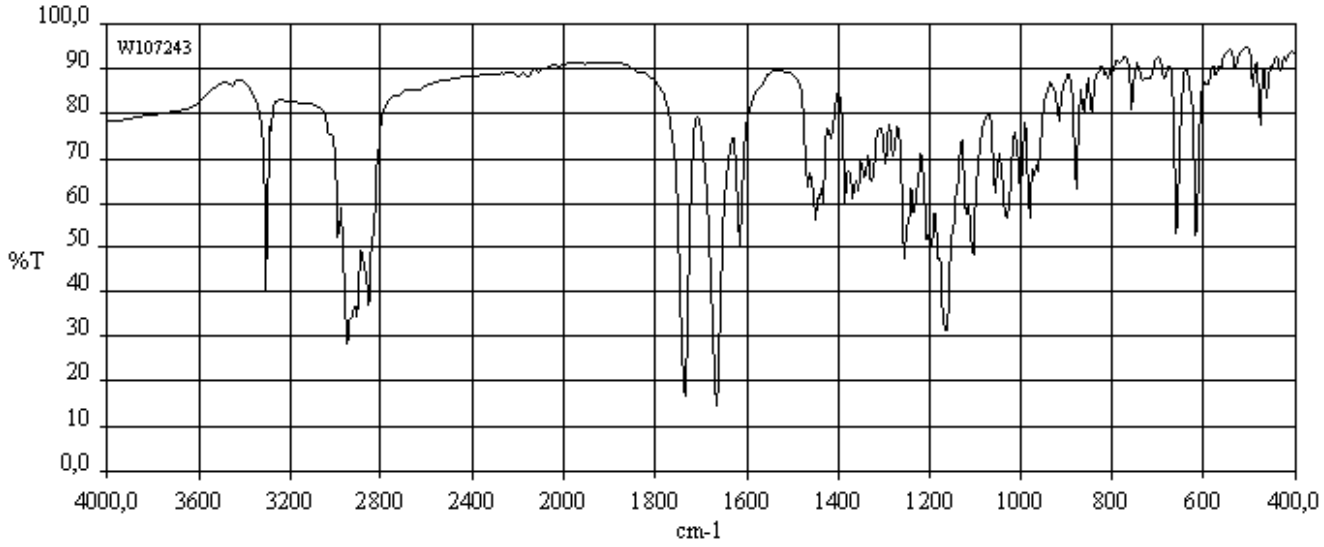
Phase: Potassium bromide disc



Norethisterone enantate (RS0088)

Instrument: Fourier Transform

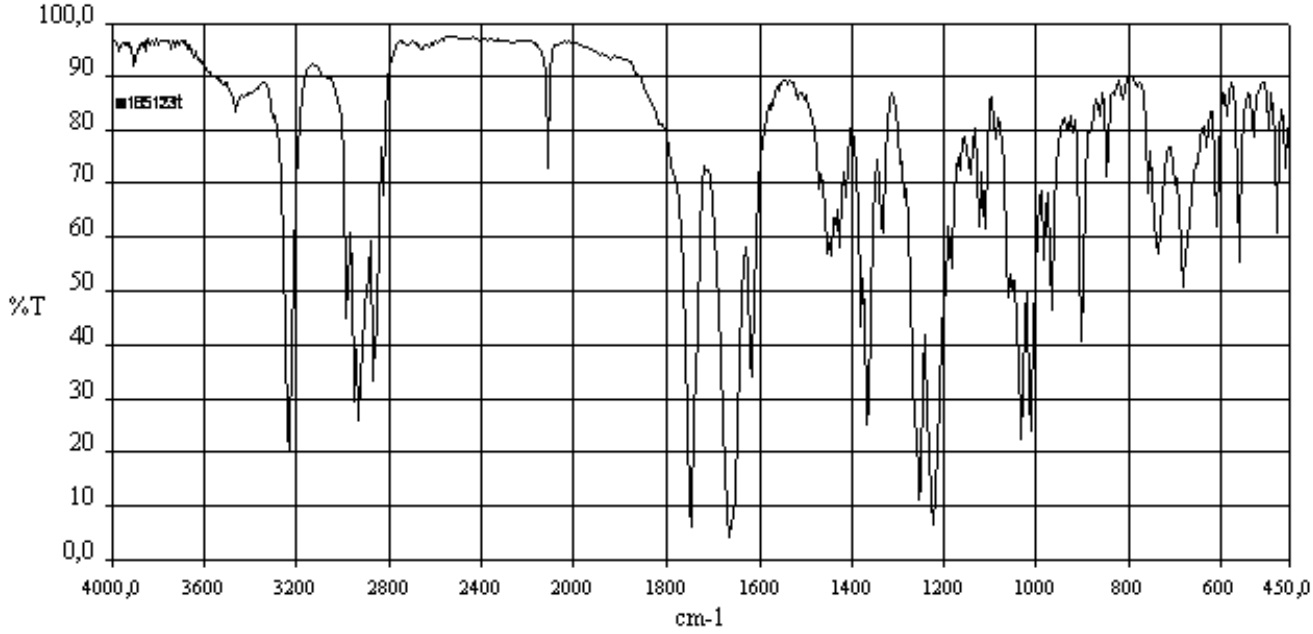
Phase: Potassium bromide disc



Norethisterone acetate (RS0087)

Instrument: Fourier Transform

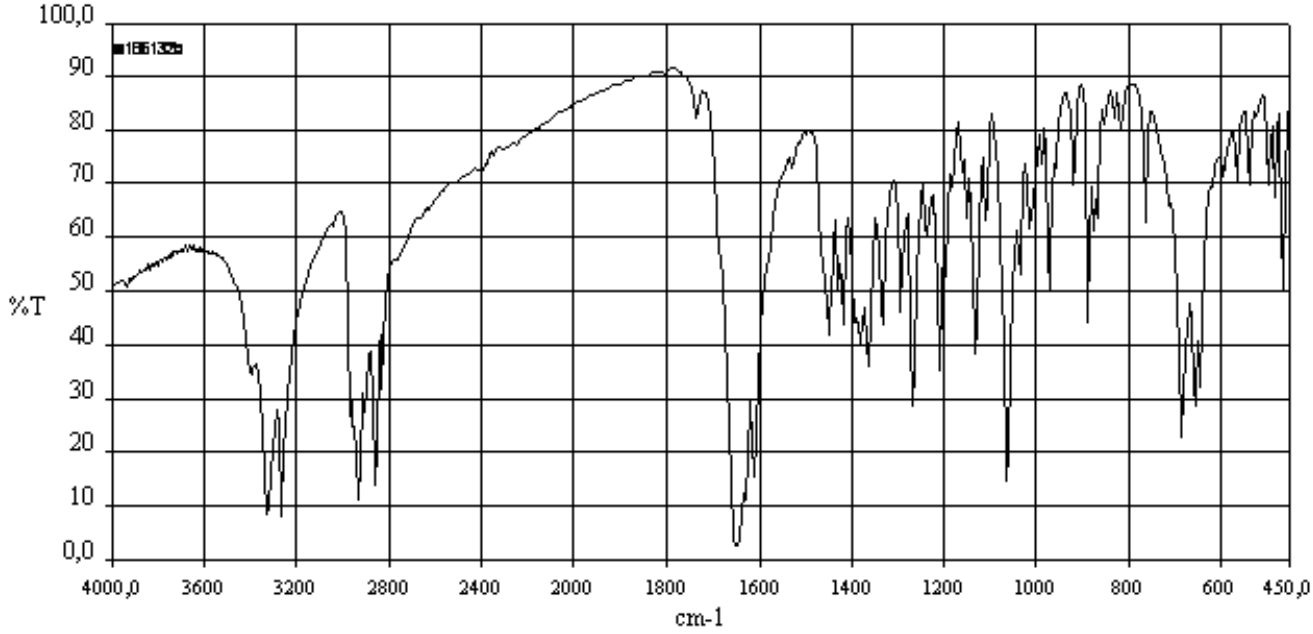
Phase: Potassium bromide disc



Norethisterone (RS0086)

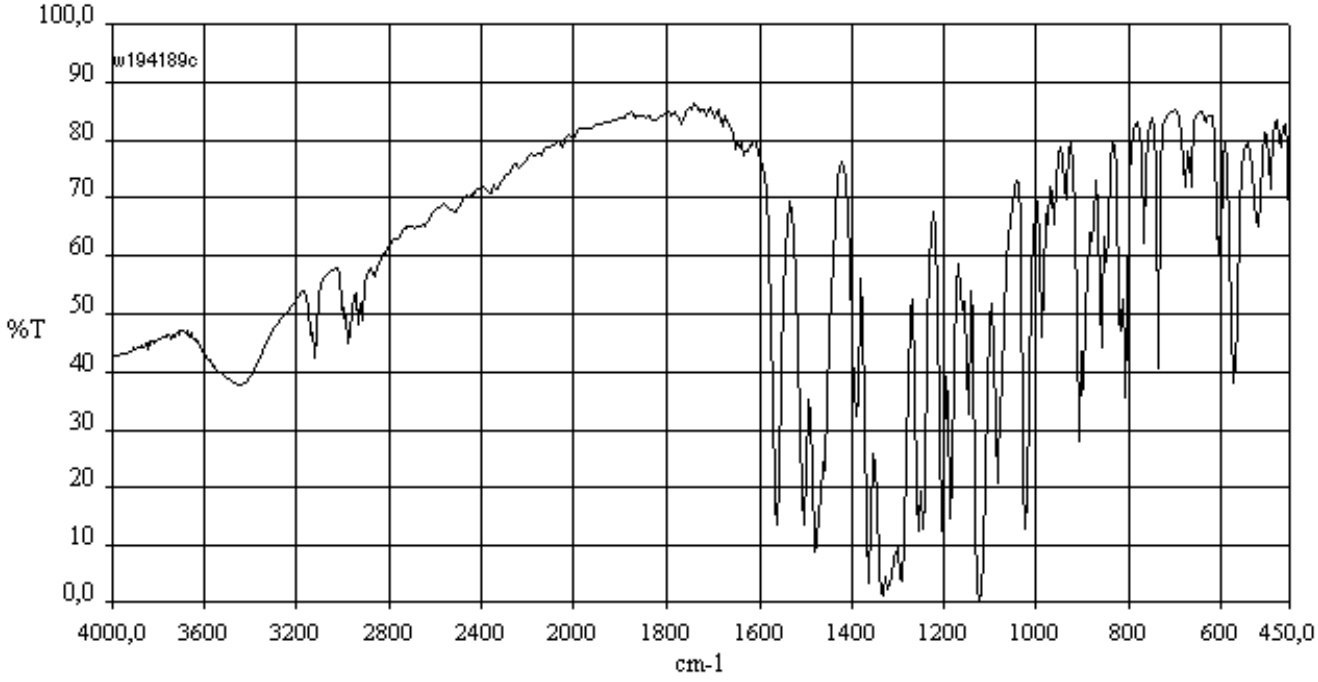
Instrument: Fourier Transform

Phase: Potassium bromide disc



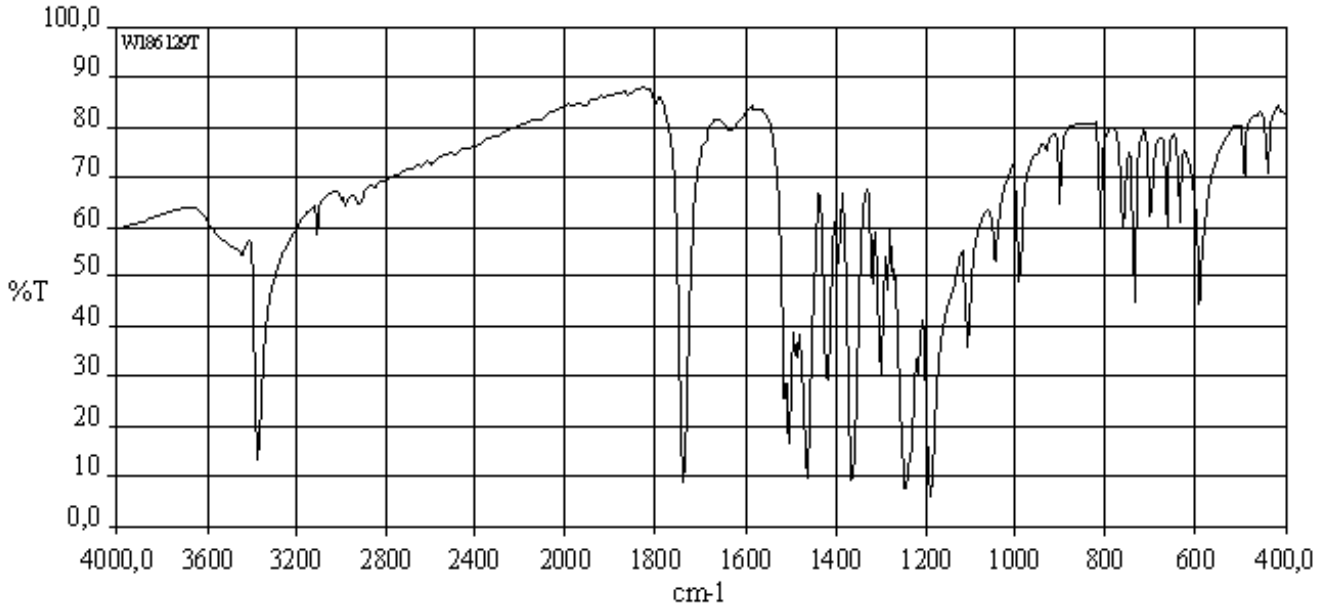
Nifurtimox (RS0084)

Instrument: Fourier Transform
Phase: Potassium bromide disc



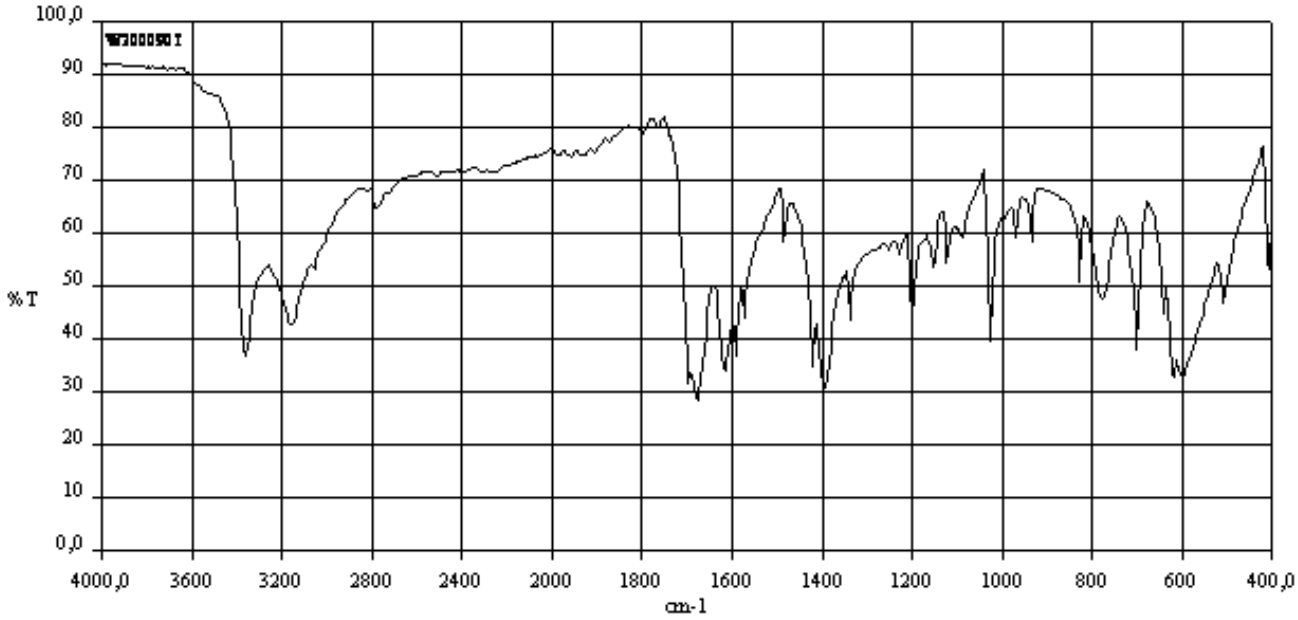
Niridazole (RS0085)

Instrument: Fourier Transform
Phase: Potassium bromide disc



Nicotinamide (RS0083)

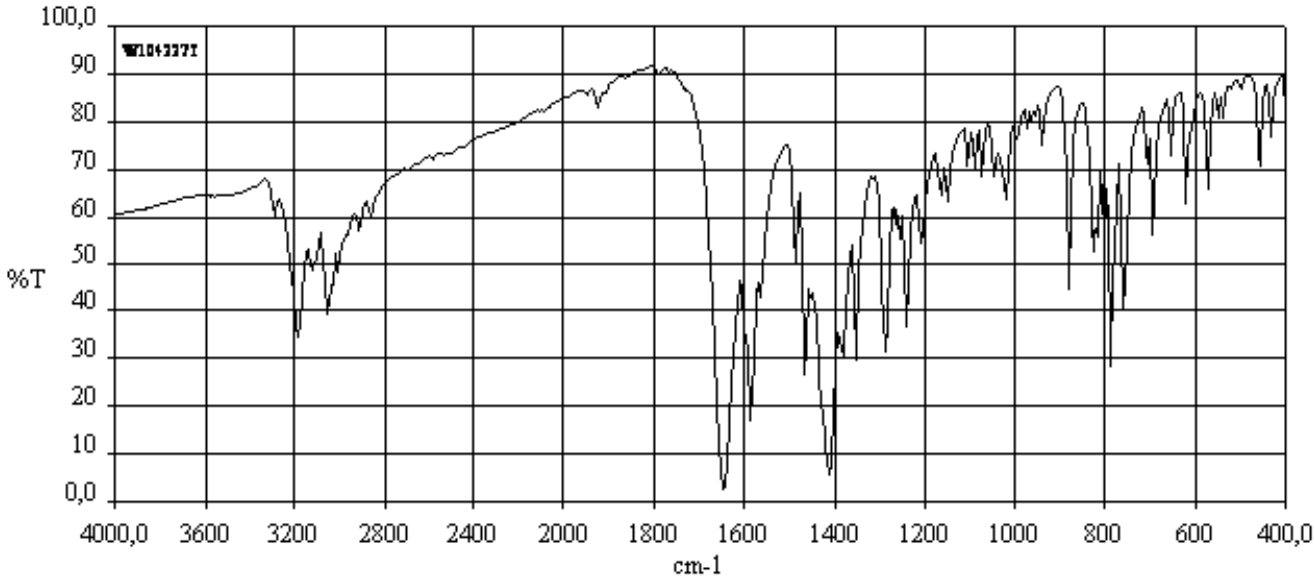
Instrument: Fourier Transform
Phase: Potassium bromide disc



Nevirapine anhydrous (RS0082)

Instrument: Fourier Transform

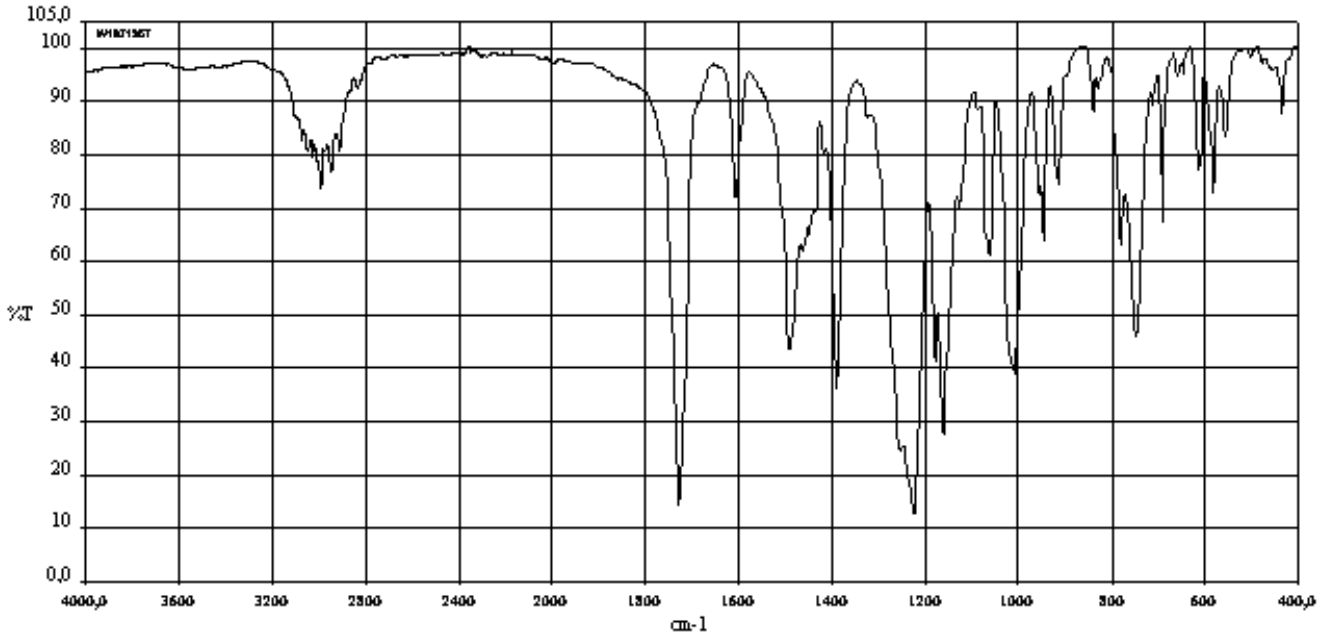
Phase: Potassium bromide disc



Neostigmine metilsulfate (RS0081)

Instrument: Fourier Transform

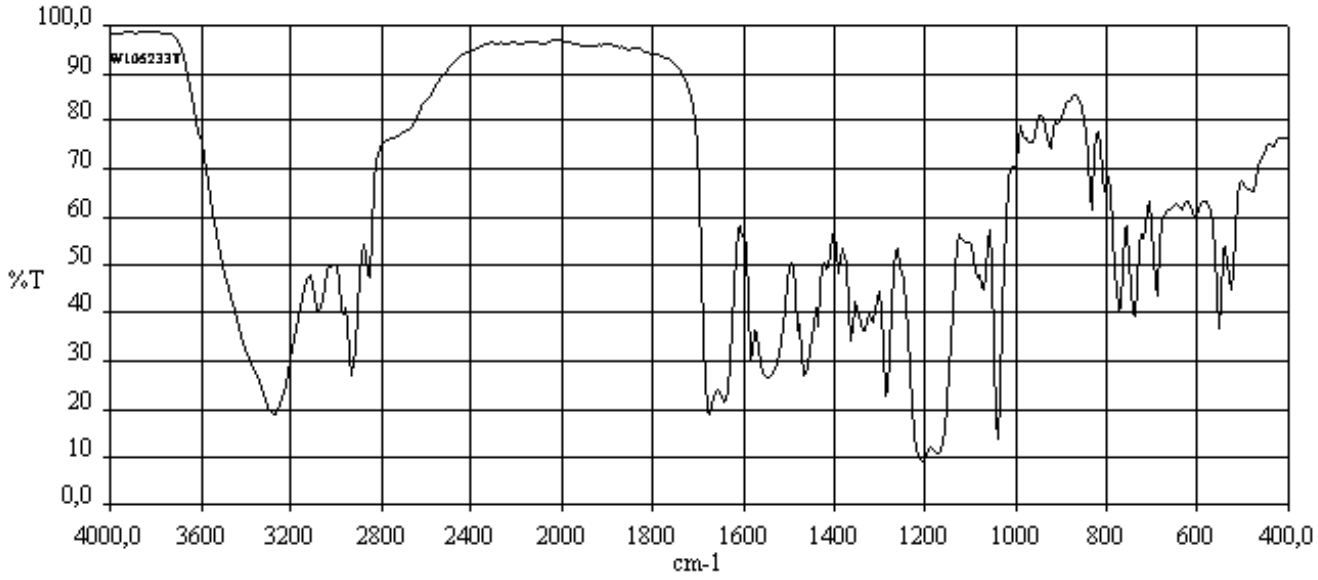
Phase: Potassium bromide disc



Nelfinavir mesilate (RS0080)

Instrument: Fourier Transform

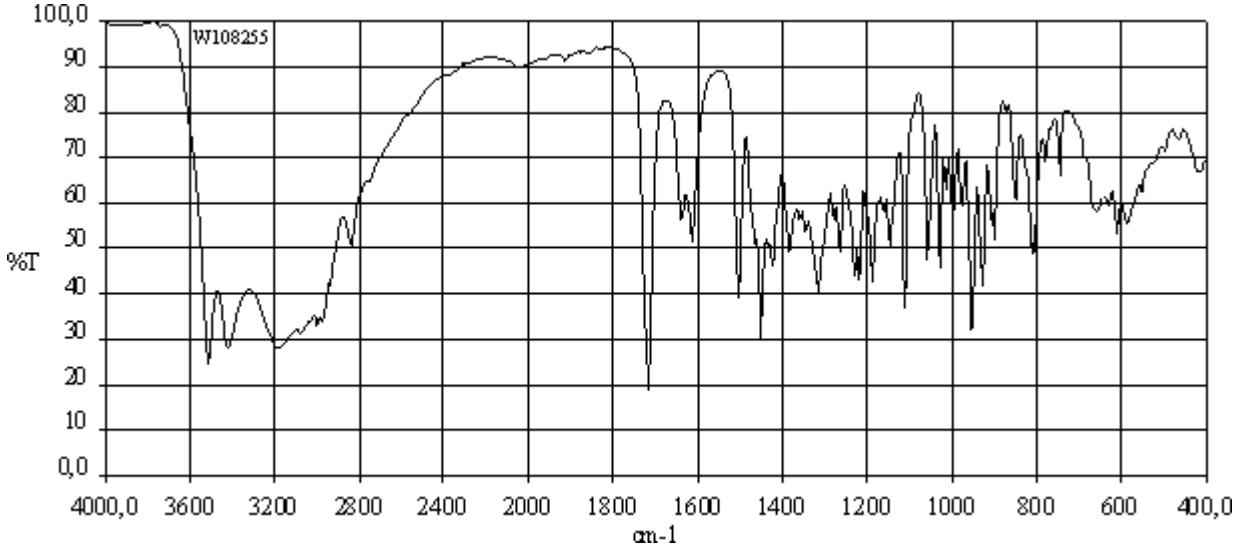
Phase: Potassium bromide disc



Naloxone hydrochloride dihydrate (RS00146)

Instrument: Fourier Transform

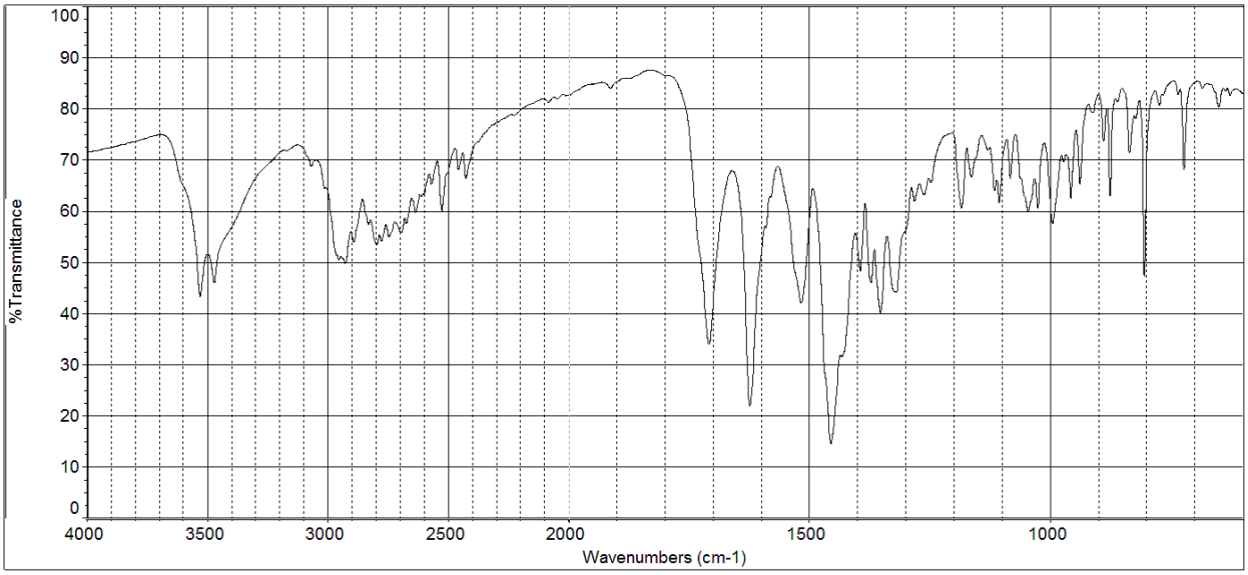
Phase: Potassium bromide disc



Moxifloxacin hydrochloride (RS00172)

Instrument: Fourier Transform

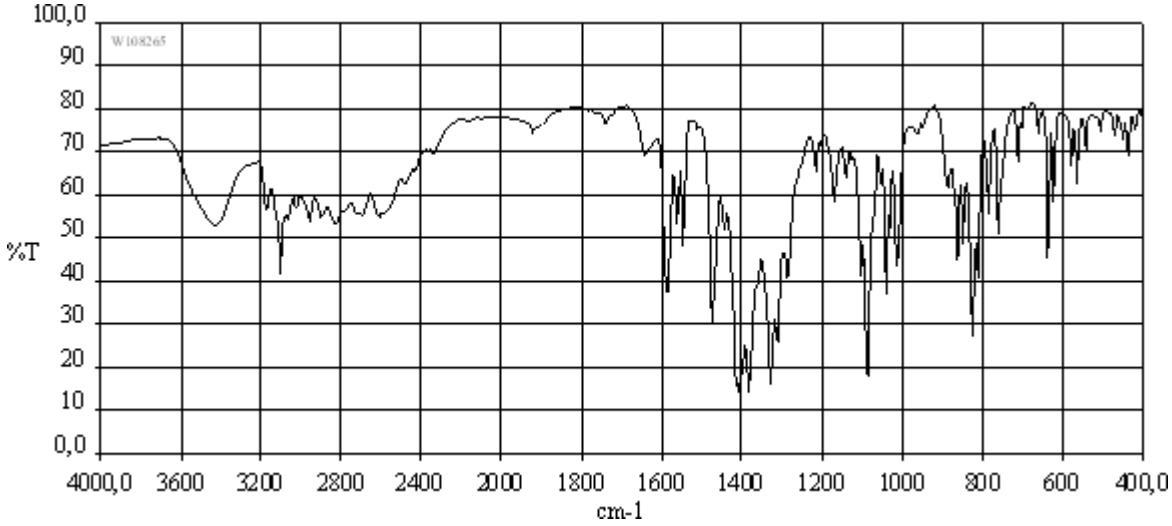
Phase: Potassium bromide disc



Miconazole nitrate (RS00145)

Instrument: Fourier Transform

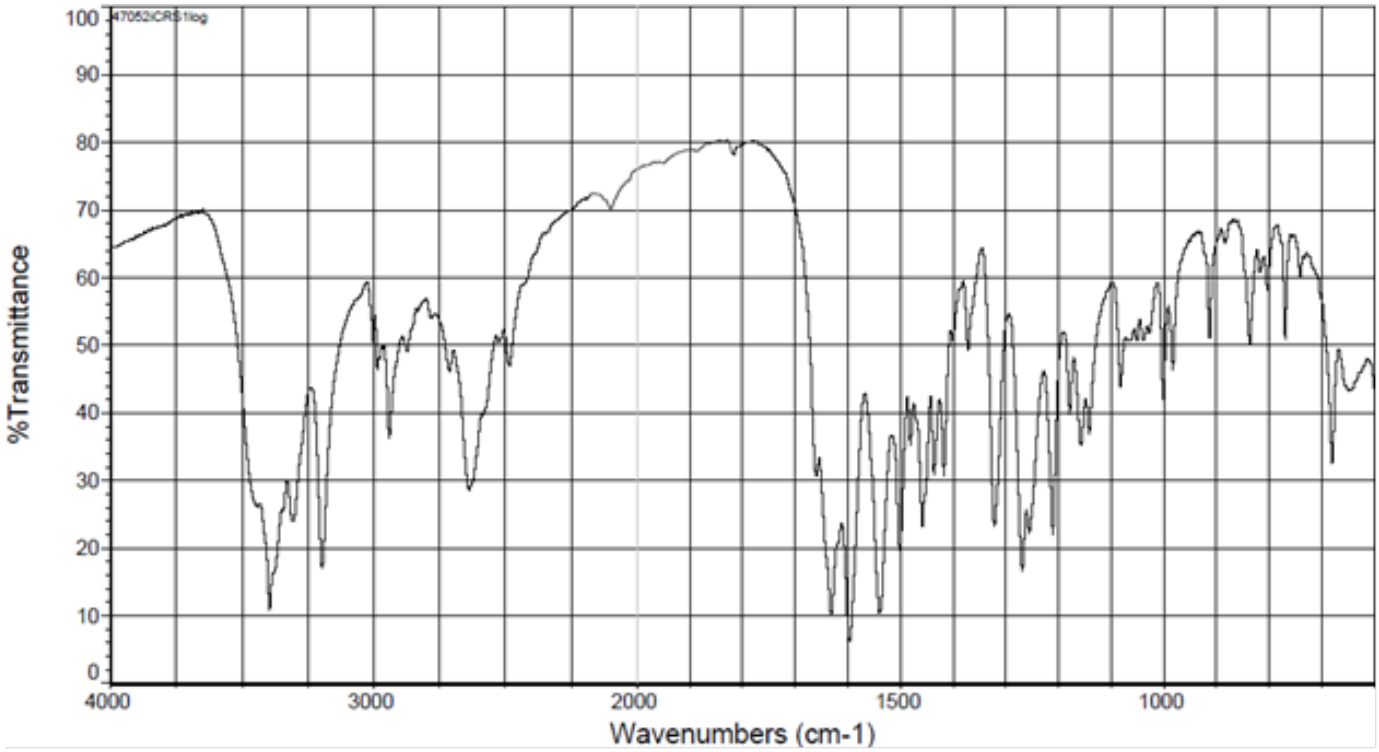
Phase: Potassium bromide disc



Metoclopramide hydrochloride (RS00166)

Instrument: Fourier Transform

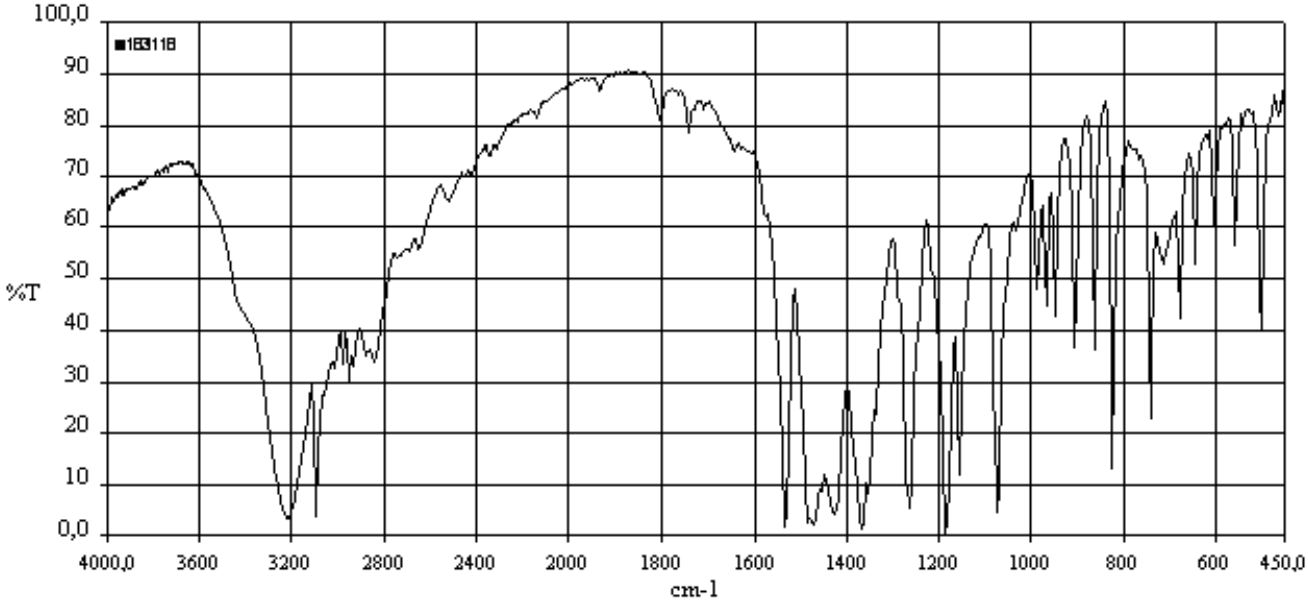
Phase: Potassium bromide disc



Metronidazole (RS0079)

Instrument: Fourier Transform

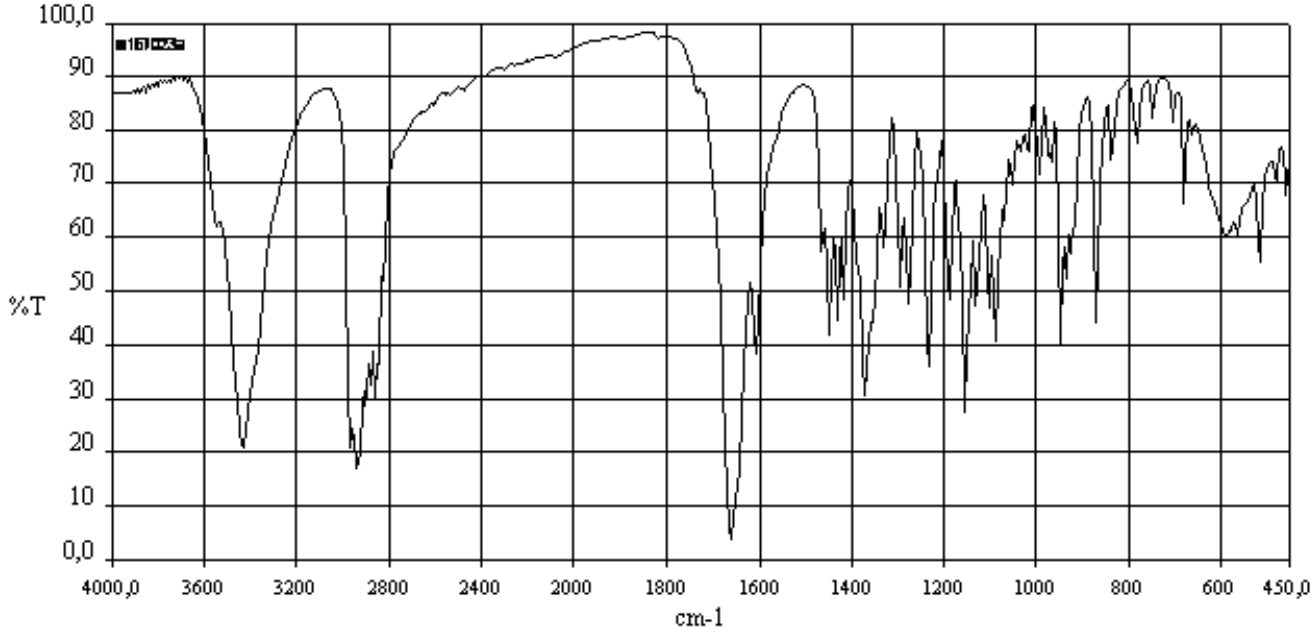
Phase: Potassium bromide disc



Methyltestosterone (RS0078)

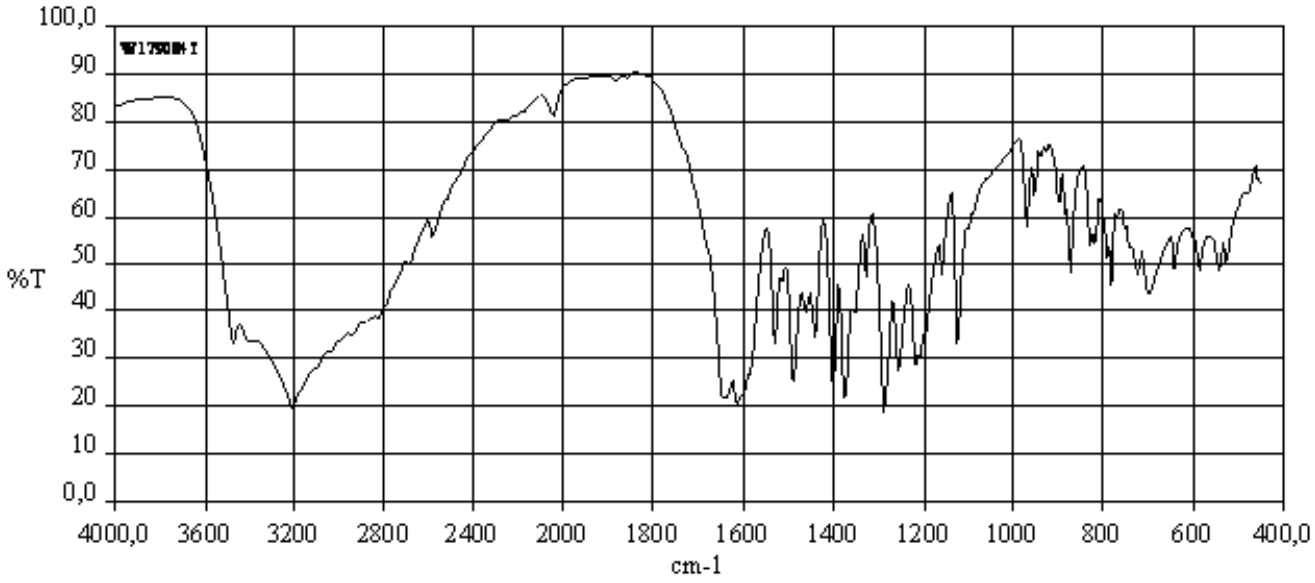
Instrument: Fourier Transform

Phase: Potassium bromide disc



Methyldopa (RS0077)

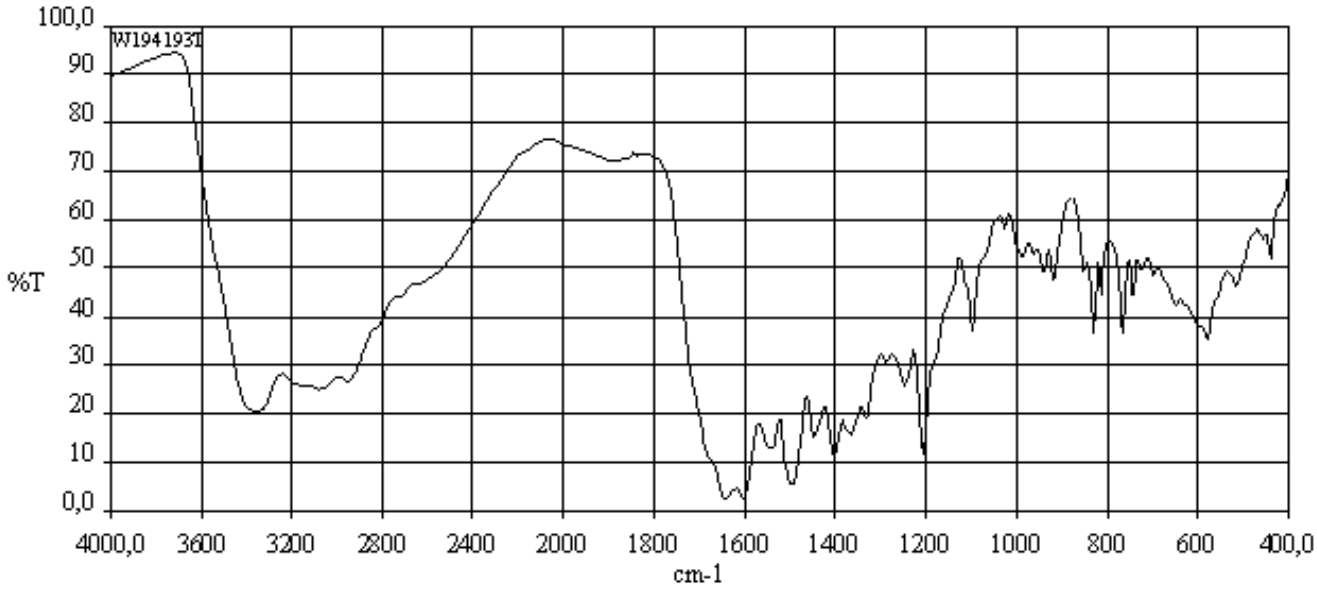
Instrument: Fourier Transform
Phase: Potassium bromide disc



Methotrexate (RS0076)

Instrument: Fourier Transform

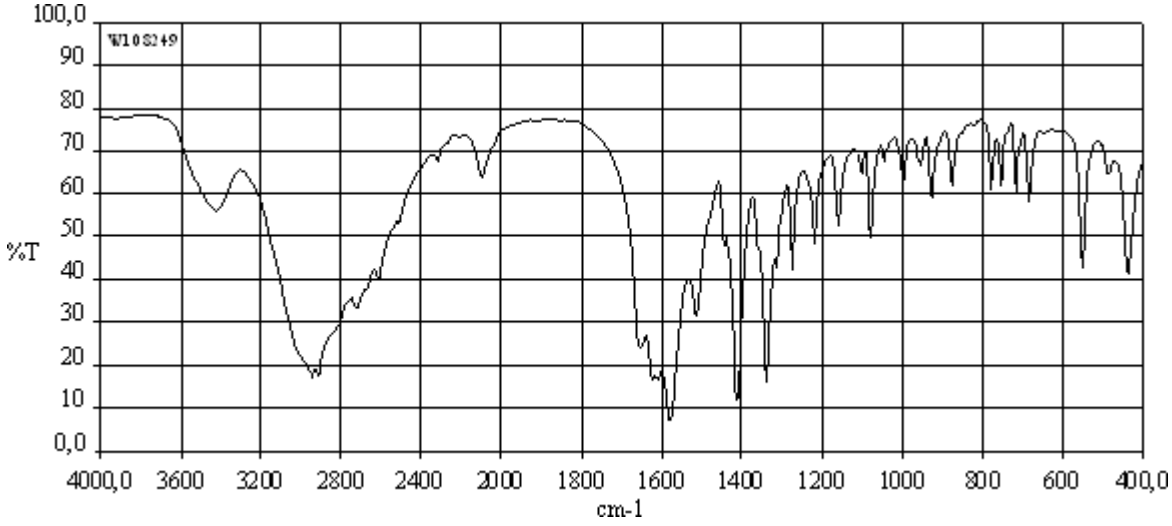
Phase: Potassium bromide disc



DL-Methionine (RS00144)

Instrument: Fourier Transform

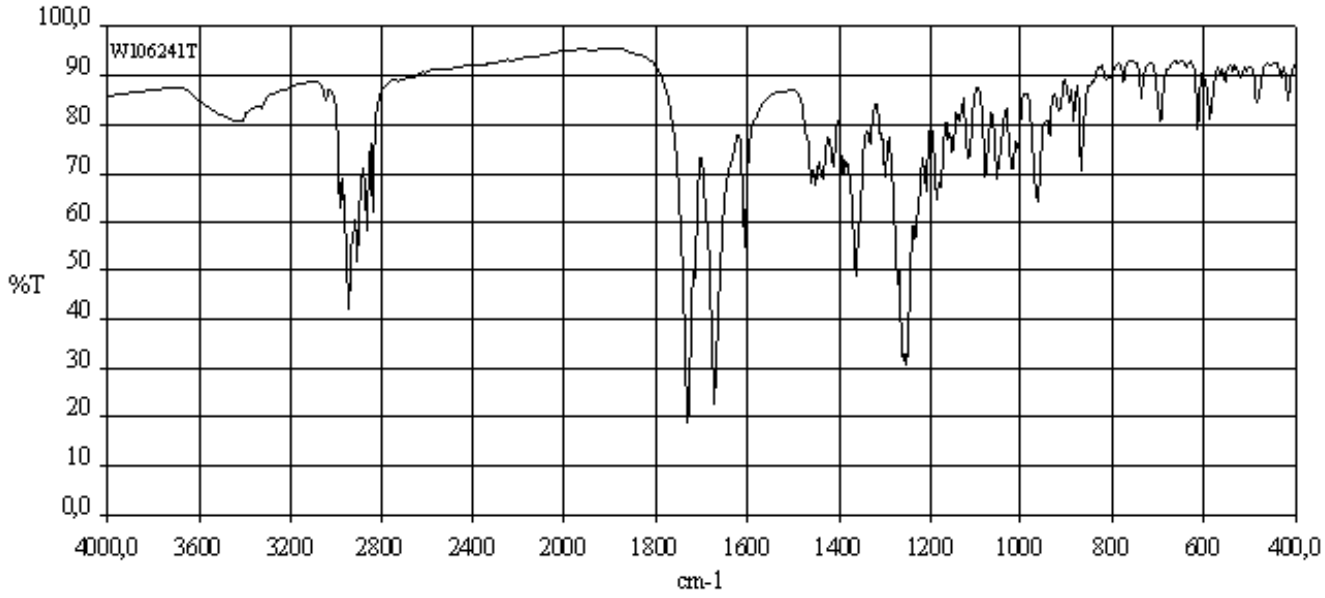
Phase: Potassium bromide disc



Medroxyprogesterone acetate (RS0075)

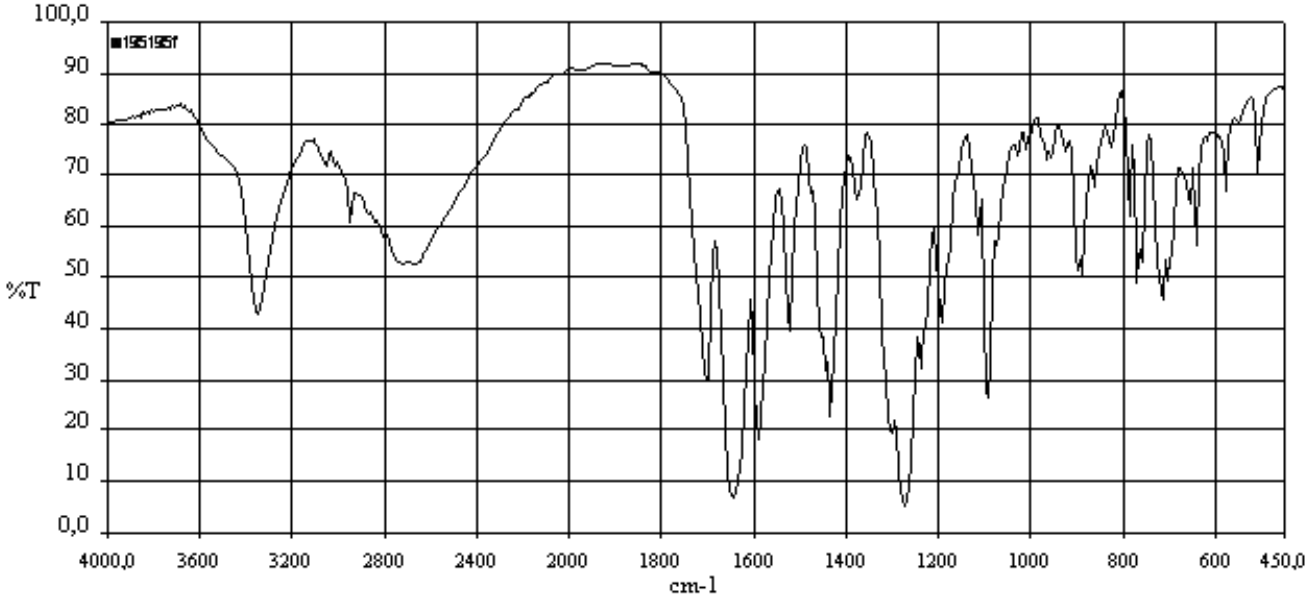
Instrument: Fourier Transform

Phase: Potassium bromide disc



Mebendazole (RS0074)

Instrument: Fourier Transform
Phase: Potassium bromide disc

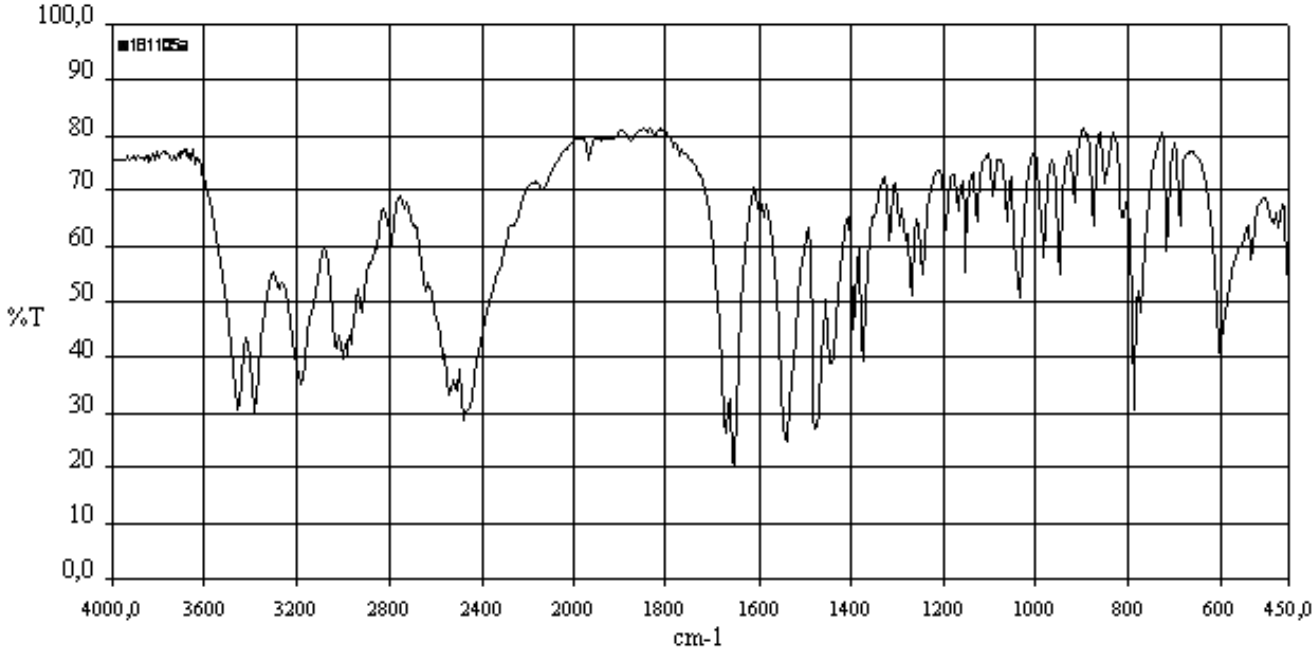


W195195f. Re-crystallized mebendazole (tetrahydrofuran).

Lidocaine hydrochloride (RS0072)

Instrument: Fourier Transform

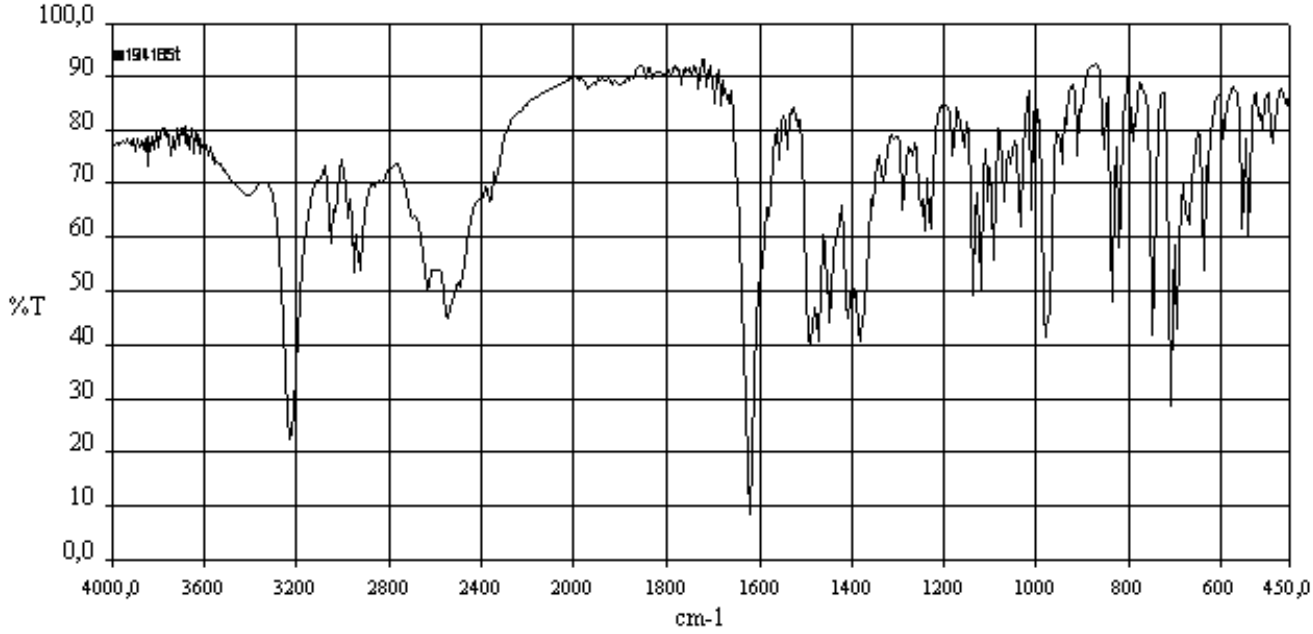
Phase: Potassium bromide disc



Loperamide hydrochloride (RS0073)

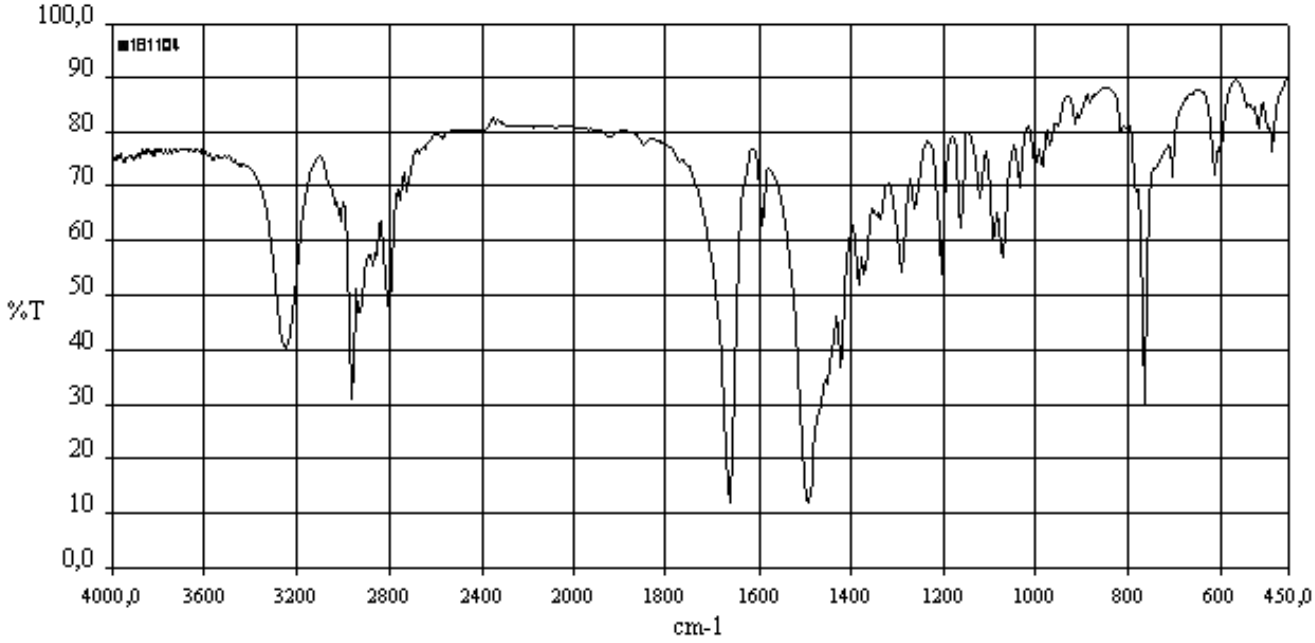
Instrument: Fourier Transform

Phase: Potassium bromide disc



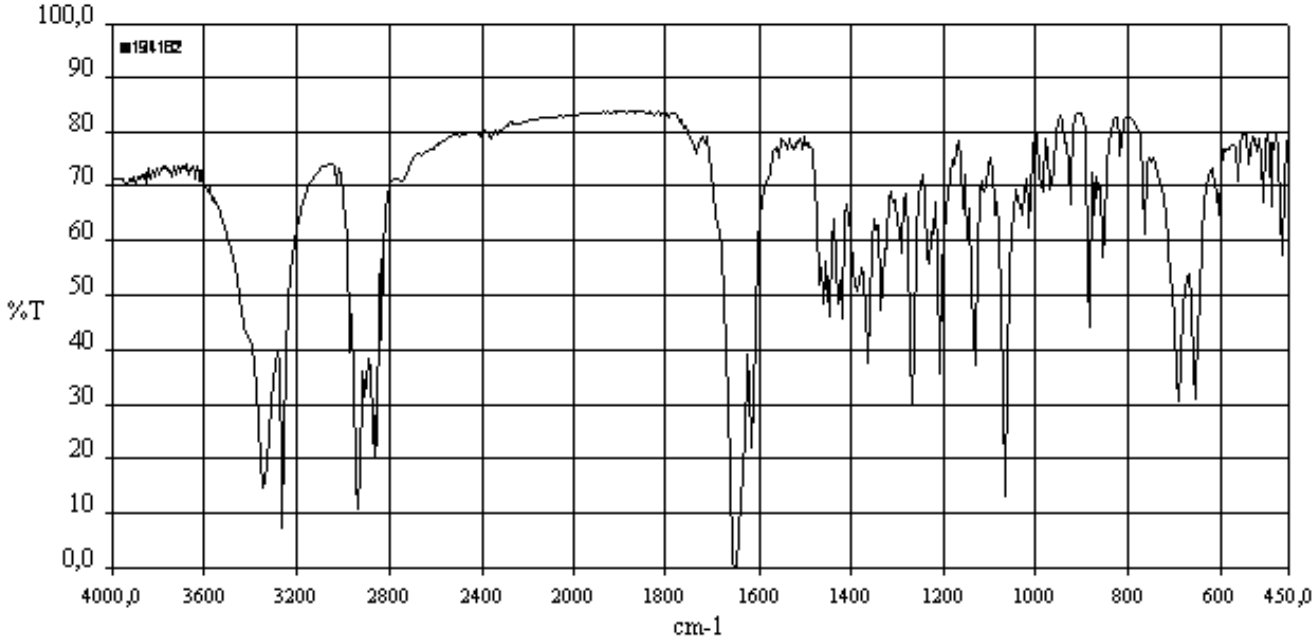
Lidocaine (RS0071)

Instrument: Fourier Transform
Phase: Potassium bromide disc



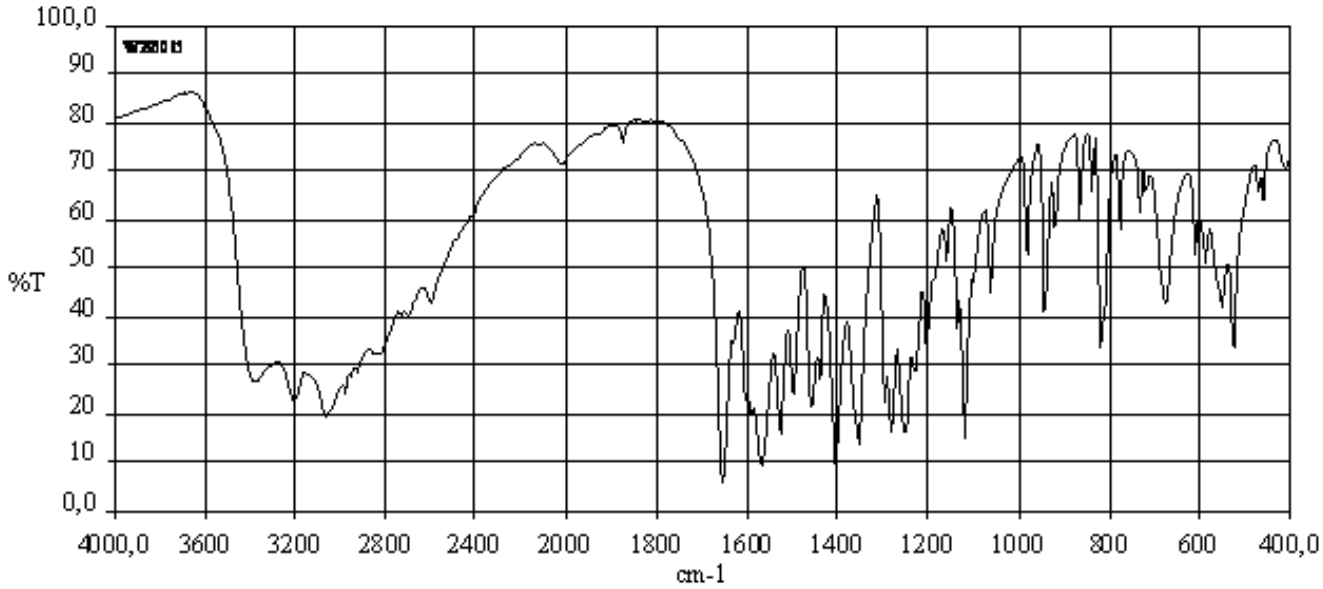
Levonorgestrel (RS0070)

Instrument: Fourier Transform
Phase: Potassium bromide disc



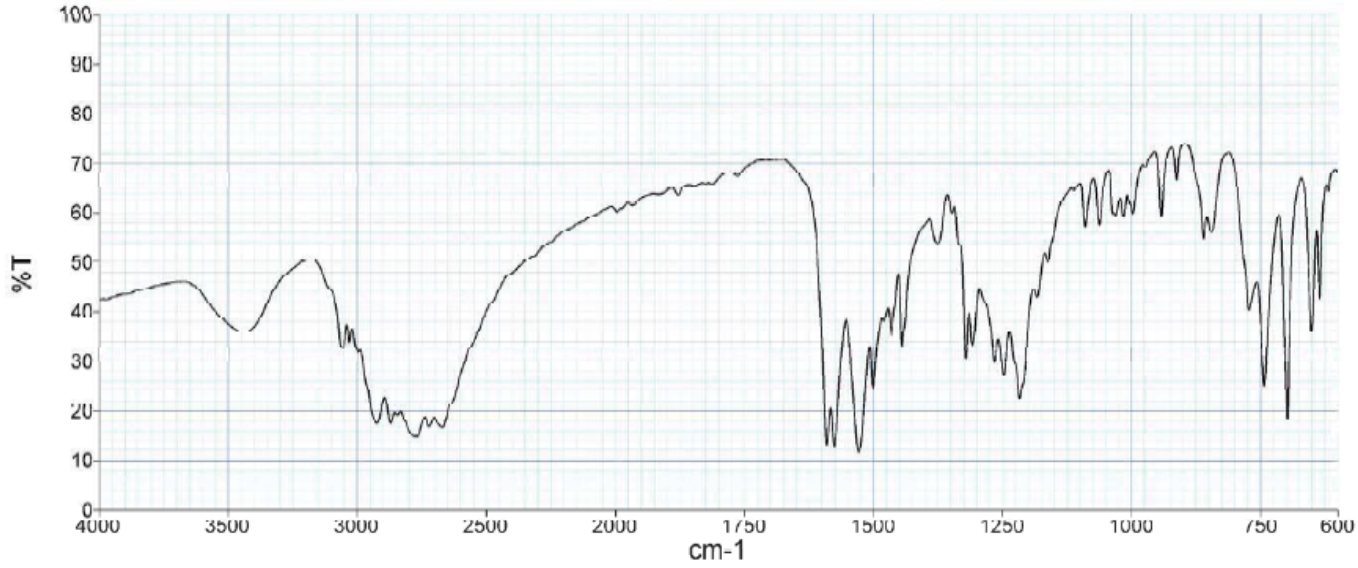
Levodopa (RS0069)

Instrument: Fourier Transform
Phase: Potassium bromide disc



Levamisole hydrochloride

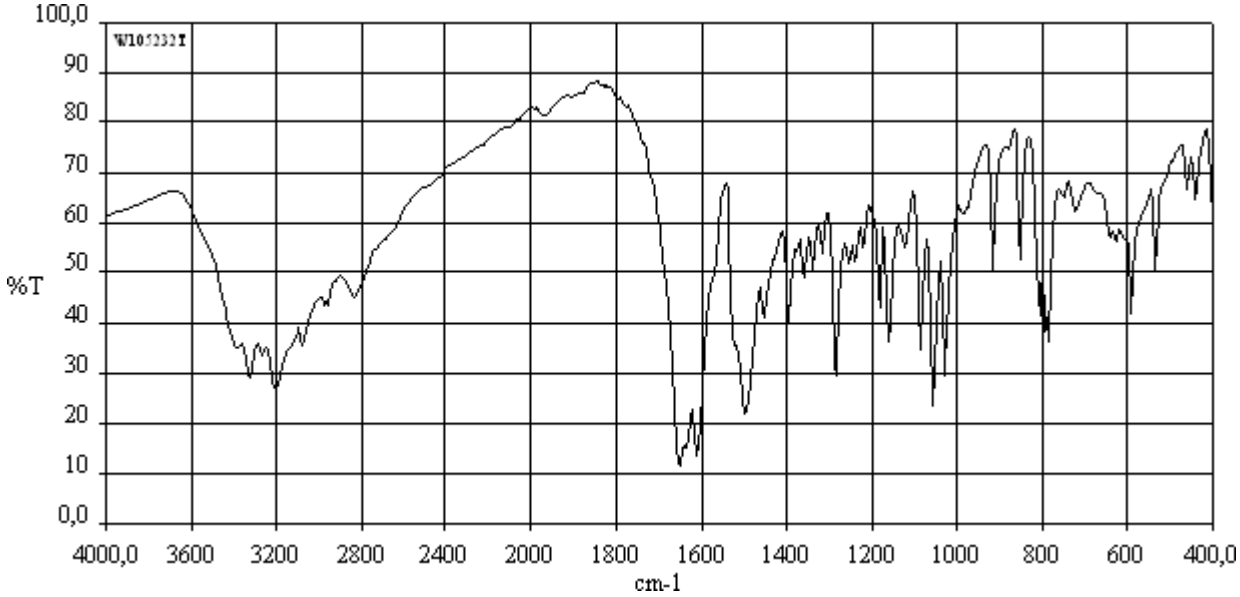
Instrument: Fourier Transform
Phase: Potassium bromide disc



Lamivudine (RS00143)

Instrument: Fourier Transform

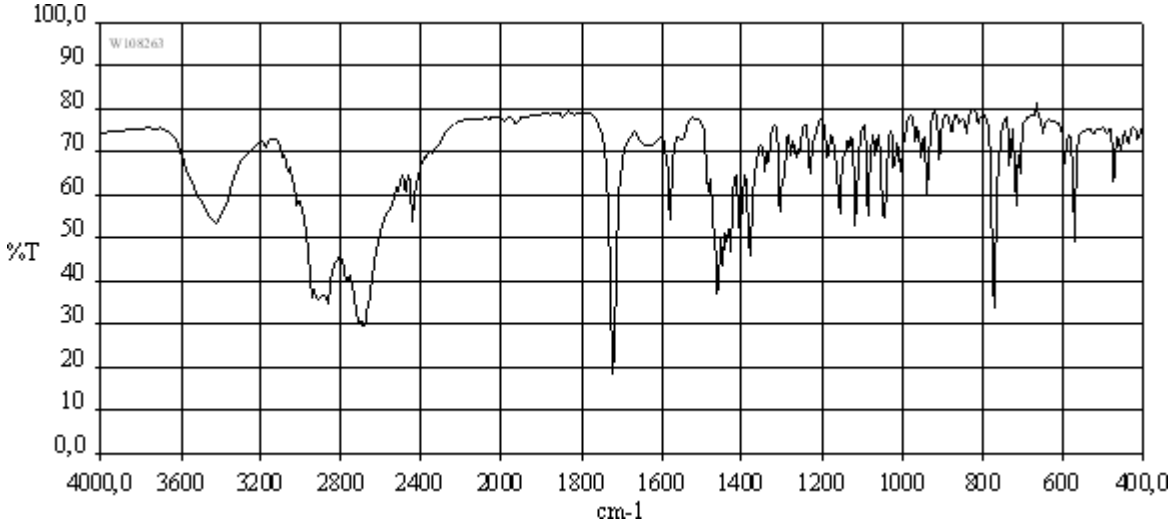
Phase: Potassium bromide disc



Ketamine hydrochloride (RS00141)

Instrument: Fourier Transform

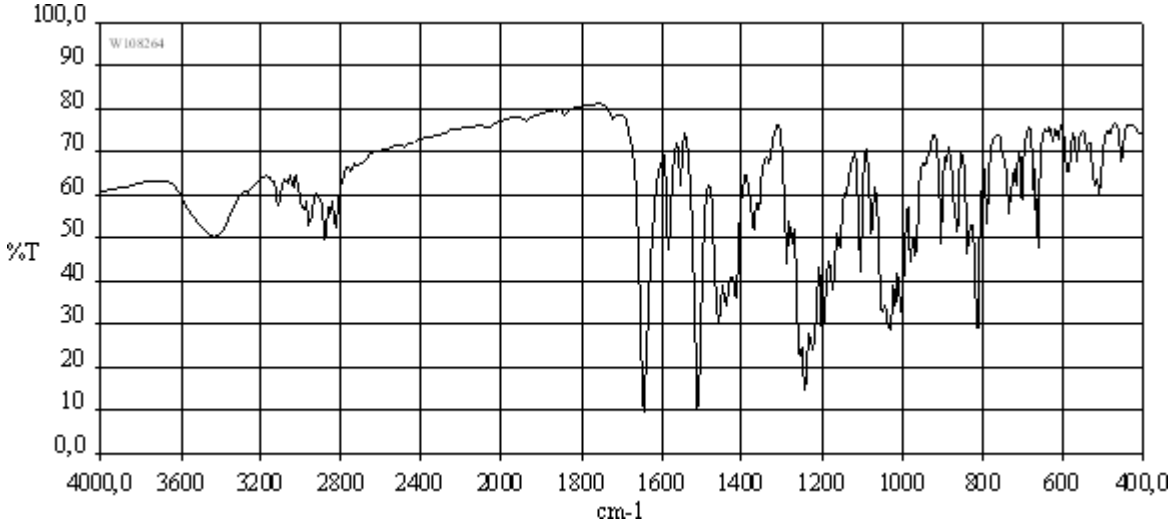
Phase: Potassium bromide disc



Ketoconazole (RS00142)

Instrument: Fourier Transform

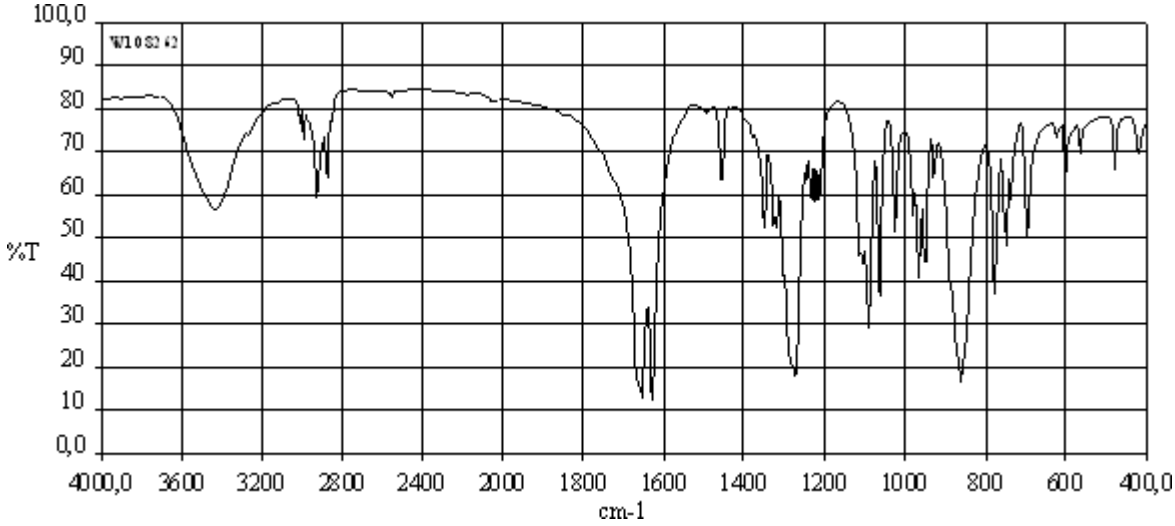
Phase: Potassium bromide disc



Isosorbide dinitrate (RS00140)

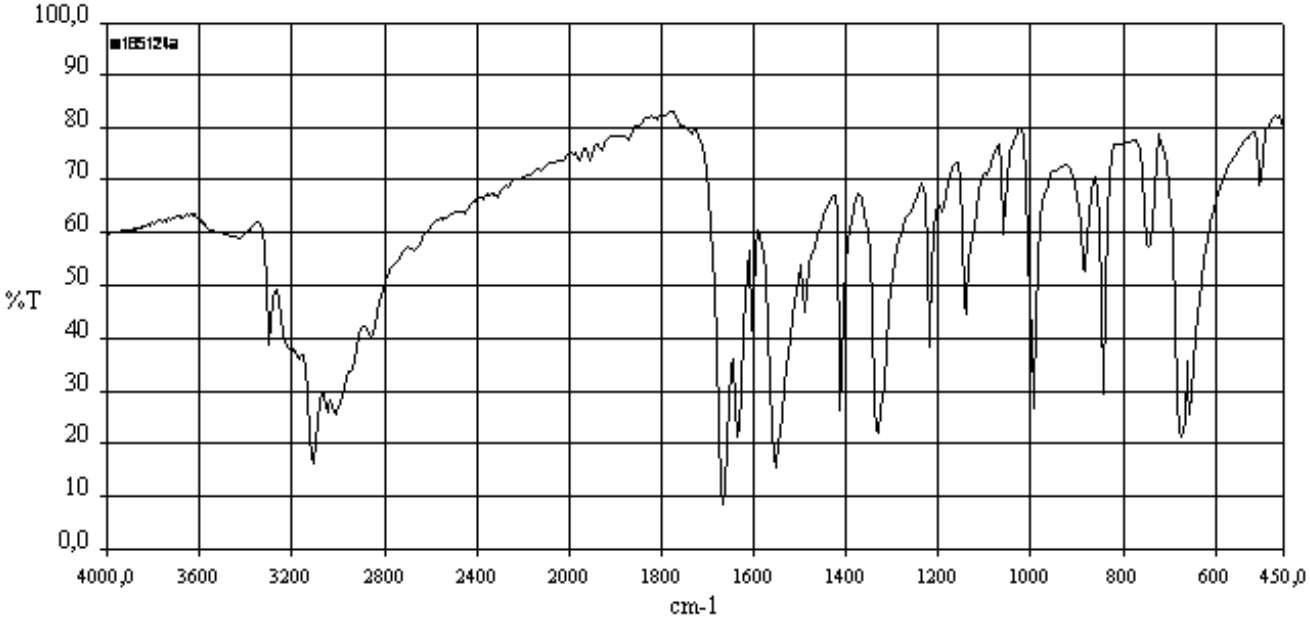
Instrument: Fourier Transform

Phase: Potassium bromide disc



Isoniazid (RS0068)

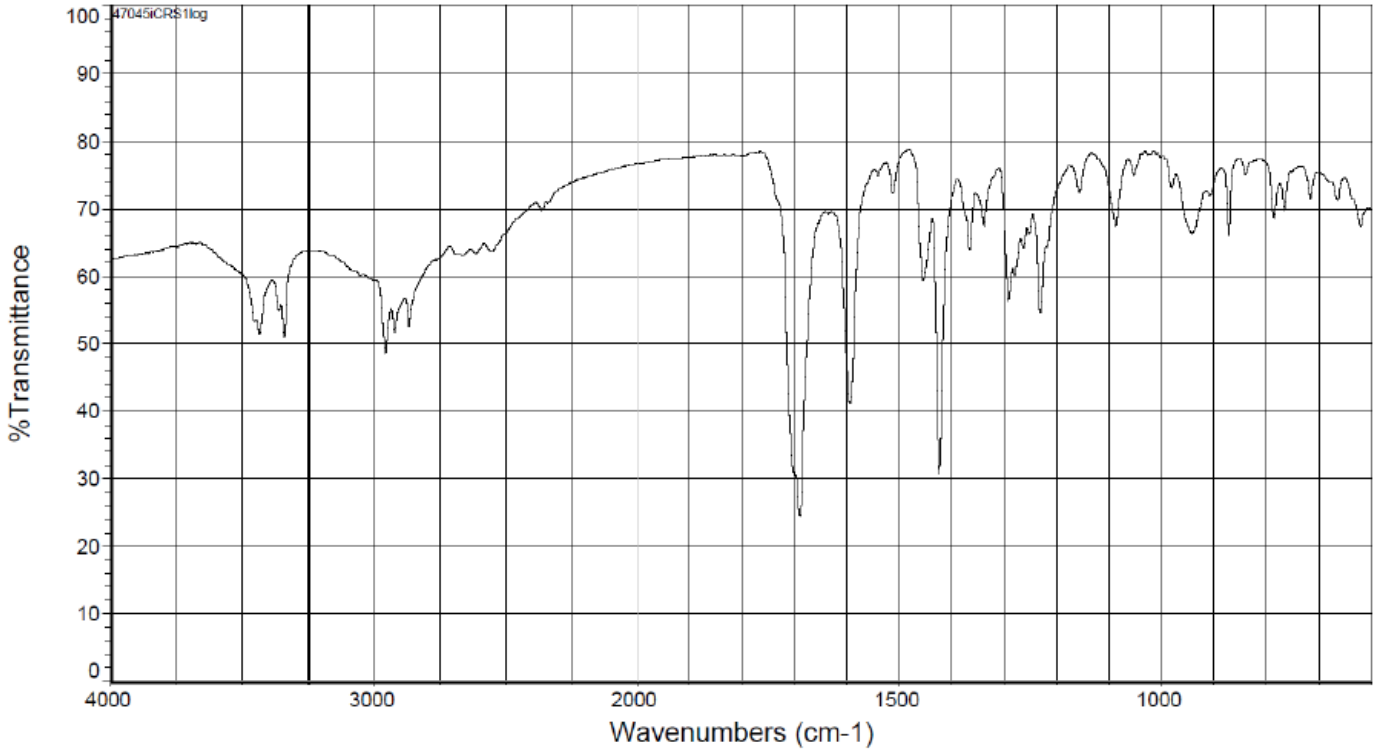
Instrument: Fourier Transform
Phase: Potassium bromide disc



Iopanoic acid

Instrument: Fourier Transform

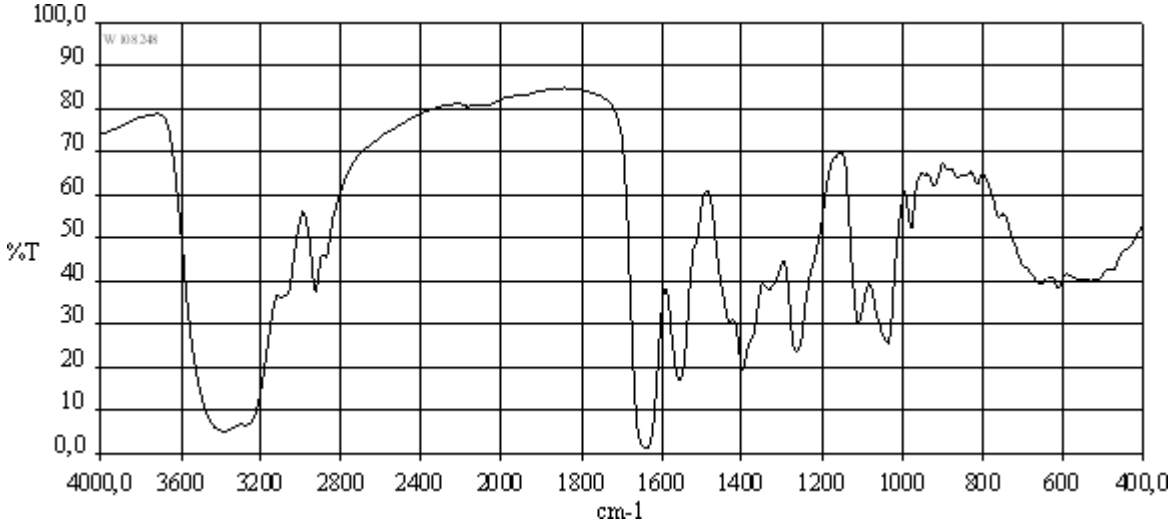
Phase: Potassium bromide disc



Iohexol (RS00139)

Instrument: Fourier Transform

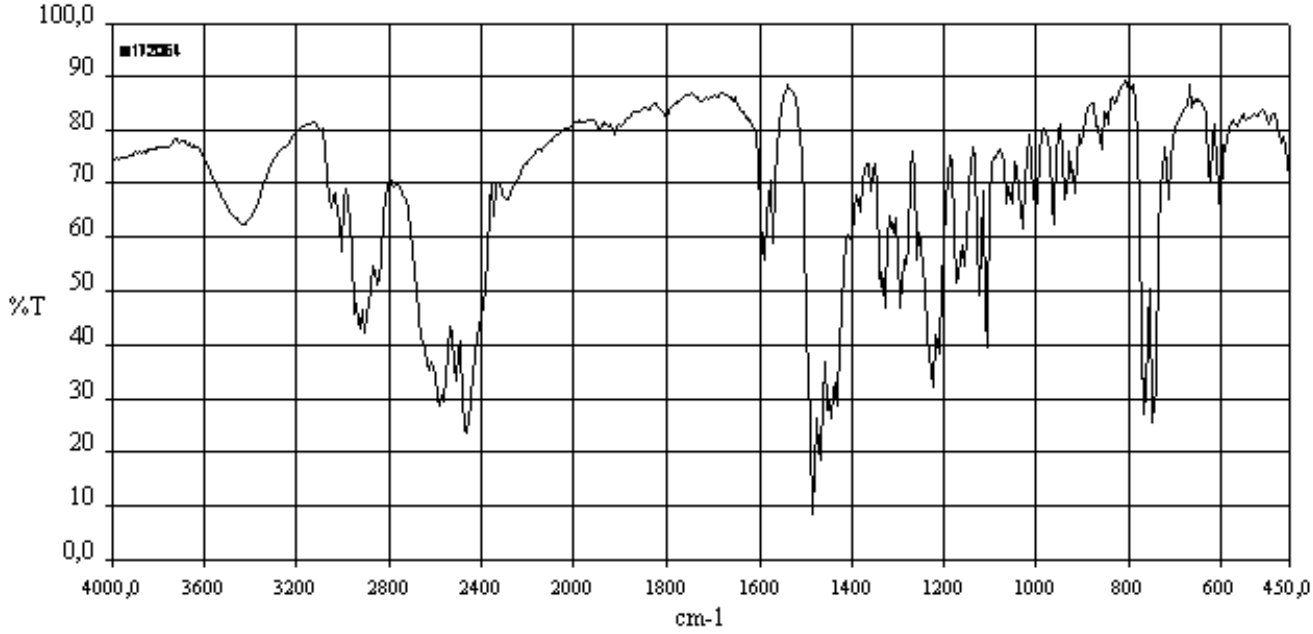
Phase: Potassium bromide disc



Imipramine hydrochloride (RS0065)

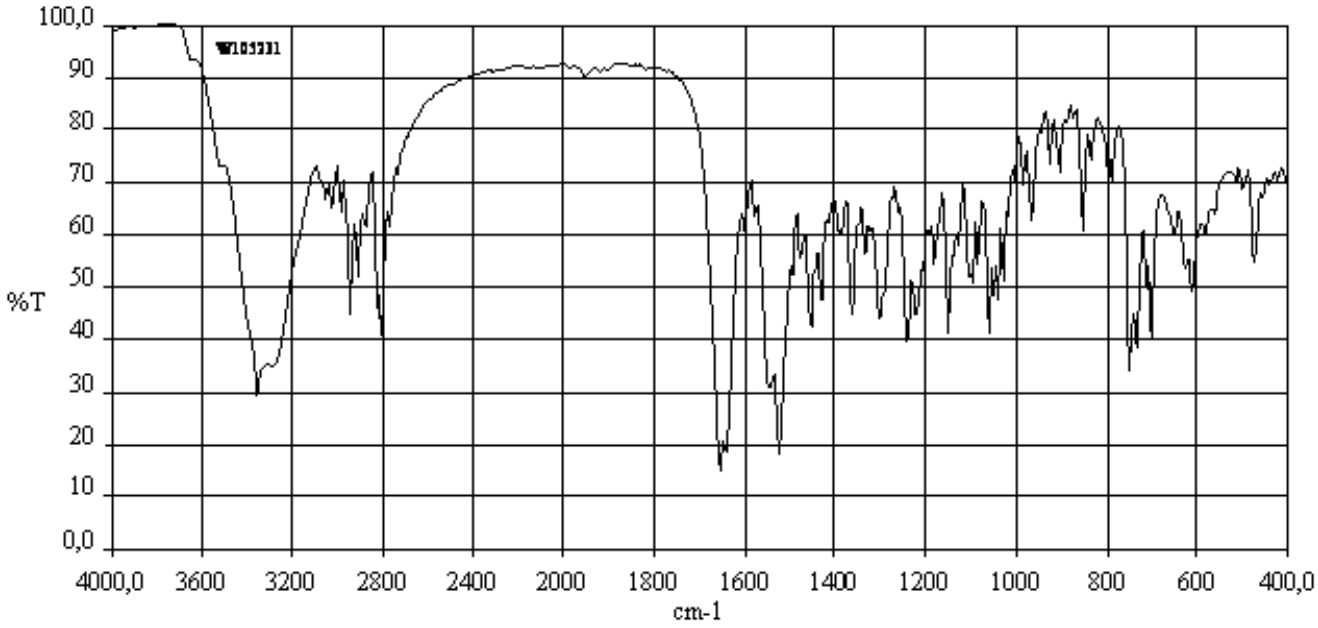
Instrument: Fourier Transform

Phase: Potassium bromide disc



Indinavir (RS0066)

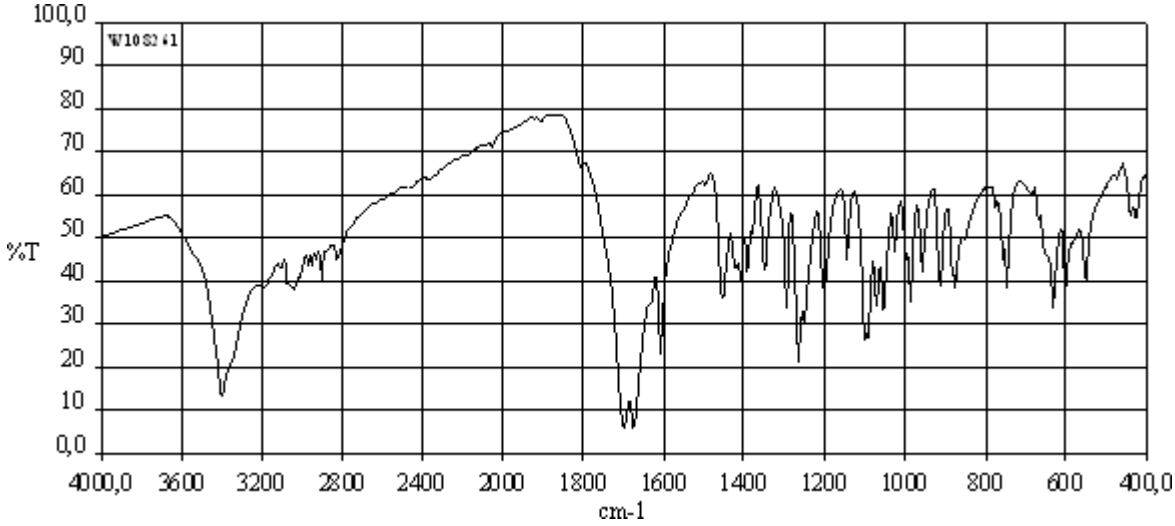
Instrument: Fourier Transform
Phase: Potassium bromide disc



Idoxuridine (RS00138)

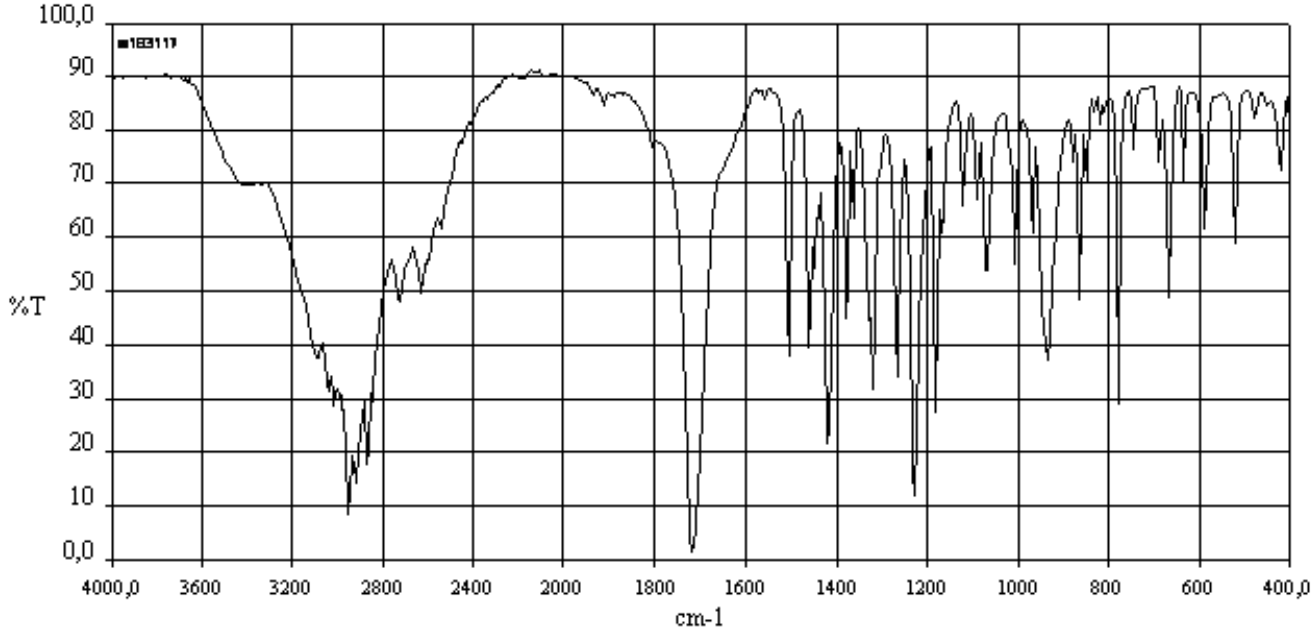
Instrument: Fourier Transform

Phase: Potassium bromide disc



Ibuprofen (RS0064)

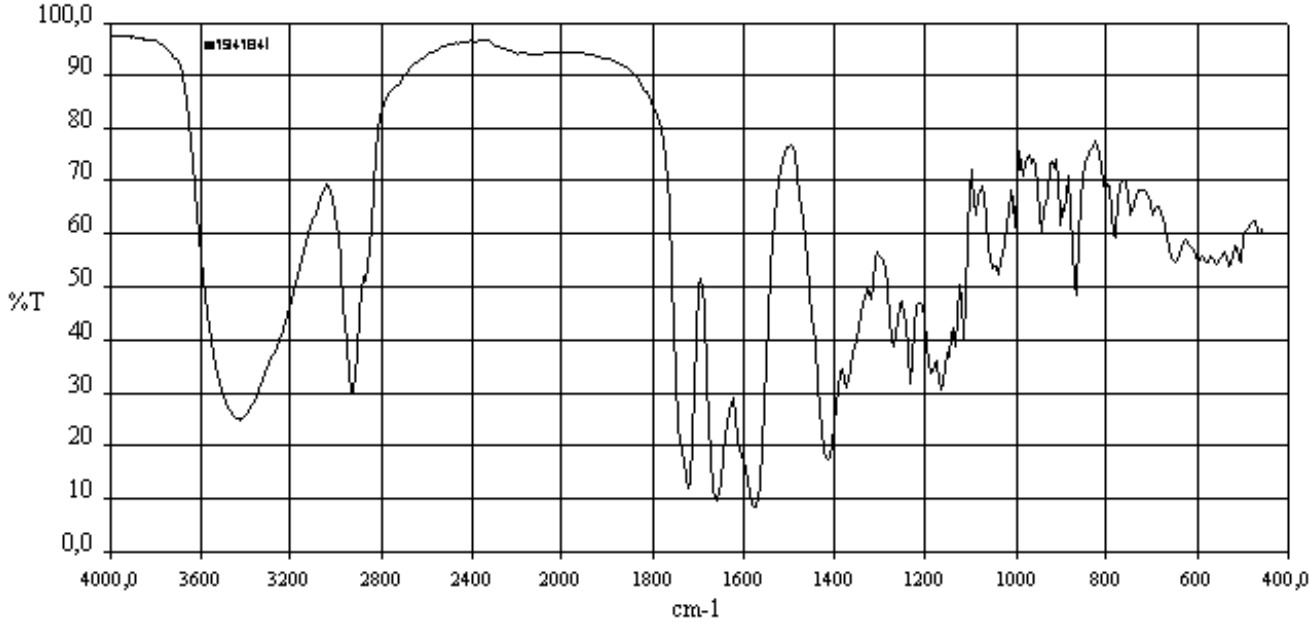
Instrument: Fourier Transform
Phase: Potassium bromide disc



Hydrocortisone sodium succinate (RS0063)

Instrument: Fourier Transform

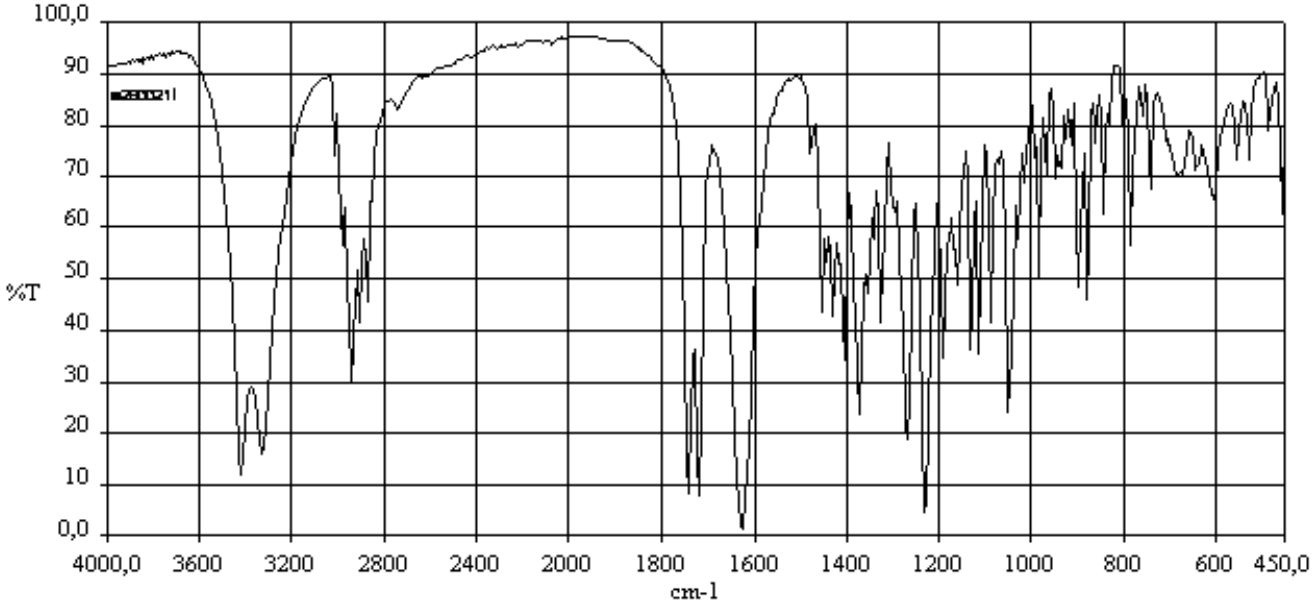
Phase: Potassium bromide disc



Hydrocortisone acetate (RS0062)

Instrument: Fourier Transform

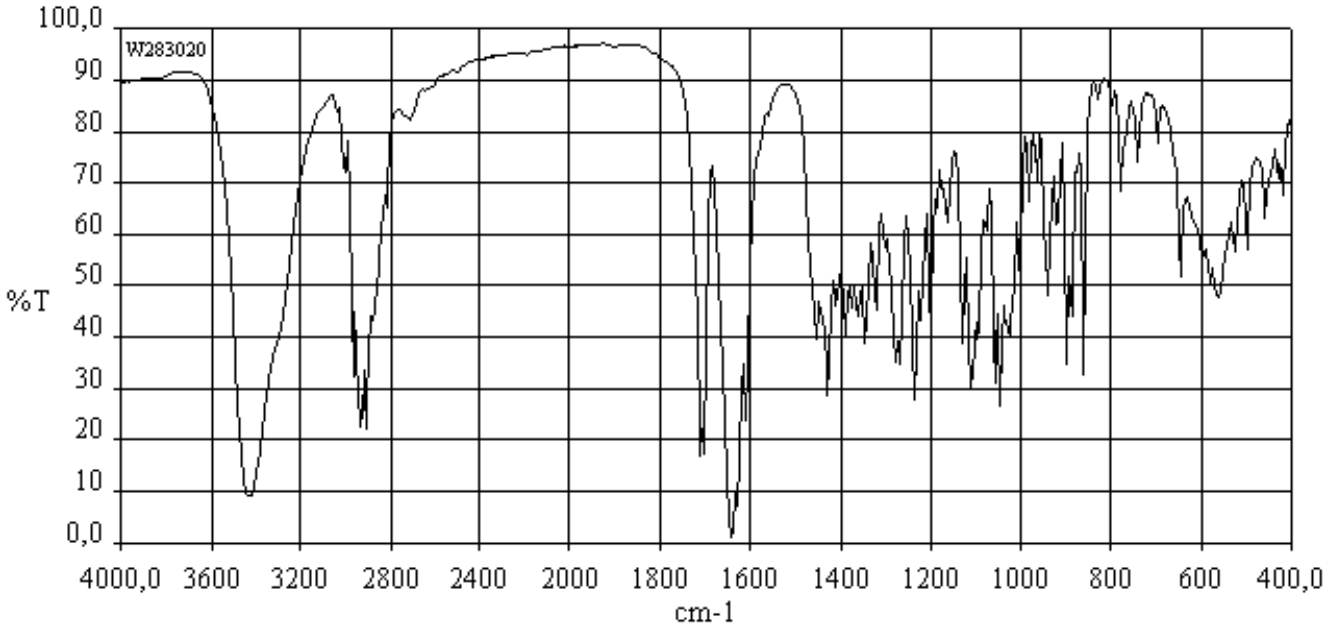
Phase: Potassium bromide disc



Hydrocortisone (RS0061)

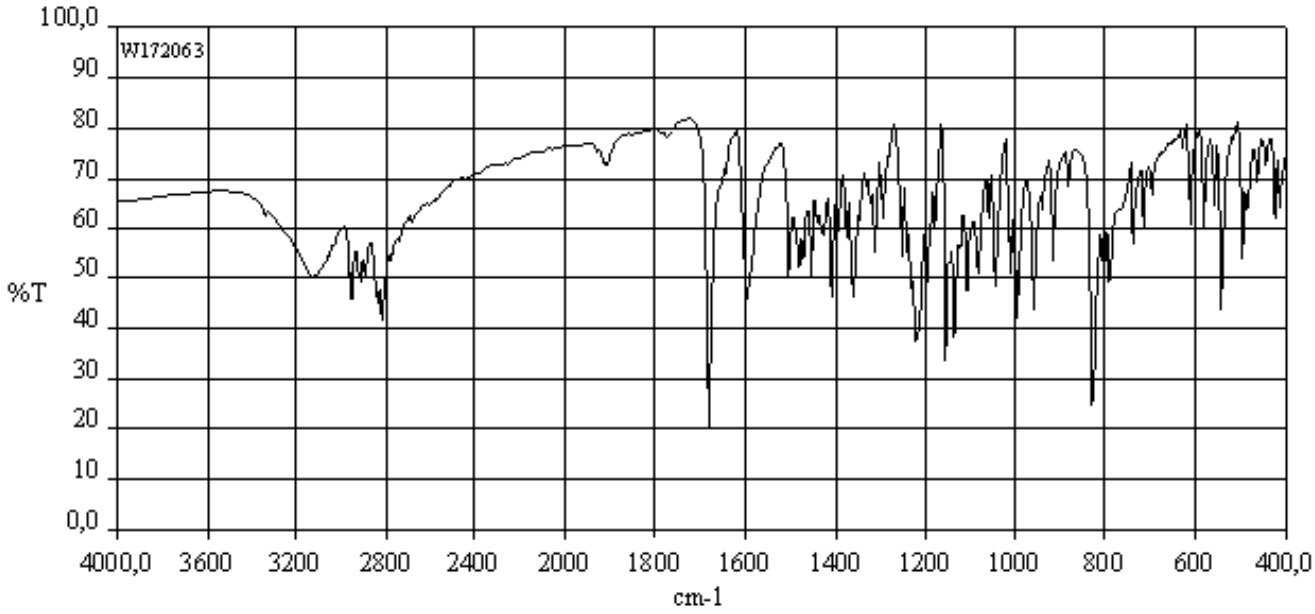
Instrument: Fourier Transform

Phase: Potassium bromide disc



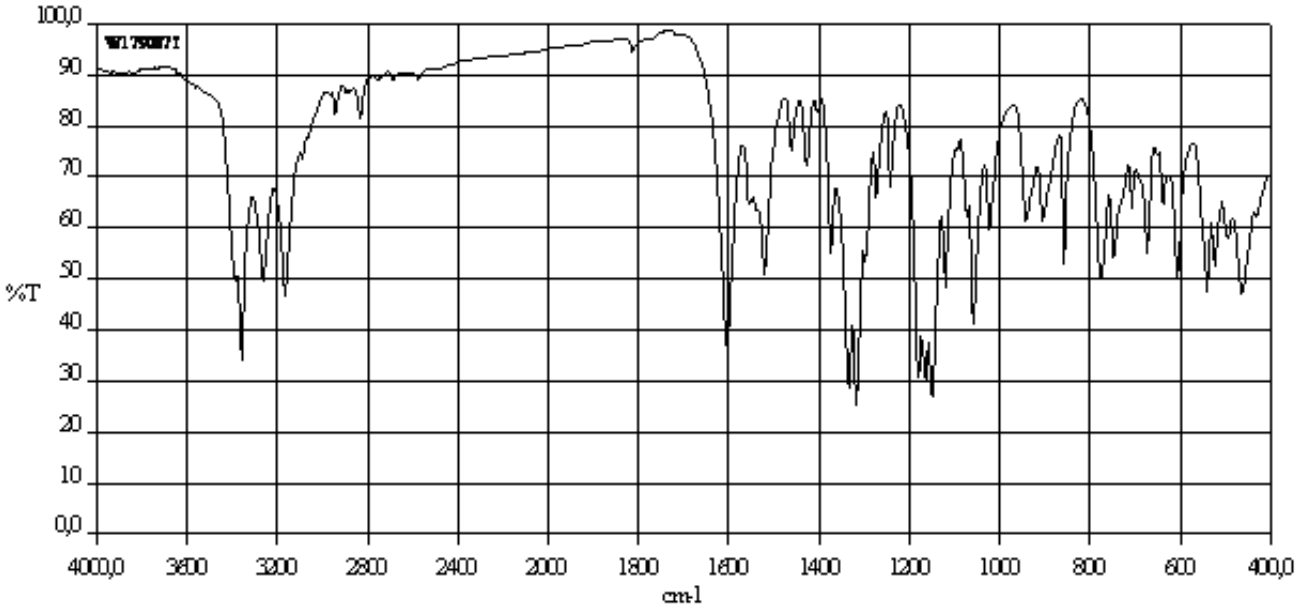
Haloperidol (RS0059)

Instrument: Fourier Transform
Phase: Potassium bromide disc



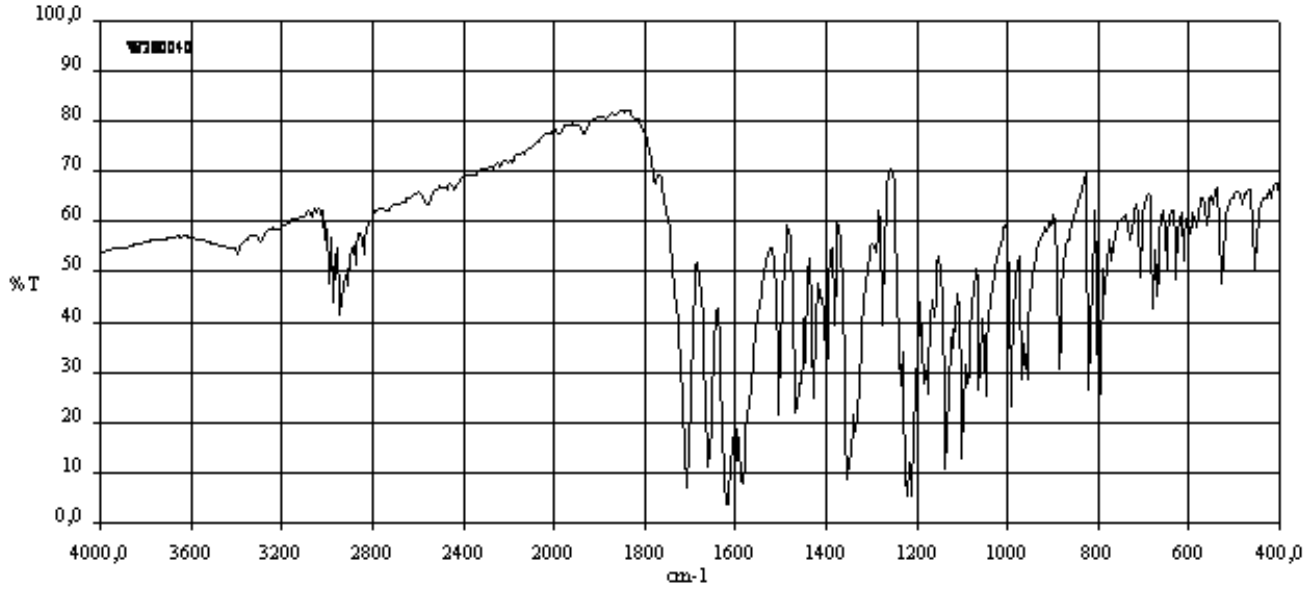
Hydrochlorothiazide (RS0060)

Instrument: Fourier Transform
Phase: Potassium bromide disc



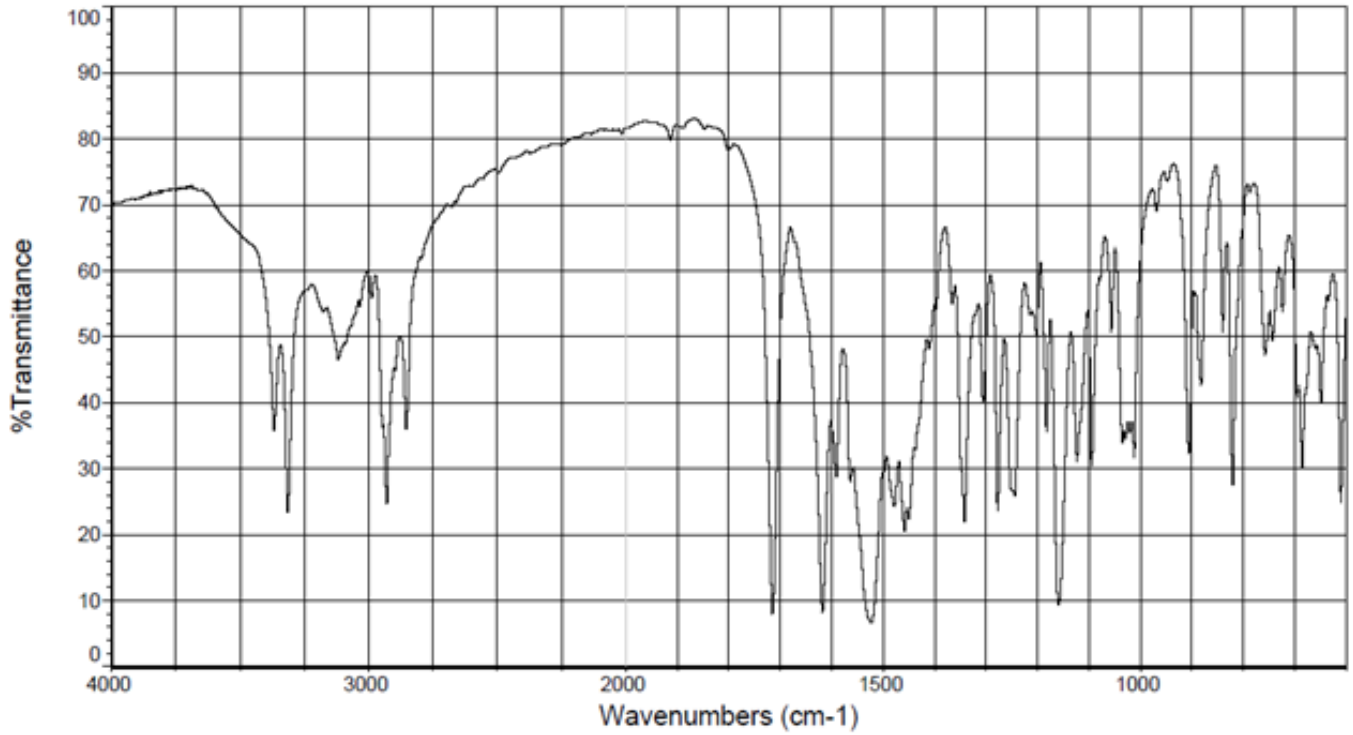
Griseofulvin (RS0058)

Instrument: Fourier Transform
Phase: Potassium bromide disc



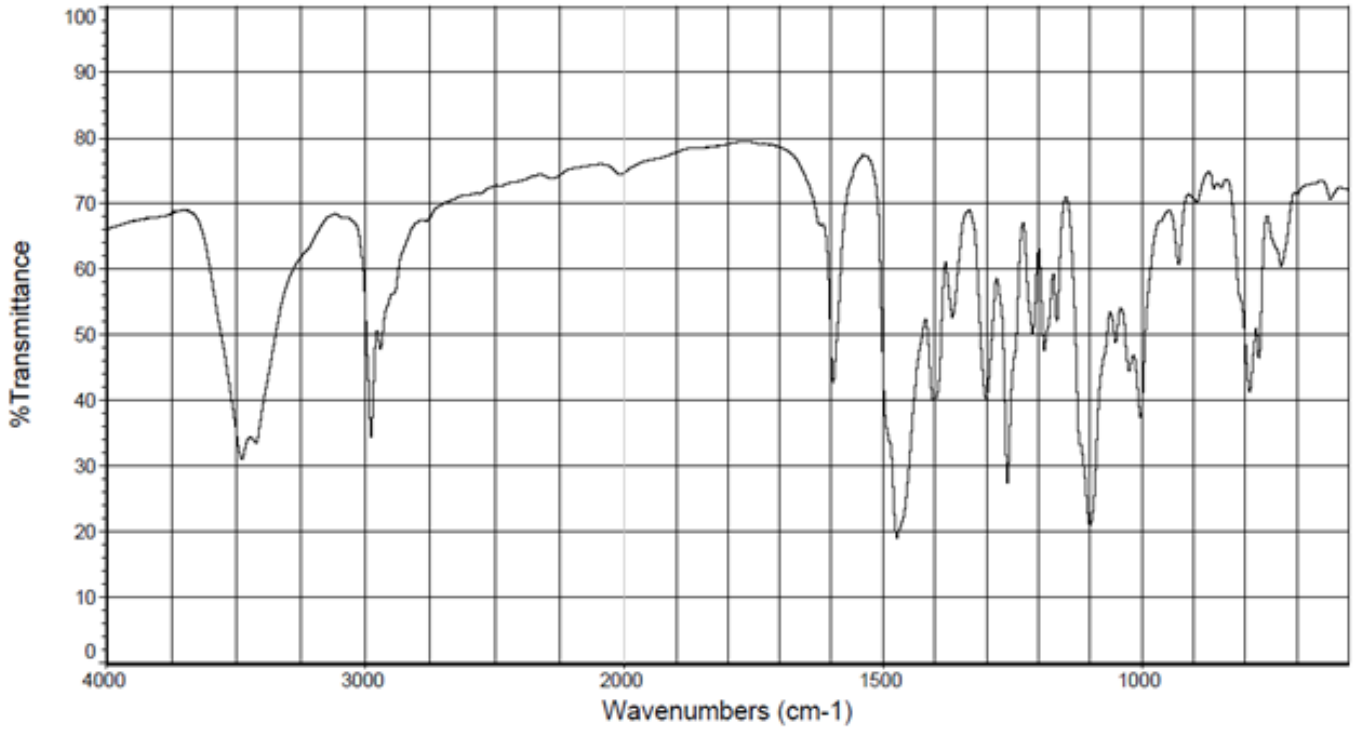
Glibenclamide (RS00161)

Instrument: Fourier Transform
Phase: Potassium bromide disc



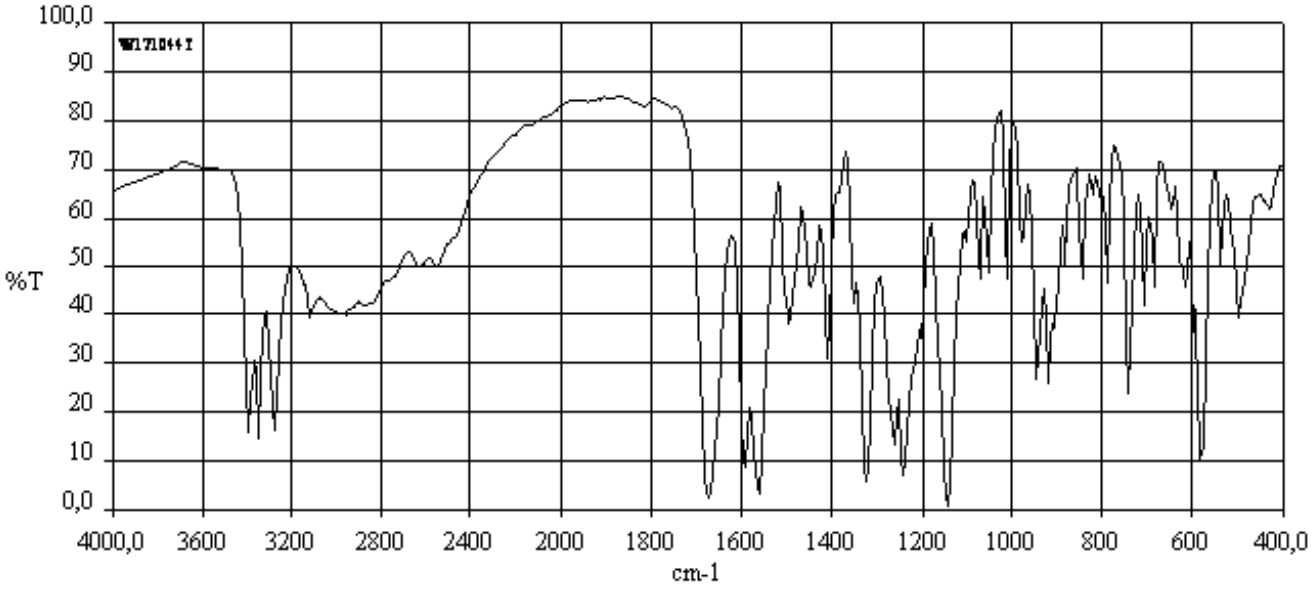
Gallamine triethiodide (RS00160)

Instrument: Fourier Transform
Phase: Potassium bromide disc



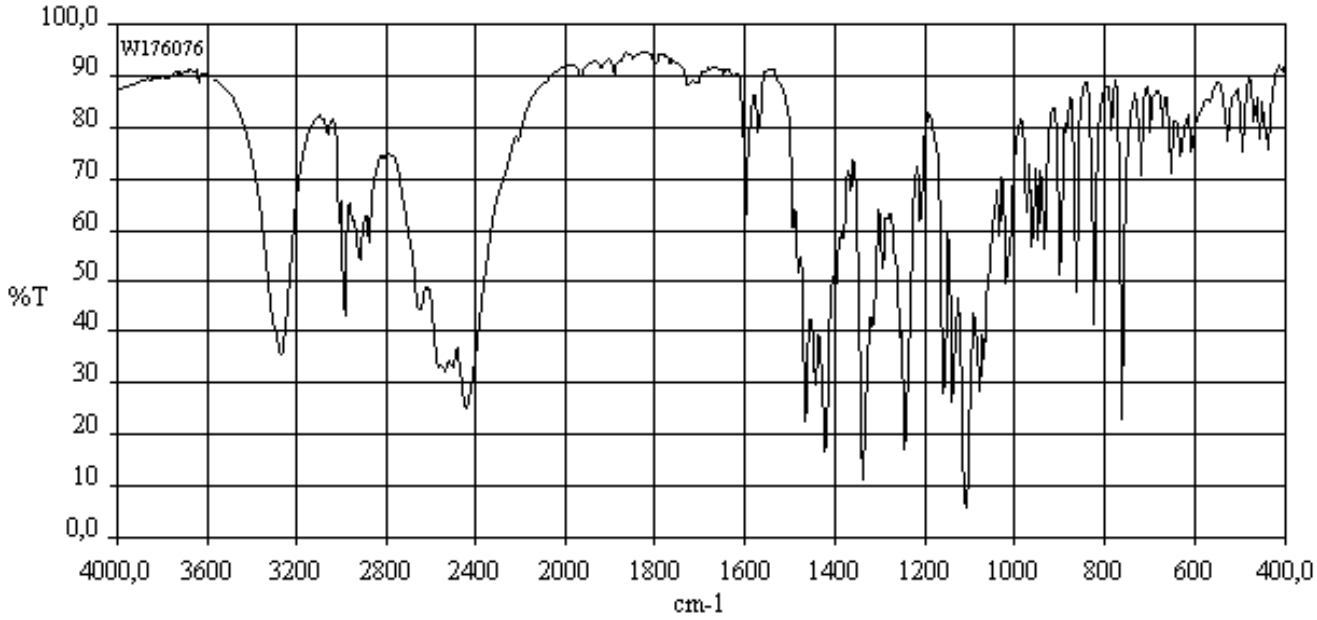
Furosemide (RS0057)

Instrument: Fourier Transform
Phase: Potassium bromide disc



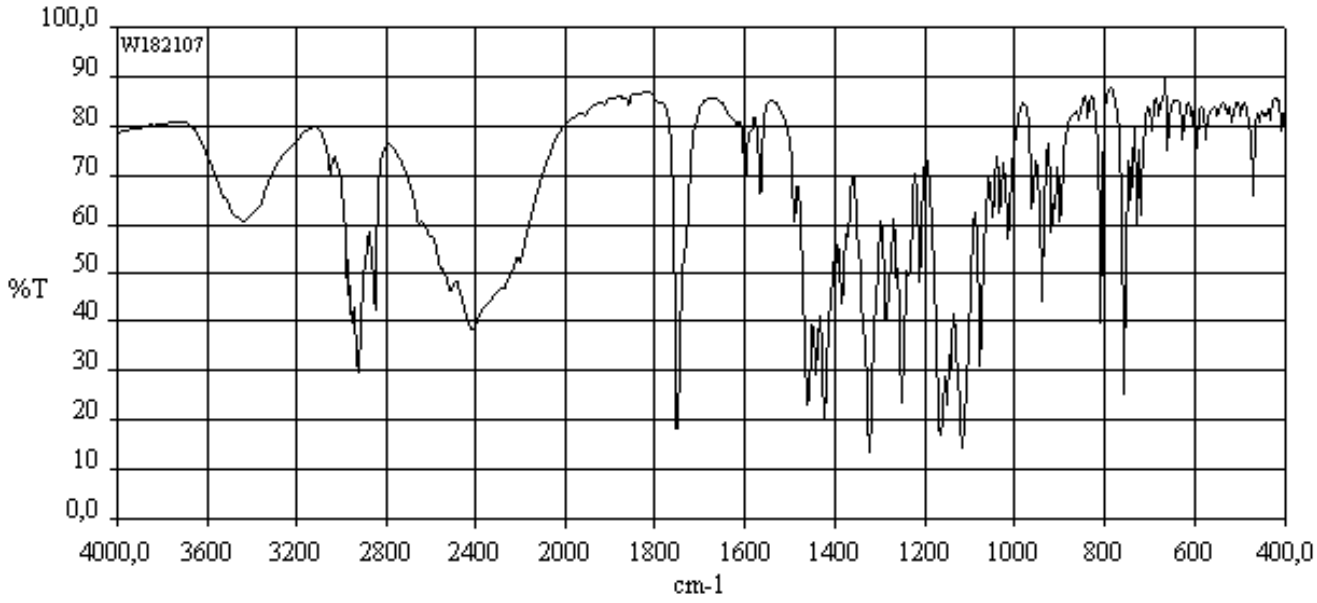
Fluphenazine hydrochloride (RS0056)

Instrument: Fourier Transform
Phase: Potassium bromide disc

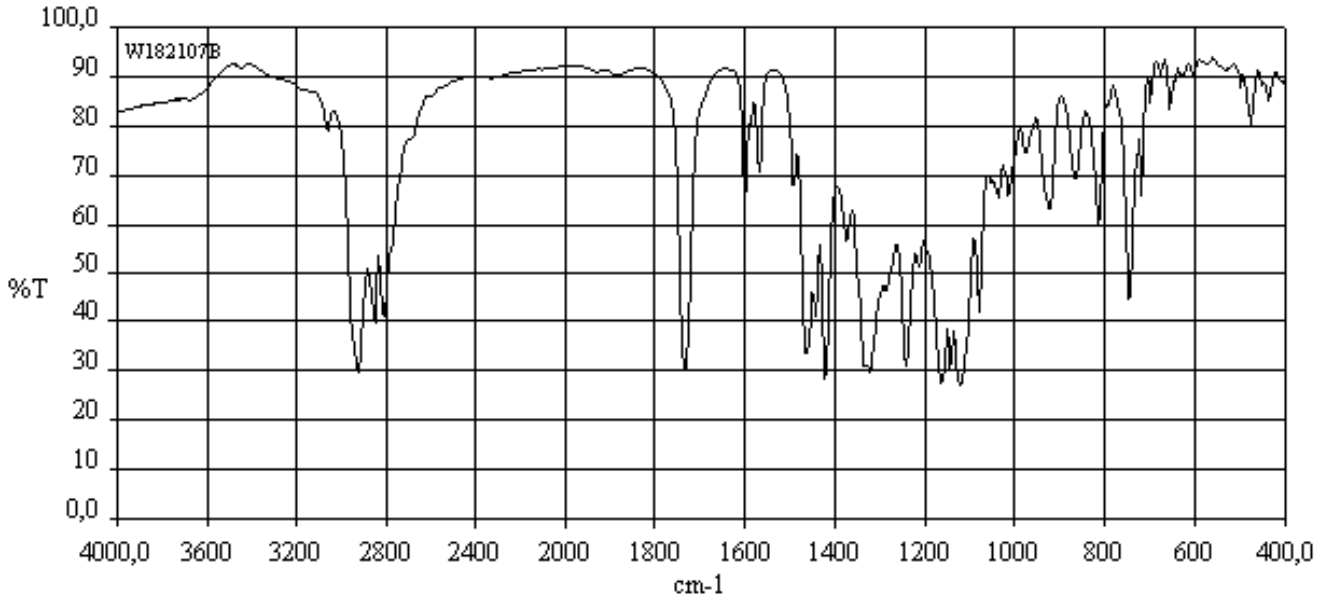


Fluphenazine decanoate dihydrochloride (RS0054)

Instrument: Fourier Transform
Phase: Potassium bromide disc



W182107. IR-spectrum of fluphenazine decanoate dihydrochloride in potassium bromide.

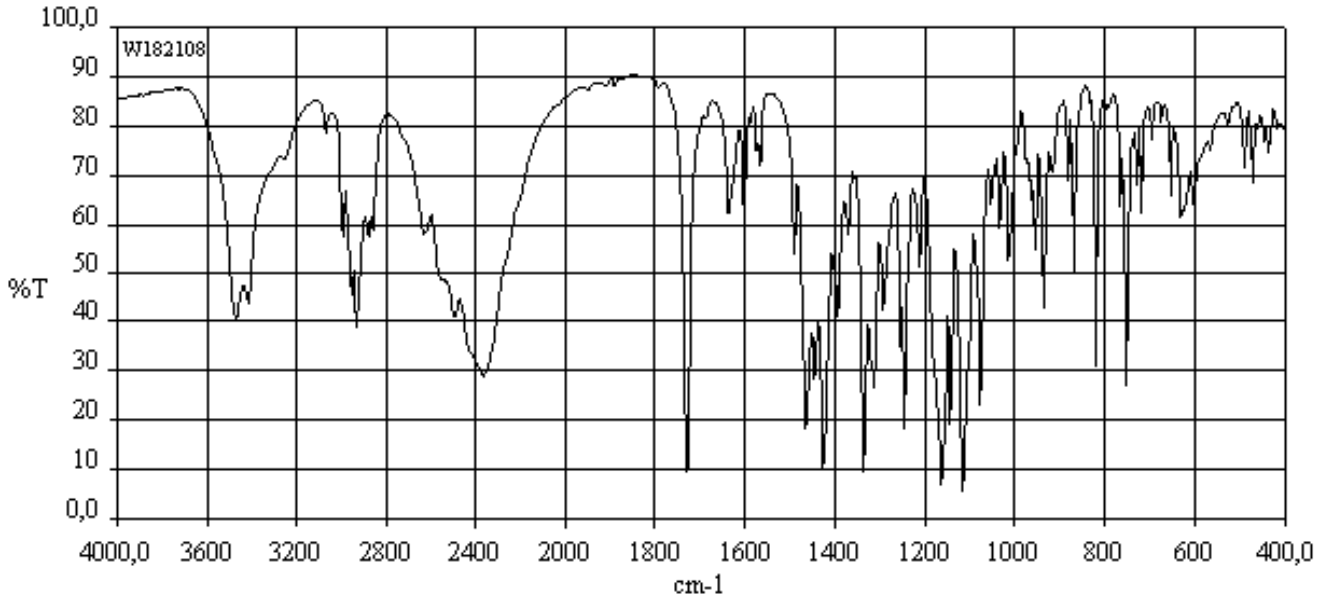


W182107B. IR-spectrum of fluphenazine decanoate base as a thin film on a potassium bromide disc.

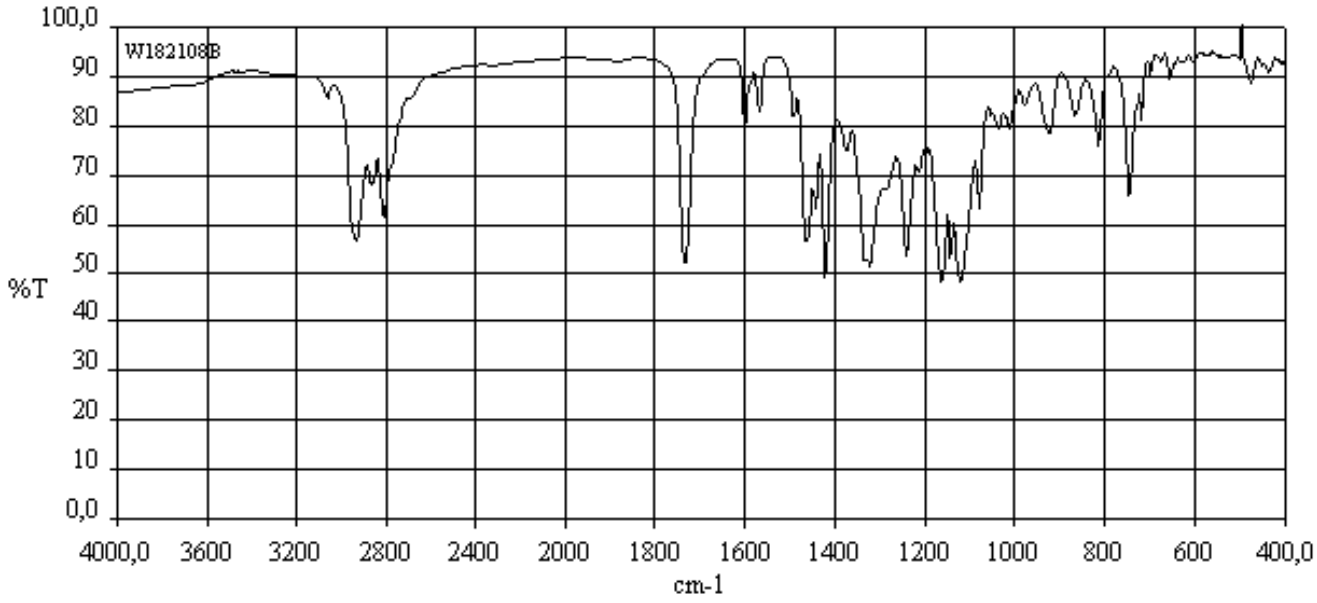
The fluphenazine decanoate base was obtained from fluphenazine decanoate dihydrochloride by the following procedure: A mixture of 0.1 g of the ICRS and 3 mL of sodium hydroxide (0.1 mol/l) was extracted with 5 mL of dichloromethane. The organic phase was filtered through 2 g of anhydrous sodium sulfate and dried with a current of nitrogen. A thin film of the base was coated on a 300 mg potassium bromide disc.

Fluphenazine enantate dihydrochloride (RS0055)

Instrument: Fourier Transform
Phase: Potassium bromide disc



W182108. IR-spectrum of fluphenazine enantate dihydrochloride in potassium bromide.

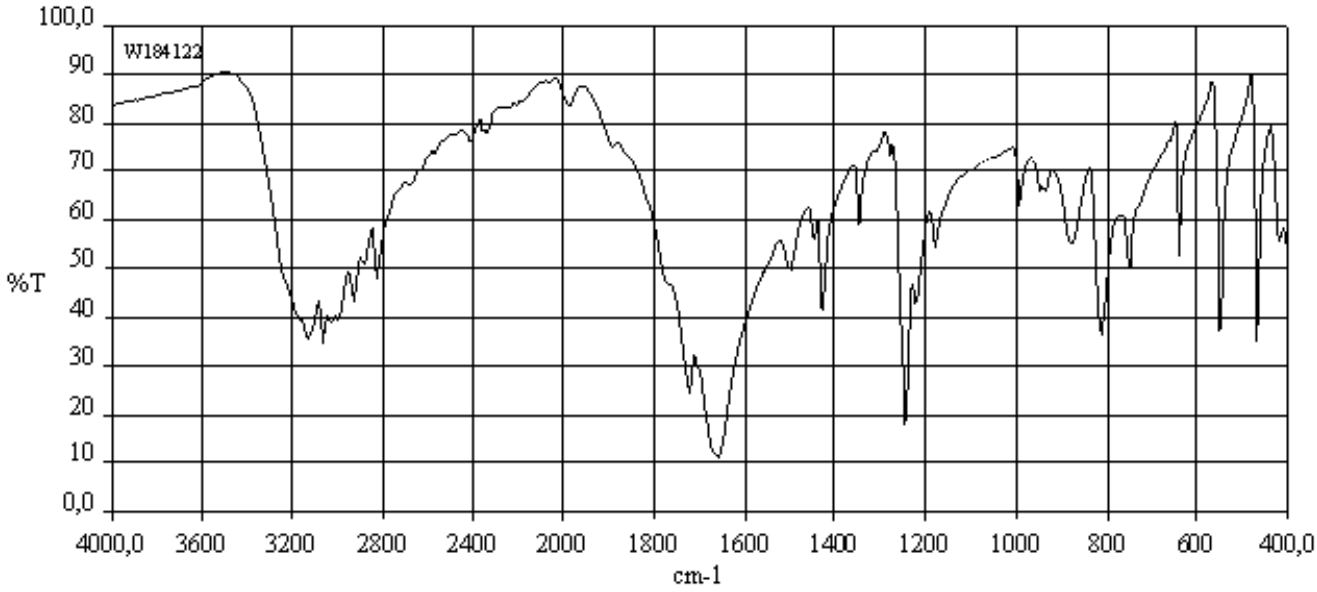


W182108B. IR-spectrum of fluphenazine enantate base as a thin film on a potassium bromide disc.

The fluphenazine enantate base was obtained from fluphenazine enantate dihydrochloride by the following procedure: A mixture of 0.1 g of the ICRS and 3 mL of sodium hydroxide (0.1 mol/l) was extracted with 5 mL of dichloromethane. The organic phase was filtered through 2 g of anhydrous sodium sulfate and dried with a current of nitrogen. A thin film of the base was coated on a 300 mg potassium bromide disc.

Fluorouracil (RS0053)

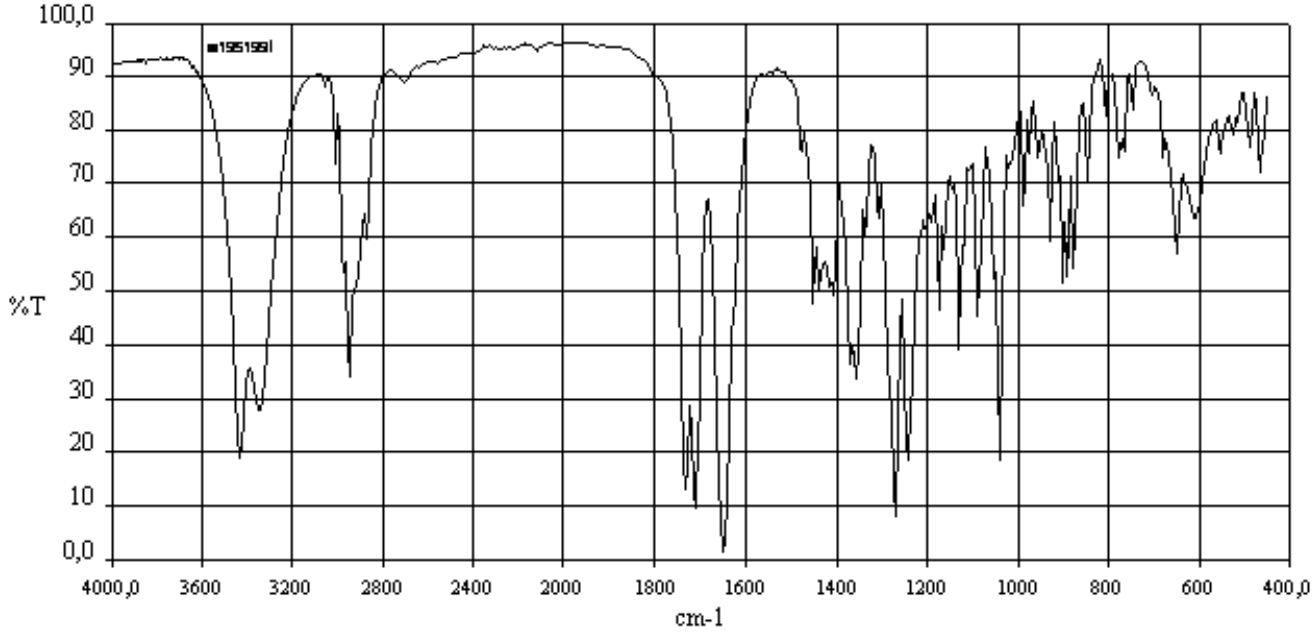
Instrument: Fourier Transform
Phase: Potassium bromide disc



Fludrocortisone acetate (RS0052)

Instrument: Fourier Transform

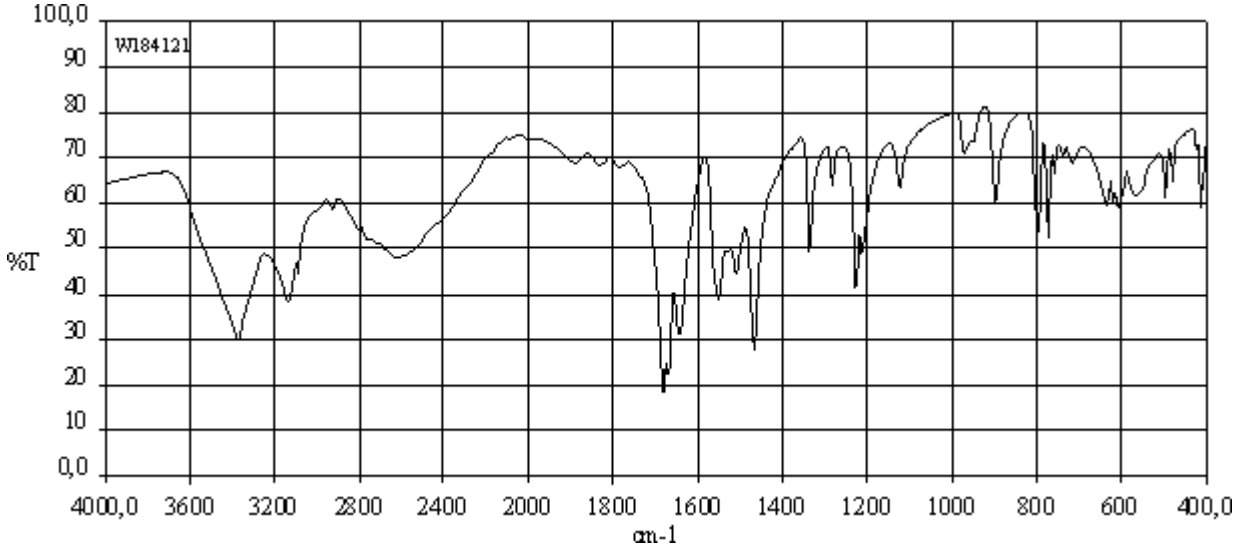
Phase: Potassium bromide disc



Flucytosine (RS00137)

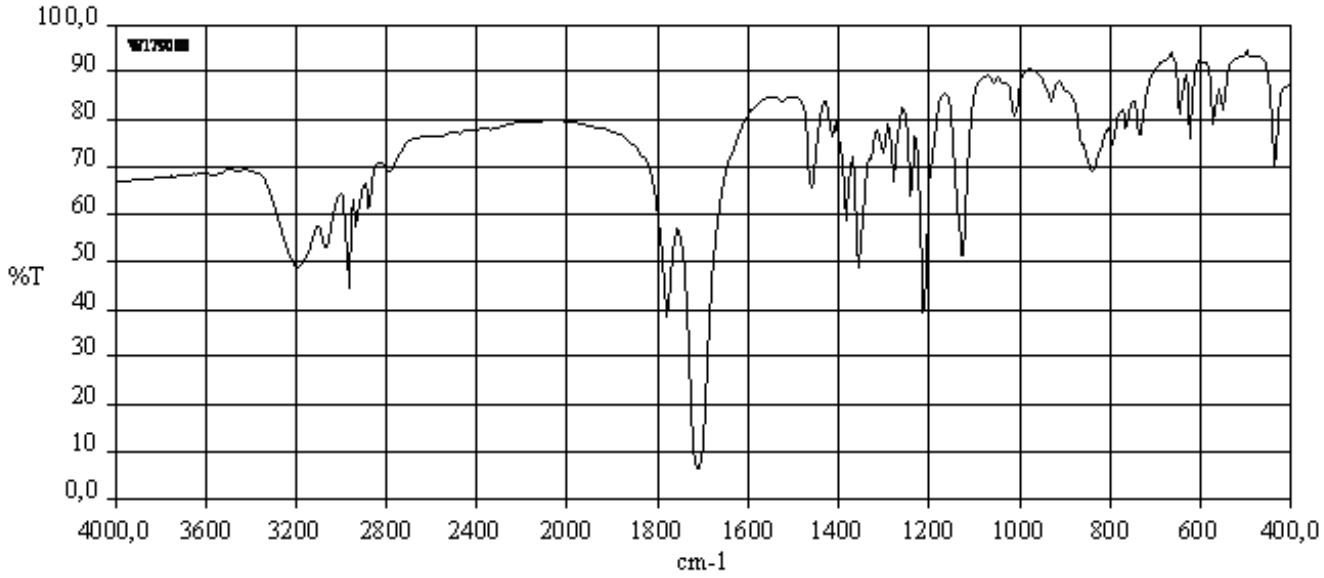
Instrument: Fourier Transform

Phase: Potassium bromide disc



Ethosuximide (RS0051)

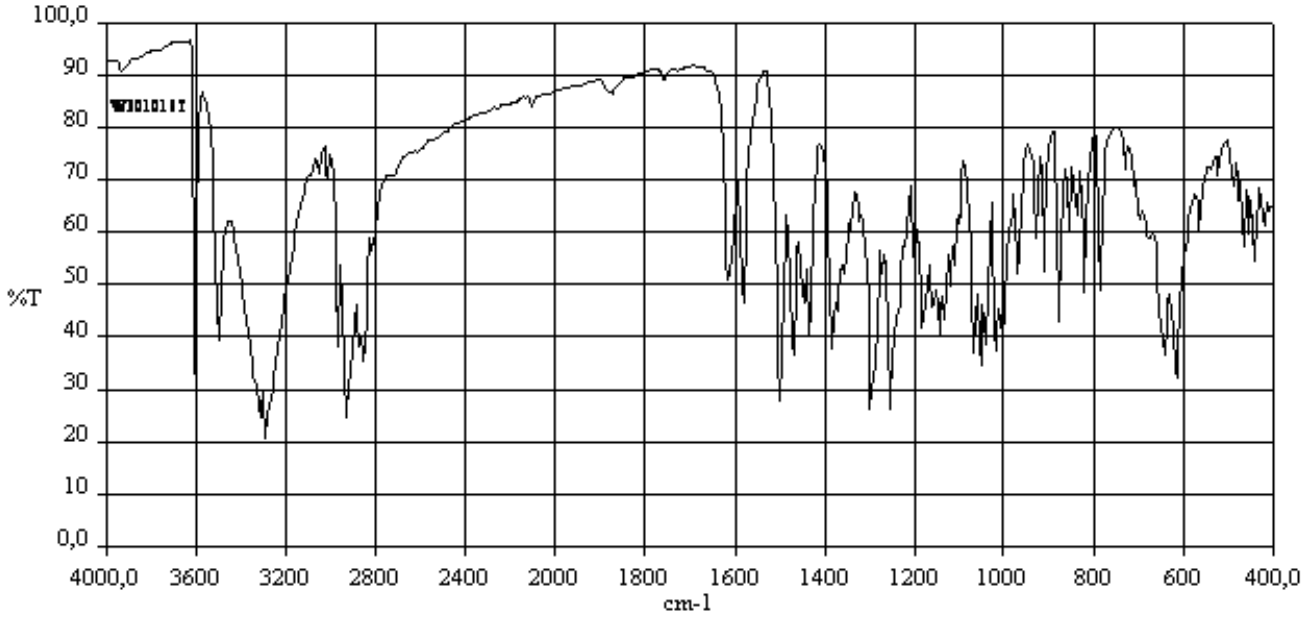
Instrument: Fourier Transform
Phase: Potassium bromide disc



Ethinylestradiol (RS0050)

Instrument: Fourier Transform

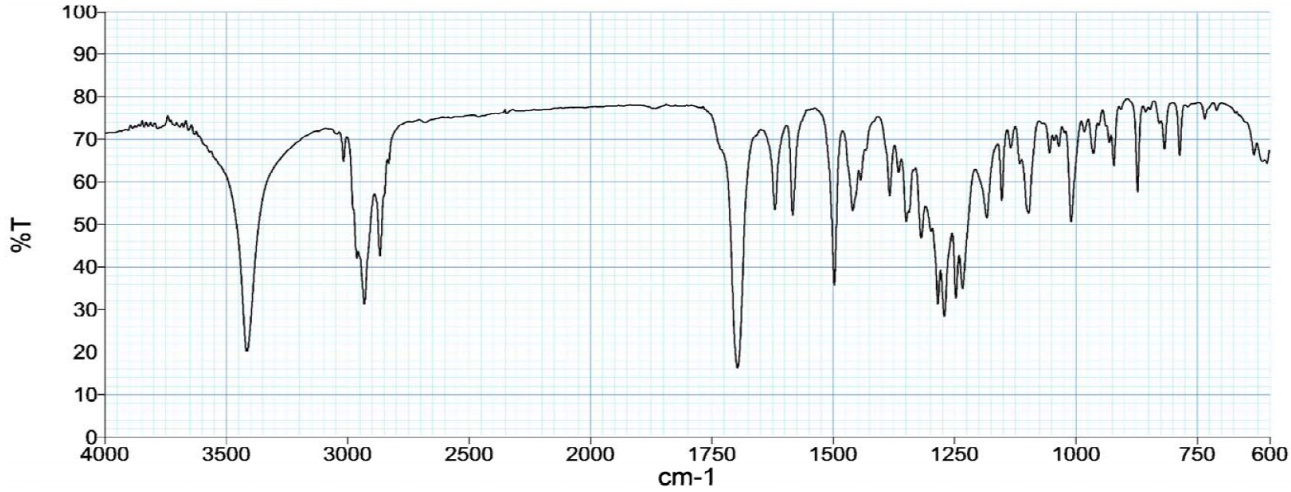
Phase: Potassium bromide disc



Estradiol valerate (RS00171)

Instrument: Fourier Transform

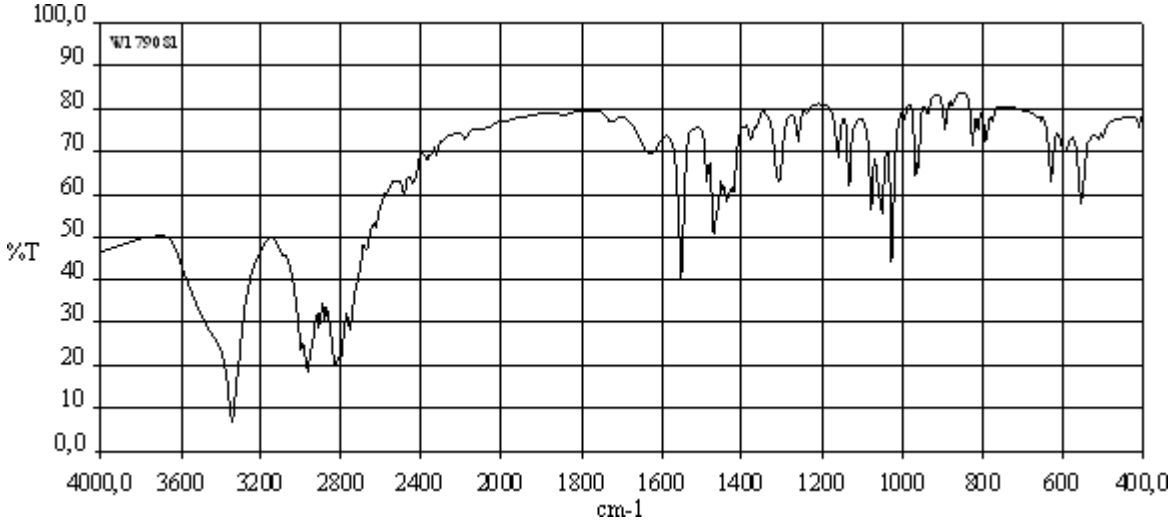
Phase: Potassium bromide disc



Ethambutanol hydrochloride (RS00136)

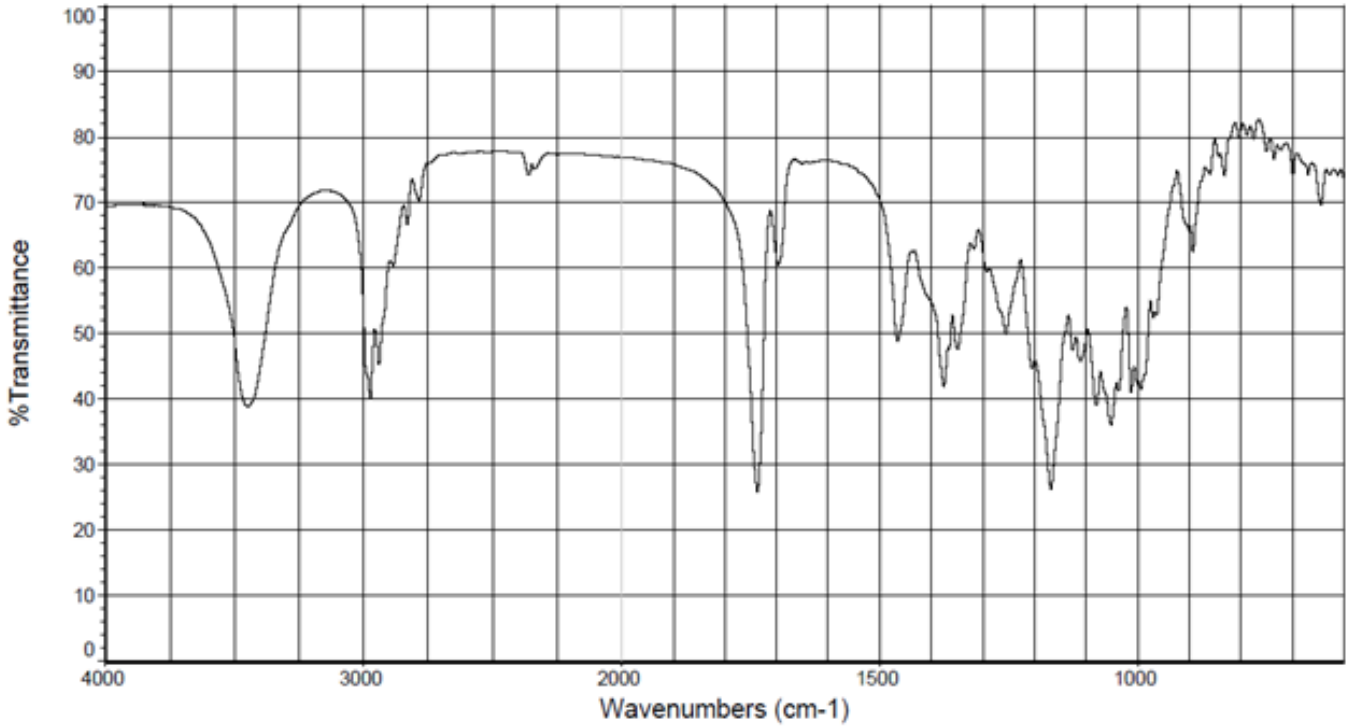
Instrument: Fourier Transform

Phase: Potassium bromide disc



Erythromycin ethylsuccinate (RS00159)

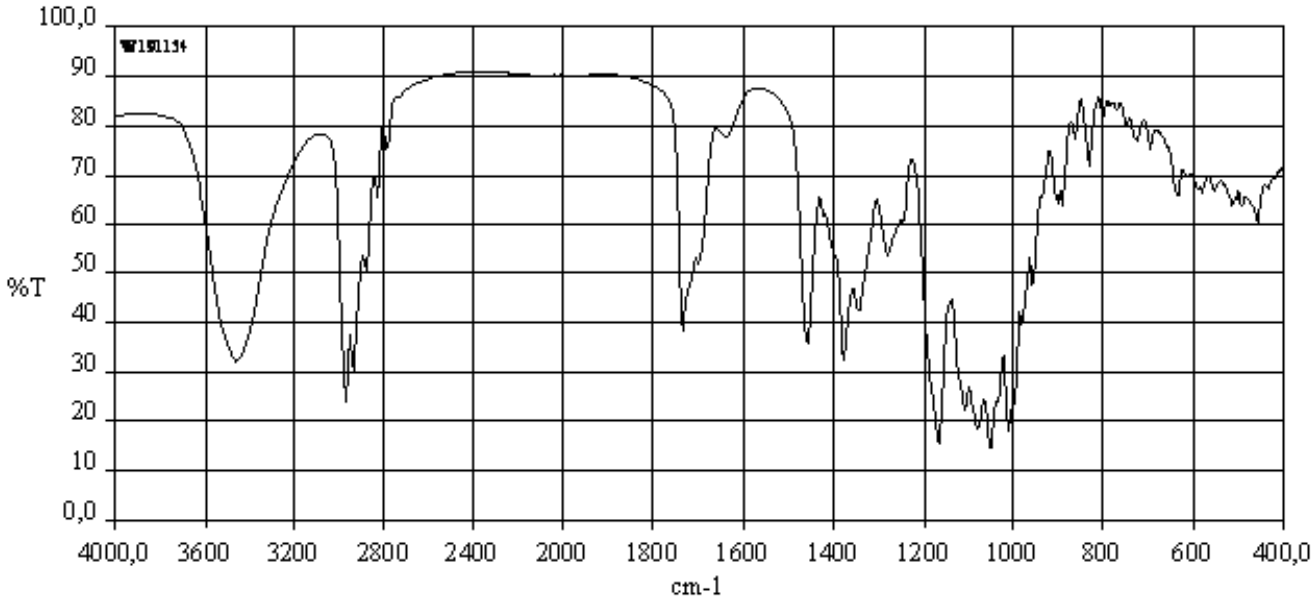
Instrument: Fourier Transform
Phase: Potassium bromide disc



Erythromycin (RS0049)

Instrument: Fourier Transform

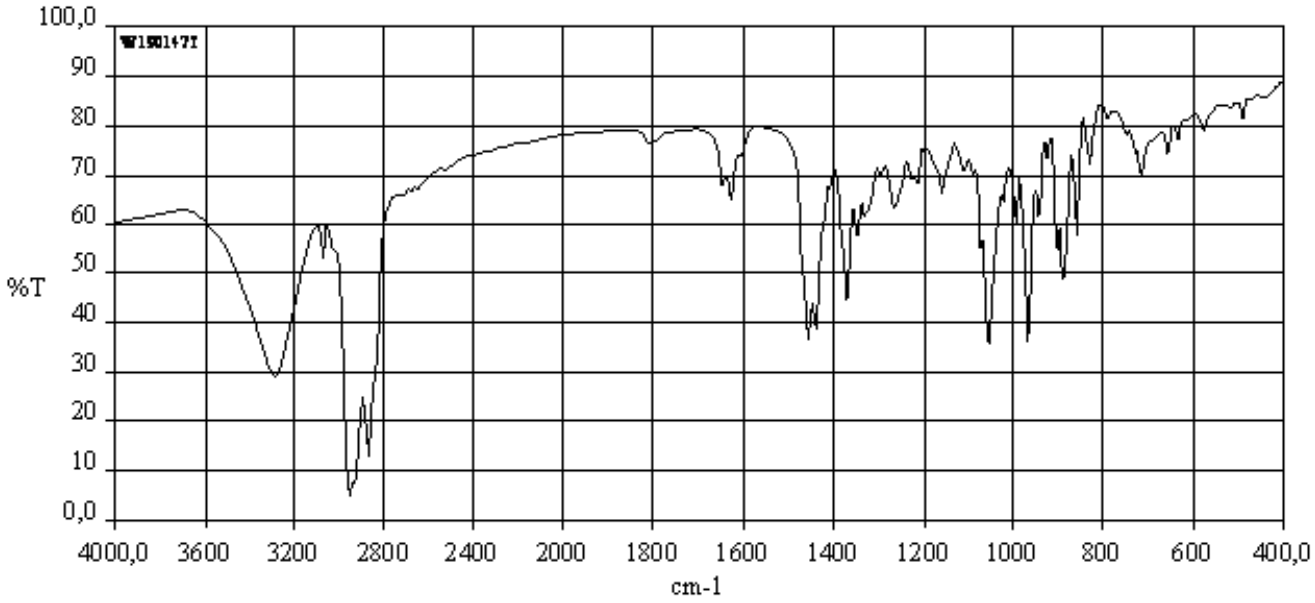
Phase: Potassium bromide disc



Ergocalciferol (RS0048)

Instrument: Fourier Transform

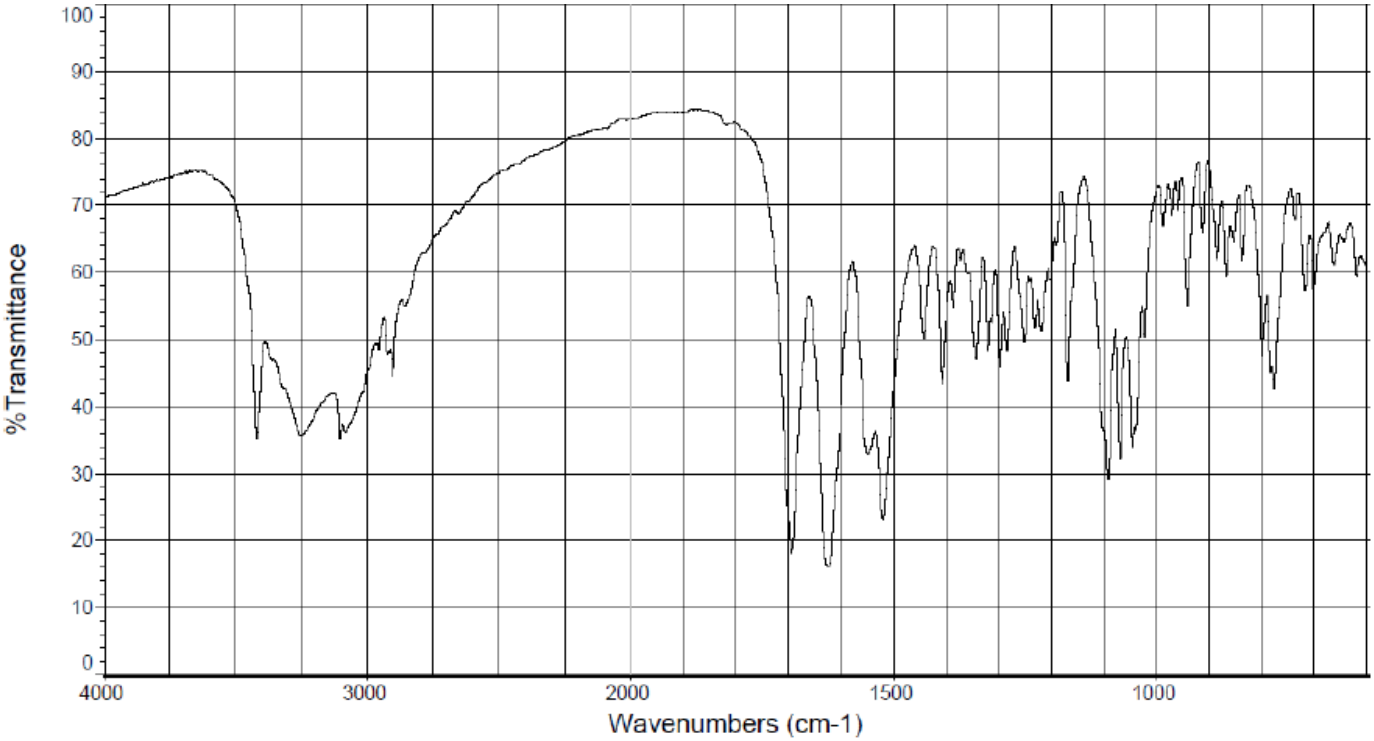
Phase: Potassium bromide disc



Emtricitabine

Instrument: Fourier Transform

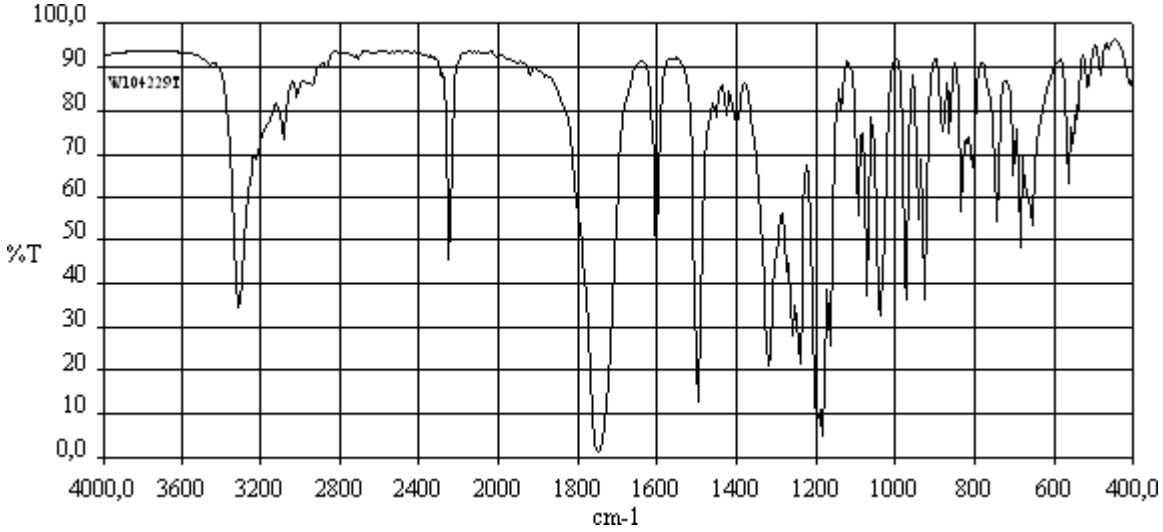
Phase: Potassium bromide disc



Efavirenz (RS00135)

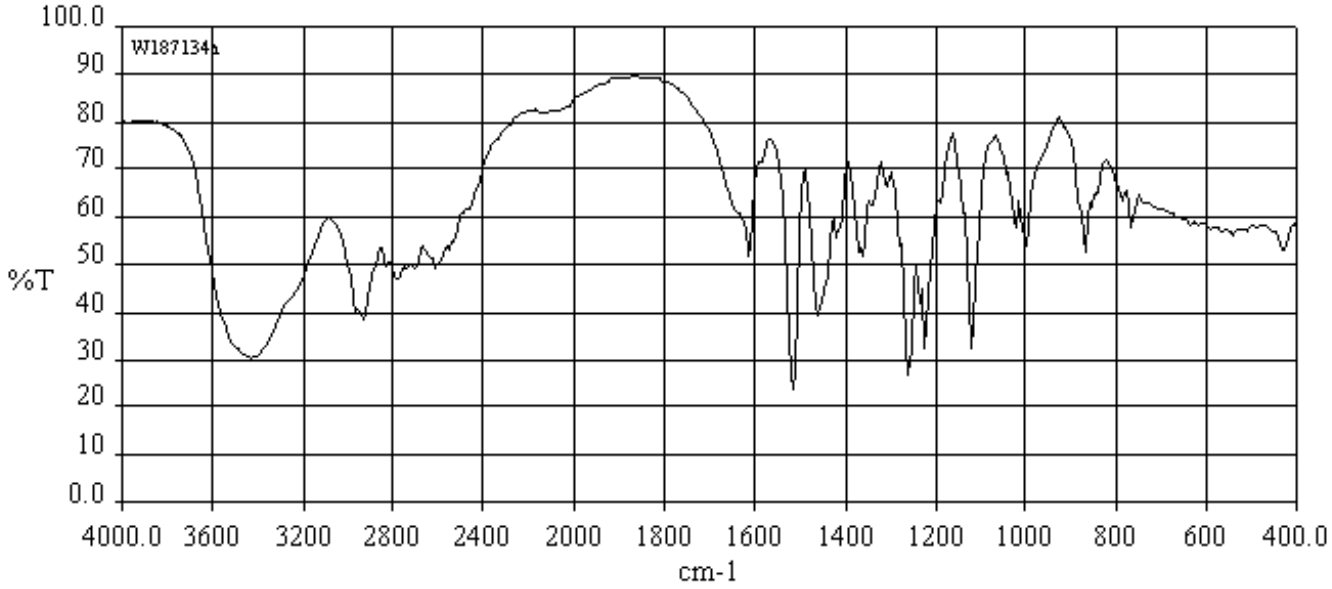
Instrument: Fourier Transform

Phase: Potassium bromide disc



Emetine hydrochloride (RS0047)

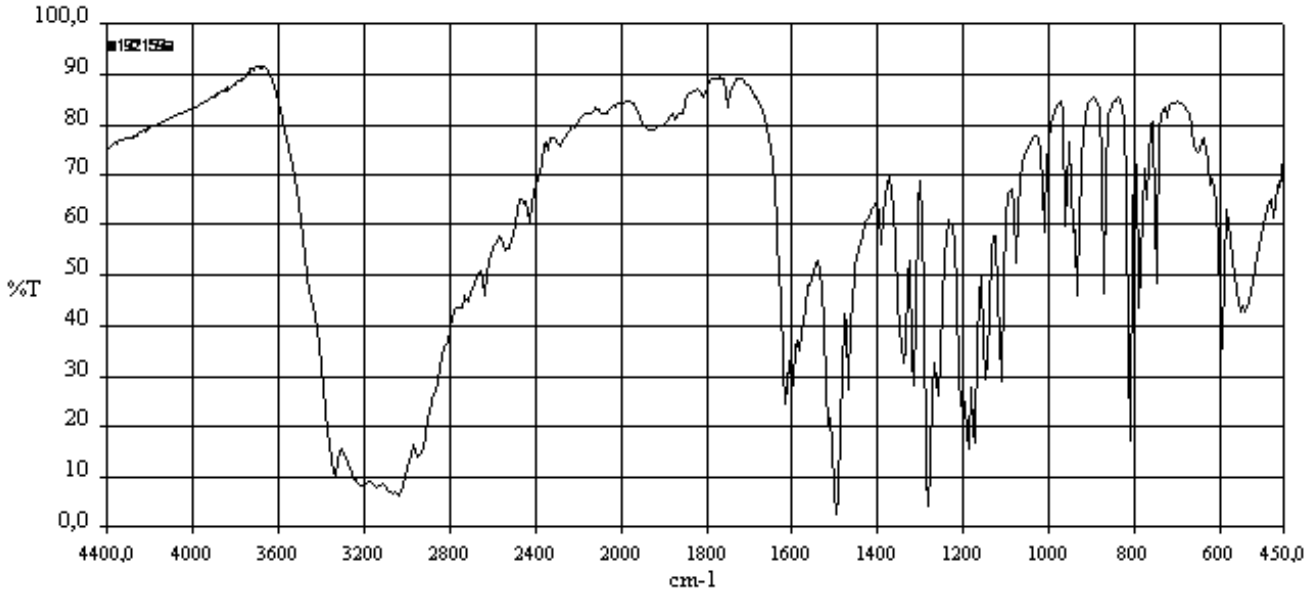
Instrument: Fourier Transform
Phase: Potassium bromide disc



Dopamine hydrochloride (RS0046)

Instrument: Fourier Transform

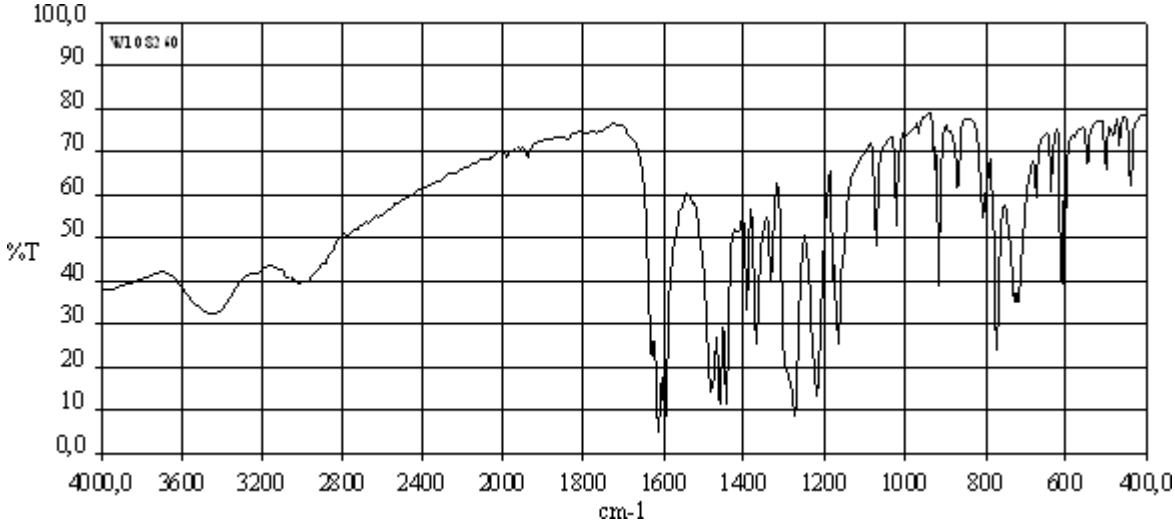
Phase: Potassium bromide disc



Dithranol (RS00134)

Instrument: Fourier Transform

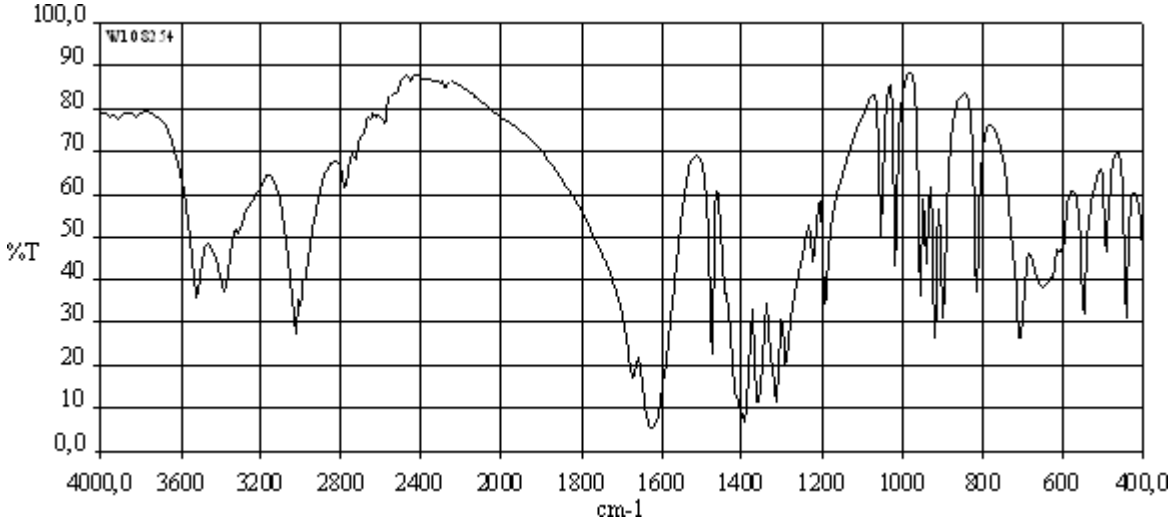
Phase: Potassium bromide disc



Disodium edetate (RS00133)

Instrument: Fourier Transform

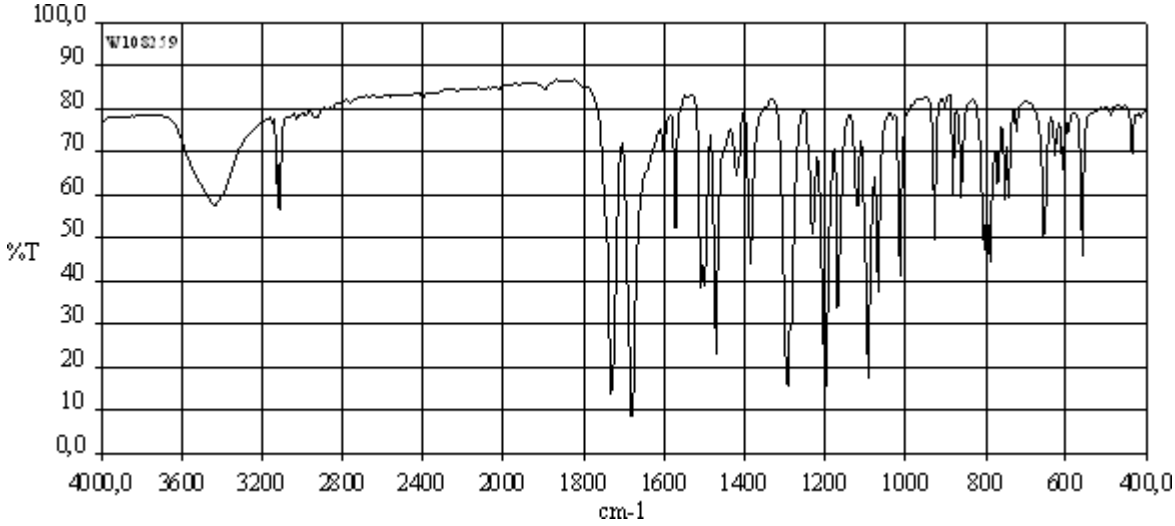
Phase: Potassium bromide disc



Diloxanide furoate (RS00132)

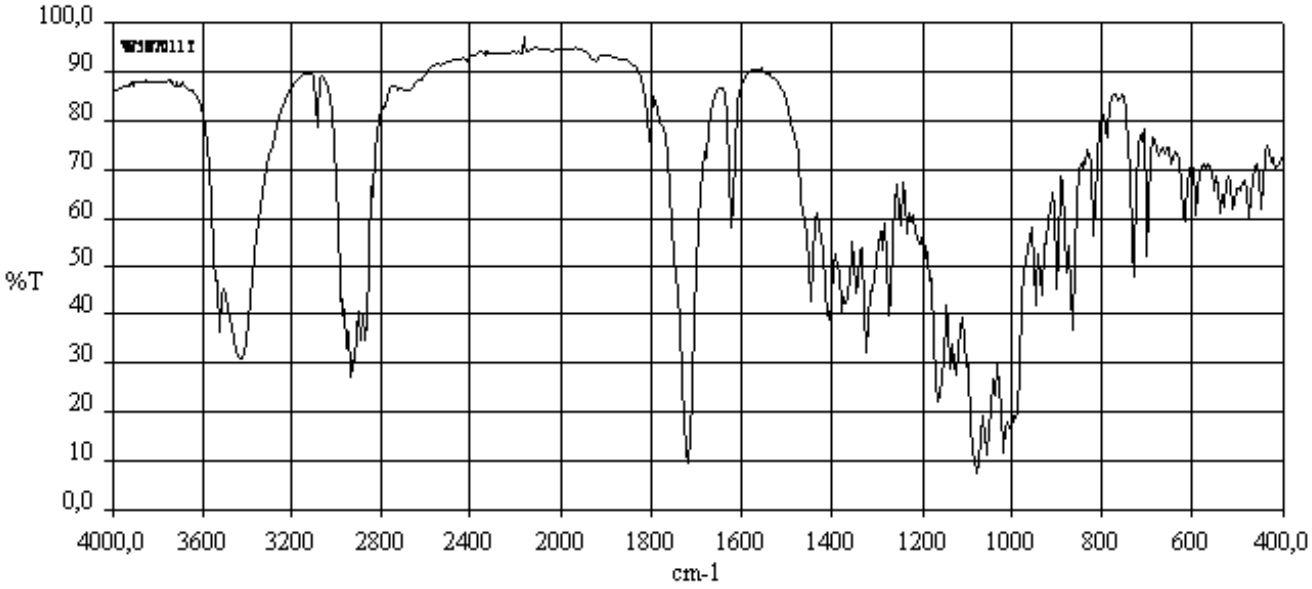
Instrument: Fourier Transform

Phase: Potassium bromide disc



Digoxin (RS0045)

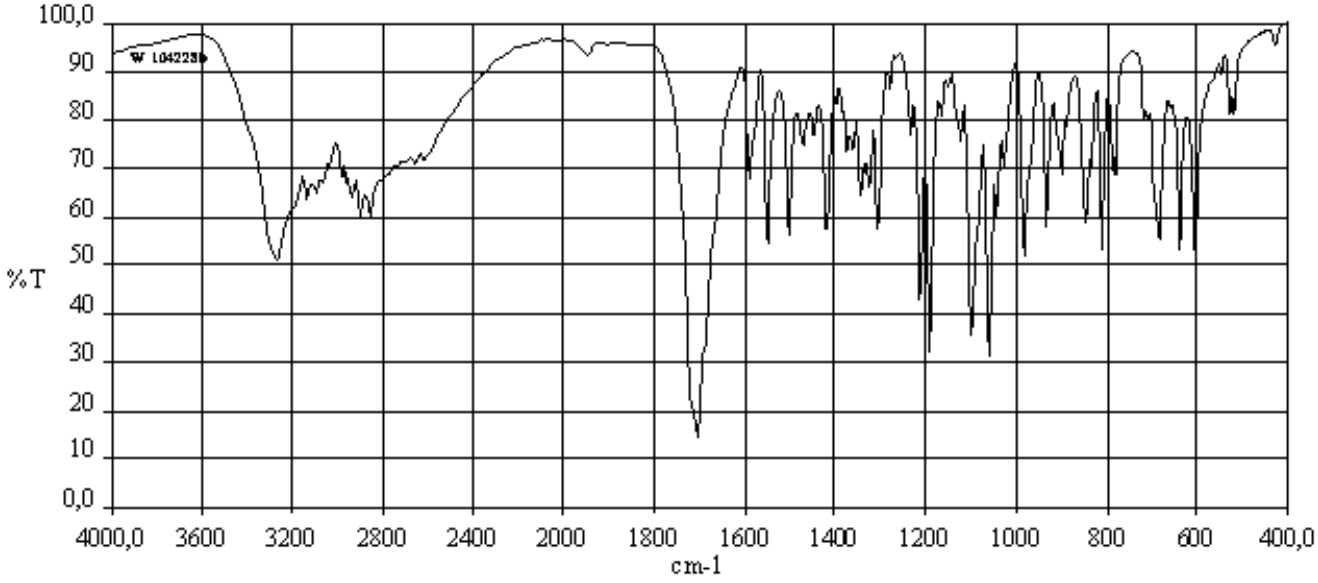
Instrument: Fourier Transform
Phase: Potassium bromide disc



Didanosine (RS0043)

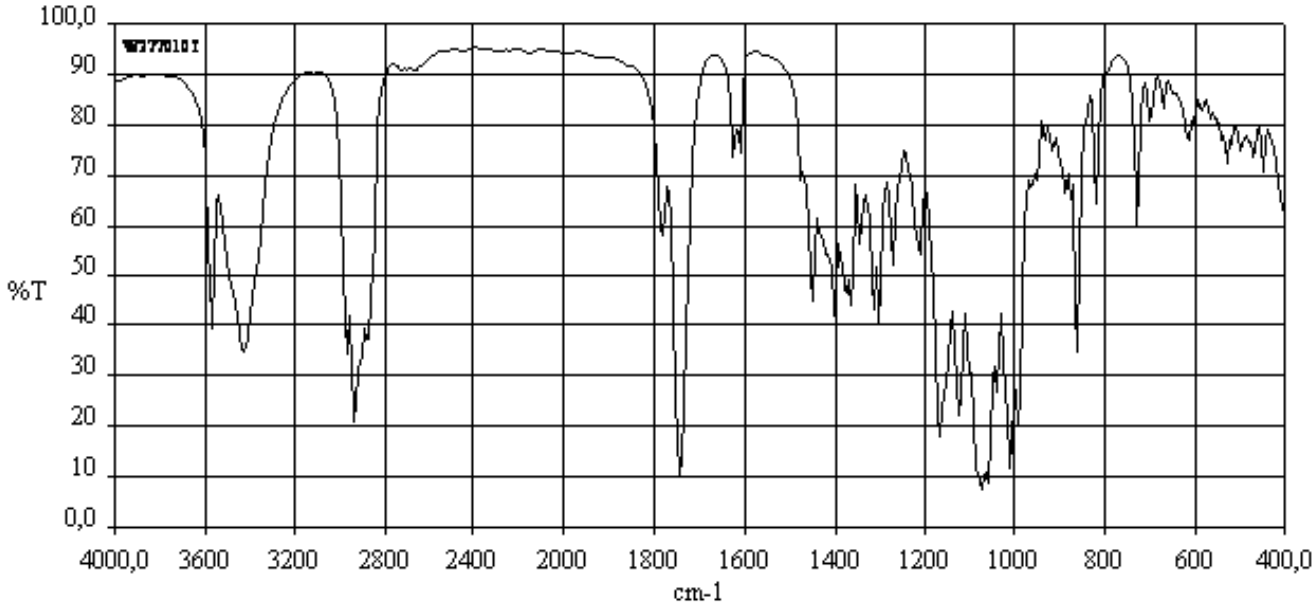
Instrument: Fourier Transform

Phase: Potassium bromide disc



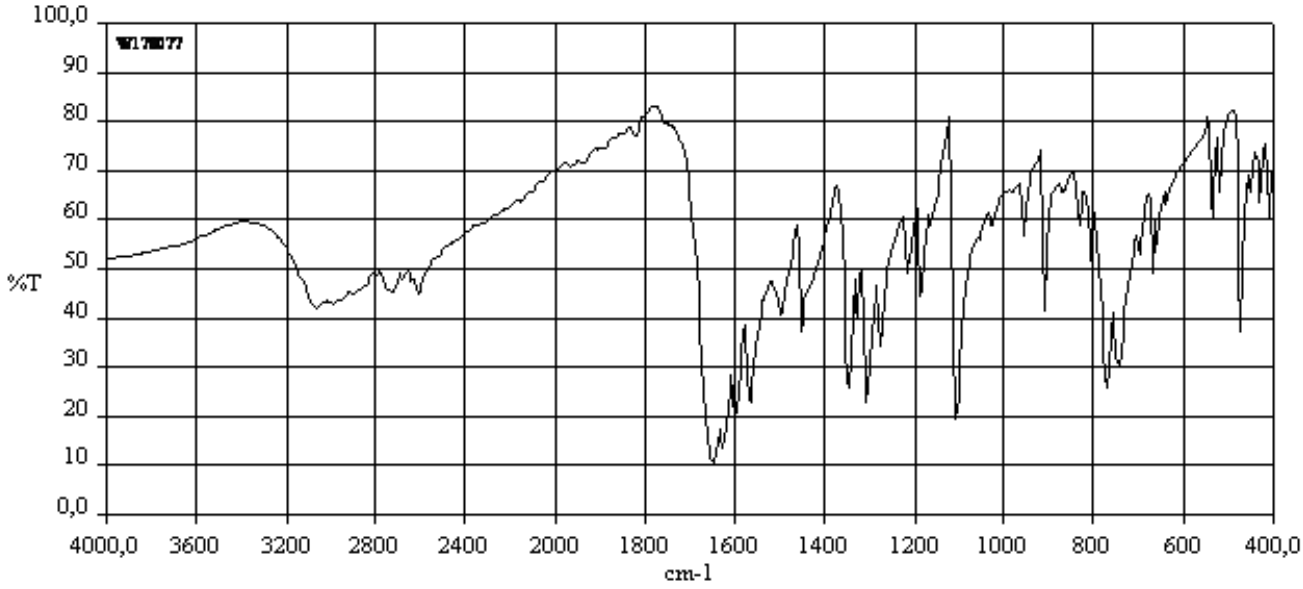
Digitoxin (RS0044)

Instrument: Fourier Transform
Phase: Potassium bromide disc



Dicoumarol (RS0042)

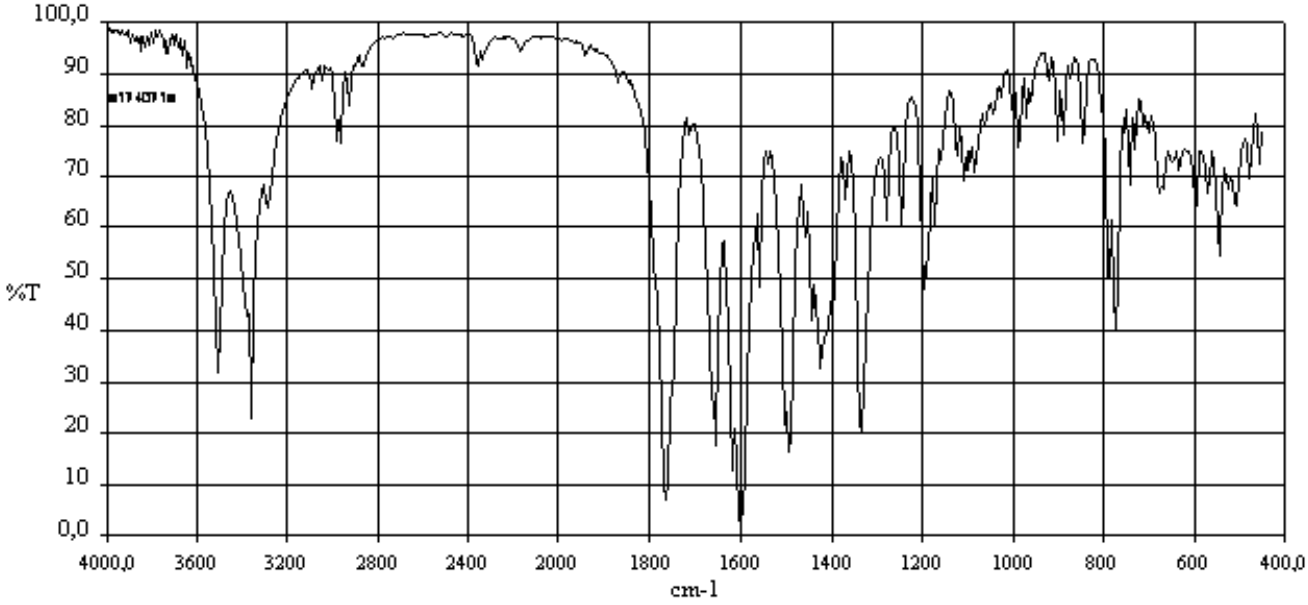
Instrument: Fourier Transform
Phase: Potassium bromide disc



Dicloxacillin sodium (RS0041)

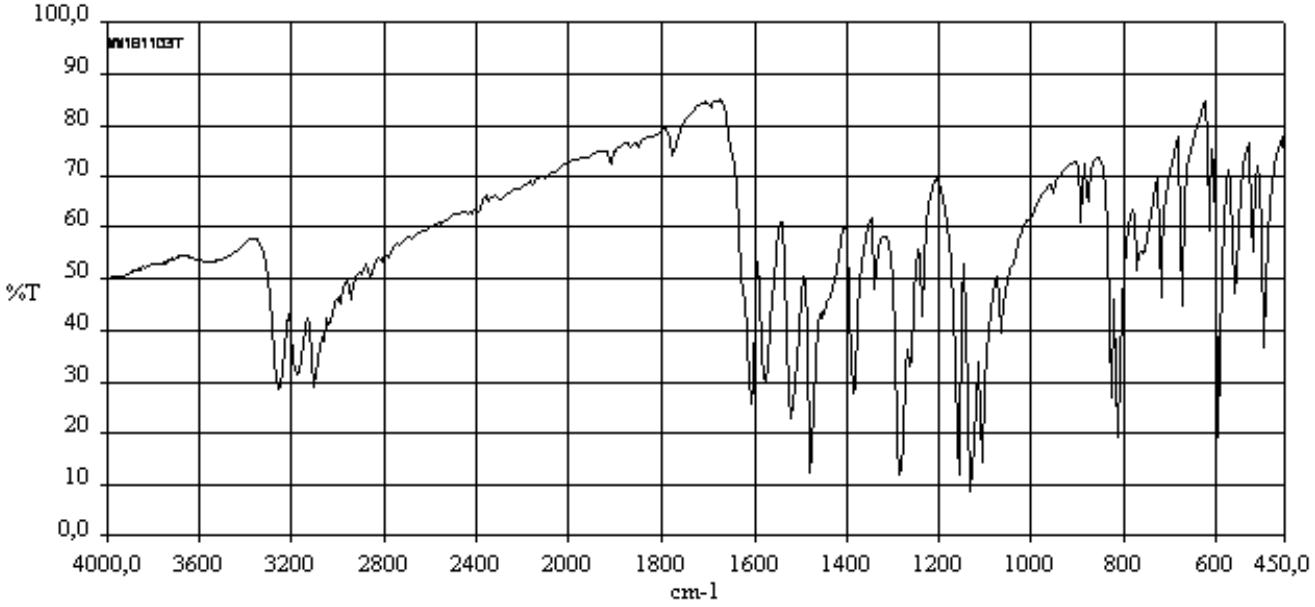
Instrument: Fourier Transform

Phase: Potassium bromide disc



Diazoxide (RS0040)

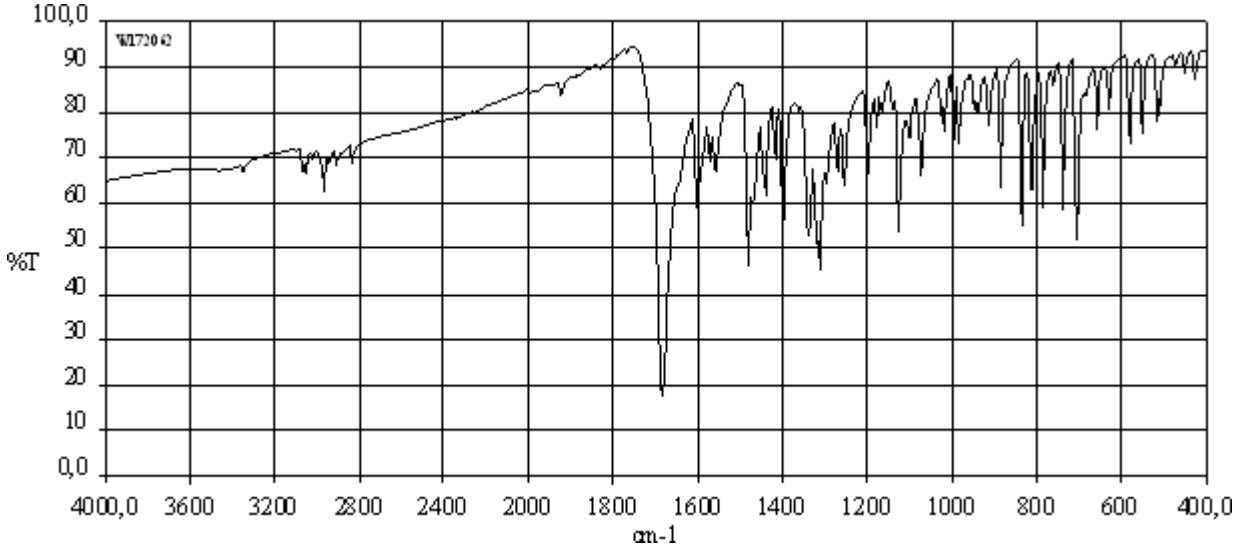
Instrument: Fourier Transform
Phase: Potassium bromide disc



Diazepam (RS00131)

Instrument: Fourier Transform

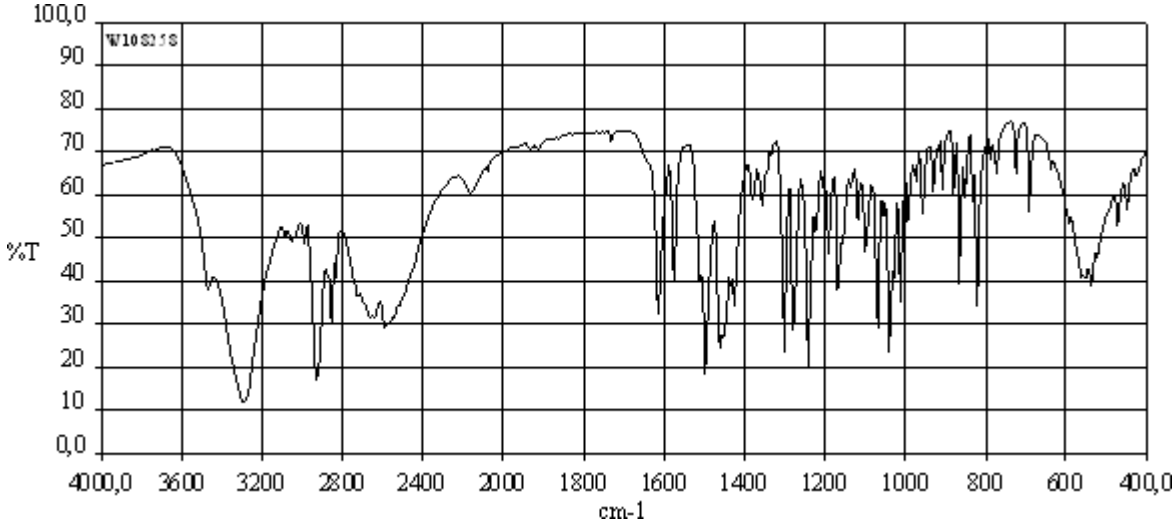
Phase: Potassium bromide disc



Dextromethorphan hydrobromide (RS00130)

Instrument: Fourier Transform

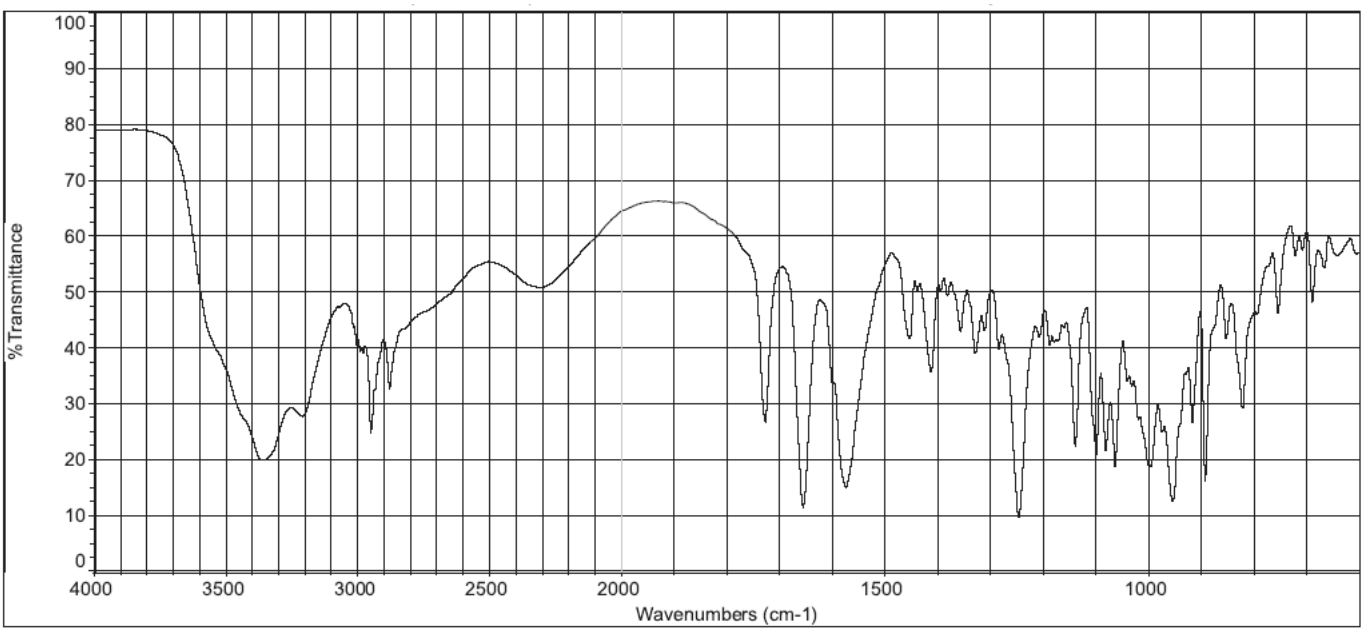
Phase: Potassium bromide disc



Dexamethasone sodium phosphate

Instrument: Fourier Transform

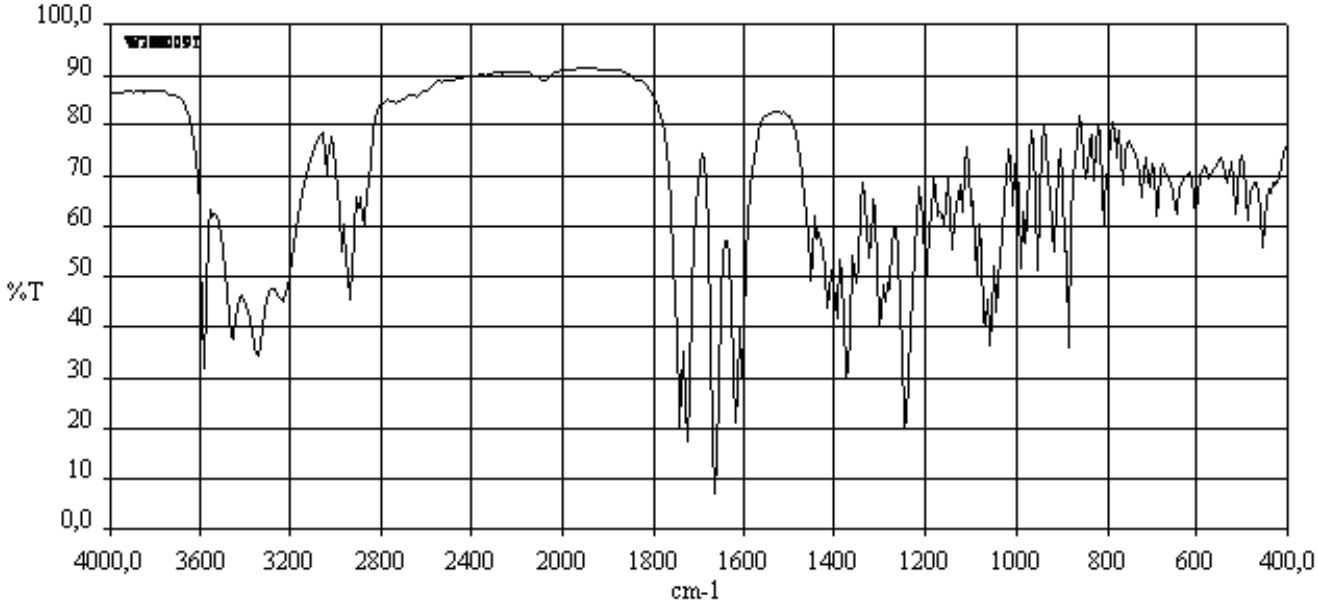
Phase: Potassium bromide disc



Dexamethasone acetate (RS0039)

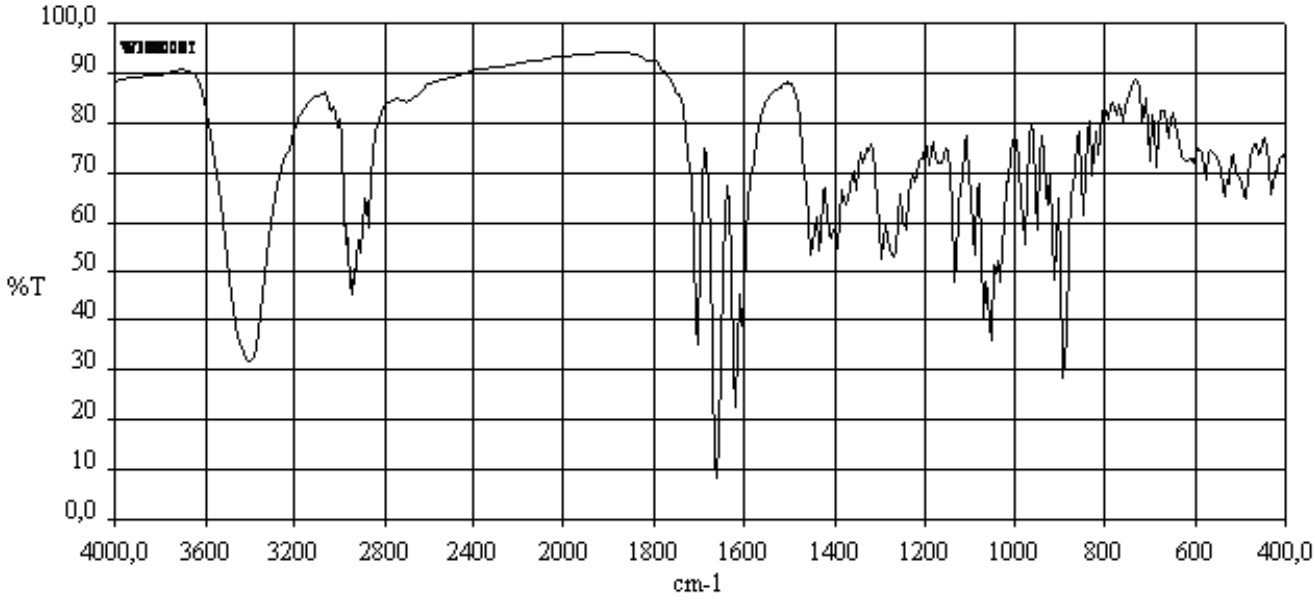
Instrument: Fourier Transform

Phase: Potassium bromide disc



Dexamethasone (RS0038)

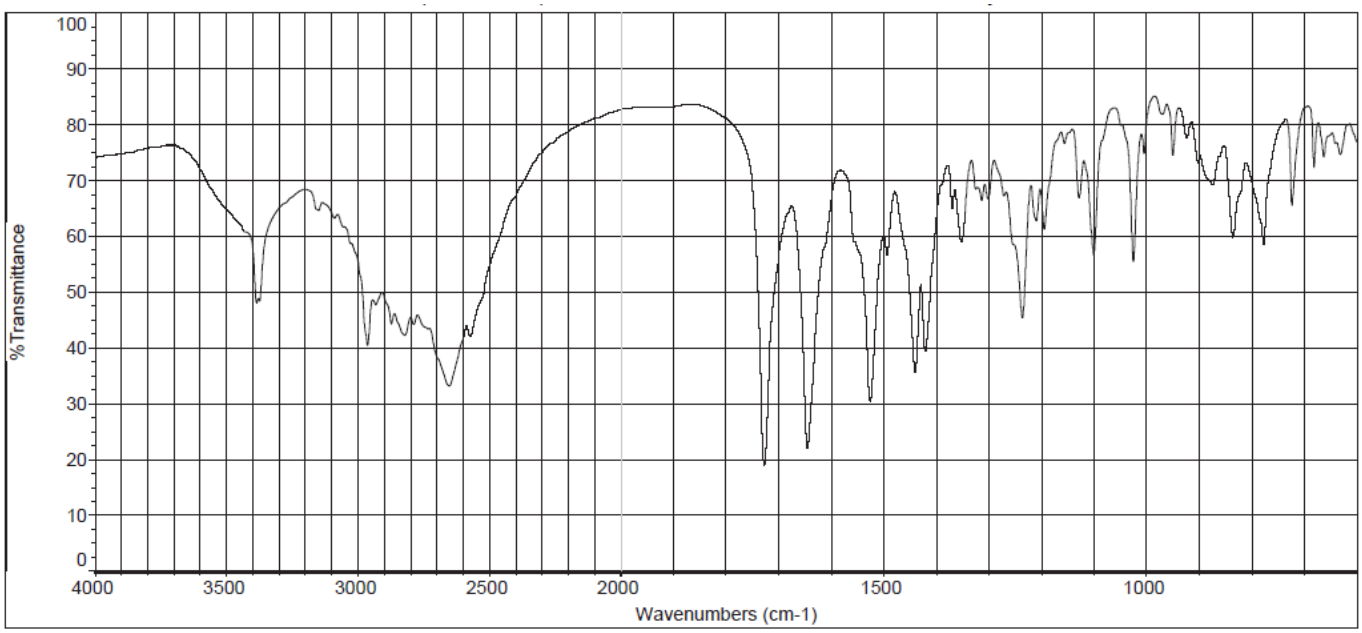
Instrument: Fourier Transform
Phase: Potassium bromide disc



Daclatasvir dihydrochloride

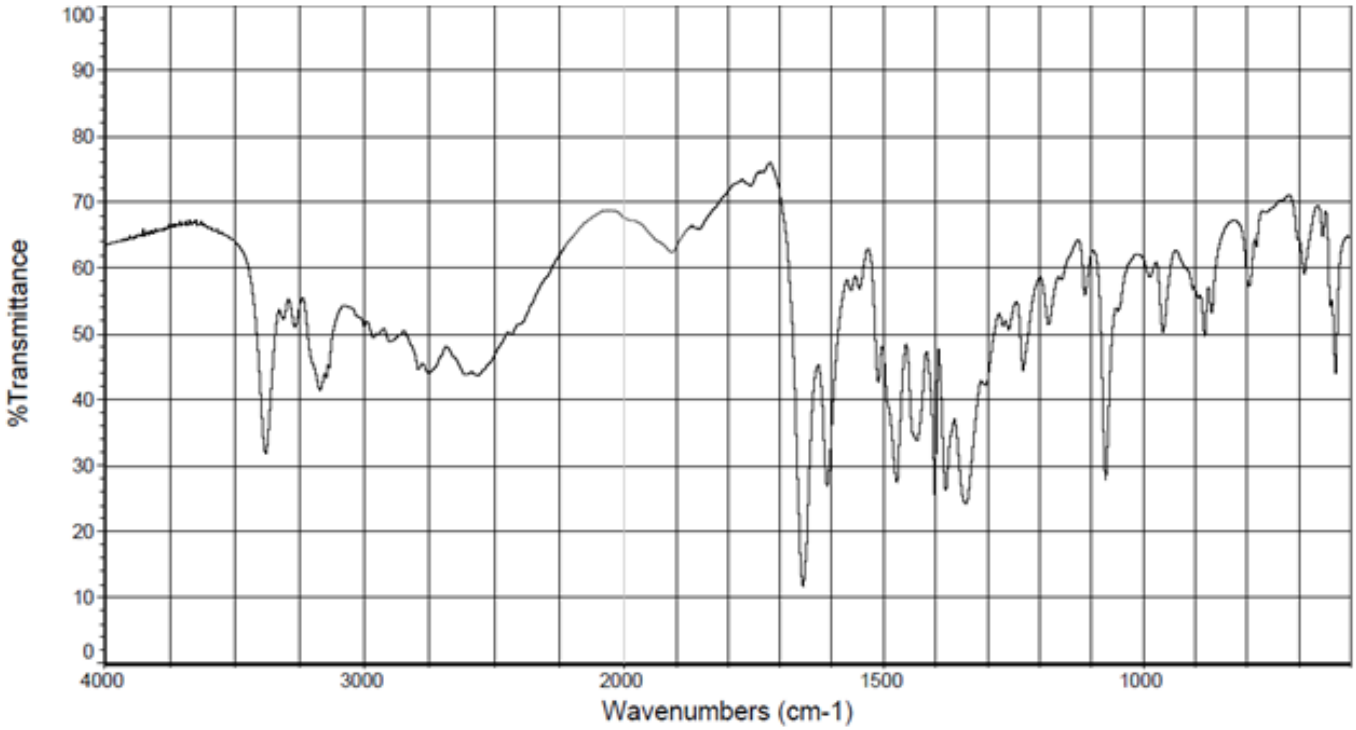
Instrument: Fourier Transform

Phase: Potassium bromide disc



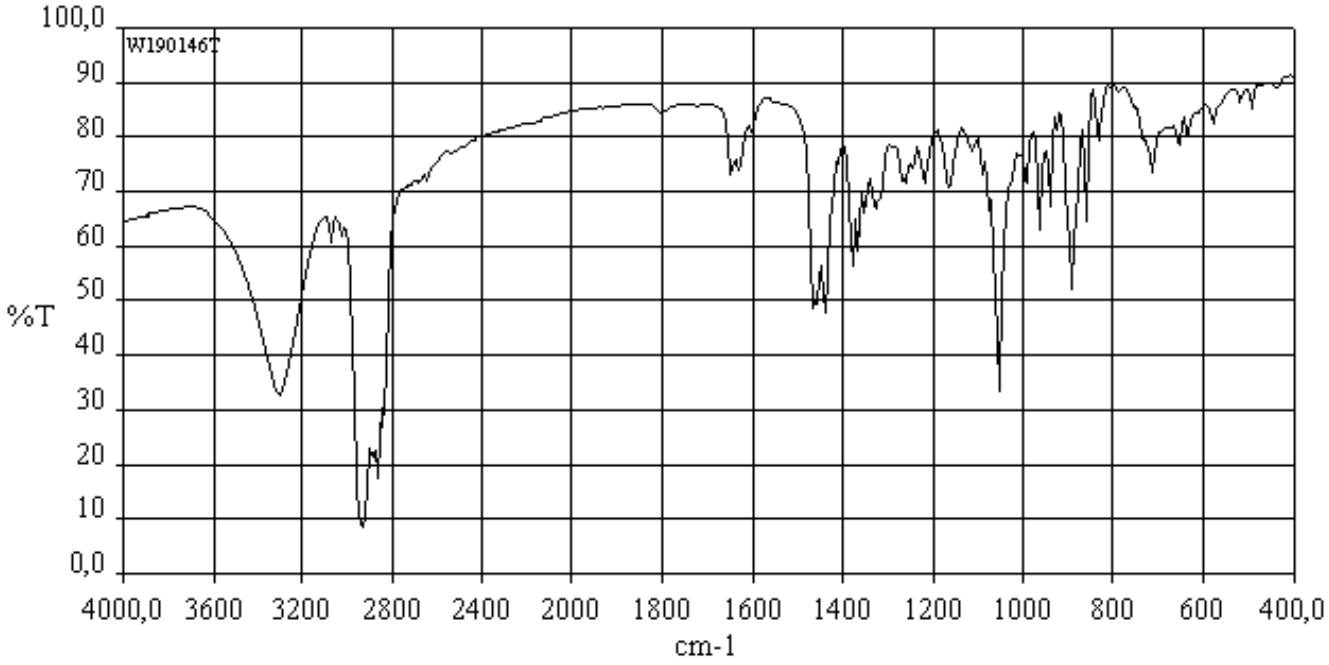
Dacarbazine (RS00158)

Instrument: Fourier Transform
Phase: Potassium bromide disc



Colecalciferol (RS0037)

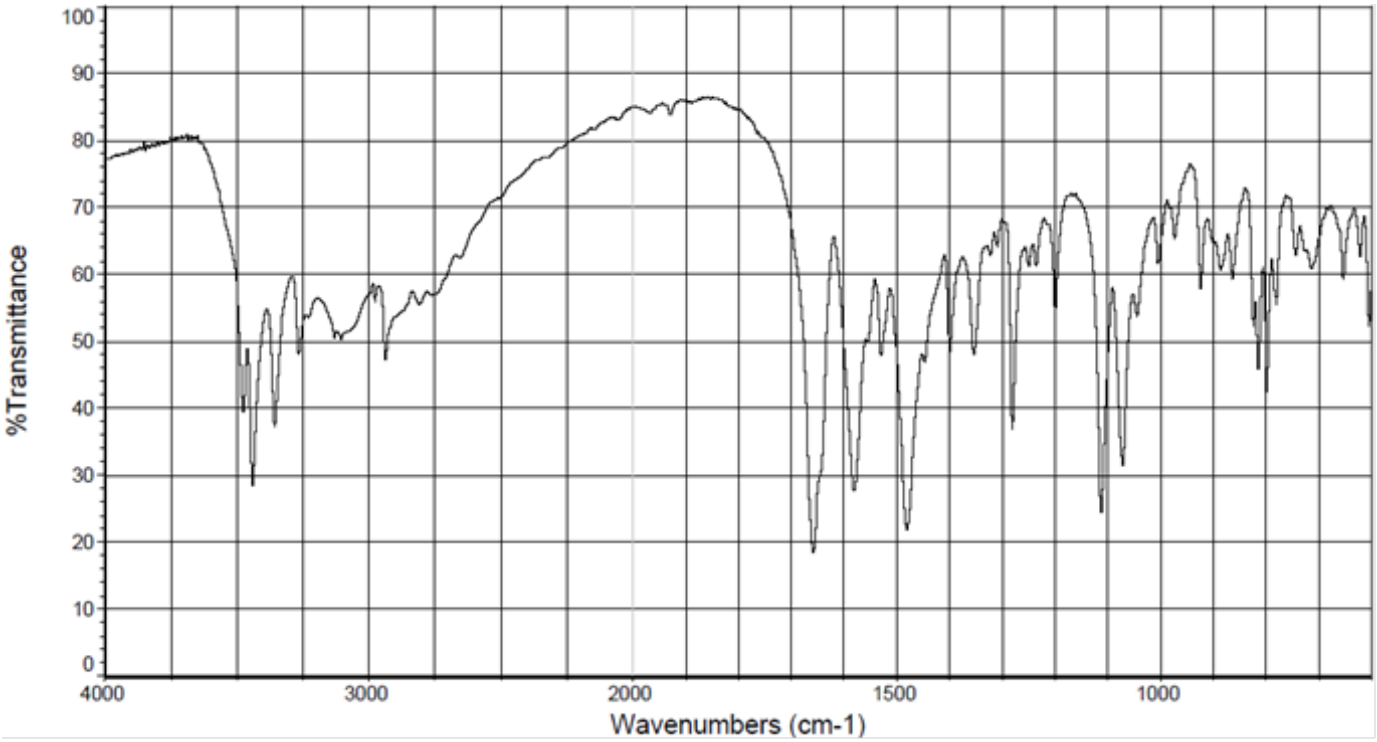
Instrument: Fourier Transform
Phase: Potassium bromide disc



Cytarabine (RS00165)

Instrument: Fourier Transform

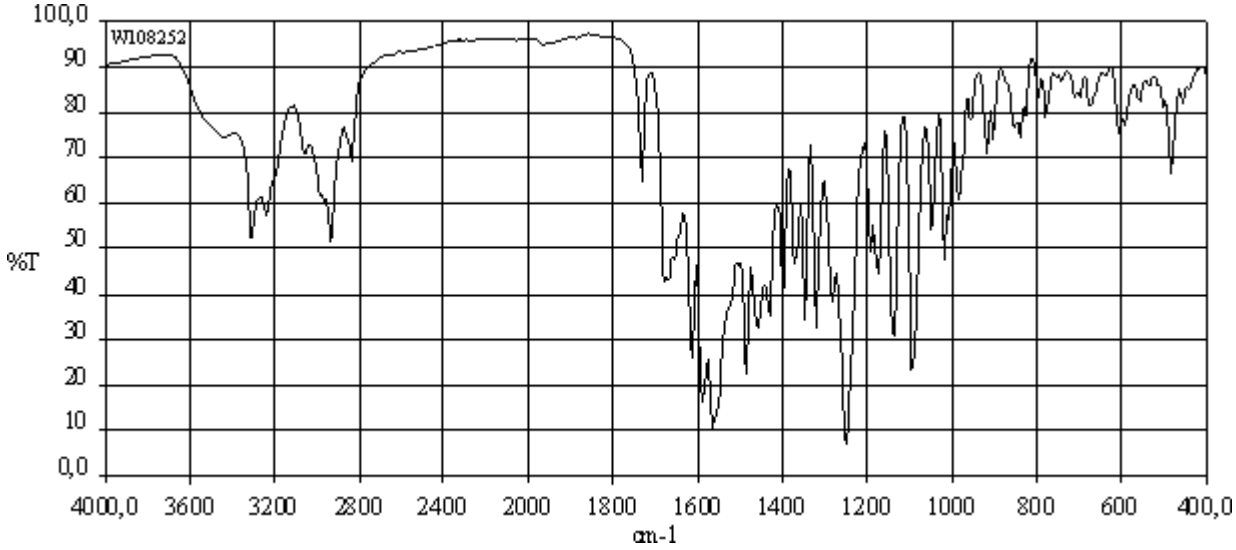
Phase: Potassium bromide disc



Colchicine (RS00129)

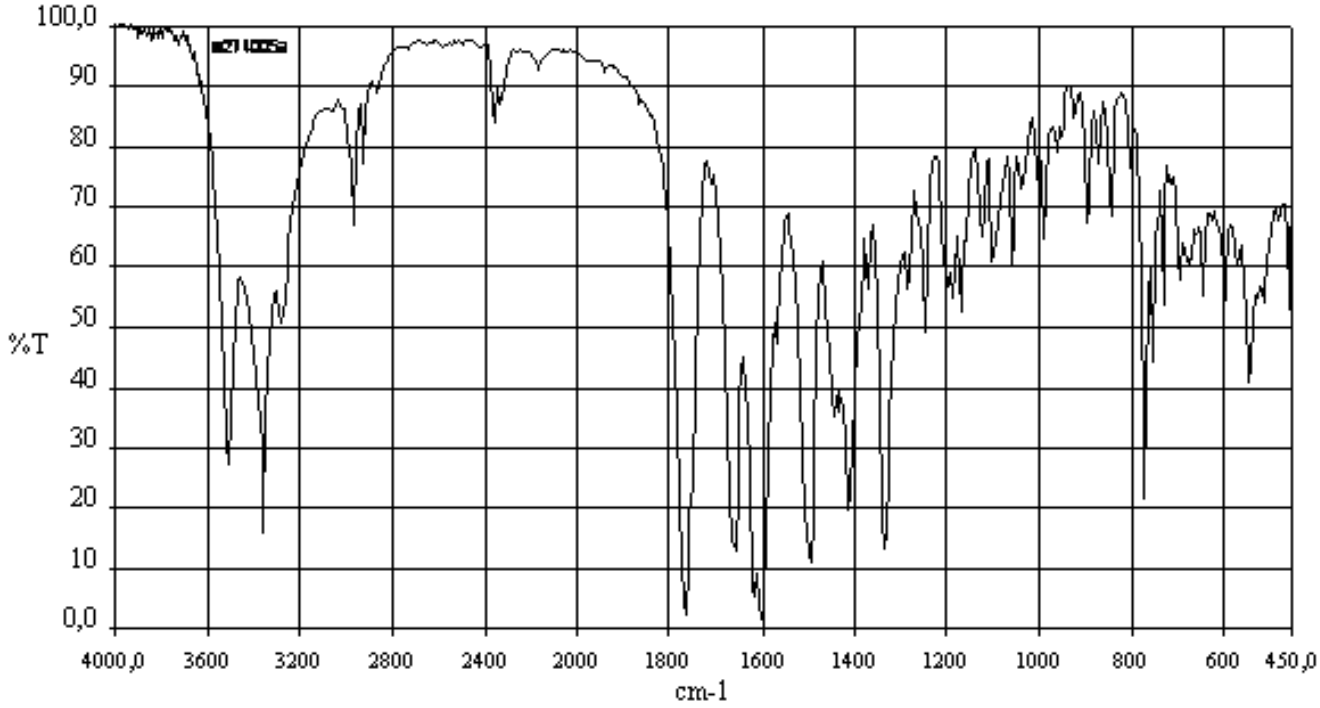
Instrument: Fourier Transform

Phase: Potassium bromide disc



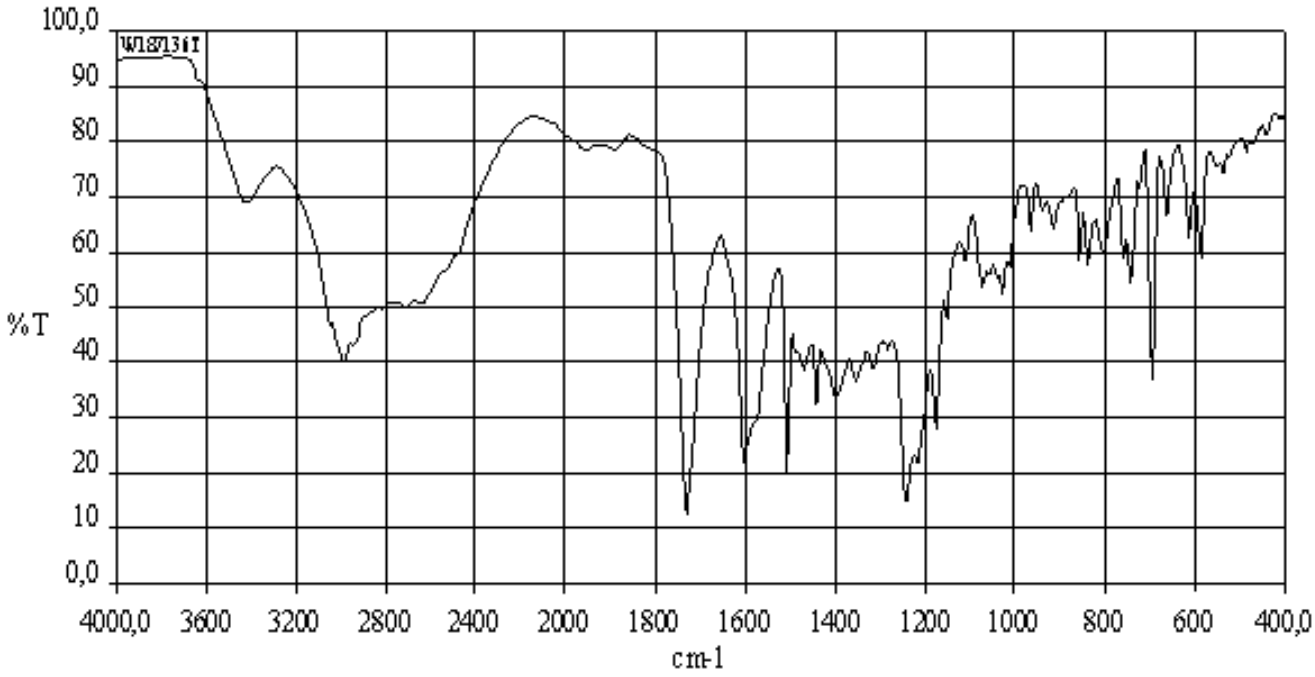
Cloxacillin sodium (RS0036)

Instrument: Fourier Transform
Phase: Potassium bromide disc



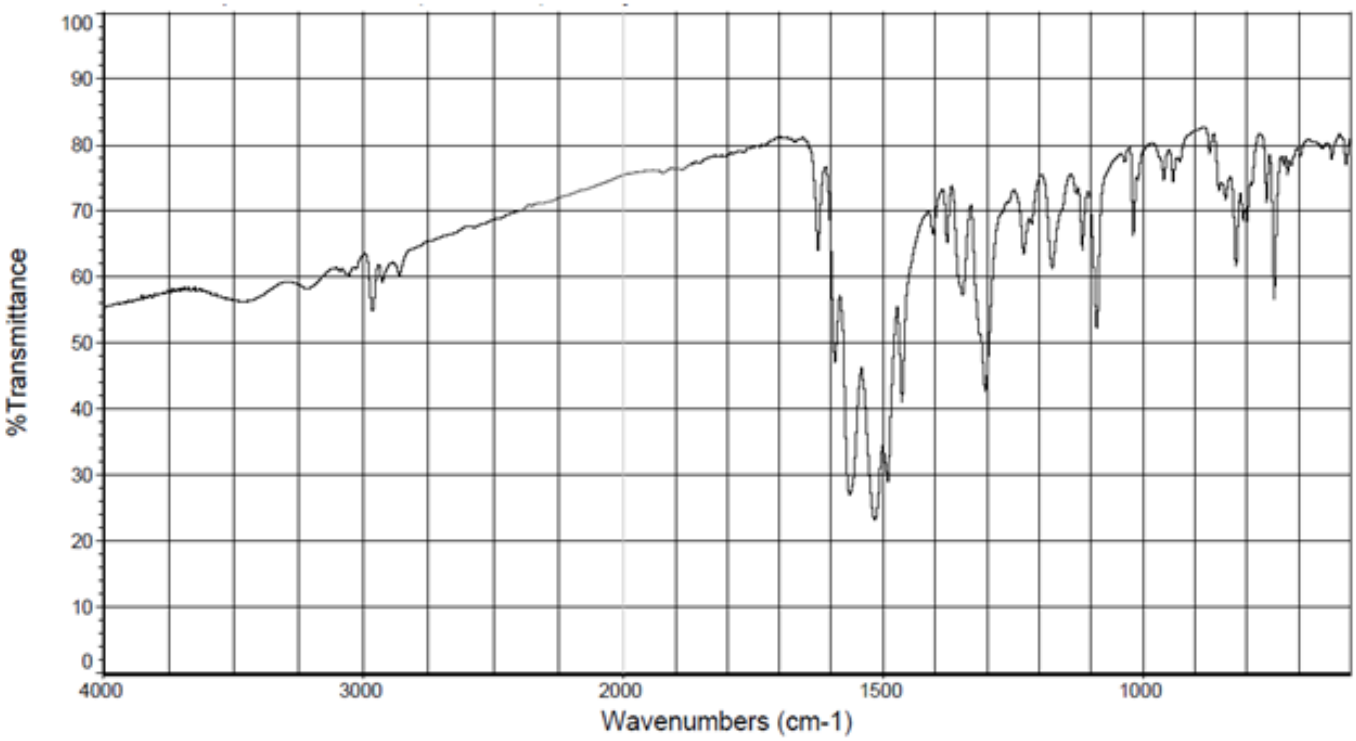
Clomifene citrate (RS0035)

Instrument: Fourier Transform
Phase: Potassium bromide disc



Clofazimine (RS00156)

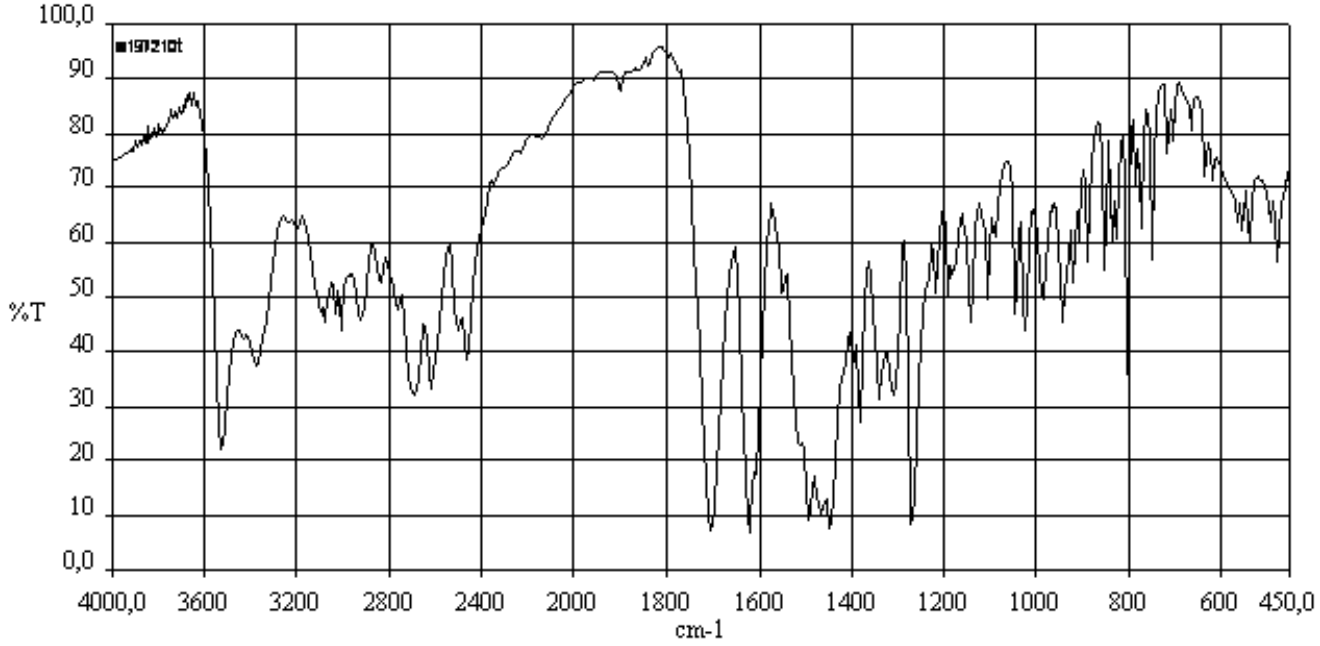
Instrument: Fourier Transform
Phase: Potassium bromide disc



Ciprofloxacin hydrochloride (RS0033)

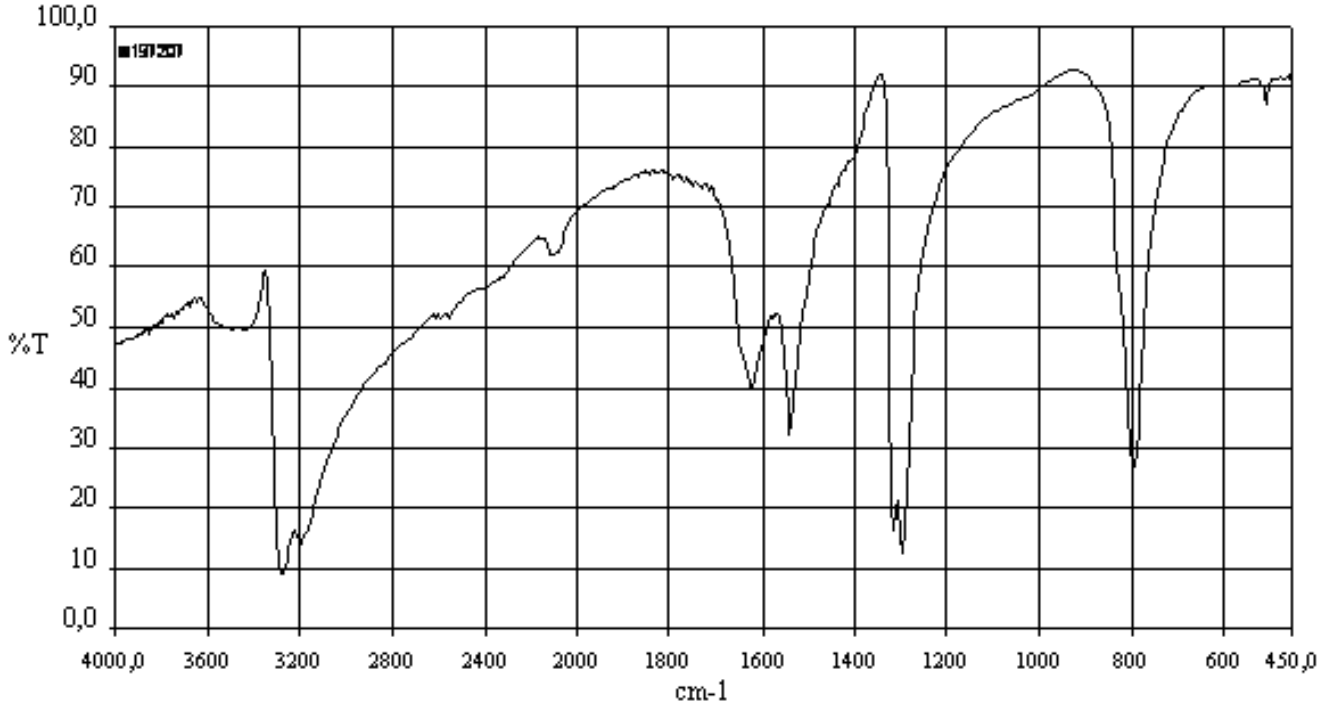
Instrument: Fourier Transform

Phase: Potassium bromide disc



Cisplatin (RS0034)

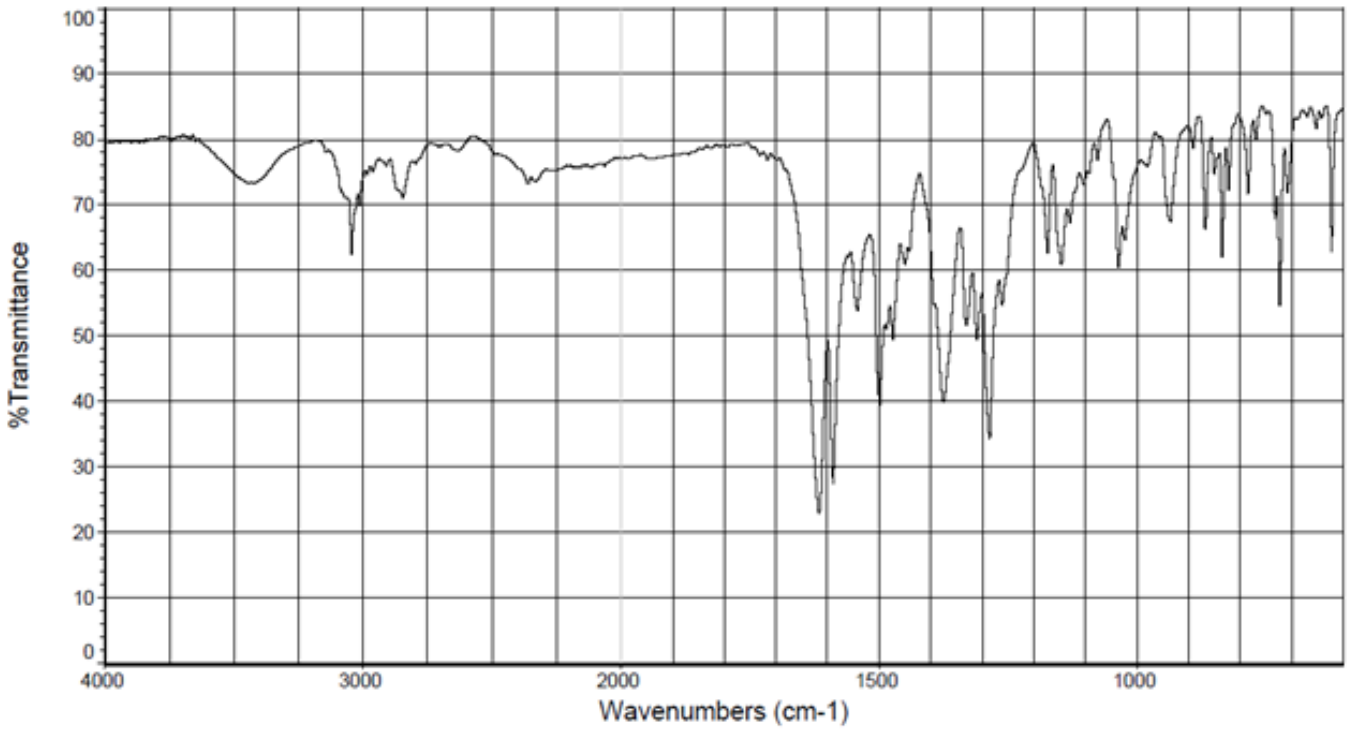
Instrument: Fourier Transform
Phase: Potassium bromide disc



Ciprofloxacin (RS00157)

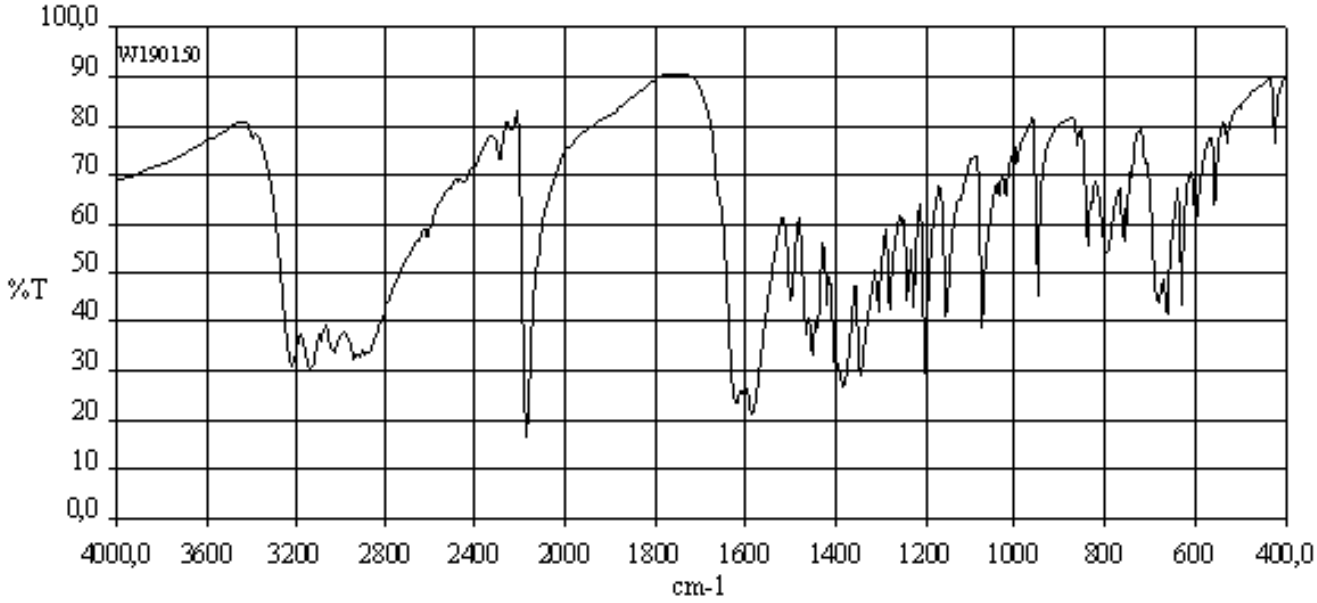
Instrument: Fourier Transform

Phase: Potassium bromide disc



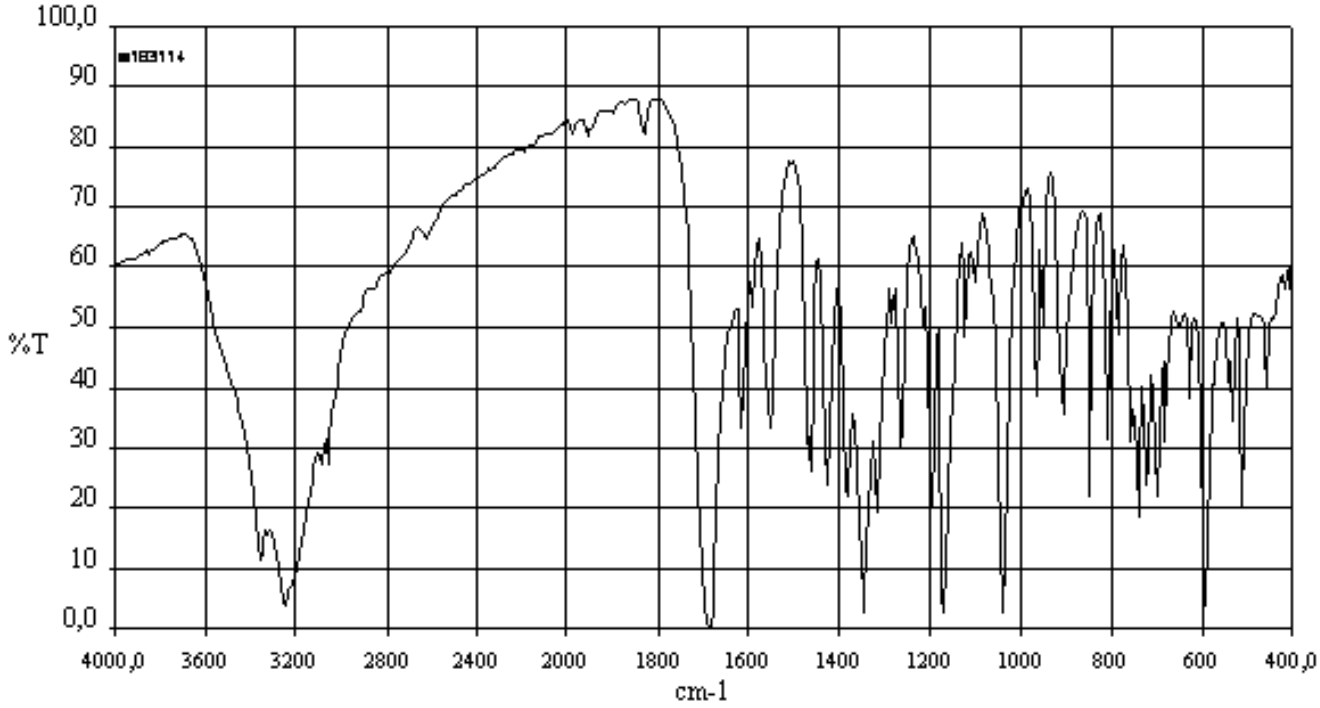
Cimetidine (RS0032)

Instrument: Fourier Transform
Phase: Potassium bromide disc



Chlortalidone (RS0031)

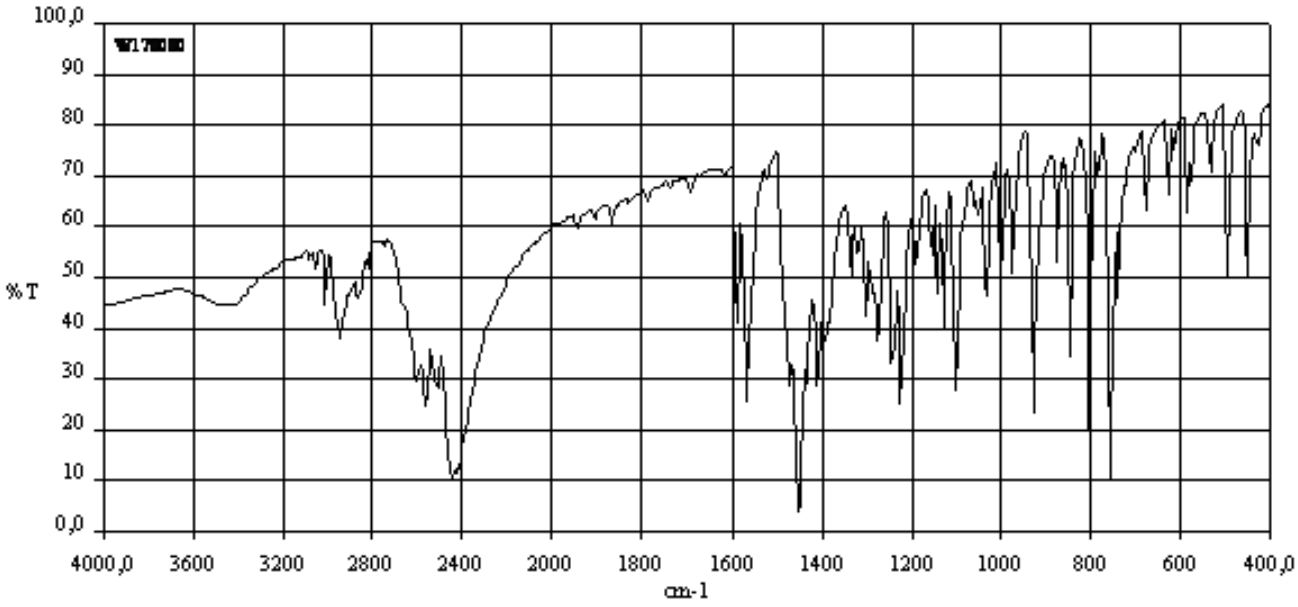
Instrument: Fourier Transform
Phase: Potassium bromide disc



Chlorpromazine hydrochloride (RS0030)

Instrument: Fourier Transform

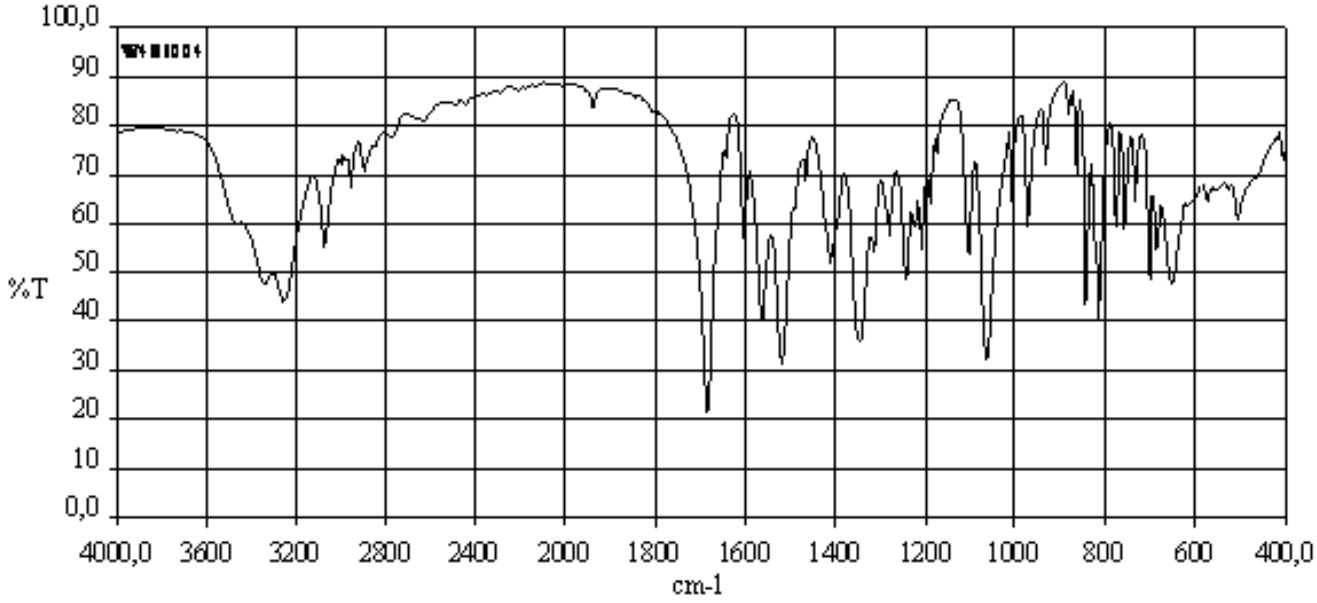
Phase: Potassium bromide disc



Chloramphenicol (RS0028)

Instrument: Fourier Transform

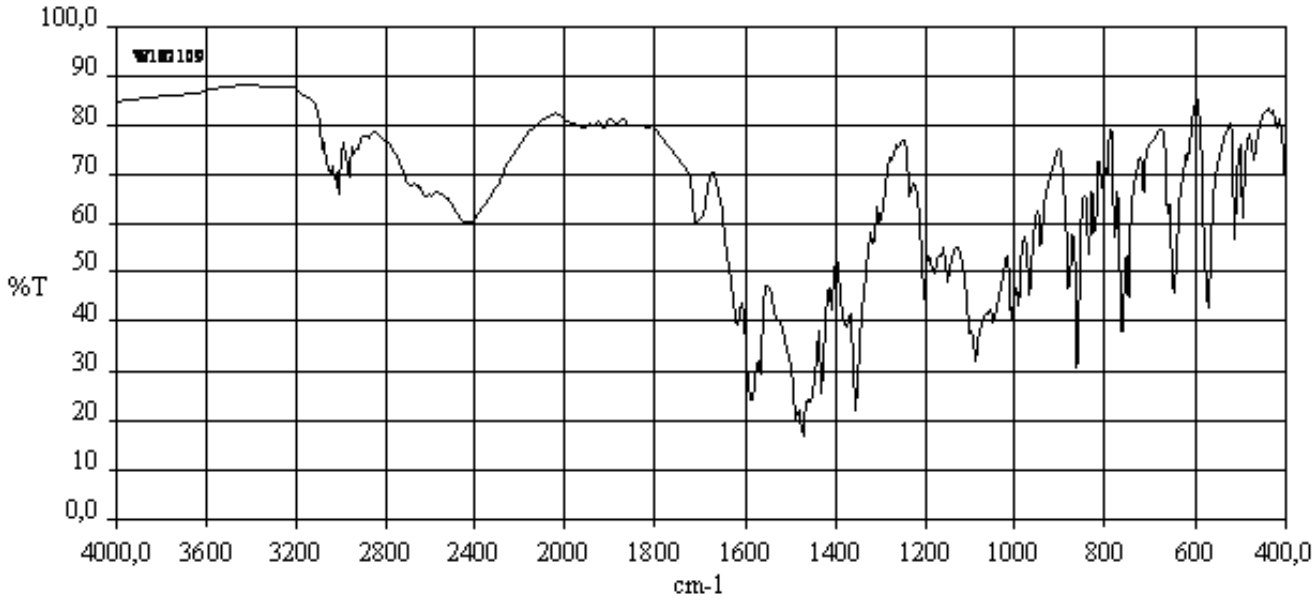
Phase: Potassium bromide disc



Chlorphenamine hydrogen maleate (RS0029)

Instrument: Fourier Transform

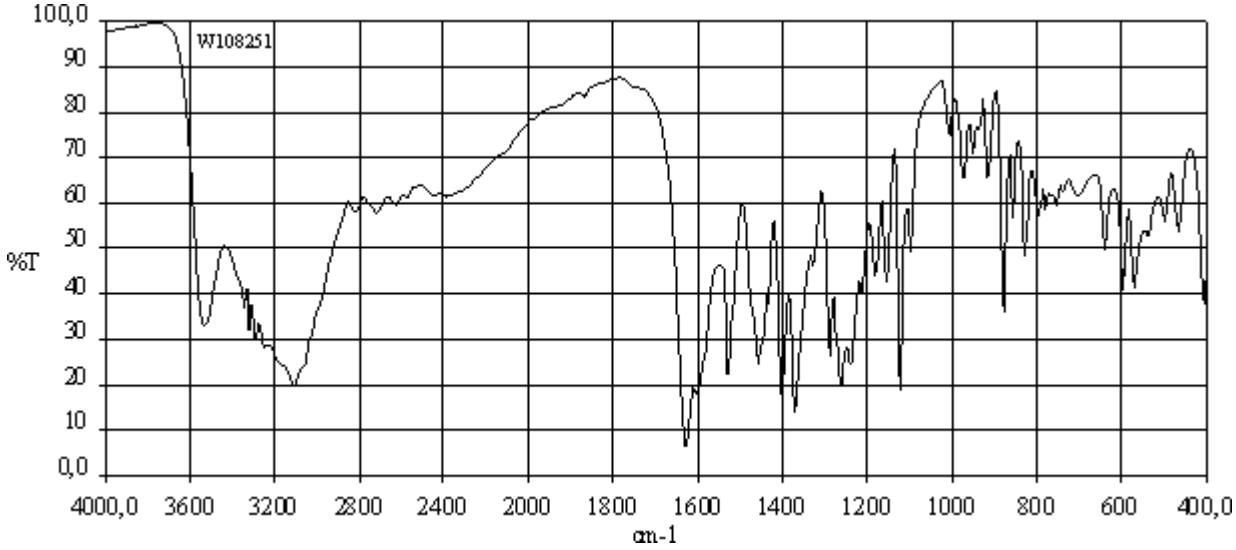
Phase: Potassium bromide disc



Carbidopa (RS00128)

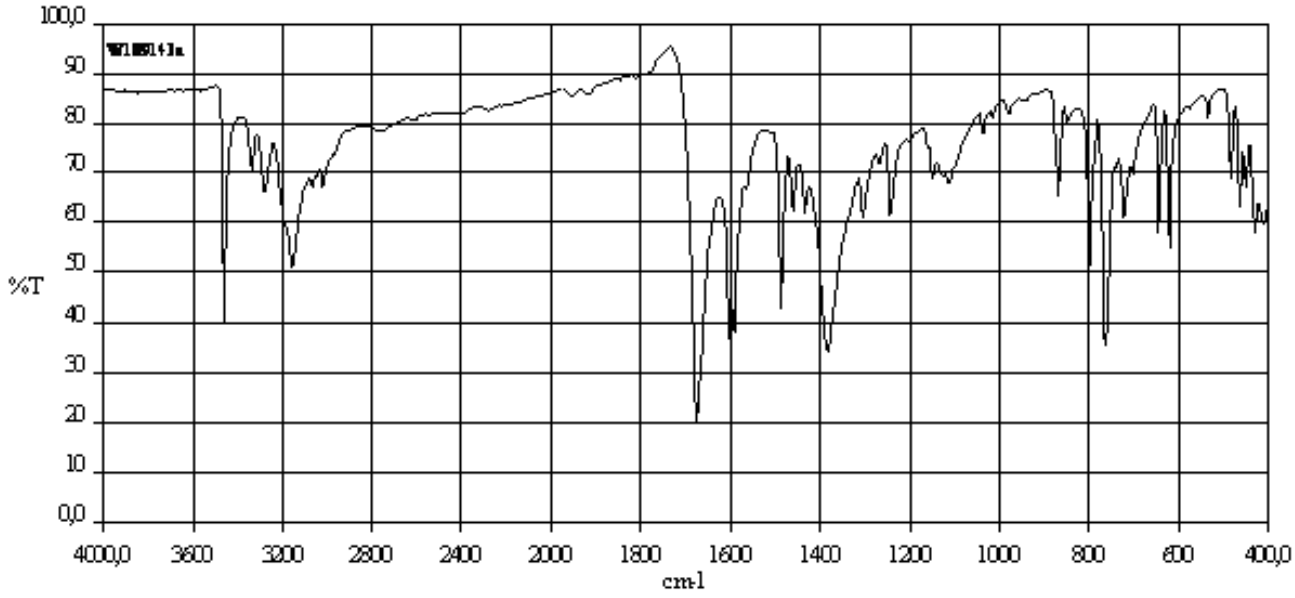
Instrument: Fourier Transform

Phase: Potassium bromide disc



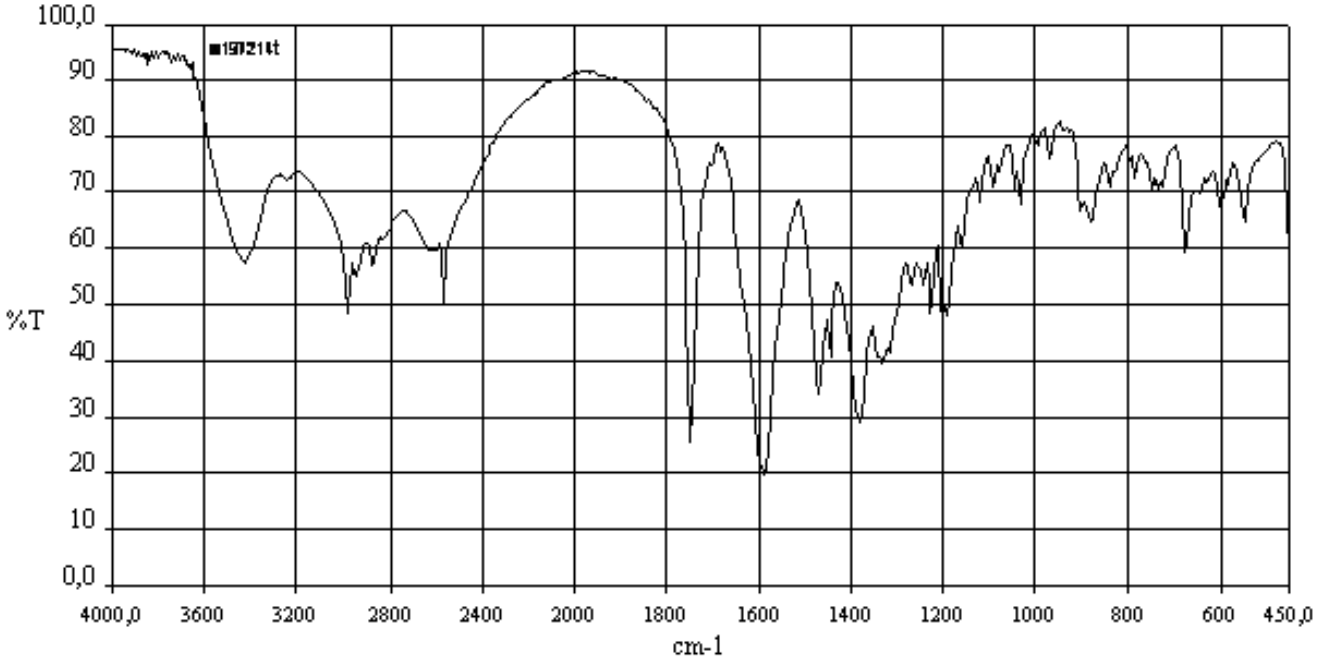
Carbamazepine (RS0027)

Instrument: Fourier Transform
Phase: Potassium bromide disc



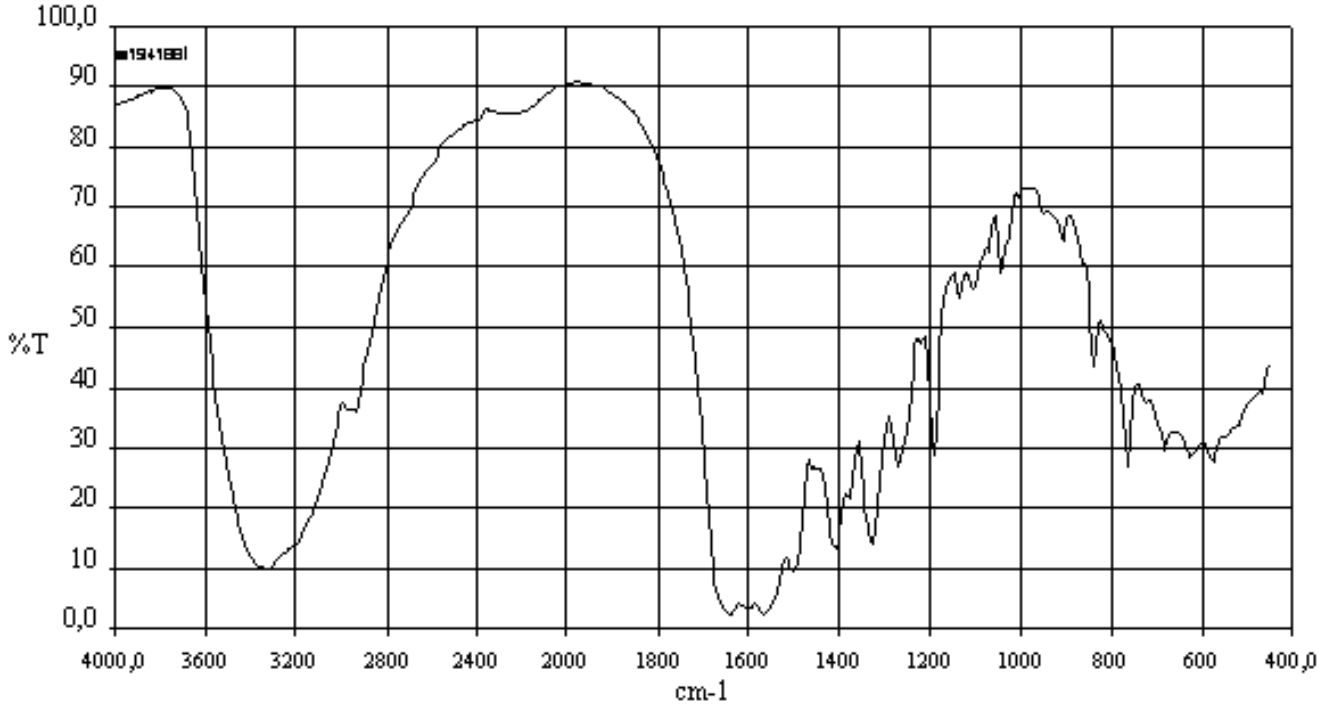
Captopril (RS0026)

Instrument: Fourier Transform
Phase: Potassium bromide disc



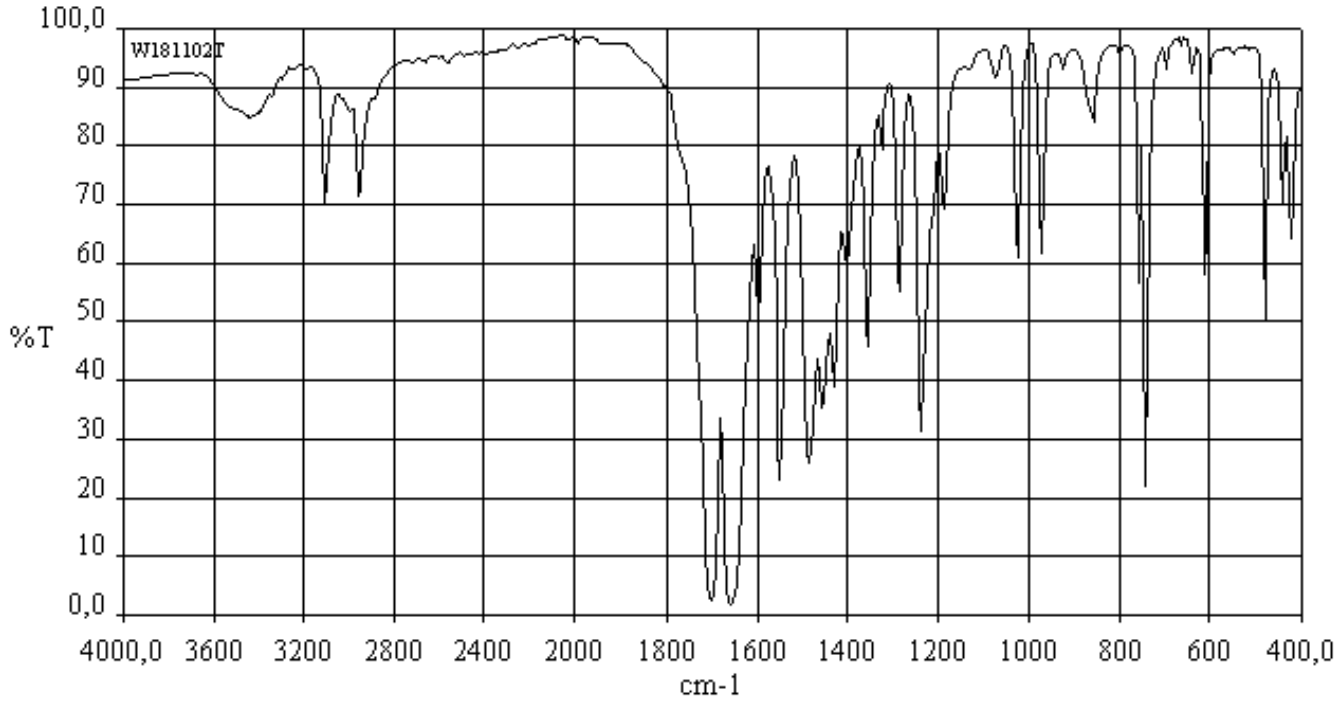
Calcium folinate (RS0025)

Instrument: Fourier Transform
Phase: Potassium bromide disc



Caffeine (RS0024)

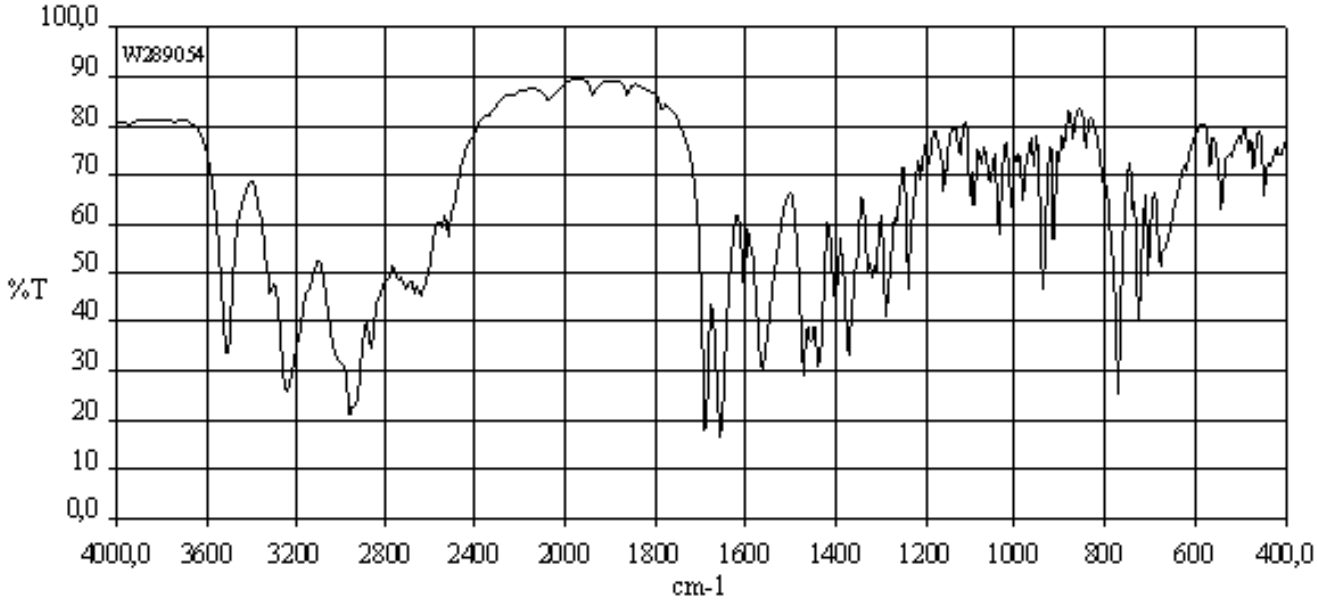
Instrument: Fourier Transform
Phase: Potassium bromide disc



Bupivacaine hydrochloride (RS0023)

Instrument: Fourier Transform

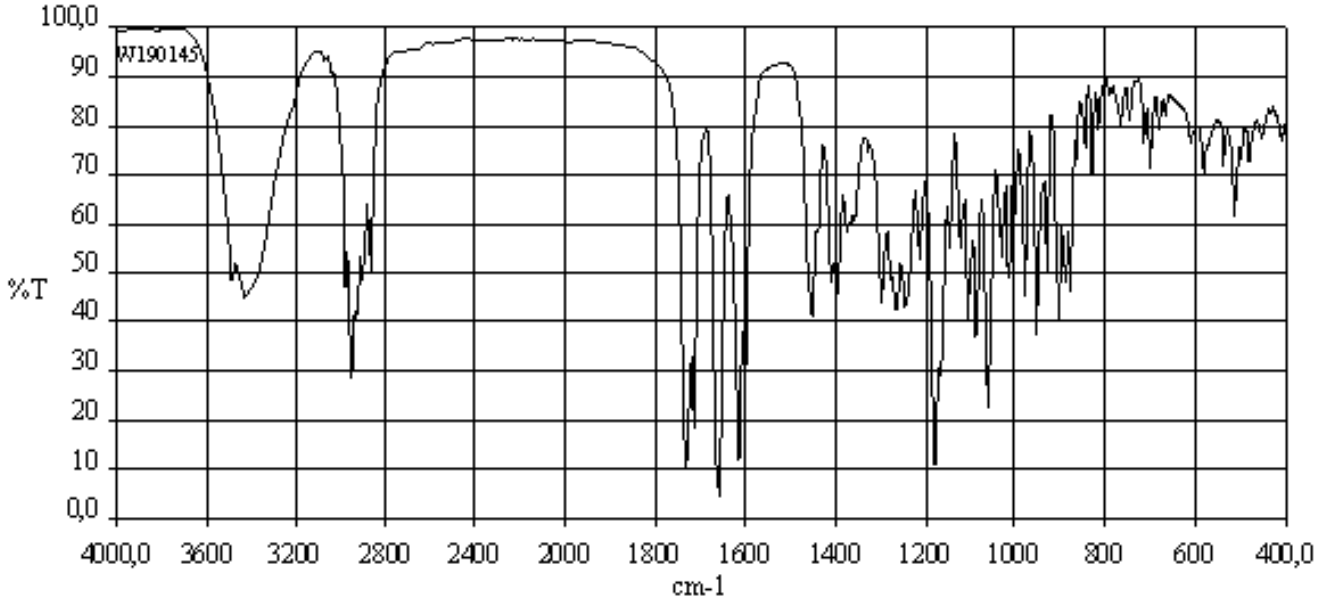
Phase: Potassium bromide disc



Betamethasone valerate (RS0022)

Instrument: Fourier Transform

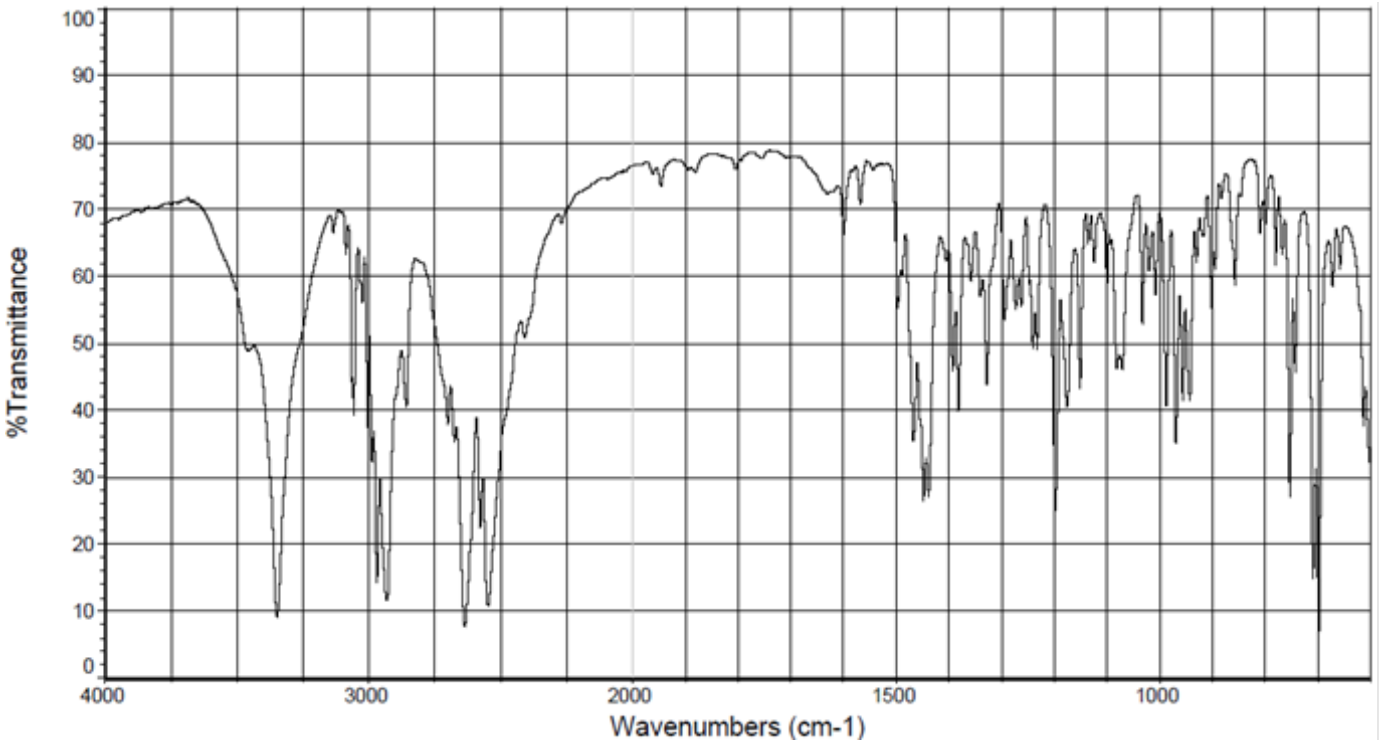
Phase: Potassium bromide disc



Biperiden hydrochloride (RS00164)

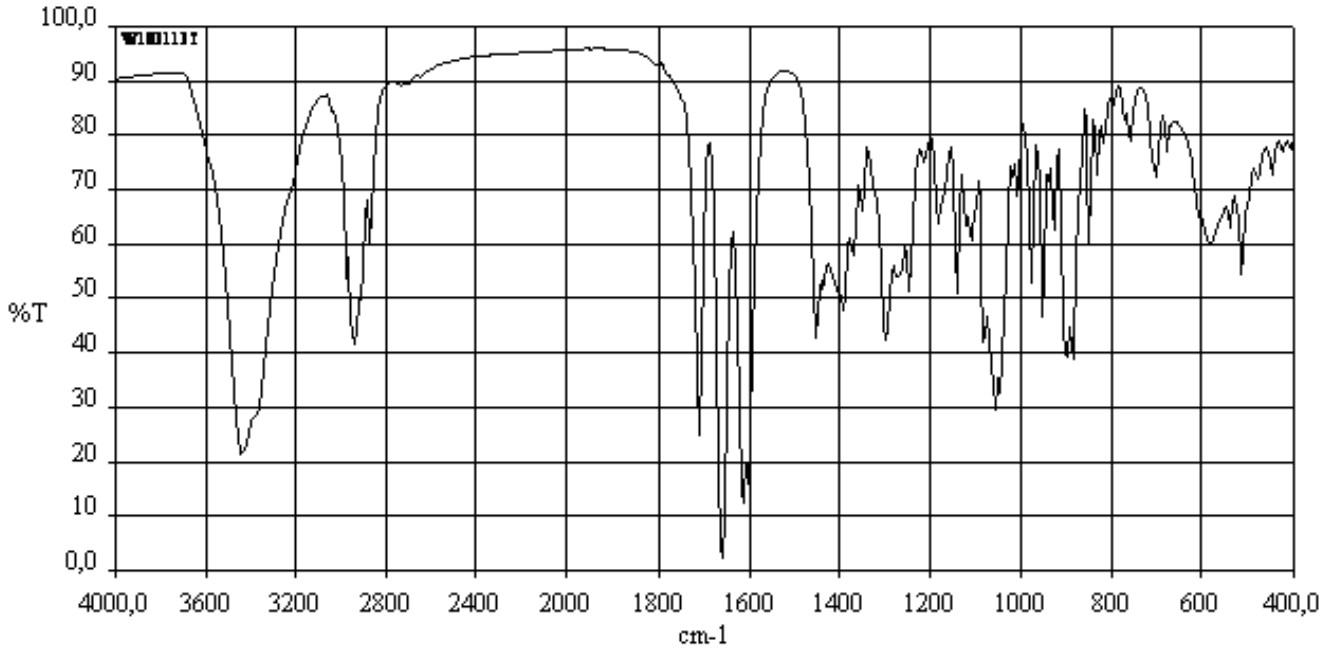
Instrument: Fourier Transform

Phase: Potassium bromide disc



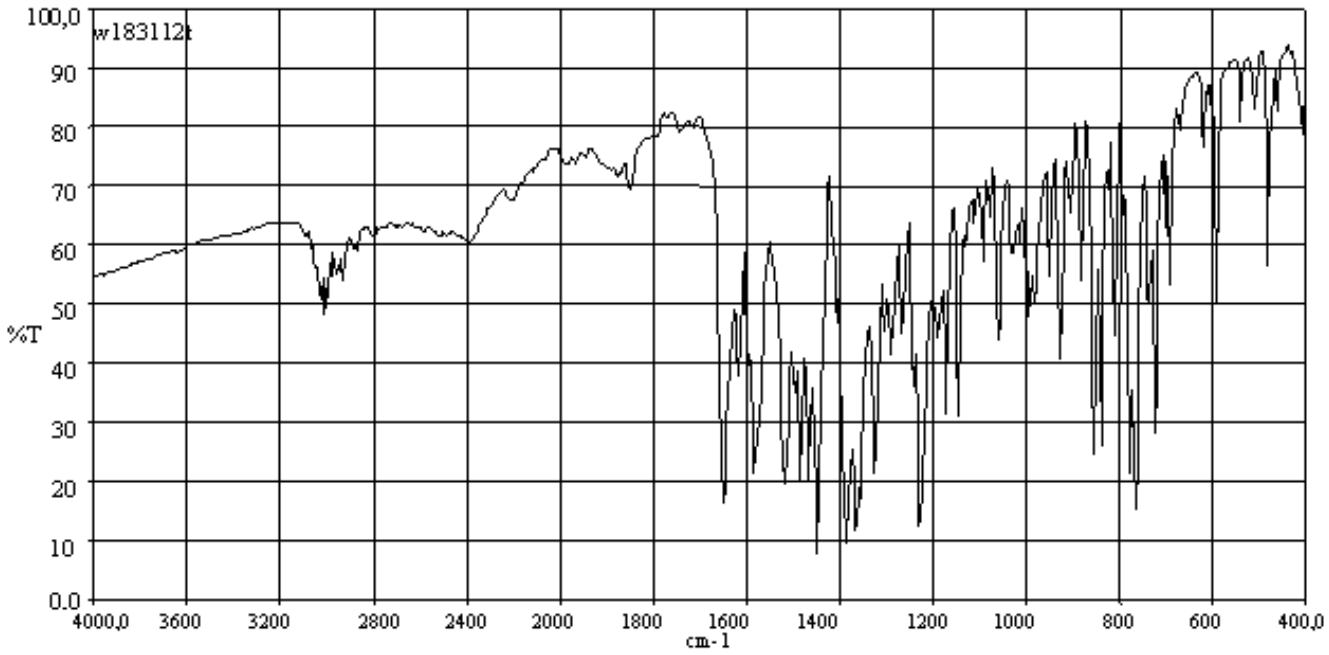
Betamethasone (RS0021)

Instrument: Fourier Transform
Phase: Potassium bromide disc



Bephenium hydroxynaphthoate (RS0020)

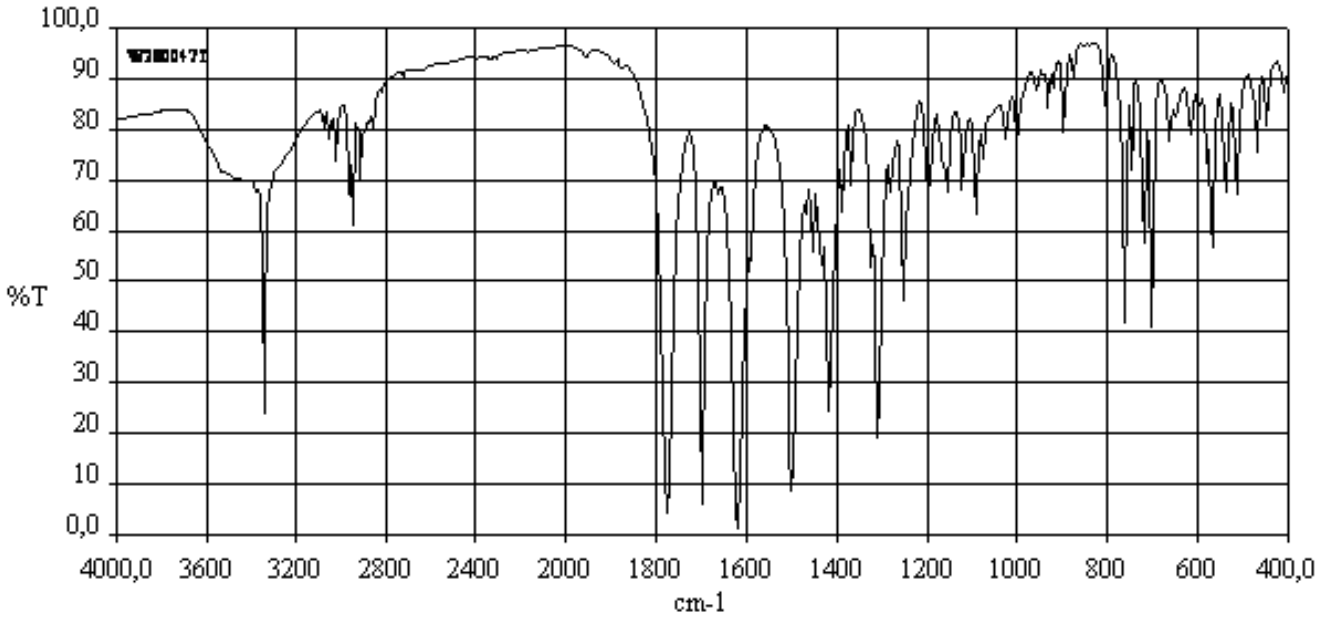
Instrument: Fourier Transform
Phase: Potassium bromide disc



Benzylpenicillin sodium (RS0019)

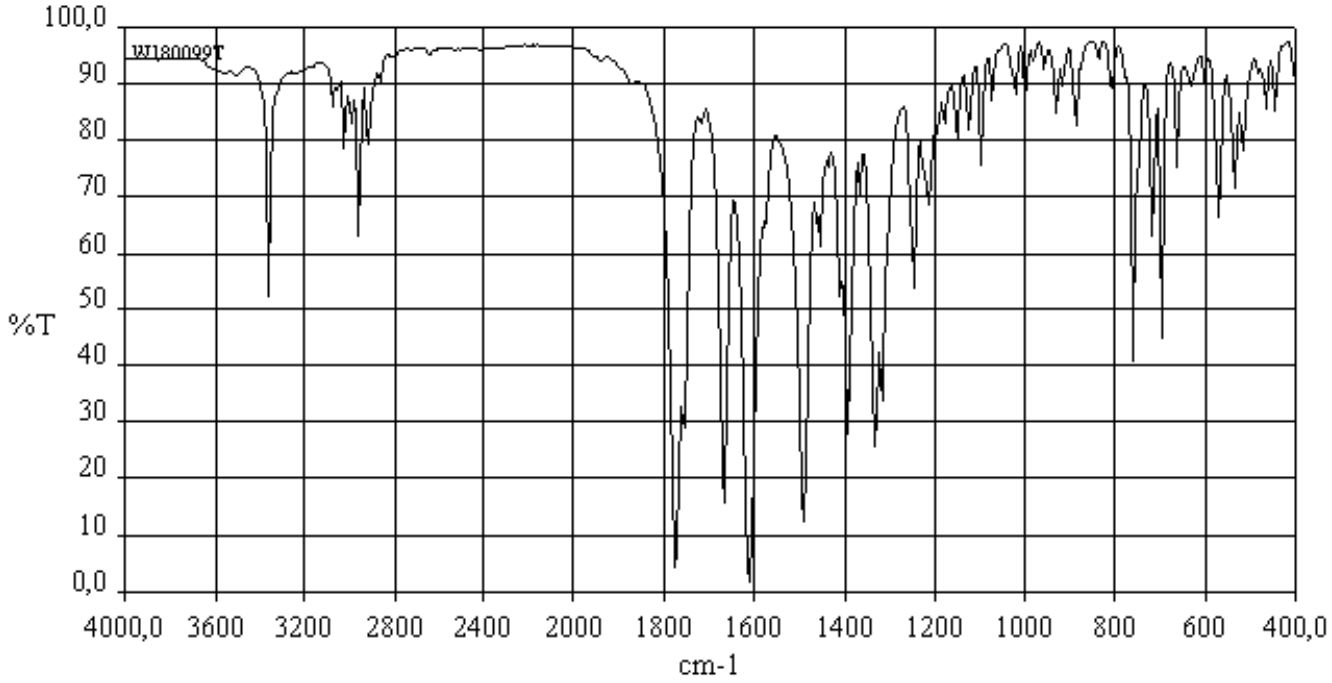
Instrument: Fourier Transform

Phase: Potassium bromide disc



Benzylpenicillin potassium (RS0018)

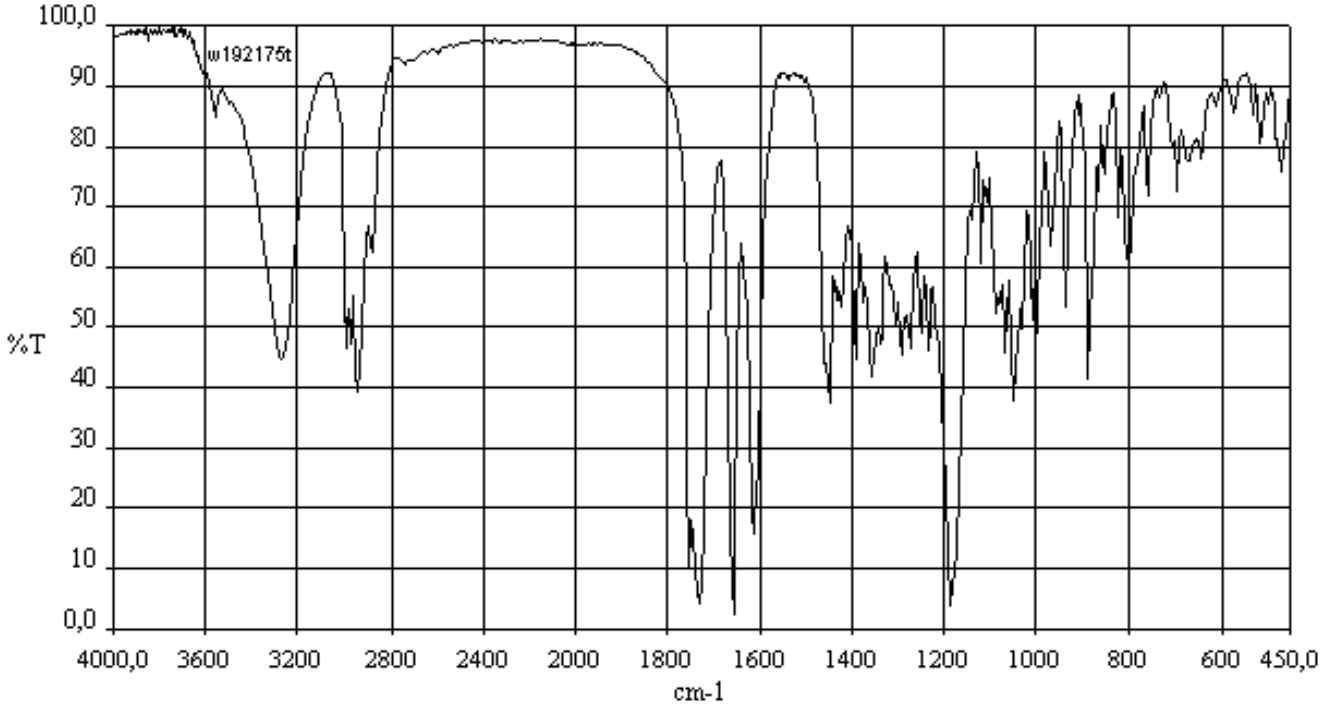
Instrument: Fourier Transform
Phase: Potassium bromide disc



Beclometasone dipropionate (RS0017)

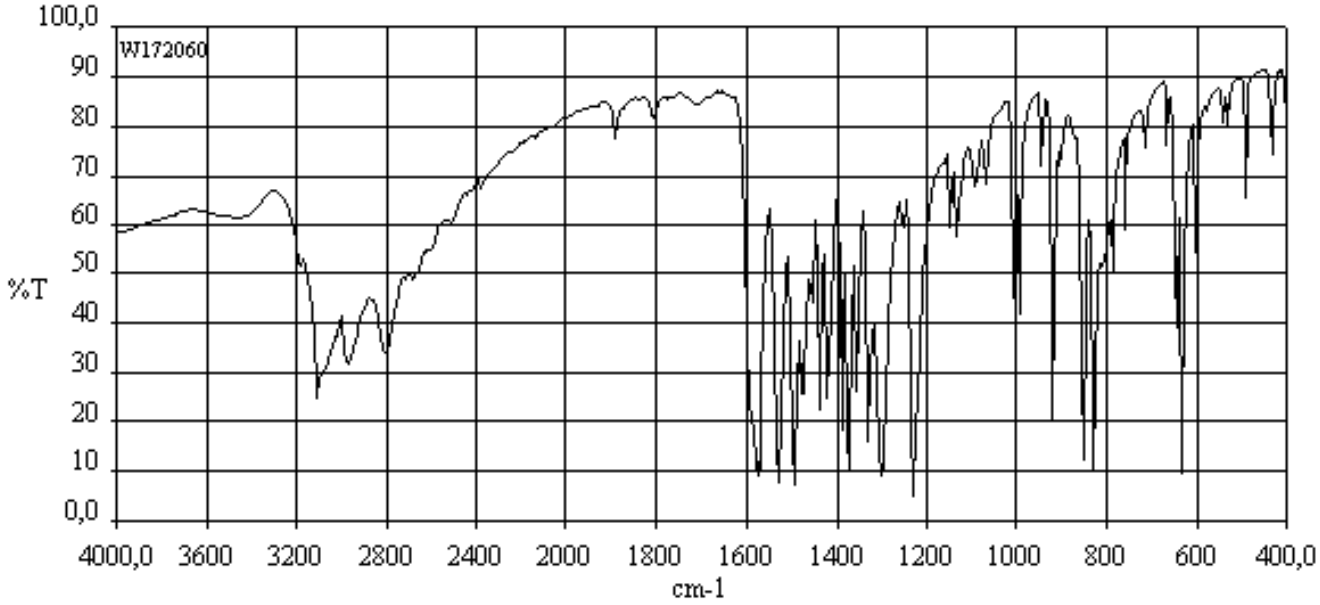
Instrument: Fourier Transform

Phase: Potassium bromide disc



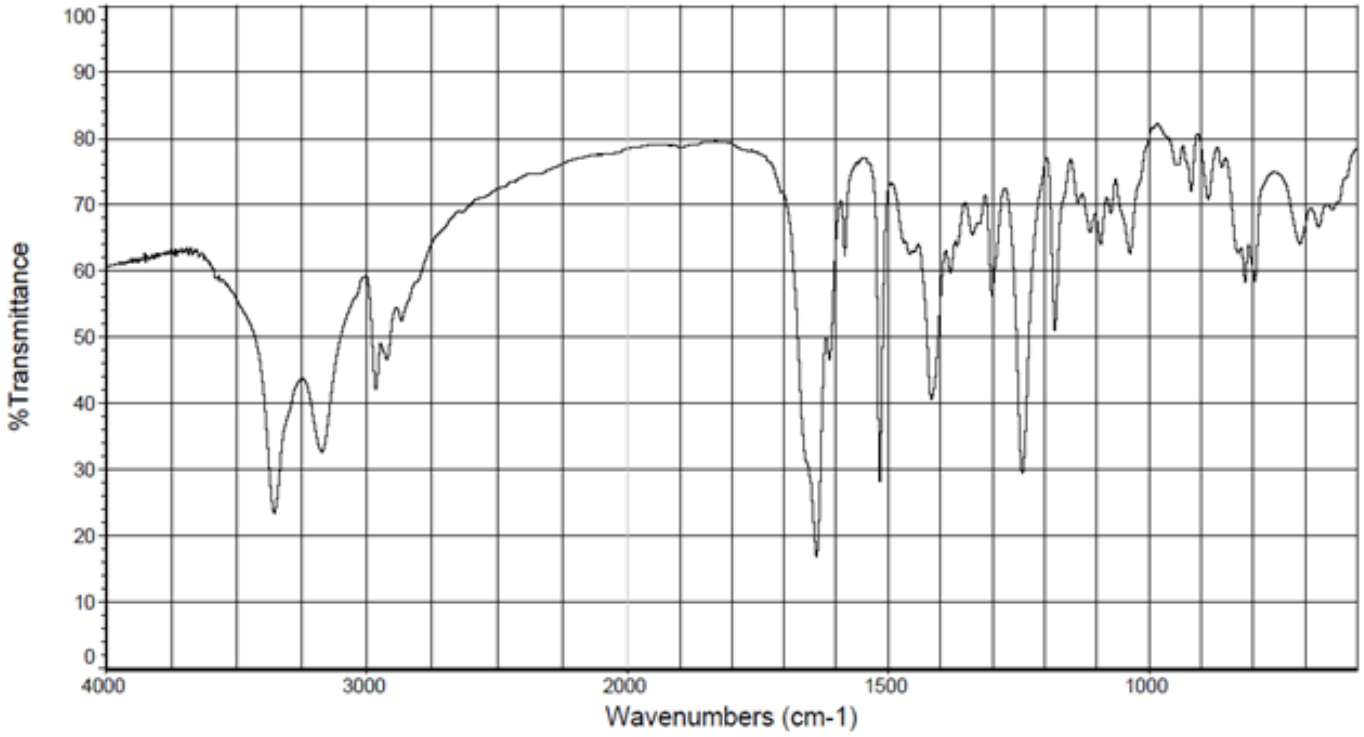
Azathioprine (RS0016)

Instrument: Fourier Transform
Phase: Potassium bromide disc



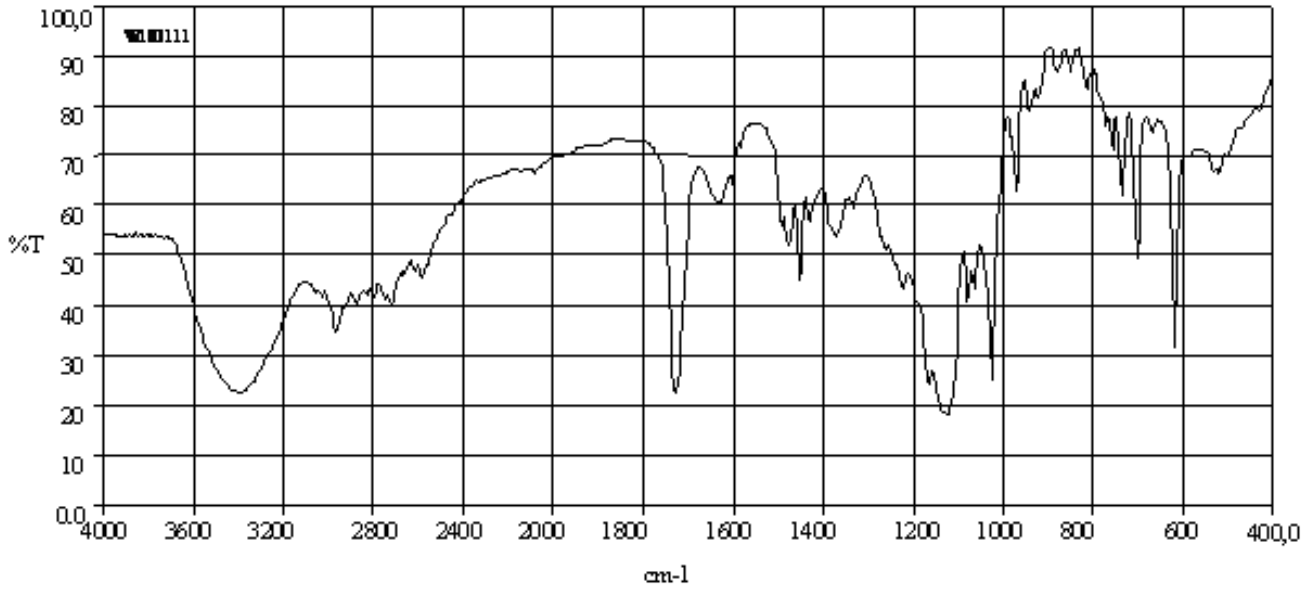
Atenolol (RS00155)

Instrument: Fourier Transform
Phase: Potassium bromide disc



Atropine sulfate (RS0015)

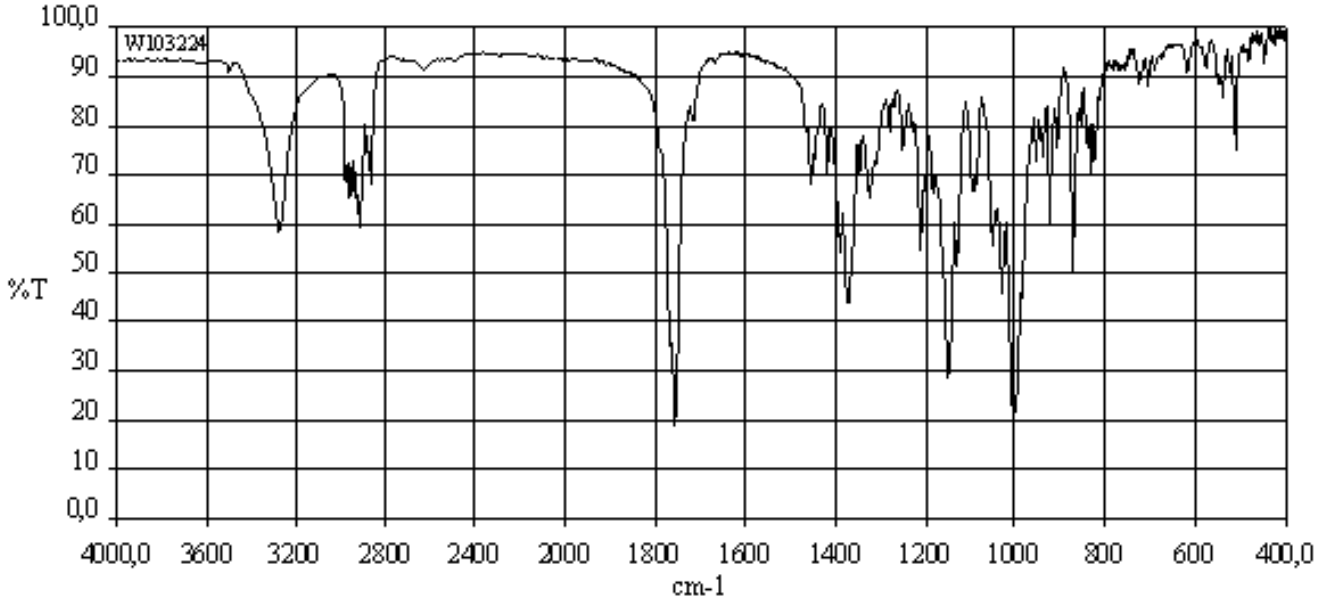
Instrument: Fourier Transform
Phase: Potassium bromide disc



Artesunate (RS0014)

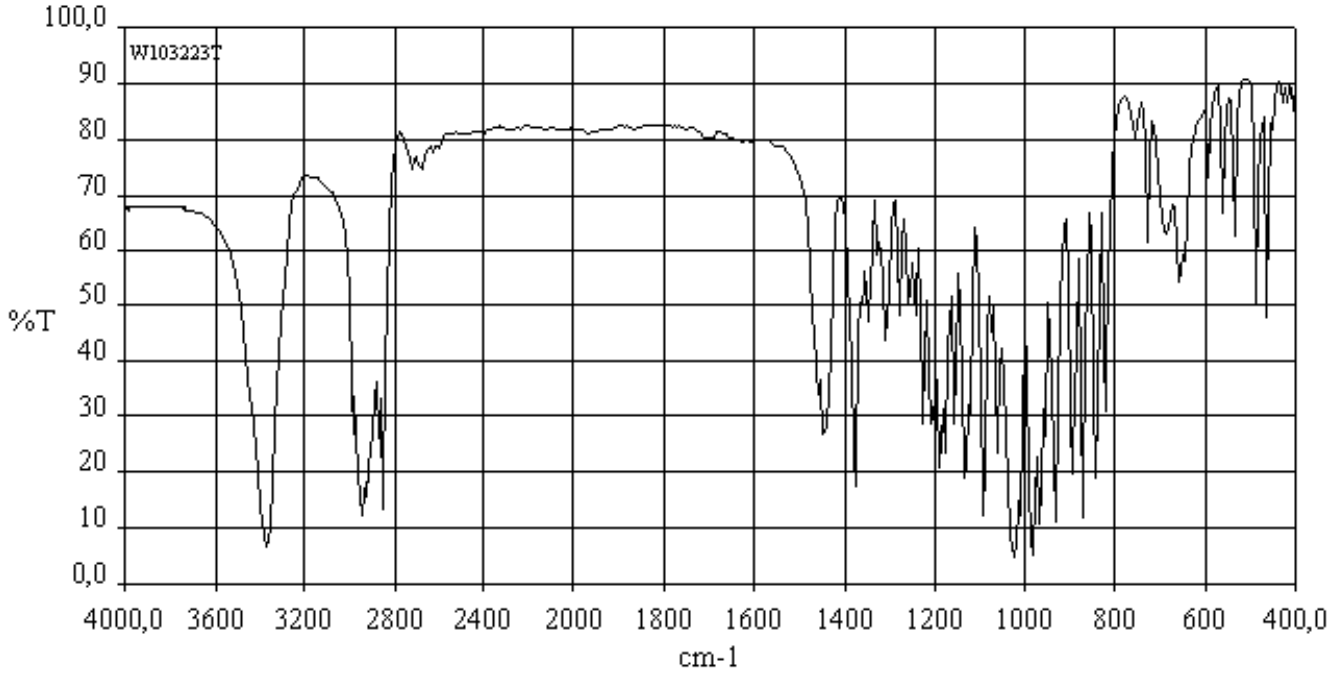
Instrument: Fourier Transform

Phase: Potassium bromide disc



Artenimol (RS0013)

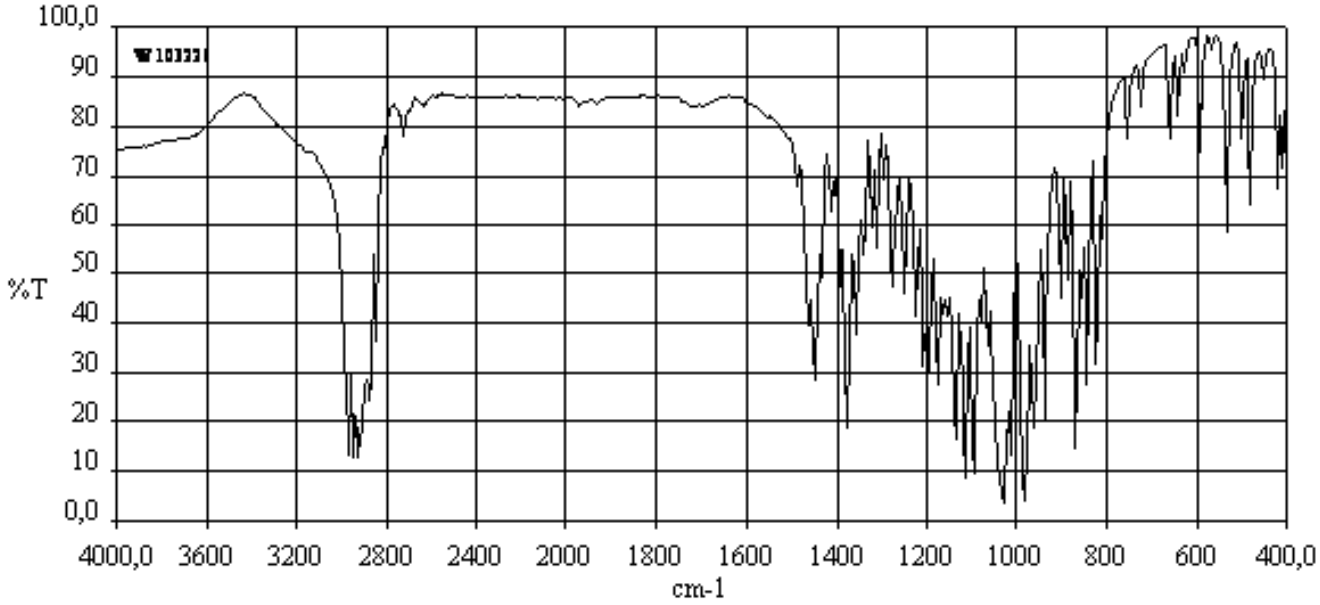
Instrument: Fourier Transform
Phase: Potassium bromide disc



Artemotil (RS0012)

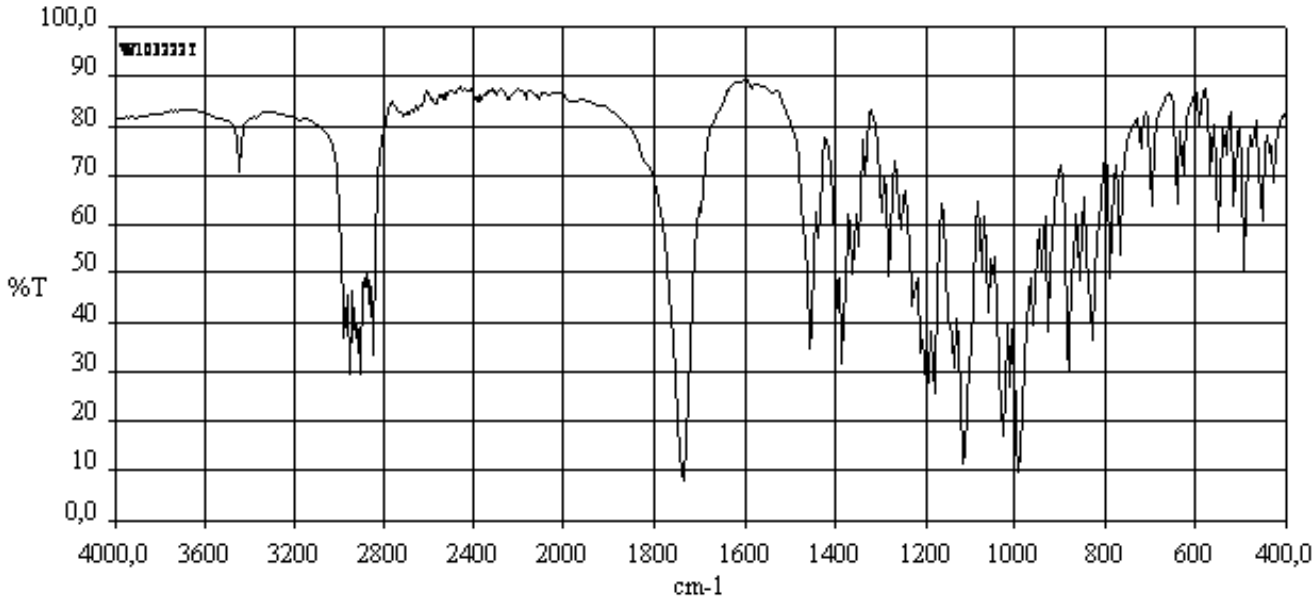
Instrument: Fourier Transform

Phase: Potassium bromide disc



Artemisinin (RS0011)

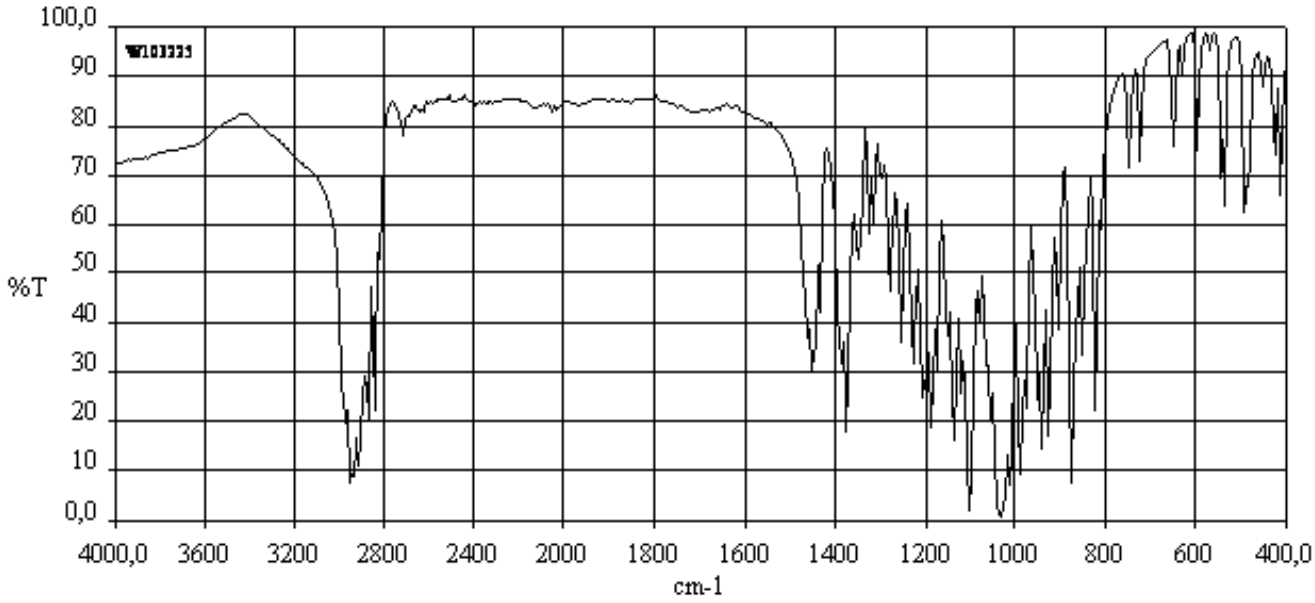
Instrument: Fourier Transform
Phase: Potassium bromide disc



Artemether (RS0010)

Instrument: Fourier Transform

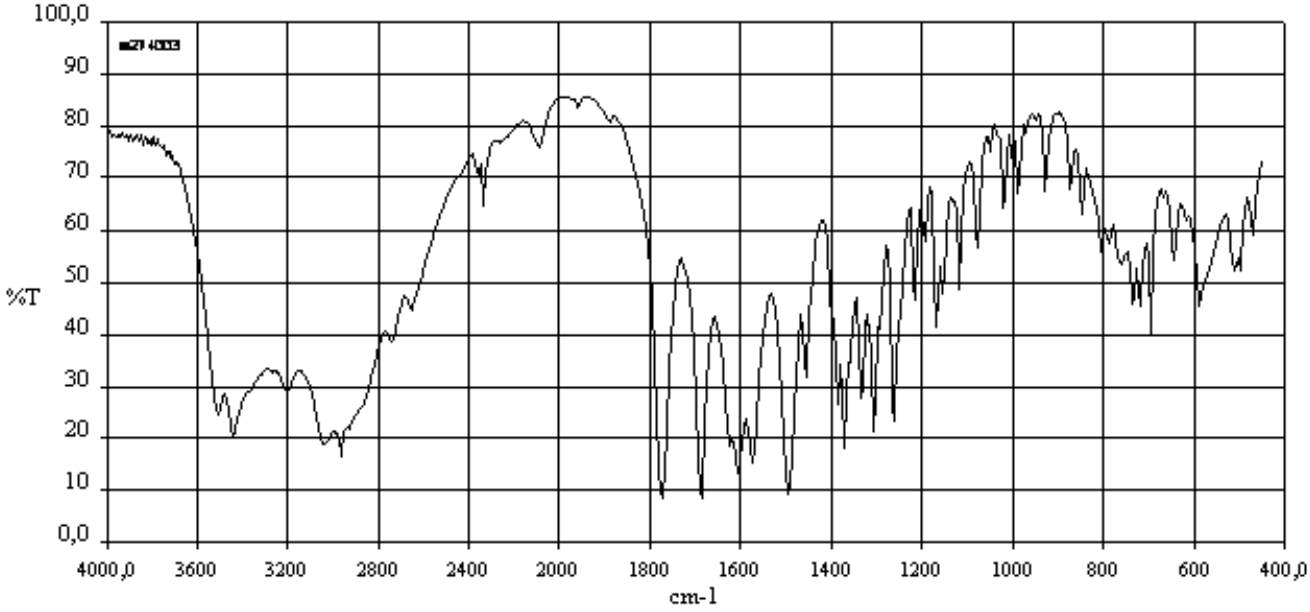
Phase: Potassium bromide disc



Ampicillin trihydrate (RS009)

Instrument: Fourier Transform

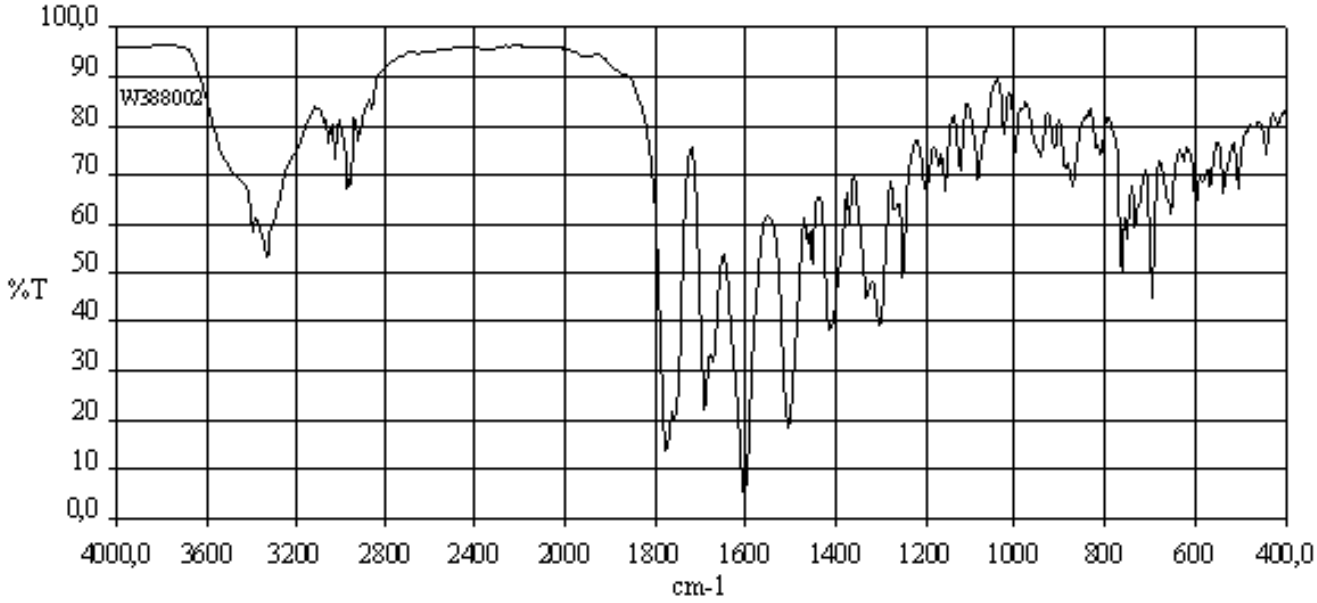
Phase: Potassium bromide disc



Ampicillin sodium (RS008)

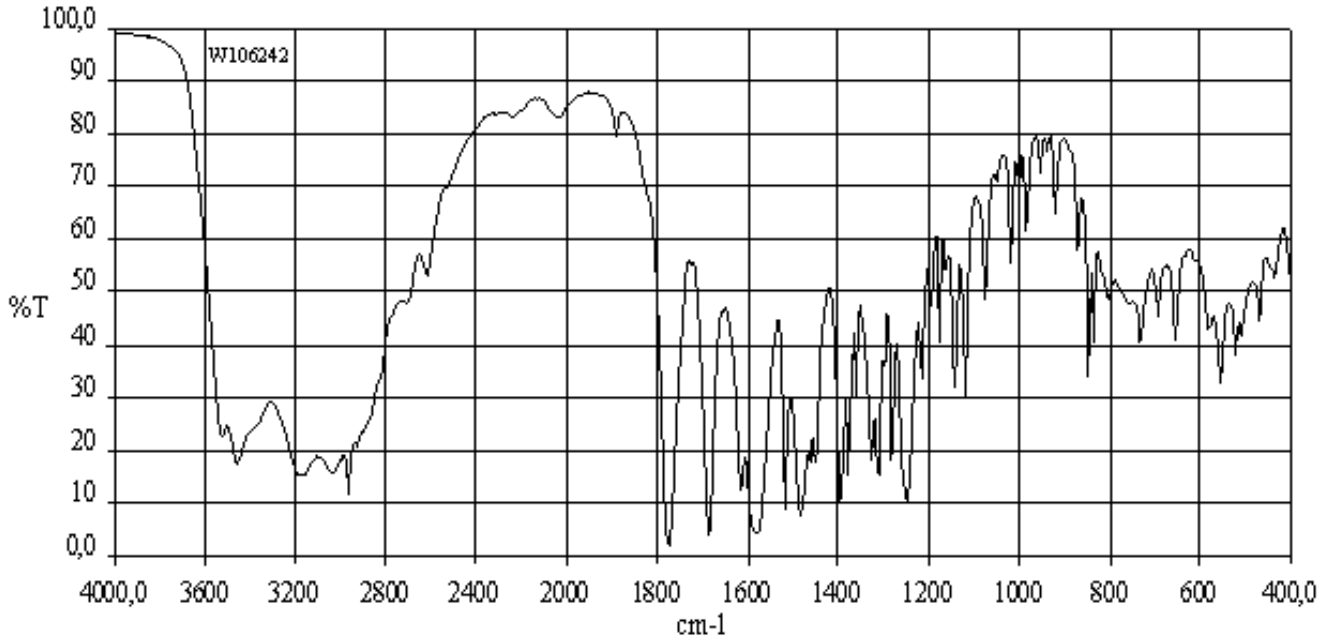
Instrument: Fourier Transform

Phase: Potassium bromide disc



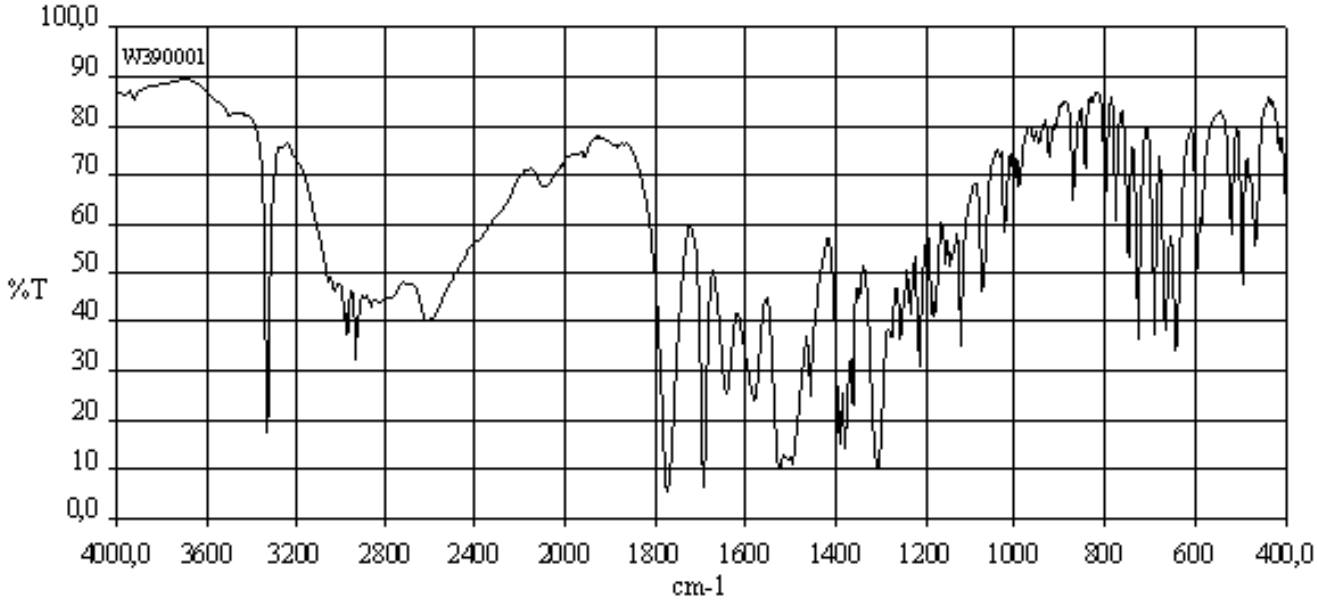
Amoxicillin trihydrate (RS006)

Instrument: Fourier Transform
Phase: Potassium bromide disc



Ampicillin (RS007)

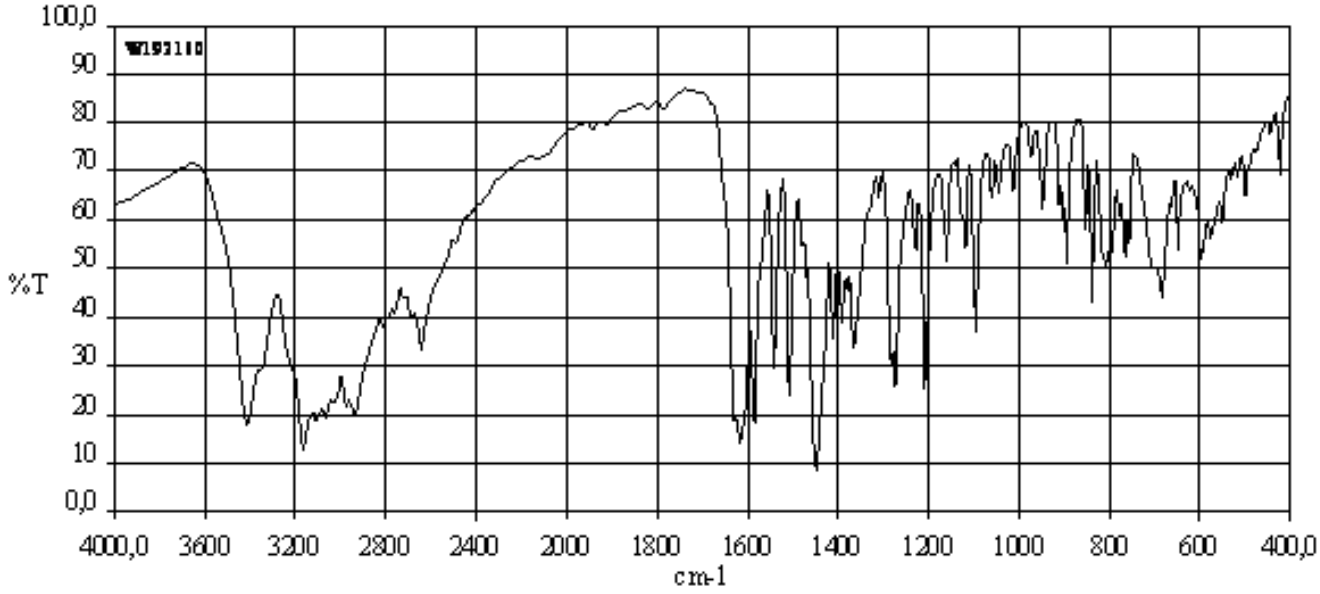
Instrument: Fourier Transform
Phase: Potassium bromide disc



Amodiaquine hydrochloride (RS005)

Instrument: Fourier Transform

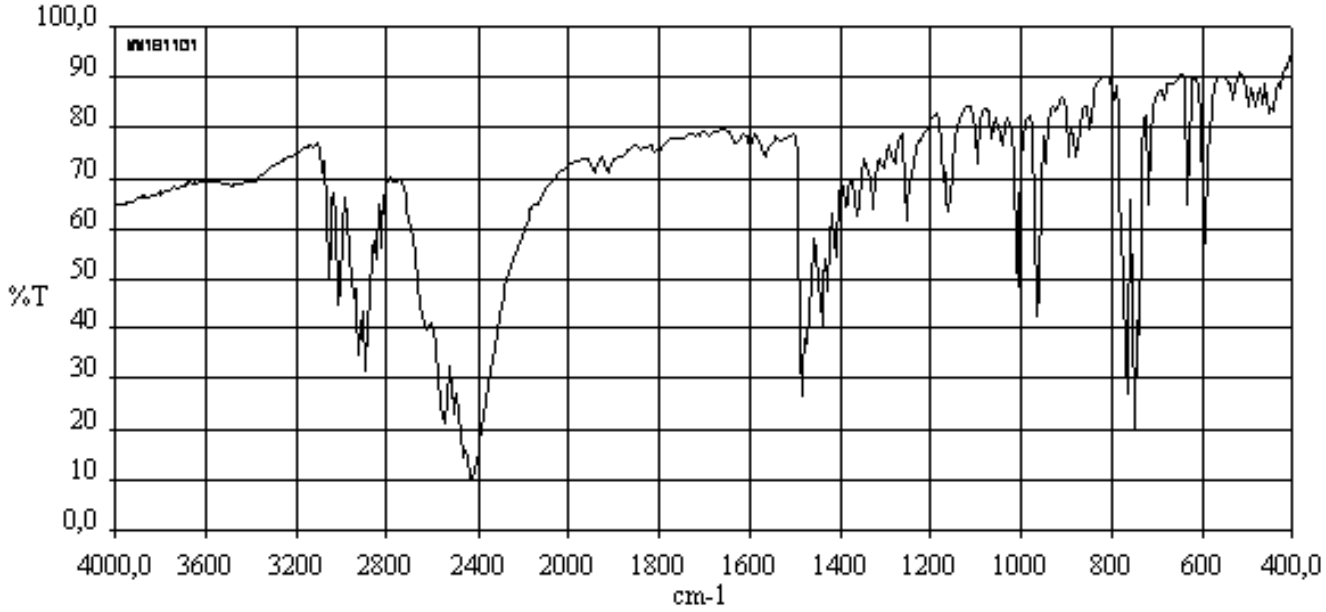
Phase: Potassium bromide disc



Amitriptyline hydrochloride (RS004)

Instrument: Fourier Transform

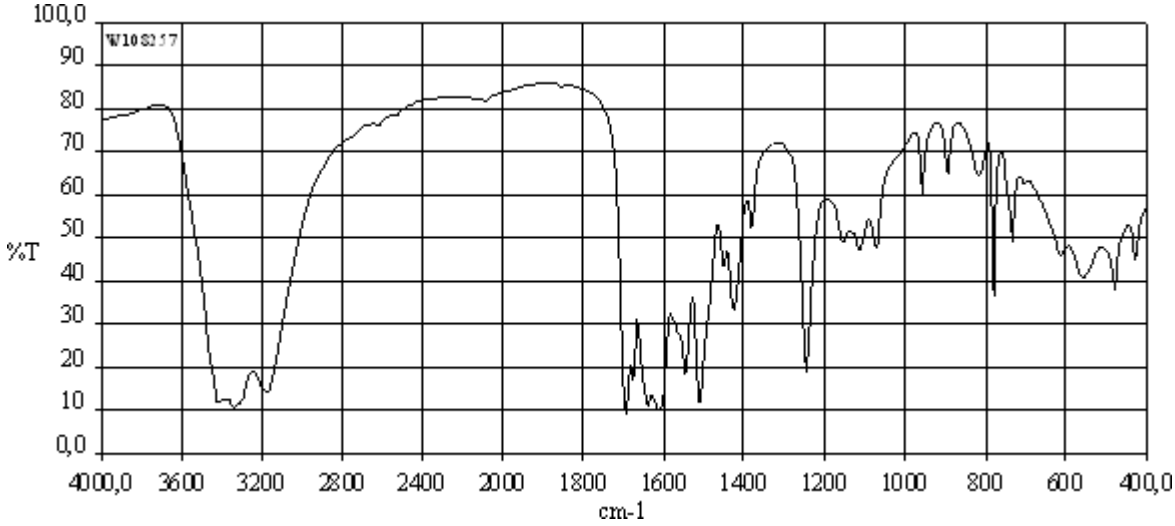
Phase: Potassium bromide disc



Amiloride hydrochloride (RS00127)

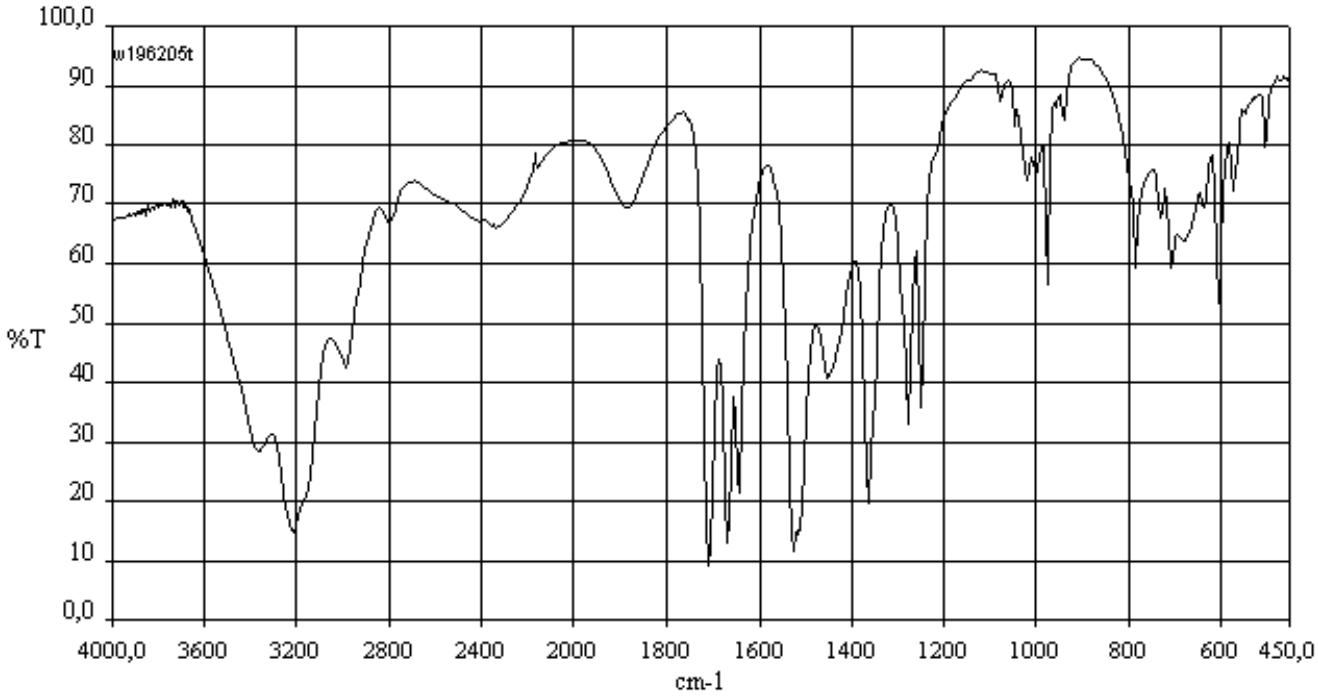
Instrument: Fourier Transform

Phase: Potassium bromide disc



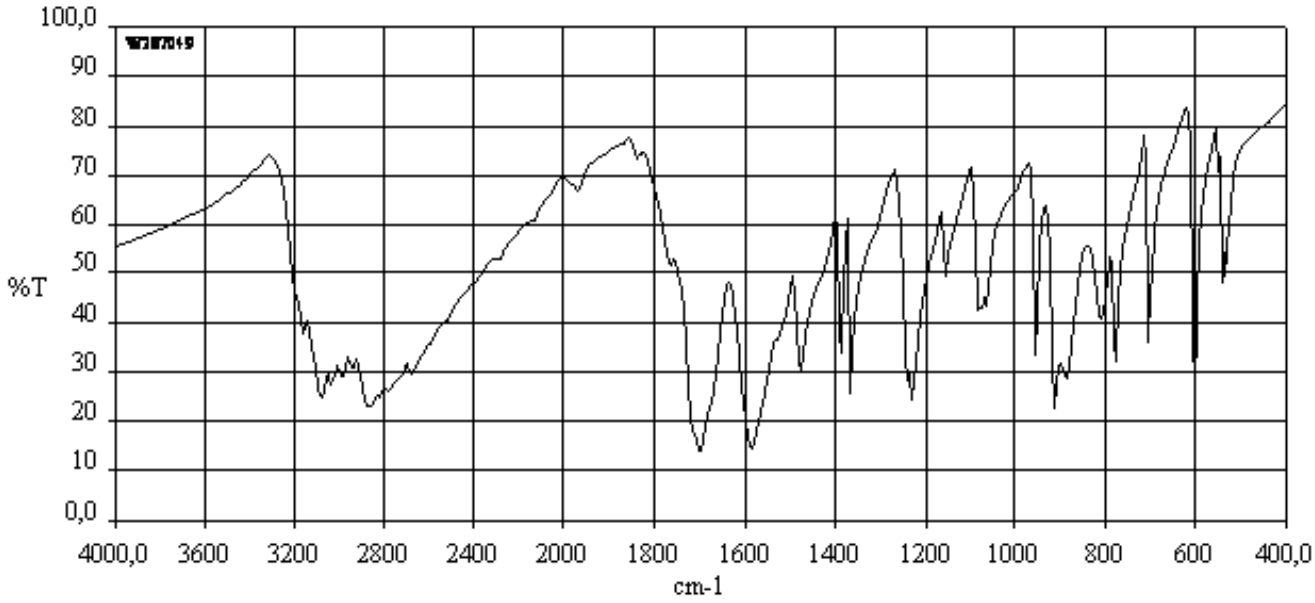
Amidotrizoic acid (RS003)

Instrument: Fourier Transform
Phase: Potassium bromide disc



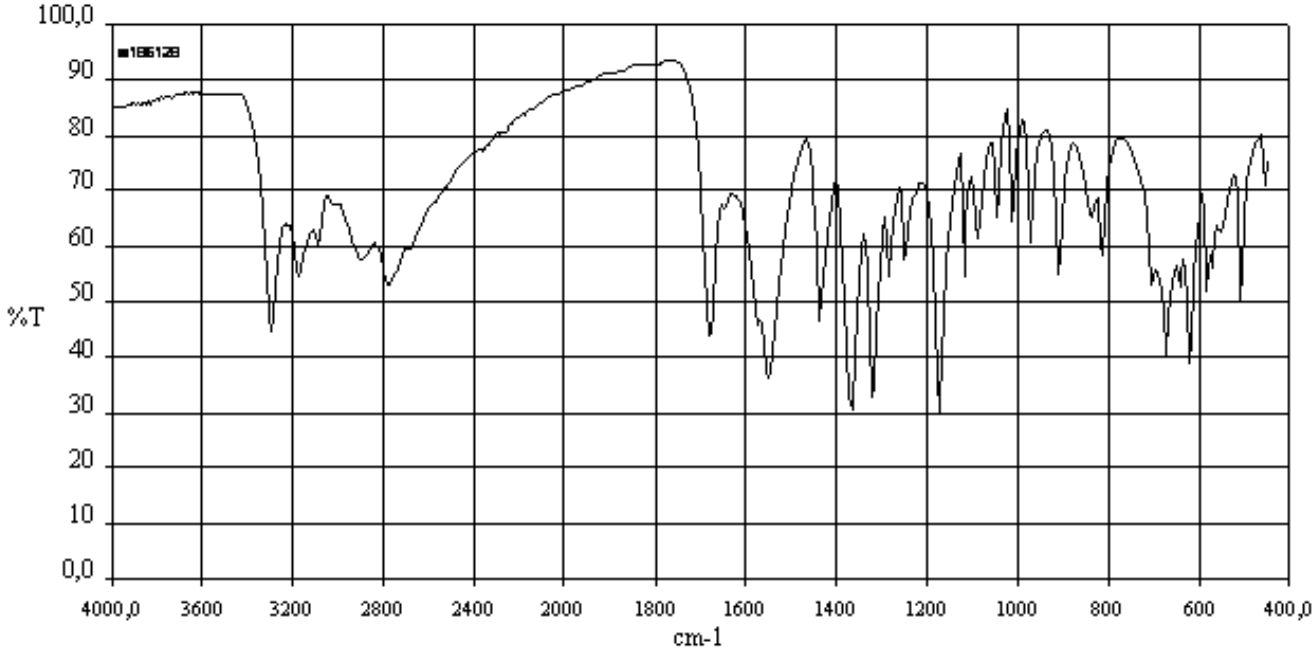
Allopurinol (RS002)

Instrument: Fourier Transform
Phase: Potassium bromide disc



Acetazolamide (RS001)

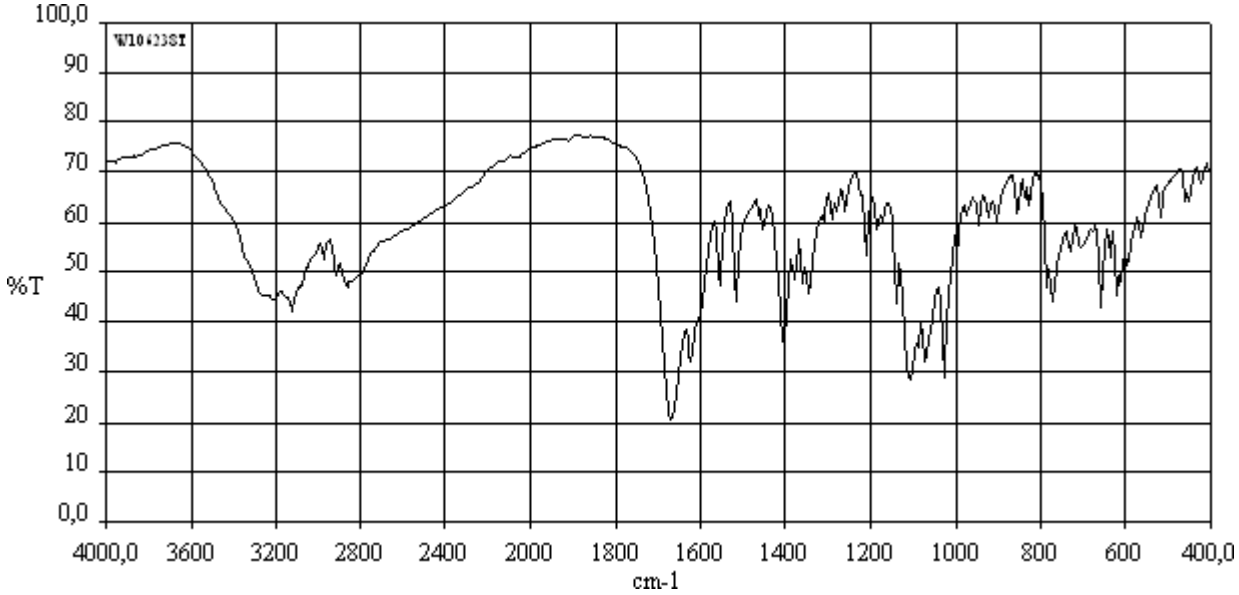
Instrument: Fourier Transform
Phase: Potassium bromide disc



Abacavir sulfate (RS00126)

Instrument: Fourier Transform

Phase: Potassium bromide disc



5.8 Methods of sterilization

Sterilization is necessary for the complete destruction or removal of all microorganisms (including spore-forming and non-spore-forming bacteria, viruses, fungi, and protozoa) that could contaminate pharmaceuticals or other materials and thereby constitute a health hazard. Since the achievement of the absolute state of sterility cannot be demonstrated, the sterility of a pharmaceutical preparation can be defined only in terms of probability. The efficacy of any sterilization process will depend on the nature of the product, the extent and type of any contamination, and the conditions under which the final product has been prepared. The requirements for Good Manufacturing Practice should be observed throughout all stages of manufacture and sterilization.

Classical sterilization techniques using saturated steam under pressure or hot air are the most reliable and should be used whenever possible. Other sterilization methods include filtration, ionizing radiation (gamma and electron-beam radiation), and gas (ethylene oxide, formaldehyde).

For products that cannot be sterilized in the final containers, aseptic processing is necessary. Materials and products that have been sterilized by one of the above processes are transferred to presterilized containers and sealed, both operations being carried out under controlled aseptic conditions.

Whatever method of sterilization is chosen, the procedure must be validated for each type of product or material, both with respect to the assurance of sterility and to ensure that no adverse change has taken place within the product. Failure to follow precisely a defined, validated process could result in a non-sterile or deteriorated product. A typical validation programme for steam or dry-heat sterilization requires the correlation of temperature measurements, made with sensory devices to demonstrate heat penetration and heat distribution, with the destruction of biological indicators, i.e. preparations of specific microorganisms known to have high resistance to the particular sterilization process. Biological indicators are also used to validate other sterilization methods (see specific methods), and sometimes for routine control of individual cycles. Periodic revalidation is recommended.

Heating in an autoclave (steam sterilization)

Exposure of microorganisms to saturated steam under pressure in an autoclave achieves their destruction by the irreversible denaturation of enzymes and structural proteins. The temperature at which denaturation occurs varies inversely with the amount of water present. Sterilization in saturated steam thus requires precise control of time, temperature, and pressure. As displacement of the air by steam is unlikely to be readily achieved, the air should be evacuated from the autoclave before admission of steam. This method should be used whenever possible for aqueous preparations and for surgical dressings and medical devices.

The recommendations for sterilization in an autoclave are 15 minutes at 121-124 °C (200 kPa).[1] The temperature should be used to control and monitor the process; the pressure is mainly used to obtain the required steam temperature. Alternative conditions, with different combinations of time and temperature, are given below.

[1] 1 atm = 101 325 Pa

Temperature (°C)	Approximate corresponding pressure (kPa)	Minimum sterilization time (min)
126-129	250 (~2.5 atm)	10
134-138	300 (~3.0 atm)	5

Minimum sterilization time should be measured from the moment when all the materials to be sterilized have reached the required temperature throughout. Monitoring the physical conditions within the autoclave during sterilization is essential. To provide the required information, temperature-monitoring probes should be inserted into representative containers, with additional probes placed in the load at the potentially coolest parts of the loaded chamber (as established in the course of the validation programme). The conditions should be within ±2 °C and ±10 kPa (±0.1 atm) of the required values. Each cycle should be recorded on a time-temperature chart or by other suitable means.

Aqueous solutions in glass containers usually reach thermal equilibrium within 10 minutes for volumes up to 100 mL and 20 minutes for volumes up to 1000 mL.

Porous loads, such as surgical dressings and related products, should be processed in an apparatus that ensures steam penetration. Most dressings are adequately sterilized by maintaining them at a temperature of 134 - 138 °C for 5 minutes.

In certain cases, glass, porcelain, or metal articles are sterilized at 121 - 124 °C for 20 minutes.

Fats and oils may be sterilized at 121 °C for 2 hours but, whenever possible, should be sterilized by dry heat.

In certain cases (e.g. thermolabile substances), sterilization may be carried out at temperatures below 121 °C, provided that the chosen combination of time and temperature has been validated. Lower temperatures offer a different level of sterilization; if this is evaluated in combination with the known microbial burden of the material before sterilization, the lower temperatures may be

satisfactory. Specific conditions of temperature and time for certain preparations are stated in individual monographs.

The bioindicator strain proposed for validation of this sterilization process is: spores of *Bacillus stearothermophilus* (e.g. ATCC 7953 or CIP 52.81) for which the D-value (i.e. 90% reduction of the microbial population) is 1.5-2 minutes at 121 °C, using about 10^6 spores per indicator.

Dry-heat sterilization

In dry-heat processes, the primary lethal process is considered to be oxidation of cell constituents. Dry-heat sterilization requires a higher temperature than moist heat and a longer exposure time. The method is, therefore, more convenient for heat-stable, non-aqueous materials that cannot be sterilized by steam because of its deleterious effects or failure to penetrate. Such materials include glassware, powders, oils, and some oil-based injectables.

Preparations to be sterilized by dry heat are filled in units that are either sealed or temporarily closed for sterilization. The entire content of each container is maintained in the oven for the time and at the temperature given in the table below. Other conditions may be necessary for different preparations to ensure the effective elimination of all undesirable microorganisms.

Temperature (°C)	Minimum sterilization time (min)
160	180
170	60
180	30

Specific conditions of temperature and time for certain preparations are stated in individual monographs.

The oven should normally be equipped with a forced air system to ensure even distribution of heat throughout all the materials processed. This should be controlled by monitoring the temperature. Containers that have been temporarily closed during the sterilization procedure are sealed after sterilization using aseptic techniques to prevent microbial recontamination.

The bioindicator strain proposed for validation of the sterilization process is: spores of *Bacillus subtilis* (e.g. *var. niger* ATCC 9372 or CIP 77.18) for which the D-value is 5-10 minutes at 160 °C using about 10^6 spores per indicator.

Filtration

Sterilization by filtration is employed mainly for thermolabile solutions. These may be sterilized by passage through sterile bacteria-retaining filters, e.g. membrane filters (cellulose derivatives, etc.), plastic, porous ceramic, or suitable sintered glass filters, or combinations of these. Asbestos-containing filters should not be used.

Appropriate measures should be taken to avoid loss of solute by adsorption onto the filter and to prevent the release of contaminants from the filter. Suitable filters will prevent the passage of microorganisms, but the filtration must be followed by an aseptic transfer of the sterilized solution to the final containers which are then immediately sealed with great care to exclude any recontamination.

Usually, membranes of not greater than 0.22 µm nominal pore size should be used. The effectiveness of the filtration method must be validated if larger pore sizes are employed.

To confirm the integrity of filters, both before and after filtration, a bubble point or similar test should be used, in accordance with the filter manufacturer's instructions. This test employs a prescribed pressure to force air bubbles through the intact membrane previously wetted with the product, with water, or with a hydrocarbon liquid.

All filters, tubes, and equipment used "downstream" must be sterile. Filters capable of withstanding heat may be sterilized in the assembly before use by autoclaving at 121 °C for 15 - 45 minutes depending on the size of the filter assembly. The effectiveness of this sterilization should be validated. For filtration of a liquid in which microbial growth is possible, the same filter should not be used for procedures lasting longer than one working day.

Exposure to ionizing radiation

Sterilization of certain active ingredients, drug products, and medical devices in their final container or package may be achieved by exposure to ionizing radiation in the form of gamma radiation from a suitable radioisotopic source such as ^{60}Co (cobalt 60) or of electrons energized by a suitable electron accelerator. Laws and regulations for protection against radiation must be respected.

Gamma radiation and electron beams are used to effect ionization of the molecules in organisms. Mutations are thus formed in the DNA and these reactions alter replication. These processes are very dangerous and only well-trained and experienced staff should decide upon the desirability of their use and should ensure monitoring of the processes. Specially designed and purpose-built installations and equipment must be used.

It is usual to select an absorbed radiation level of 25 kGy[1] (2.5 Mrad)[2], although other levels may be employed provided that they have been validated.

[1] kilogray

[2] megarad

Radiation doses should be monitored with specific dosimeters during the entire process. Dosimeters should be calibrated against a standard source on receipt from the supplier and at appropriate intervals thereafter. The radiation system should be reviewed and validated whenever the source material is changed and, in any case, at least once a year.

The bioindicator strains proposed for validation of this sterilization process are: spores of *Bacillus pumilus* (e.g. ATCC 27142 or CIP 77.25) with 25 kGy (2.5 Mrad) for which the D-value is about 3 kGy (0.3 Mrad) using 10^7-10^8 spores per indicator; for higher doses, spores of *Bacillus cereus* (e.g. SSI C 1/1) or *Bacillus sphaericus* (e.g. SSI C_1A) are used.

Gas sterilization

The active agent of the gas sterilization process can be ethylene oxide or another highly volatile substance. The highly flammable and potentially explosive nature of such agents is a disadvantage unless they are mixed with suitable inert gases to reduce their highly toxic properties and the possibility of toxic residues remaining in treated materials. The whole process is difficult to control and should only be considered if no other sterilization procedure can be used. It must only be carried out under the supervision of highly skilled staff.

The sterilizing efficiency of ethylene oxide depends on the concentration of the gas, the humidity, the time of exposure, the temperature, and the nature of the load. In particular, it is necessary to ensure that the nature of the packaging is such that the gas exchange can take place. It is also important to maintain sufficient humidity during sterilization. Records of gas concentration and of temperature and humidity should be made for each cycle. Appropriate sterilization conditions must be determined experimentally for each type of load.

After sterilization, time should be allowed for the elimination of residual sterilizing agents and other volatile residues, which should be confirmed by specific tests.

Because of the difficulty of controlling the process, efficiency must be monitored each time using the proposed bioindicator strains: spores of *Bacillus subtilis* (e.g. *var. niger* ATCC 9372 or CIP 77.18) or of *Bacillus stearothermophilus,* (e.g. ATCC 7953 or CIP 52.81). The same quantity of spores should be used as for "Heating in an autoclave" and "Dry-heat sterilization".

5.6 Extractable volume for parenteral preparations

This text is based on the internationally-harmonized texts developed by the Pharmacopoeial Discussion Group (PDG). Some editorial modifications have been made in order to be in line with the style used in The International Pharmacopoeia.

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

Single-dose containers

Select one container if the nominal volume is 10 mL or more, three containers if the nominal volume is more than 3 mL and less than 10 mL or five containers if the nominal volume is 3 mL or less. Take up individually the total contents of each container selected into a dry syringe of a capacity not exceeding three times the volume to be measured and fitted with a 21-gauge needle not less than 2.5 cm in length. Expel any air bubbles from the syringe and needle, then discharge the contents of the syringe without emptying the needle into a standardized dry cylinder (graduated to contain rather than to deliver the designated volumes) of such size that the volume to be measured occupies at least 40% of its graduated volume. Alternatively the volume of the contents in millilitres may be calculated as the mass in grams divided by the density.

For containers with a nominal volume of 2 mL or less the contents of a sufficient number of containers may be pooled to obtain the volume required for the measurement, provided that a separate, dry syringe assembly is used for each container. The contents of containers holding 10 mL or more may be determined by opening them and emptying the contents directly into the graduated cylinder or tared beaker.

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

Multidose containers

For injections in multidose containers labelled to yield a specific number of doses of a stated volume, select one container and proceed as directed for single-dose containers, using the same number of separate syringe assemblies as the number of doses specified.

The volume is such that each syringe delivers not less than the stated dose.

Cartridges and prefilled syringes

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

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

Parenteral infusions

Select one container. Transfer the contents into a dry measuring cylinder of such a capacity that the volume to be determined occupies at least 40% of the nominal volume of the cylinder. Measure the volume transferred.

The volume is not less than the nominal volume.

5.7 Tests for particulate contamination

Particulate contamination of injections and parenteral infusions consists of extraneous, mobile, undissolved particles, other than gas bubbles, unintentionally present in the solutions.

The types of preparations for which compliance with these tests are required are stated in respective general monographs.

5.7.1 Subvisible particles

This chapter is based on the internationally-harmonized texts developed by the Pharmacopoeial Discussion Group (PDG). Some editorial modifications have been made in order to be in line with the style used in The International Pharmacopoeia *. It should be noted, however, that acceptance criteria of parenteral preparations having a nominal volume of 100 mL were exempted from the PDG harmonization. For the purpose of* The International Pharmacopoeia*, 100 mL is classified as a small-volume parenteral preparation and the criteria are set accordingly.*

For the determination of particulate contamination two procedures, Method A (Light Obscuration Particle Count Test) and Method B (Microscopic Particle Count Test), are specified hereinafter. When examining injections and parenteral infusions for subvisible particles Method A is preferably applied. However, it may be necessary to test some preparations by the light obscuration particle count test followed by the microscopic particle count test to reach a conclusion on conformance to the requirements.

Not all parenteral preparations can be examined for subvisible particles by one or both of these methods. When Method A is not applicable, e.g. in the case of preparations having reduced clarity or increased viscosity, the test should be carried out according to Method B. Emulsions, colloids and liposomal preparations are examples. Similarly, products that produce air or gas bubbles when drawn into the sensor may also require microscopic particle count testing. If the viscosity of the preparation to be tested is sufficiently high so as to preclude its examination by either test method, a quantitative dilution with an appropriate diluent may be made to decrease viscosity, as necessary, to allow the analysis to be performed.

The results obtained in examining a discrete unit or group of units for particulate contamination cannot be extrapolated with certainty to other units that remain untested. Thus, statistically-sound sampling plans must be developed if valid inferences are to be drawn from observed data to characterize the level of particulate contamination in a large group of units.

METHOD A. LIGHT OBSCURATION PARTICLE COUNT TEST

Use a suitable apparatus based on the principle of light blockage which allows an automatic determination of the size of particles and the number of particles according to size.

The apparatus is calibrated using dispersions of spherical particles of known sizes between 10 µm and 25 µm. These standard particles are dispersed in particle-free water R. Care must be taken to avoid aggregation of particles during dispersion.

General precautions

The test is carried out under conditions limiting particulate contamination, preferably in a unidirectional airflow cabinet.

Very carefully wash the glassware and filtration equipment used, except for the membrane filters, with a warm detergent solution and rinse with abundant amounts of water to remove all traces of detergent. Immediately before use, rinse the equipment from top to bottom, outside and then inside, with particle-free water R.

Take care not to introduce air bubbles into the preparation to be examined, especially when fractions of the preparation are being transferred to the container in which the determination is to be carried out.

In order to check that the environment is suitable for the test, that the glassware is properly cleaned and that the water to be used is particle-free, the following test is carried out: determine the particulate contamination of 5 samples of particle-free water R, each of 5 mL, according to the method described below. If the number of particles of 10 µm or greater size exceeds 25 for the combined 25 mL, the precautions taken for the test are not sufficient. The preparatory steps must be repeated until the environment, glassware and water are suitable for the test.

Method

Mix the contents of the sample by slowly inverting the container 20 times successively. If necessary, cautiously remove the sealing closure. Clean the outer surfaces of the container opening using a jet of particle-free water R and remove the closure, avoiding any contamination of the contents. Eliminate gas bubbles by appropriate measures such as allowing to stand for 2 min or sonicating.

For large-volume parenterals, single units are tested. For small-volume parenterals less than 25 mL in volume, the contents of 10 or more units are combined in a cleaned container to obtain a volume of not less than 25 mL; where justified and authorized, the test solution may be prepared by mixing the contents of a suitable number of vials and diluting to 25 mL with particle-free water R or with an appropriate solvent without contamination of particles when particle-free water R is not suitable. Small-volume parenterals having a volume of 25 mL or more may be tested individually.

Powders for parenteral use are reconstituted with particle-free water R or with an appropriate solvent without contamination of particles when particle-free water R is not suitable.

The number of test specimens must be adequate to provide a statistically sound assessment. For large-volume parenterals or for small-volume parenterals having a volume of 25 mL or more, fewer than 10 units may be tested, based on an appropriate sampling plan.

Remove four portions, each of not less than 5 mL, and count the number of particles equal to or greater than 10 µm and 25 µm. Disregard the result obtained for the first portion and calculate the mean number of particles for the preparation to be examined.

Evaluation

For preparations supplied in containers with a nominal volume of more than 100 mL, apply the criteria of test A.1.

For preparations supplied in containers with a nominal volume of 100 mL or less, apply the criteria of test A.2.

If the average number of particles exceeds the limits, test the preparation by the Microscopic Particle Count Test.

Test A.1. Solutions for parenteral infusion or solutions for injection supplied in containers with a nominal content of more than 100 mL.

The preparation complies with the test if the average number of particles present in the units tested does not exceed 25 per millilitre equal to or greater than 10 µm and does not exceed 3 per millilitre equal to or greater than 25 µm.

Test A.2. Solutions for parenteral infusion or solutions for injection supplied in containers with a nominal content of 100 mL or less.

The preparation complies with the test if the average number of particles present in the units tested does not exceed 6000 per container equal to or greater than 10 µm and does not exceed 600 per container equal to or greater than 25 µm.

METHOD B. MICROSCOPIC PARTICLE COUNT TEST

Use a suitable binocular microscope, filter assembly for retaining particulate contamination and membrane filter for examination.

The microscope is equipped with an ocular micrometer calibrated with an objective micrometer, a mechanical stage capable of holding and traversing the entire filtration area of the membrane filter, two suitable illuminators to provide episcopic illumination in addition to oblique illumination and is adjusted to 100 ± 10 magnifications.

The ocular micrometer is a circular diameter graticule (see Figure 1) and consists of a large circle divided by crosshairs into quadrants, transparent and black reference circles 10 µm and 25 µm in diameter at 100 magnifications and a linear scale graduated in 10 µm increments. It is calibrated using a stage micrometer that is certified by either a national, regional or international standard institution. A relative error of the linear scale of the graticule within ± 2% is acceptable. The large circle is designated the graticule field of view (GFOV).

Two illuminators are required. One is an episcopic bright-field illuminator internal to the microscope, the other is an external, focusable auxiliary illuminator adjustable to give reflected oblique illumination at an angle of 10° to 20°.

The filter assembly for retaining particulate contamination consists of a filter holder made of glass or other suitable material and is equipped with a vacuum source and a suitable membrane filter.

The membrane filter is of suitable size, black or dark grey in colour, non-gridded or gridded and 1.0 µm or finer in nominal pore size.

General precautions

The test is carried out under conditions limiting particulate contamination, preferably in a laminar-flow cabinet.

Very carefully wash the glassware and filter assembly used, except for the membrane filter, with a warm detergent solution and rinse with abundant amounts of water to remove all traces of detergent. Immediately before use rinse both sides of the membrane filter and the equipment from top to bottom, outside and then inside, with particle-free water R.

In order to check that the environment is suitable for the test, that the glassware and the membrane filter are properly cleaned and that the water to be used is particle-free, the following test is carried out: determine the particulate contamination of a 50 mL volume of particle-free water R according to the method described below. If more than 20 particles 10 µm or larger in size or if more than 5 particles 25 µm or larger in size are present within the filtration area, the precautions taken for the test are not sufficient. The preparatory steps must be repeated until the environment, glassware, membrane filter and water are suitable for the test.

Method

Mix the contents of the samples by slowly inverting the container 20 times successively. If necessary, cautiously remove the sealing closure. Clean the outer surfaces of the container opening using a jet of particle-free water R and remove the closure, avoiding any contamination of the contents.

For large-volume parenterals, single units are tested. For small-volume parenterals less than 25 mL in volume, the contents of 10 or more units are combined in a cleaned container; where justified and authorized, the test solution may be prepared by mixing the contents of a suitable number of vials and diluting to 25 mL with particle-free water R or with an appropriate solvent without contamination of particles when particle-free water R is not suitable. Small-volume parenterals having a volume of 25 mL or more may be tested individually.

Powders for parenteral use are constituted with particle-free water R or with an appropriate solvent without contamination of particles when particle-free water R is not suitable.

The number of test specimens must be adequate to provide a statistically sound assessment. For large-volume parenterals or for small-volume parenterals having a volume of 25 mL or more, fewer than 10 units may be tested, based on an appropriate sampling plan.

Wet the inside of the filter holder fitted with the membrane filter with several millilitre of particle-free water R. Transfer to the filtration funnel the total volume of a solution pool or of a single unit and apply vacuum. If needed add stepwise a portion of the solution until the entire volume is filtered. After the last addition of solution, begin rinsing the inner walls of the filter holder by using a jet of particle-free water R. Maintain the vacuum until the surface of the membrane filter is free from liquid. Place the filter in a Petri dish and allow the filter to air-dry with the cover slightly ajar. After the filter has been dried, place the Petri dish on the stage of the microscope, scan the entire membrane filter under the reflected light from the illuminating device and count the number of particles that are equal to or greater than 10 µm and the number of particles that are equal to or greater than 25 µm. Alternatively, partial filter count and determination of the total filter count by calculation is allowed. Calculate the mean number of particles for the preparation to be examined.

The particle sizing process with the use of the circular diameter graticule is carried out by transforming mentally the image of each particle into a circle and then comparing it to the 10 µm and 25 µm graticule reference circles. Thereby the particles are not moved from their initial locations within the graticule field of view and are not superimposed on the reference circles for comparison. The inner diameter of the transparent graticule reference circles is used to size white and transparent particles, while dark particles are sized by using the outer diameter of the black opaque graticule reference circles.

In performing the microscopic particle count test do not attempt to size or enumerate amorphous, semiliquid or otherwise morphologically indistinct materials that have the appearance of a stain or discoloration on the membrane filter. These materials show little or no surface relief and present a gelatinous or film-like appearance. In such cases the interpretation of enumeration may be aided by testing a sample of the solution by the light obscuration particle count test.

Evaluation

For preparations supplied in containers with a nominal volume of more than 100 mL, apply the criteria of test B.1.

For preparations supplied in containers with a nominal volume of 100 mL or less, apply the criteria of test B.2.

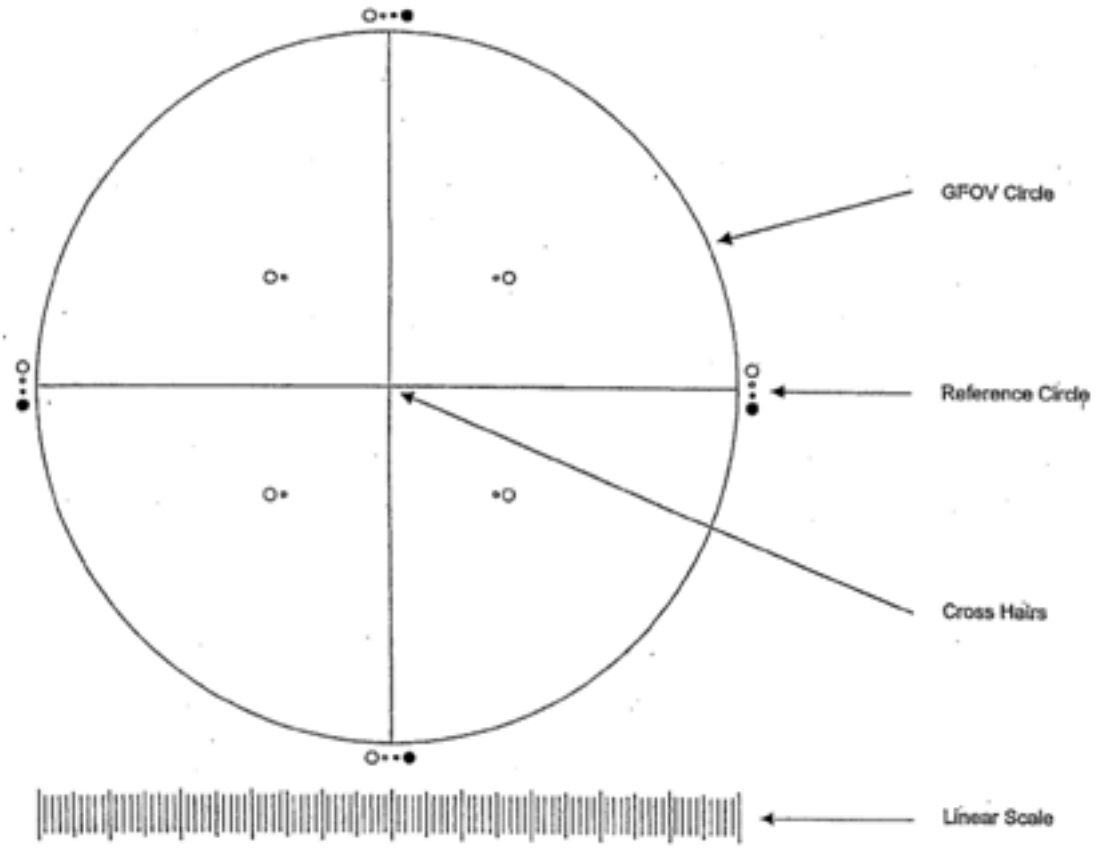
Test B.1. Solutions for parenteral infusion or solutions for injection supplied in containers with a nominal content of more than 100 mL.

The preparation complies with the test if the average number of particles present in the units tested does not exceed 12 per millilitre equal to or greater than 10 µm and does not exceed 2 per millilitre equal to or greater than 25 µm.

Test B.2. Solutions for parenteral infusion or solutions for injection supplied in containers with a nominal content of 100 mL or less.

The preparation complies with the test if the average number of particles present in the units tested does not exceed 3000 per container equal to or greater than 10 µm and does not exceed 300 per container equal to or greater than 25 µm.

Figure 1. Circular diameter graticule

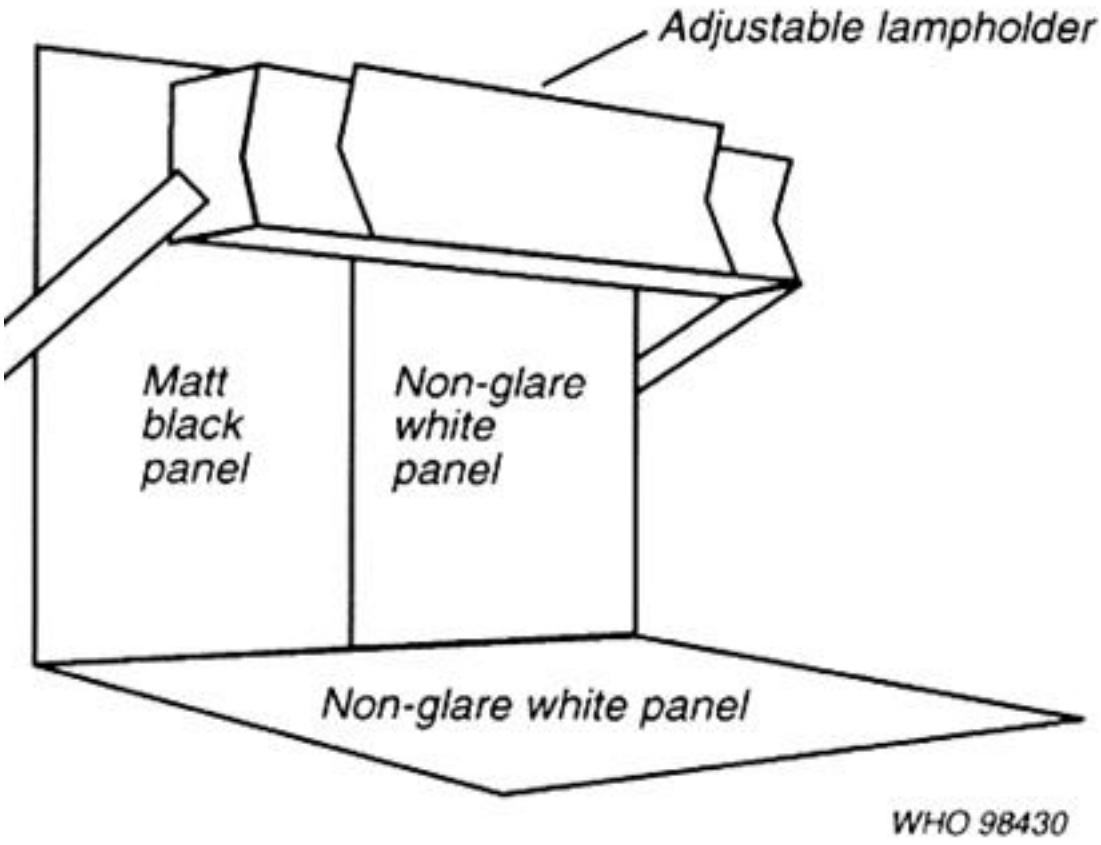


5.7.2 Visible particles

This test provides a simple method for the detection of visible particles. It is performed in accordance with the provisions of good manufacturing practices. The test is not intended for use by a manufacturer for batch release purposes. To ensure that a product will meet pharmacopoeial specifications with respect to visible particulate matter, if and when tested, manufacturers should carry out a 100% inspection and rejection of unsatisfactory items prior to release or use other appropriate means.

Subvisible particles and the nature of the particles are not identified by this method.

Figure 2. Apparatus for visible particles



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Apparatus[1]

The apparatus (Figure 2) consists of a viewing station comprising:

- -a matt black panel of appropriate size held in a vertical position;
- -a non-glare white panel of appropriate size held in a vertical position next to the black panel;
- -an adjustable lamp holder fitted with a shaded, white-light source and with a light diffuser (a viewing illuminator containing two 13-W fluorescent tubes, each 525 mm in length is suitable). The intensity of illumination at the viewing point is maintained between 2000 lux and 3750 lux for clear glass ampoules. Higher values are preferable for coloured glass and plastic containers.

Recommended procedure

Remove any adherent labels from the container and wash and dry the outside. Gently swirl or invert each individual container, making sure that no air bubbles are introduced and observe for about 5 seconds in front of the white panel. Repeat the procedure in front of the black panel.

Record the presence of any particles. Repeat the procedure for a further 19 containers.

The preparation fails the test if one or more particles are found in more than one container.

When the test is applied to reconstituted solutions from powder for injections, the test fails if particles are found in more than two containers.

[1] This method was developed by WHO in collaboration with Group 12 of the European Pharmacopoeia Commission.

5.5 Dissolution test for oral dosage forms

2022-01

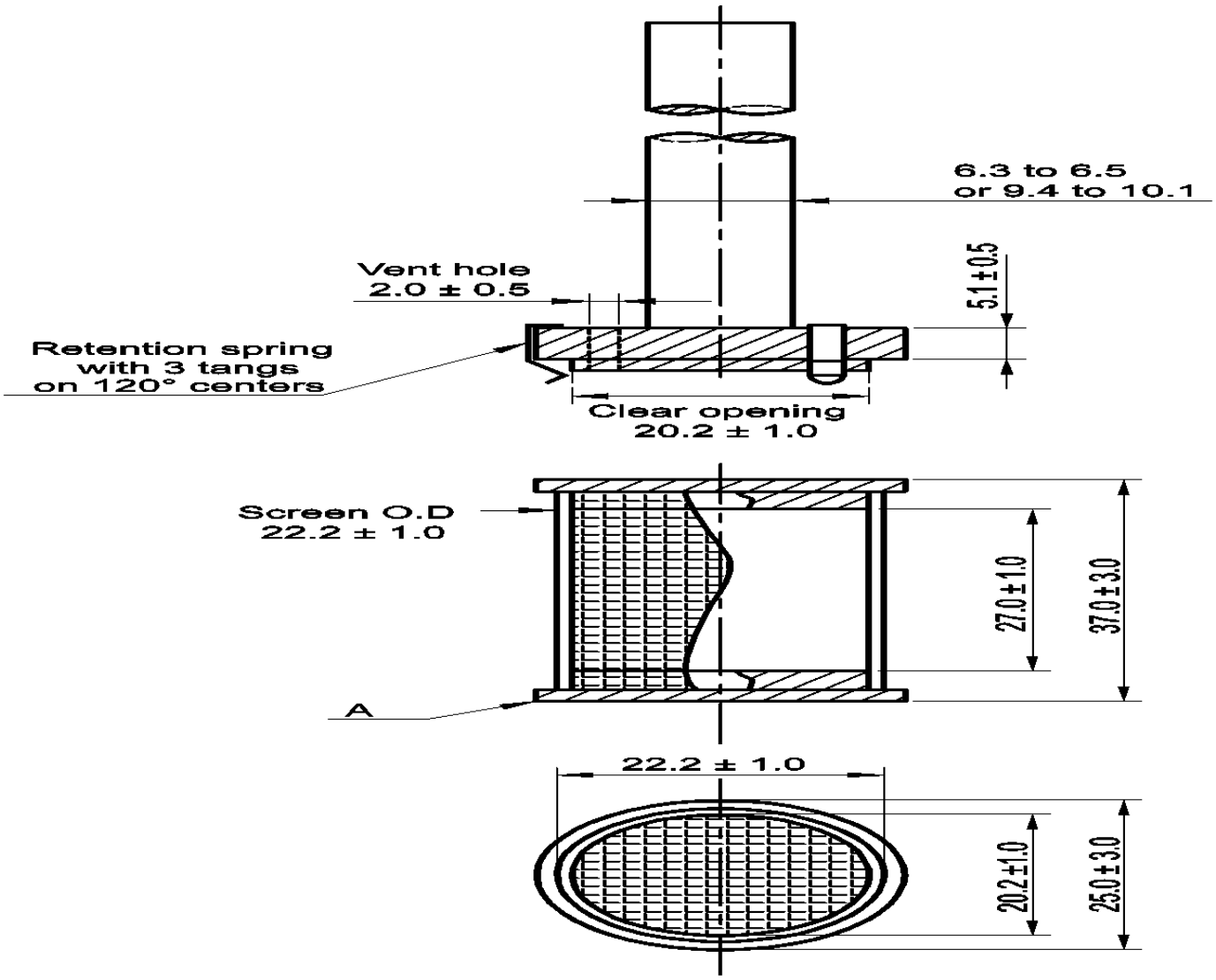
This text is based on the internationally-harmonized texts developed by the Pharmacopoeial Discussion Group (PDG). It has been developed in line with the style and requirements used in The International Pharmacopoeia*. The additional sections on* Oral suspensions, powders for oral suspension and granules for oral suspenion *and on* Monographs of The International Pharmacopoeia *are not part of the PDG text.*

For further guidance, see also the chapter Dissolution testing of tablets and capsules *in the* Supplementary Information *section.*

This test determines the amount of active ingredient(s) released from an oral dosage form, under controlled conditions using a known volume of dissolution medium within a predetermined length of time.

Basket apparatus. The assembly consists of the following: a vessel, which may be covered, made of glass or other inert, transparent material, which should not sorb, react or interfere with the preparation to be tested; a motor; a drive shaft; and a cylindrical basket (stirring element). The vessel is partially immersed in a suitable water bath of any convenient size or heated by a suitable device such as a heating jacket. The water bath or heating device permits maintaining the temperature inside the vessel at 37 ± 0.5 ºC during the test. No part of the assembly, including the environment in which the assembly is placed, contributes significant motion, agitation or vibration beyond that due to the smoothly rotating stirring element. An apparatus that permits observation of the preparation and stirring element during the test is preferable. The vessel is cylindrical with a hemispherical bottom and a capacity of 1 litre. Its height is 160–210 mm and its inside diameter is 98–106 mm. Its sides are flanged at the top. A fitted cover may be used to retard evaporation. If a cover is used, it provides sufficient openings to allow ready insertion of the thermometer and withdrawal of samples. The shaft is positioned so that its axis is not more than 2 mm at any point from the vertical axis of the vessel and rotates smoothly and without significant wobble that could affect the results. A speed-regulating device is used that allows the shaft rotation speed to be selected and maintained at a specified rate within ± 4%.

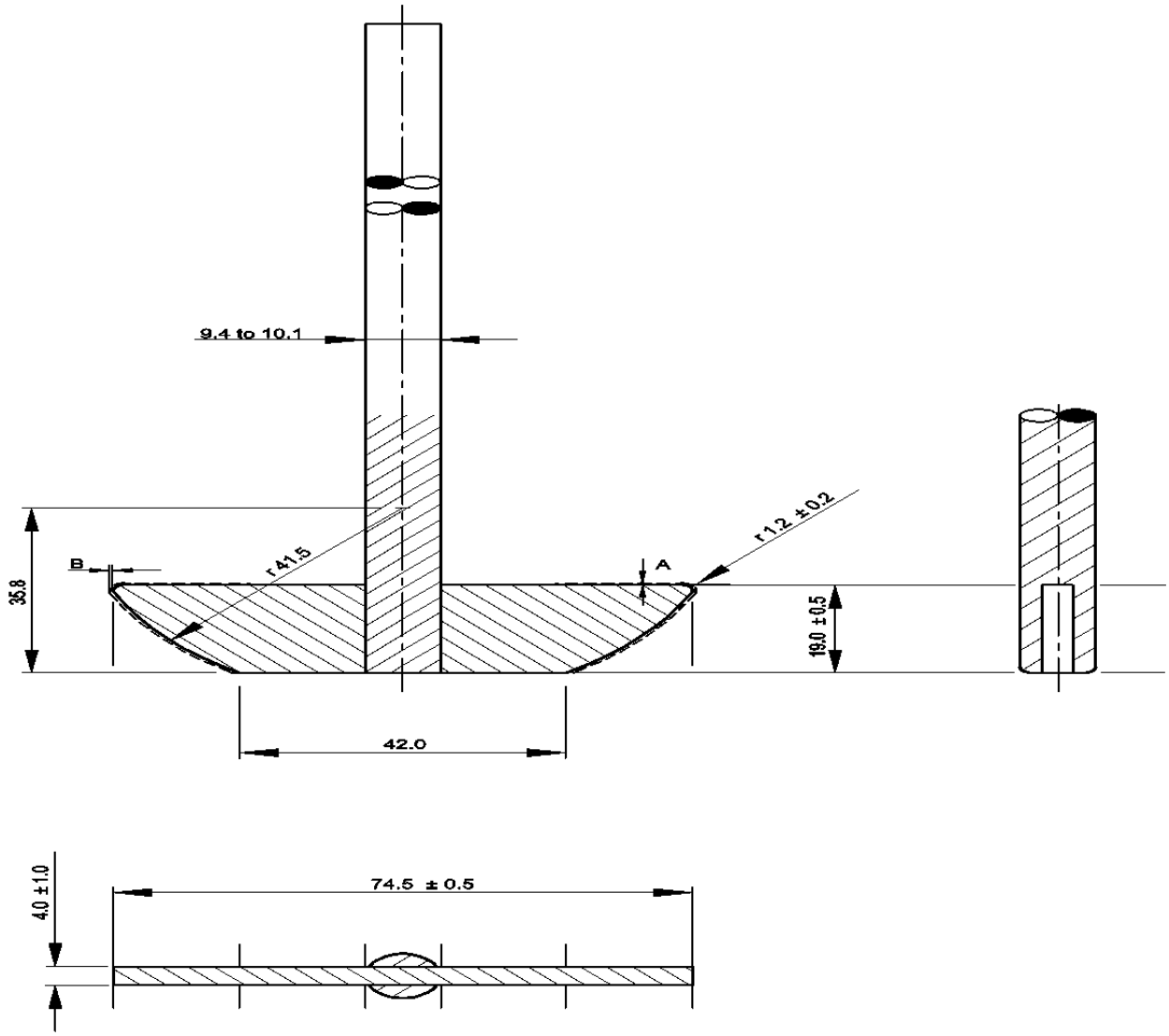
Shaft and basket components of the stirring element are fabricated of stainless steel, type 316 or equivalent, to the specifications shown in Figure 1. A basket having a gold coating of about 2.5 µm (0.0001 inch) thick may be used. The dosage unit is placed in a dry basket at the beginning of each test. The distance between the inside bottom of the vessel and the bottom of the basket is maintained at 25 ± 2 mm during the test.



1. Screen with welded seam: 0.22–0.31 mm wire diameter with wire opening of 0.36–0.44 mm. After welding, the screen may be slightly altered.

2. Maximum allowable runout at "A" is 1.0 mm when the part is rotated on centre line axis with basket mounted.

Figure 1. Basket stirring element; dimensions in millimetres.



A and B dimensions do not vary more than 0.5 mm when part is rotated on centre line axis. Tolerances are ± 1.0 mm unless otherwise stated.

Figure 2. Paddle stirring element; dimensions in millimetres.

Paddle apparatus. Use the assembly from the basket apparatus except that a paddle formed from a blade and a shaft is used as the stirring element. The shaft is positioned so that its axis is not more than 2 mm from the vertical axis of the vessel at any point and rotates smoothly without significant wobble that could affect the results. The vertical centre line of the blade passes through the axis of the shaft so that the bottom of the blade is flush with the bottom of the shaft. The paddle conforms to the specifications shown in Figure 2. The distance of 25 ± 2 mm between the bottom of the blade and the inside bottom of the vessel is maintained during the test. The metallic or suitably inert, rigid blade and shaft comprise a single entity. A suitable two-part detachable design may be used provided the assembly remains firmly engaged during the test. The paddle blade and shaft may be coated with a suitable coating so as to make them inert. The dosage unit is allowed to sink to the bottom of the vessel before rotation of the blade is started. A small, loose piece of non-reactive material, such as not more than a few turns of wire helix, may be attached to dosage units that would otherwise float. An alternative sinker device is shown in Figure 3. Other validated sinker devices may be used.

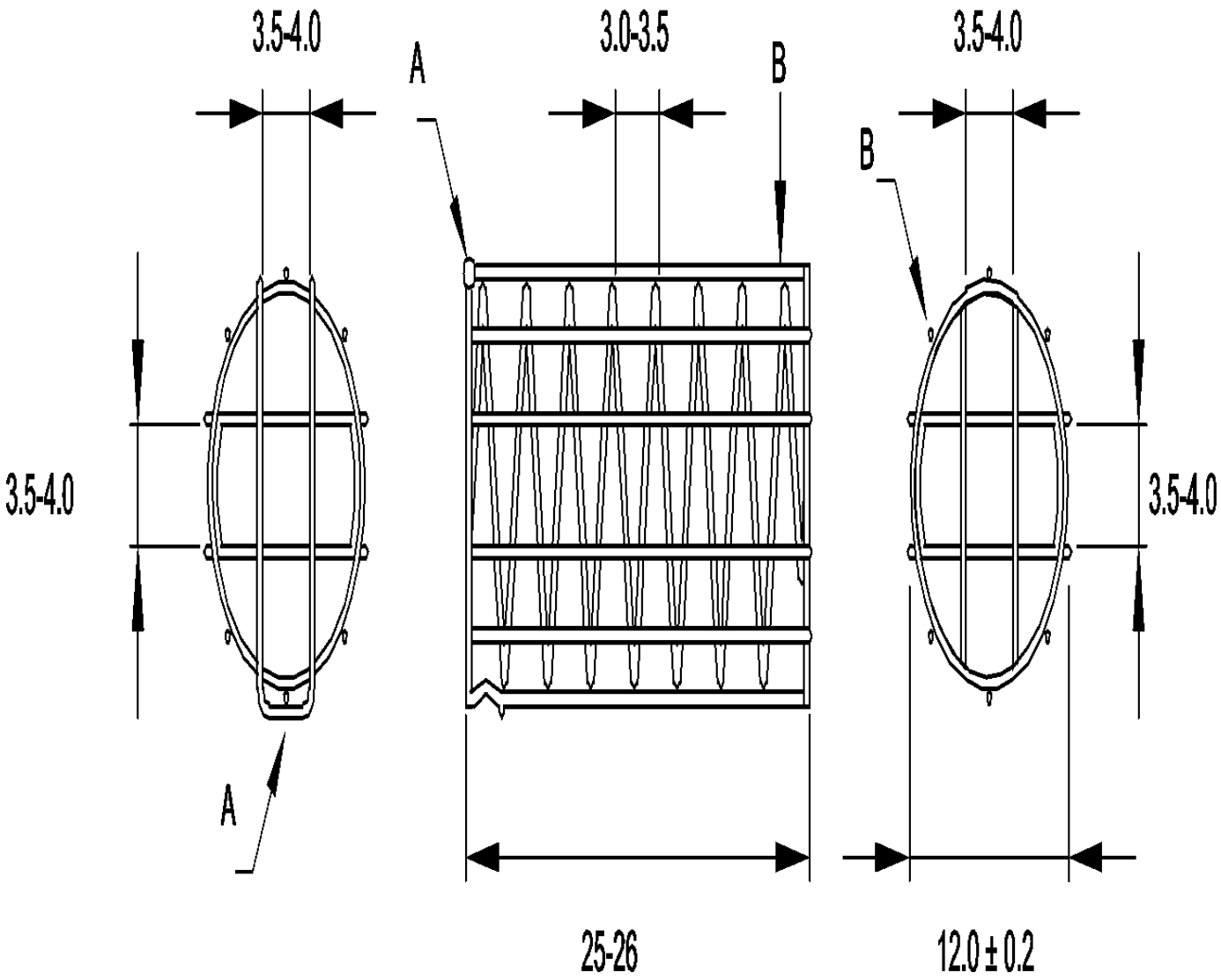


Figure 3. Alternative sinker. A: acid-resistant wire clasp; B: acid-resistant wire support; dimensions in millimeters.

Recommended procedure

Conventional-release (or immediate-release) dosage forms

Procedure. Place the stated volume of the dissolution medium (± 1%) in the vessel of the specified apparatus. Assemble the apparatus, equilibrate the dissolution medium to 37 ± 0.5 °C and remove the thermometer. The test may also be carried out with the thermometer in place, provided it is shown that results equivalent to those obtained without the thermometer are obtained. Place one dosage unit in the apparatus taking care to exclude air bubbles from the surface of the dosage unit. Operate the apparatus at the specified rate. Within the time interval specified, or at each of the times stated, withdraw a sample from a zone midway between the surface of the dissolution medium and the top of the rotating basket or blade not less than 1 cm from the vessel wall. Agitation/stirring should continue during sampling. Where multiple sampling times are specified, replace the samples withdrawn for analysis with equal volumes of fresh dissolution medium at 37 °C or, where it can be shown that replacement of the medium is not necessary, correct for the volume change in the calculation. Keep the vessel covered for the duration of the test and verify the temperature (37 ± 0.5 °C) of the medium at suitable times. Perform the analysis as directed in the individual monograph using a suitable assay method. The samples are filtered immediately upon sampling, preferably by using in-line filtration or a filter in the tip of the sampling probe or both, unless filtration is demonstrated to be unnecessary. Use an inert filter that does not cause adsorption of the active ingredient or contain extractable substances that would interfere with the analysis. Centrifugation is not recommended unless validated for the specific test. The test is to be conducted with six dosage form units in parallel.

If automated equipment is used for sampling, or the apparatus is otherwise modified, verification is necessary that the modified apparatus will produce results equivalent to those obtained with the apparatus described in this chapter.

Dissolution medium. A suitable dissolution medium is used. The volume specified refers to measurements made between 20 °C and 25 °C. If the dissolution medium is a buffered solution, adjust the solution so that its pH is within 0.05 units of the specified pH. Dissolved gases can cause bubbles to form which may change the results of the test. In such cases, dissolved gases must

be removed prior to testing. [1]

Time. Where a single time specification is given, the test may be concluded in a shorter period if the requirement for minimum amount dissolved is met. Samples are to be withdrawn only at the stated times, within a tolerance of ± 2%.

Determine the quantity of active ingredient dissolved at the specified time(s) indicated in the individual monograph. The result should be expressed as a percentage of the content stated on the label of the dosage form.

Sustained-release (or extended-/prolonged-release) dosage forms

Procedure. Proceed as described for conventional-release dosage forms.

Dissolution medium. Proceed as described for conventional-release dosage forms.

Time. The test-time points, generally three, are expressed in hours.

Delayed-release dosage forms

Procedure. Use methods A or B.

Method A

Acid stage. Place 750 mL *hydrochloric acid (0.1 mol/L) VS* in the vessel and assemble the apparatus. Allow the medium to equilibrate to a temperature of 37 ± 0.5 °C. Place one dosage unit in the apparatus, cover the vessel and operate the apparatus at the specified rate. After 2 hours of operation in *hydrochloric acid (0.1 mol/L) VS*, withdraw a sample of the fluid and proceed immediately as directed under buffer stage. Perform an analysis of the sample using a suitable assay method.

Buffer stage. Complete the operations of adding and adjusting the pH within 5 minutes. With the apparatus operating at the rate specified, add to the fluid in the vessel 250 mL of a 0.2 M solution of *trisodium orthophosphate R* that has been equilibrated to 37 ± 0.5 °C. Adjust, if necessary, with *hydrochloric acid (~70 g) TS* or *sodium hydroxide (~80 g/L) TS* to a pH of 6.8 ± 0.05. Continue to operate the apparatus for 45 minutes or for the specified time. At the end of the time period, withdraw a sample of the fluid and perform the analysis using a suitable assay method.

Method B

Acid stage. Place 1000 mL of *hydrochloric acid (0.1 mol/L) VS* in the vessel and assemble the apparatus. Allow the medium to equilibrate to a temperature of 37 ± 0.5 °C. Place one dosage unit in the apparatus, cover the vessel and operate the apparatus at the specified rate. After 2 hours of operation in *hydrochloric acid (0.1 mol/L) VS*, withdraw a sample of the fluid and proceed immediately as directed under buffer stage. Perform an analysis of the sample using a suitable assay method.

Buffer stage. For this stage of the procedure, use buffer that has previously been equilibrated to a temperature of 37 ± 0.5 °C. Drain the acid from the vessel and add 1000 mL of pH 6.8 phosphate buffer, prepared by mixing three volumes of *hydrochloric acid (0.1 mol/L) VS* with one volume of a 0.20 M solution of trisodium orthophosphate R and adjusting, if necessary, with hydrochloric acid (~70 g/L) TS or *sodium hydroxide (~80 g/L) TS* to a pH of 6.8 ± 0.05. This may also be accomplished by removing from the apparatus the vessel containing the acid and replacing it with another vessel containing the buffer and transferring the dosage unit to the vessel containing the buffer. Continue to operate the apparatus for 45 minutes or for the specified time. At the end of the time period, withdraw a sample of the fluid and perform the analysis using a suitable assay method.

Time. All test times stated are to be observed within a tolerance of ± 2%, unless otherwise specified.

Oral suspensions, powders for oral suspension and granules for oral suspension

Proceed as described in the test for conventional-release, sustained-release or delayed-release dosage forms, as appropriate, using the oral-suspension of a suspension prepared from the powder or granules for oral suspension in accordance with the directions given on the labelling. Shake the suspension immediately before taking a sample. Avoid introducing air bubbles into the sample to ensure the suspension is homogeneous.

Transfer to the dissolution vessel the volume of sample indicated in the monograph. If no volume is given, use a volume that is equivalent to a unit dose or, where the product has different doses depending on body weight or age, the volume of sample that corresponds to the highest unit dose to be administered at one time. If the product is presented as a single-dose preparation, take each sample from a different container. Otherwise, each sample may come from the same container.

Acceptance criteria

Conventional-release (or immediate-release) dosage forms

Unless otherwise specified in the individual monograph, the requirements are met if the quantities of active ingredient(s)

dissolved from the dosage forms tested conform to Table 1. Continue testing through the three levels unless the results conform at either S_1 or S_2. The quantity, *Q*, is the specified amount of dissolved active ingredient expressed as a percentage of the labelled content; the 5%, 15% and 25% values in the acceptance table are percentages of the labelled content so that these values and *Q* are in the same terms.

Table 1

Level	Samples tested	Acceptance criteria
S_1	6	Each value is not less than *Q* + 5%.
S_2	6	Average value of the 12 dosage units (S_1 + S_2) is equal to or greater than *Q* and no unit is less than *Q* - 15%.
S_3	12	Average value of 24 dosage units (S_1 + S_2 + S_3) is equal to or greater than *Q*; not more than 2 units are less than *Q* - 15%; no unit is less than *Q* - 25%.

Sustained-release (or extended-/prolonged-release) dosage forms

Unless otherwise specified in the individual monograph, the requirements are met if the quantities of active ingredient(s) dissolved from the dosage forms tested conform to Table 2. Continue testing through the three levels unless the results conform at either L_1 or L_2. Limits on the amounts of active ingredient(s) dissolved are expressed in terms of the labelled content. The limits embrace each value of Q_i , the amount dissolved at each specified fractional dosing interval. Where more than one range is specified, the acceptance criteria apply individually to each range.

Table 2

Level	Samples tested	Acceptance criteria
L_1	6	No individual value lies outside each of the stated ranges and no individual value is less than the stated amount at the final test time.
L_2	6	The average value of the 12 dosage units (L_1 + L_2) lies within each of the stated ranges and is not less than the stated amount at the final test time; none is more than 10% of the labelled content outside each of the stated ranges; and none is more than 10% of labelled content below the stated amount at the final test time.
L_3	12	The average value of the 24 dosage units (L_1 + L_2 + L_3) lies within the stated ranges and is not less than the stated amount at the final test time; not more than 2 of the 24 dosage units are more than 10% of labelled content outside each of the stated ranges; not more than 2 of the 24 dosage units are more than 10% of labelled content below the stated amount at the final test time; and none is more than 20% of labelled content outside each of the stated ranges or more than 20% of labelled content below the stated amount at the final test time.

Delayed-release dosage forms

Acid stage. Unless otherwise stated in the individual monograph, the requirements of this part of the test are met if the quantities, based on the percentage of the labelled content of active ingredient(s) dissolved from the dosage units tested conform to Table 3. Continue testing through the three levels unless the results of both acid and buffer stages conform at an earlier level.

Table 3

Level	Samples tested	Acceptance criteria
A_1	6	No individual value exceeds 10% dissolved.
A_2	6	Average value of the 12 dosage units (A_1 + A_2) is not more than 10% dissolved, and no individual value is greater than 25% dissolved.
A_3	12	Average value of 24 dosage units (A_1 + A_2 + A_3) is not more than 10% dissolved, and no individual value is greater than 25% dissolved.

Buffer stage. Unless otherwise specified in the individual monograph, the requirements are met if the quantities of active ingredients dissolved from the units tested conform to Table 4. Continue testing through the three levels unless the results of both stages conform at an earlier level. The value of Q in Table 4 is 75% dissolved unless otherwise specified. The quantity, Q, is the specified total amount of active ingredient dissolved in both the acid and buffer stages, expressed as a percentage of the labelled content. The 5%, 15% and 25% values in the table are percentages of the labelled content so that these values and Q are in the same terms.

Table 4

Level	Samples tested	Acceptance criteria
B_1	6	No value is less than Q + 5%.
B_2	6	Average value of the 12 dosage units (B_1 + B_2) is equal to or greater than Q, and no unit is less than Q - 15%.
B_3	12	Average value of the 24 dosage units (B_1 + B_2 + B_3) is equal to or greater than Q; not more than 2 units are less than Q - 15%, and no unit is less than Q - 25%.

Monographs of *The International Pharmacopoeia*

The following additional statements apply to the individual monographs of The International Pharmacopoeia.

Qualification of dissolution test equipment and verification of system performance [2]

Periodically qualify the equipment utilizing an "enhanced mechanical calibration", such as the procedure described in the international standard procedure ASTM 2503 or a combination of a mechanical calibration to determine conformance of the dissolution apparatus to the dimensions and tolerances as given above and the analysis of suitable reference tablets to verify the performance of the testing system.

Test conditions

The following specifications are given in the individual monographs:

the apparatus to be used;

the composition and volume of the dissolution medium;

the rotation speed of the paddle or basket;

the preparation of the test and reference solutions;

the time, the method and the amount of sample to be withdrawn or the conditions for continuous monitoring; the preparation of the sample and the reference solution;

the method of analysis; and

the limits of the quantity or quantities of active pharmaceutical ingredient(s) required to dissolve within a prescribed time.

Dissolution media

If a buffer is added to the dissolution medium, adjust its pH to within ± 0.05 units of the prescribed value.

In specific cases, and subject to approval by the relevant regional or national authority, dissolution media may contain enzymes and/or surfactants. The addition of enzymes may be considered, for example, for formulations containing gelatin when dissolution failures can be ascribed to the cross-linking of this excipient (e.g. hard and soft gelatin capsules; gelatin containing tablets). For the testing of preparations containing poorly aqueous-soluble active substances, modification of the medium may be necessary. A surfactant may be added only when the active pharmaceutical ingredient is insoluble over the entire physiological pH range, pH 1.2 to 6.8. In such circumstances, a low concentration of surfactant may be prescribed.

Below are some examples of dissolution media:

-**Dissolution buffer pH 1.2, TS**

Dissolve 2 g of *sodium chloride R* in 800 mL of *water R*, adjust the pH to 1.2 with *hydrochloric acid (~70 g/L) TS* and dilute to 1000 mL with *water R*.

-**Dissolution buffer pH 2.5, TS**

Dissolve 2 g of *sodium chloride R* in 800 mL of *water R*, adjust the pH to 2.5 with *hydrochloric acid (~70 g/L) TS* and dilute to 1000 mL with *water R*.

-**Dissolution buffer pH 3.5, TS**

Dissolve 7.507 g of *glycine R* and 5.844 g of *sodium chloride R* in 800 mL of *water R*, adjust the pH to 3.5 with hydrochloric acid (~70 g/L) TS and dilute to 1000 mL with water R.

-**Dissolution buffer pH 4.5, TS1**

Dissolve 2.99 g of sodium acetate R in 900 mL of water R, adjust the pH to 4.5 by adding about 14 mL of acetic acid (~120 g/L) TS and dilute to 1000 mL with water R.

-**Dissolution buffer pH 4.5, TS2**

Dissolve 6.8 g of *potassium dihydrogen phosphate R* in 900 mL of*water R*, adjust the pH to 4.5 either with*hydrochloric acid (~70 g/L) TS* or*sodium hydroxide (~80 g/L) TS* and dilute to 1000 mL with *water R*.

-**Dissolution buffer, pH 6.8, TS**

Dissolve 6.9 g of *sodium dihydrogen phosphate R* and 0.9 g of *sodium hydroxide R* in 800 mL of *water R*, adjust the pH to 6.8 with *sodium hydroxide (~80g/L) TS* and dilute to 1000 mL with *water R*.

-**Dissolution buffer, pH 6.8, 0.25% SDS TS**

Dissolve 6.9 g of *sodium dihydrogen phosphate R*, 0.9 g of *sodium hydroxide R* and 2.5 g of *sodium dodecyl sulfate R* in 800 mL of *water R*, adjust the pH to 6.8 with*sodium hydroxide (~80g/L) TS* and dilute to1000 mL with *water R*.

-**Dissolution buffer, pH 6.8, 0.5% SDS TS**

Dissolve 6.9 g of *sodium dihydrogen phosphate R*, 0.9 g of*sodium hydroxide R* and 5.0 g of *sodium dodecyl sulfate R* in 800 mL of water R, adjust the pH to 6.8 with *sodium hydroxide (~80g/L) TS* and dilute to1000 mL with water R.

-**Dissolution buffer pH 7.2, TS**

Dissolve 9.075 g of *potassium dihydrogen phosphate R* in*water R* to produce 1000 mL (solution A). Dissolve 11.87 g of *disodium hydrogen phosphate R* in sufficient *water R* to produce 1000 mL (solution B). Mix 300 mL of solution A with 700 mL of solution B.

-**Gastric fluid, simulated, TS**

Dissolve 2.0 g of *sodium chloride R* and 3.2 g of *pepsin R* in 7.0 mL of *hydrochloric acid (~420 g/L) TS* and sufficient *water R* to produce 1000 mL. This test solution has a pH of about 1.2.

-**Intestinal fluid pH 6.8, simulated, TS**

Mix 77.0 mL of s*odium hydroxide (0.2 mol/L) VS*, 250.0 mL of a solution containing 6.8 g *potassium dihydrogen phosphate R* and 500 mL of *water R*. Add 10.0 g *pancreatin R,* mix and adjust the pH with the buffer components to 6.8 ± 0.1. Dilute to 1000 mL with *water R.*

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[1] One appropriate method of deaeration is as follows: heat the medium, while stirring gently, to about 41 °C, immediately filter under vacuum using a filter having a pore size of 0.45 µm or less, with vigorous stirring and continue stirring under vacuum for at least 5 minutes, preferably 15 minutes, until no more bubbles are observed. Other validated deaeration techniques for removal of dissolved gases may be used.

[2] See also Supplementary Guidelines on Good Manufacturing Practices: Validation. World Health Organization. WHO Technical Report Series, No. 937, 2006.

5.4 Disintegration test for suppositories and rectal capsules

2015-01

The disintegration test determines whether suppositories disintegrate or soften within a prescribed time when placed in an immersion fluid using the experimental conditions described below.

Disintegration is considered to be achieved when:

- dissolution is complete;

- the components of the suppositories have separated, e.g. melted fatty substances have collected on the surface of the liquid, insoluble powders have fallen to the bottom and soluble components have dissolved or are distributed in one or more of the ways described in Methods 1 and 2;

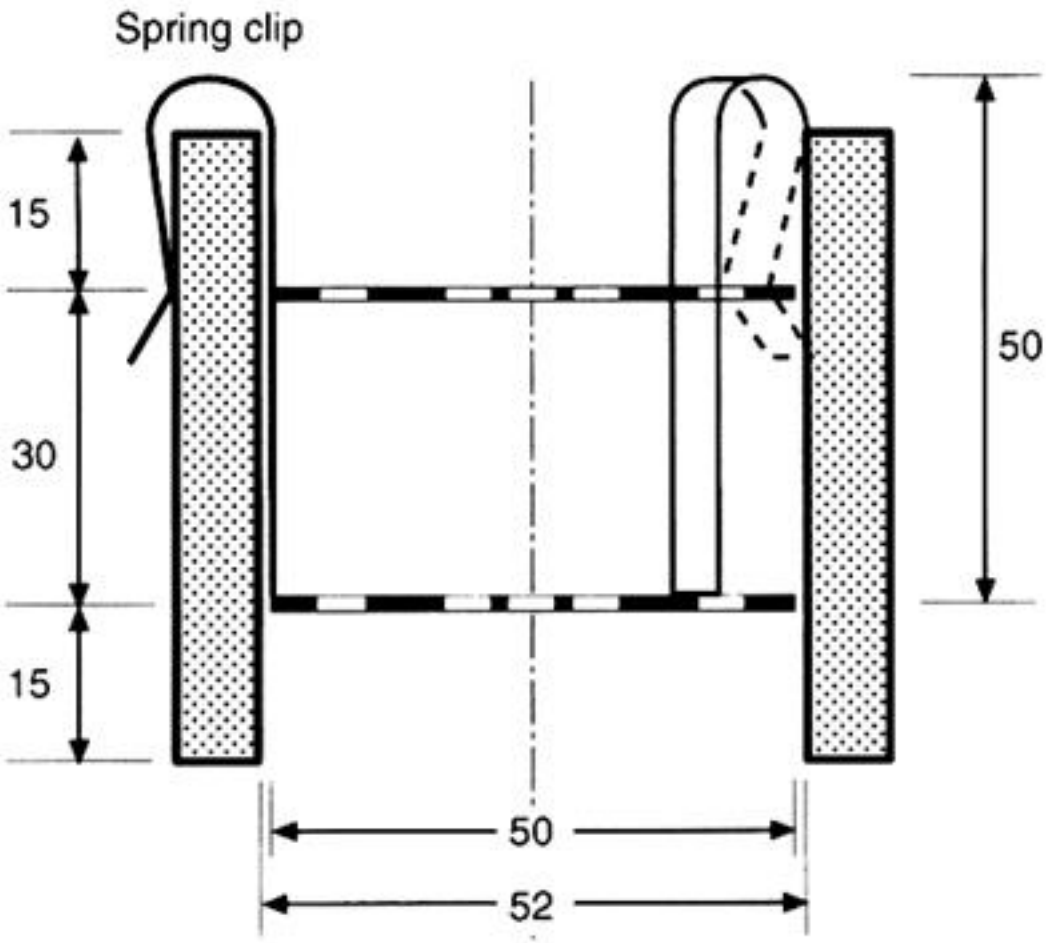
- there is softening of the test sample, usually accompanied by an appreciable change of shape without complete separation of the components. The softening process is such that a solid core no longer exists when pressure is applied with a glass rod; rupture of the gelatin shell or rectal capsule occurs resulting in release of the contents.

Method 1 (for water-soluble, hydrodispersible and fat-based suppositories and rectal capsules)

This test measures the time elapsed for a suppository or rectal capsule placed in water to disintegrate.

Apparatus

The apparatus (Figure 1) consists of a 60 mm long cylinder of glass or transparent plastic and a metal device consisting of two perforated stainless steel discs held about 30 mm apart. These discs each have 39 holes, 4 mm in diameter, which are evenly spaced in a concentric pattern. The diameter of the discs is marginally inferior to that of the interior of the cylinder. Once inserted into the cylinder, the metal device is attached to the rim of the cylinder by means of three spring clips. The test is carried out using three such apparatuses each containing a single test sample. Each apparatus is placed in a beaker with a minimum capacity of 4 litres filled with water unless otherwise prescribed. The beaker is fitted with a slow stirrer and a support that holds the apparatus vertically 90 mm below the surface of the water so that it can be inverted without emerging from the water.



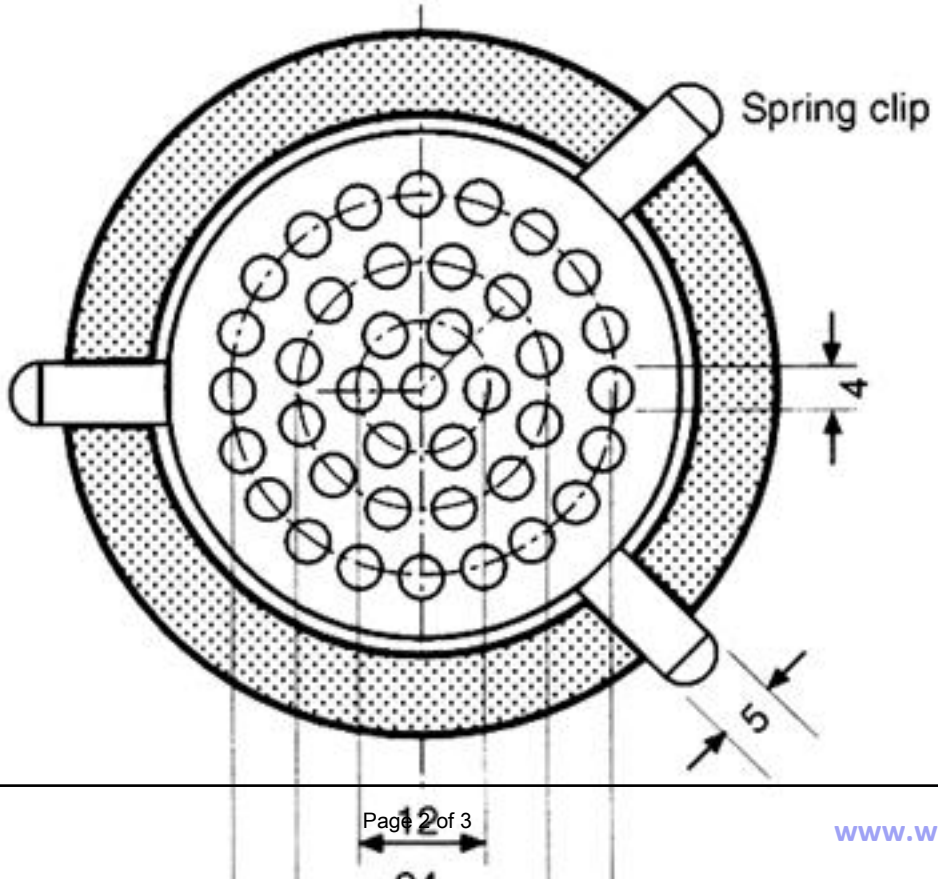


Figure 1. Apparatus for water-soluble, hydrodispersible and fat-based suppositories
A. Horizontal view. B. Vertical view. Dimensions in millimetres.

Procedure

Unless otherwise described in the individual monograph use water maintained at a temperature of 36–37 °C as the immersion fluid. The test requires three suppositories or rectal capsules and the procedure is applied to each of them.

Place the sample on the lower disc of the metal device and then insert it into the cylinder. Place the apparatus into the beaker and invert it every 10 minutes without removing it from the liquid. Repeat the operation with the remaining two suppositories or rectal capsules. Record the time required for the disintegration of the suppositories or rectal capsules.

Unless otherwise stated in the individual monograph for each of the three suppositories or rectal capsules examine the state of the sample after 30 minutes for fat-based suppositories and rectal capsules and after 60 minutes for water-soluble suppositories.

Method 2 (alternative for fat-based suppositories)

This test measures the time elapsed for a suppository placed in water to soften to the extent that it no longer offers resistance when a defined weight is applied. The softening time is determined according to the text *Softening time determination of lipophilic suppositories*, published in the *Supplementary information* section.

Apply the procedure to three suppositories and examine the state of each sample after 30 minutes unless otherwise stated in the individual monograph.

5.3 Disintegration test for tablets and capsules

2015-01

This test is provided to determine whether tablets or capsules disintegrate within the prescribed time when placed in a liquid medium under the experimental conditions presented below.

For the purposes of this test disintegration does not imply complete dissolution of the unit or even of its active constituent. Complete disintegration is defined as that state in which any residue of the unit, except fragments of insoluble coating or capsule shell, remaining on the screen of the test apparatus or adhering to the lower surface of the discs, if used, is a soft mass having no palpably firm core.

Use apparatus A for tablets and capsules that are not greater than 18 mm. For larger tablets and capsules use apparatus B.

Test A. Tablets and capsules of normal size

This text is based on the internationally-harmonized texts developed by the Pharmacopoeial Discussion Group (PDG). Some editorial modifications have been made in order to be in line with the style used in The International Pharmacopoeia.

Apparatus. The apparatus (Figure 1) consists of a basket-rack assembly, a 1000 mL, low-form beaker, 138–160 mm in height and having an inside diameter of 97–115 mm for the immersion fluid, a thermostatic arrangement for heating the fluid between 35 °C and 39 °C and a device for raising and lowering the basket in the immersion fluid at a constant frequency rate between 29 and 32 cycles per minute, through a distance of not less than 53 mm and not more than 57 mm. The volume of the fluid in the vessel is such that at the highest point of the upward stroke the wire mesh remains at least 15 mm below the surface of the fluid and descends to not less than 25 mm from the bottom of the vessel on the downward stroke. At no time should the top of the basket-rack assembly become submerged. The time required for the upward stroke is equal to the time required for the downward stroke and the change in stroke direction is a smooth transition rather than an abrupt reversal of motion. The basket-rack assembly moves vertically along its axis. There is no appreciable horizontal motion or movement of the axis from the vertical.

Basket-rack assembly . The basket-rack assembly consists of six open-ended transparent tubes each 75.0–80.0 mm long and having an internal diameter of 20.70–23.00 mm and a wall 1.0–2.8 mm thick; the tubes are held in a vertical position by two plates, each 88–92 mm in diameter and 5.00–8.50 mm in thickness, with six holes, each 22–26 mm in diameter, equidistant from the centre of the plate and equally spaced from one another. Attached to the lower surface of the lower plate is a woven stainless steel wire mesh which has a plain square weave with 1.8–2.2 mm apertures and with a wire diameter of 0.570–0.660 mm. The parts of the apparatus are assembled and rigidly held by means of three bolts passing through the two plates. A suitable means is provided to suspend the basket-rack assembly from the raising and lowering device using a point on its axis.

The design of the basket-rack assembly may be varied somewhat provided the specifications for the glass tubes and the screen mesh size are maintained. The basket-rack assembly conforms to the dimensions shown in Figure 1.

Discs. The use of discs is permitted only where specified or allowed. Each tube is provided with a cylindrical disc 9.35–9.65 mm thick and 20.55–20.85 mm in diameter. The disc is made of a suitable, transparent plastic material having a specific gravity of 1.18–1.20. Five parallel 1.9–2.1 mm holes extend between the ends of the cylinder. One of the holes is centered on the cylindrical axis. The other holes are centered 5.8–6.2 mm from the axis on imaginary lines perpendicular to the axis and parallel to each other. Four identical trapezoidal-shaped planes are cut into the wall of the cylinder nearly perpendicular to the ends of the cylinder. The trapezoidal shape is symmetrical; its parallel sides coincide with the ends of the cylinder and are parallel to an imaginary line connecting the centres of two adjacent holes 6 mm from the cylindrical axis. The parallel side of the trapezoid on the bottom of the cylinder has a length of 1.5–1.7 mm and its bottom edges lie at a depth of 1.50–1.80 mm from the cylinder's circumference. The parallel side of the trapezoid on the top of the cylinder has a length of 9.2–9.6 mm and its centre lies at a depth of 2.5–2.7 mm from the cylinder's circumference. All surfaces of the disc are smooth. If the use of discs is specified add a disc to each tube and operate the apparatus as directed under procedure. The discs conform to the dimensions found in Figure 1.

The use of automatic detection employing modified discs is permitted where the use of discs is specified or allowed. Such discs must comply with the requirements of density and dimension given in this chapter.

Procedure. Place one dosage unit in each of the six tubes of the basket and if specified add a disc. Operate the apparatus using water as the immersion fluid unless another liquid is specified and maintain its temperature at 35–39 °C. At the end of the specified time lift the basket from the fluid and observe the dosage units: all of the dosage units have disintegrated completely. If one or two dosage units fail to disintegrate repeat the test on 12 additional dosage units. The requirements of the test are met if not less than 16 of the 18 dosage units tested are disintegrated.

Test B – Large tablets and large capsules

This test is reproduced from The European Pharmacopoeia *with permission and with appropriate editorial modifications.*

Apparatus. The main part of the apparatus (Figure 2) is a rigid basket-rack assembly supporting 3 cylindrical transparent tubes 77.5 ± 2.5 mm long, 33.0 mm ± 0.5 mm in internal diameter and with a wall thickness of 2.5 ± 0.5 mm. Each tube is provided with a cylindrical disc 31.4 ± 0.13 mm in diameter and 15.3 ± 0.15 mm thick, made of transparent plastic with a relative density of

1.18–1.20. Each disc is pierced by 7 holes, each 3.15 ± 0.1 mm in diameter, 1 in the centre and the other 6 spaced equally on a circle of radius 4.2 mm from the centre of the disc. The tubes are held vertically by 2 separate and superimposed rigid plastic plates 97 mm in diameter and 9 mm thick with 3 holes. The holes are equidistant from the centre of the plate and equally spaced. Attached to the underside of the lower plate is a piece of woven gauze made from stainless steel wire 0.63 ± 0.03 mm in diameter and having mesh apertures of 2.0 ± 0.2 mm. The plates are held rigidly in position and 77.5 mm apart by vertical metal rods at the periphery. A metal rod is also fixed to the centre of the upper plate to enable the assembly to be attached to a mechanical device capable of raising and lowering it smoothly at a constant frequency of between 29 and 32 cycles per minute, through a distance of 55 ± 2 mm.

The assembly is suspended in the specified liquid medium in a suitable vessel preferably a 1 litre beaker. The volume of the liquid is such that when the assembly is in the highest position the wire mesh is at least 15 mm below the surface of the liquid, and when the assembly is in the lowest position the wire mesh is at least 25 mm above the bottom of the beaker and the upper open ends of the tubes remain above the surface of the liquid. A suitable device maintains the temperature of the liquid at 35–39 °C.

The design of the basket-rack assembly may be varied provided the specifications for the tubes and wire mesh are maintained.

Method. Test 6 tablets or capsules either by using 2 basket-rack assemblies in parallel or by repeating the procedure. In each of the 3 tubes place 1 tablet or capsule and, if prescribed, add a disc; suspend the assembly in the beaker containing the specified liquid. Operate the apparatus using water as the immersion fluid unless another liquid is specified for the prescribed period, withdraw the assembly and examine the state of the tablets or capsules. To pass the test all 6 of the tablets or capsules must have disintegrated.

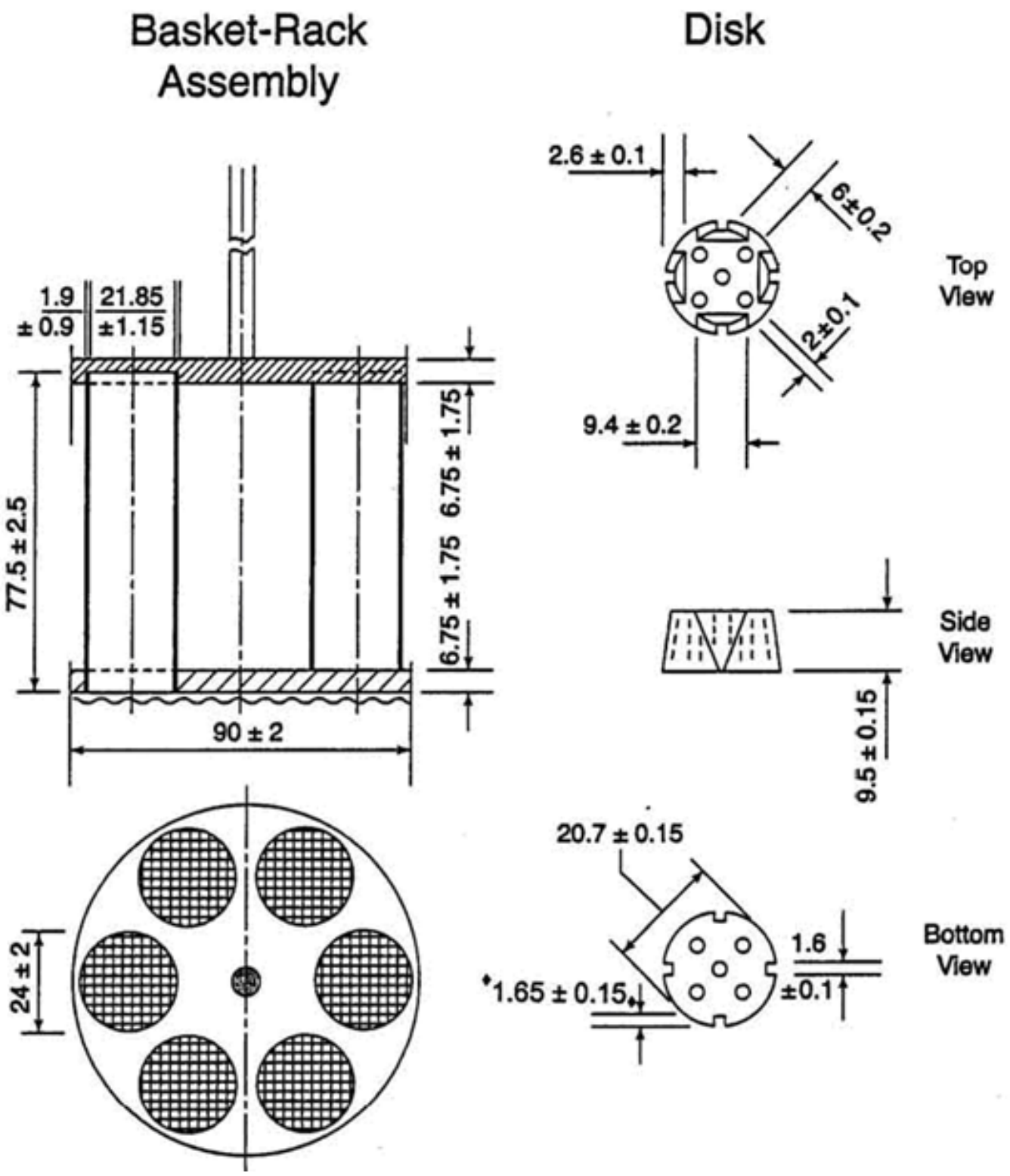


Figure 1. Diagram for disintegration apparatus A (dimensions are expressed in millimeters).

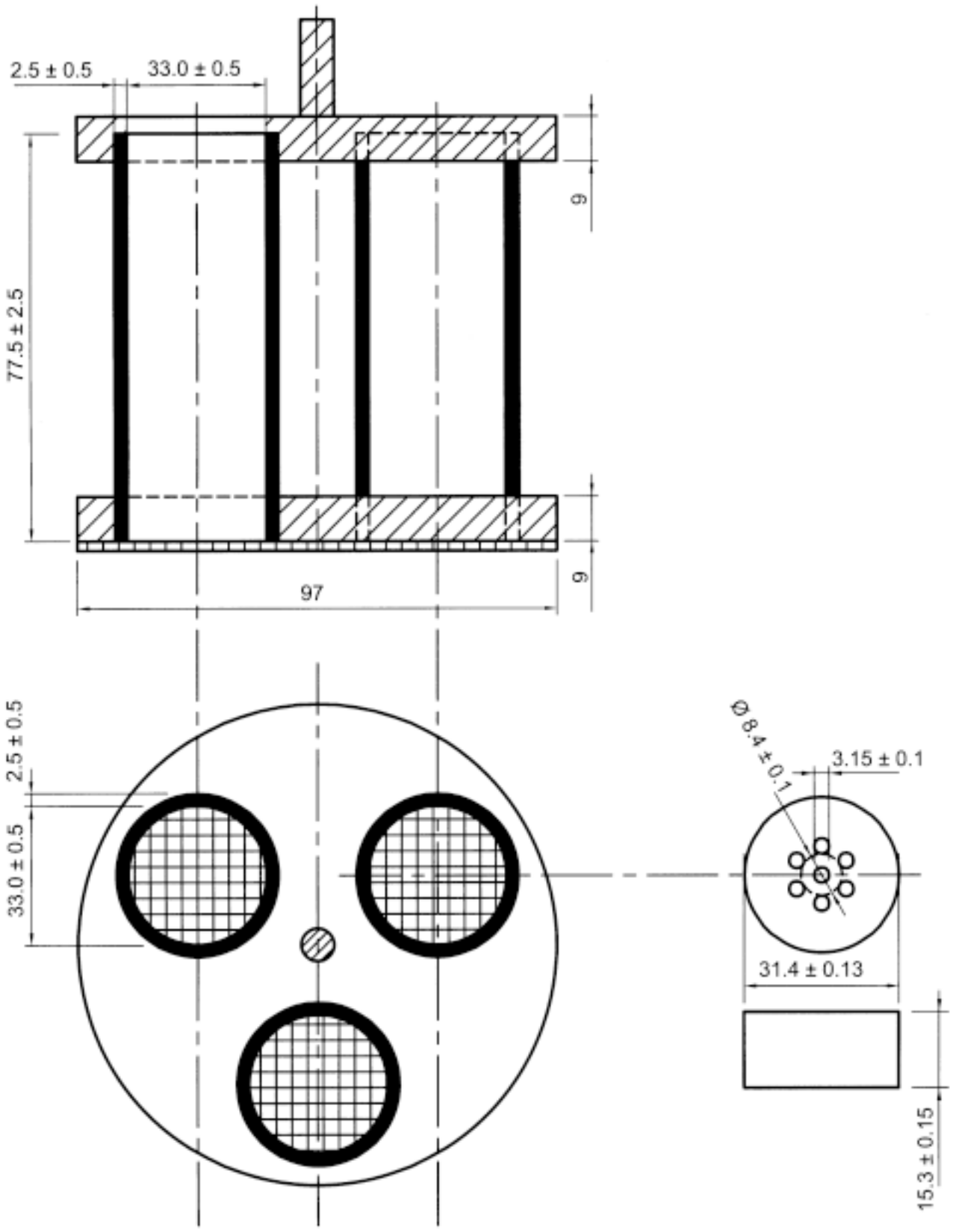


Figure 2.9.1.-2. – *Disintegration apparatus B*
Dimensions in millimetres

Figure 2. Diagram for disintegration apparatus B (dimensions are expressed in millimeters).

5.2 Uniformity of mass for single-dose preparations

Tablets

Uncoated tablets and film-coated tablets formulated to contain 5% or more of the active ingredient should comply with the following test.

Recommended procedure

Weigh 20 tablets and calculate the average mass. When weighed singly, the deviation of individual masses from the average mass should not exceed the limits given below.

Average mass of tablet	Deviation %	Number of tablets
less than 80 mg	±10.0	minimum 18
	±20.0	maximum 2
80 mg to 250 mg	±7.5	minimum 18
	±15.0	maximum 2
more than 250 mg	±5.0	minimum 18
	±10.0	maximum 2

If film-coated tablets fail this test it may be because of variability in the thickness (mass) of the coatings. In such a case, a test for 5.1 Uniformity of content for single-dose preparations should be applied; if the tablets meet the requirement of this test, they can be considered acceptable.

Capsules

Recommended procedure

Weigh 20 intact capsules individually, and calculate the average mass. The mass of each capsule should be within ±10% of the average mass. If all the capsules do not fall within these limits, weigh the 20 capsules again, taking care to preserve the identity of each capsule, and remove the contents as completely as possible. For soft gelatin capsules, wash the shell with ether or some other suitable solvent and allow it to stand until the odour of the solvent is no longer perceptible. Other means, such as a jet of compressed air, may be used to remove the contents.

Weigh the emptied shells individually and calculate for each capsule the net mass of its contents by subtracting the mass of the shell from the gross mass. Determine the average net content from the sum of the individual net masses. Then determine the difference between each individual net content and the average net content. Deviation of individual net mass from the average net mass should not exceed the limits given below.

Net mass of capsule contents	Deviation %	Number of capsules
less than 300 mg	± 10.0	minimum 18
	± 20.0	maximum 2
300 mg and over	± 7.5	minimum 18
	± 15.0	maximum 2

Oral powders

Weigh 20 units and calculate the average mass. The deviation of individual mass from the average mass should not exceed the limits given above under "Capsules".

Powders for injections

The test applies to powders for injections where the content is more than 40 mg. The recommended procedure is the same as described for capsules. The deviation of individual net mass from the average net mass should not exceed the limits given below.

Deviation %	Number of containers
± 10	minimum 18
± 20	maximum 2

For preparations with a content of less than 40 mg the test for 5.1 Uniformity of content for single-dose preparations applies instead.

Suppositories

Weigh 20 suppositories and calculate the average mass. When the suppositories are weighed singly, the deviation of individual mass from the average mass should not exceed the limits given below.

Deviation %	Number of suppositories
± 5	minimum 18
±10	maximum 2

5.1 Uniformity of content for single-dose preparations

This test applies only where the declared quantity of active ingredient in tablets, capsules, oral powders, single-dose oral suspensions or suppositories is 5 mg or less or is 5% or less of the total formulation or, in the case of sugar-coated and enteric-coated tablets, where the test for 5.2 Uniformity of mass for single-dose preparations does not apply, or for powders for injection or intravenous infusions for which the declared content of active ingredient is 40 mg or less. The test applies to all single-dose suspensions for injection.

For a particular tablet, capsule, oral powder, single-dose oral suspension, suppository or powder for injection or infusion with a strength higher than any indicated in the relevant individual monograph, and for which the declared quantity of active ingredient is outside the above threshold, any requirement for Uniformity of content included in the specific monograph does not apply.

Recommended procedure

Individually determine the amount of active ingredient in each of 10 units using the analytical method specified in the individual monograph under Uniformity of content.

For a particular tablet, capsule oral powder, single-dose oral suspension, suppository or powder for injection or infusion for which the declared quantity of active ingredient is within the threshold stated above, but where no requirement for Uniformity of content is included in the specific monograph, use the analytical procedure described under Assay, suitably modified, where necessary.

Requirements for tablets, single-dose suspensions for injection and single-dose powders for injections or intravenous infusions

Each single unit contains within ±15% of the average amount of the active ingredient. However, if one individual unit deviates by more than ±15% but is within ±25% of the average amount of the active ingredient, examine a further 20 units drawn from the same original sample as the first 10 units. The preparation under test complies only if the amount of active ingredient found in no more than one out of 30 units deviates by more than ±15% of the average amount. None deviates by more than ±25% of the average amount.

Requirements for capsules, single-dose oral powders, single-dose oral suspensions and suppositories

Each single unit contains within ±15% of the average amount of active ingredient. However, if up to three individual units deviate by more than ±15% but are within ±25% of the average amount of the active ingredient, examine a further 20 units drawn from the same original sample as the first 10 units. The preparation under test complies only if the amount of active ingredient found in no more than three out of 30 units deviates by more than ±15% of the average amount. None deviates by more than ±25% of the average amount.

Introduction

In addition to general methods of analysis that are invoked within the general monographs for different types of dosage forms, for example, dissolution testing, this section contains other general texts of relevance during the development and manufacture of pharmaceutical dosage forms and to which reference is made within the monographs of the *International Pharmacopoeia*.

4.6 Determination of acid value

The acid value is the number of mg of potassium hydroxide required to neutralize the free acid in 1 g of the substance.

Recommended procedure

Accurately weigh about 10 g of the substance, or the quantity specified in the monograph, into a 250-mL flask, and add 50 mL of a mixture of equal volumes of ethanol (~750 g/l) TS and ether R, which has been neutralized with potassium hydroxide (0.1 mol/l) VS after the addition of 1 mL of phenolphthalein/ethanol TS. Heat, if necessary, until the substance has completely dissolved, cool; titrate with potassium hydroxide (0.1 mol/l) VS, constantly shaking the contents of the flask until a pink colour, which persists for 15 seconds, is obtained. Note the number of mL required (*a*). Calculate the acid value from the following formula:

$$\text{Acid value} = \frac{a \times 0.00561 \times 1000}{\text{weight (in g) of substance}}$$

4.7 Determination of hydroxyl value

The hydroxyl value of a substance is the amount, in milligrams, of potassium hydroxide required to neutralize any acid when combined by acylation in 1 g of the substance under examination.

Recommended procedures

Method A

To the quantity of the substance being examined (as specified in the individual monograph) add 12 g of stearic anhydride R and 10 mL of xylene R and heat gently under a reflux condenser for 30 minutes. Allow to cool, add a mixture of 40 mL of pyridine R and 4 mL of water, and heat again under a reflux condenser for 30 minutes. Titrate the hot solution with carbonate-free sodium hydroxide (1 mol/l) VS, using phenolphthalein/ethanol TS as indicator. Repeat the procedure, omitting the substance under examination.

The hydroxyl value is calculated from the expression 56.10 *v/m,* where *v* is the difference, in mL, between the two titrations and *m* is the quantity, in g, of the substance taken.

Method B

Unless otherwise indicated in the individual monograph, weigh accurately the quantity of the substance to be examined shown in the table under 4.7 Determination of hydroxyl value, place it in a 150-mL acetylation flask fitted with an air condenser and add the corresponding volume of pyridine/acetic anhydride TS.

Presumed hydroxyl value	Quantity of substance (g)	Volume of pyridine/acetic anhydride TS (mL)
10-100	2.0	5.0
100-150	1.5	5.0
150-200	1.0	5.0
200-250	0.75	5.0
250-300	0.60 or 1.20	5.0 or 10.0
300-350	1.0	10.0
350-700	0.75	15.0
700-950	0.5	15.0

Heat the flask for 1 hour in a water-bath, maintaining the level of the water 2-3 cm above the level of the liquid in the flask. Remove the flask and condenser, allow to cool, and add 5 mL of water through the top of the condenser. If a cloudiness appears, add sufficient pyridine R to produce a clear liquid, noting the volume added. Shake the flask, place it in a water-bath for 10 minutes, remove, and allow to cool. Rinse the condenser and the walls of the flask with 5 mL of neutralized ethanol TS. Titrate with potassium hydroxide/ethanol (0.5 mol/l) VS, using 0.2 mL of phenolphthalein/ethanol TS as indicator. Repeat the procedure, omitting the substance under examination.

Calculate the hydroxyl value from the expression ($a + 28.05$) *v/m*, where *v* is the difference, in mL, between the two titrations, *a* is the acid value determined for the substance, and *m* is the quantity, in g, of the substance taken.

4.5 Determination of unsaponifiable matter

The term "unsaponifiable matter" refers to those substances present in oils or fats that are not saponified by alkali hydroxides and are extractable into ether.

Recommended procedure

Place a quantity of the test substance, accurately weighed, as specified in the monograph, in a flask provided with a reflux condenser and boil in a water-bath for 1 hour with 25 mL of potassium hydroxide/ethanol (0.5 mol/l) VS, with frequent swirling of contents. Wash the contents of the flask into a separator by means of 50 mL of water and, while the liquid is still slightly warm, extract by shaking vigorously with 3 successive quantities, each of 50 mL, of ether R, washing out the flask with the first quantity of ether R. (CAUTION: Ether should be free of peroxides.) Take care to release frequently and carefully the pressure that may build up inside the separator. Combine the ethereal solutions in another separator containing 20 mL of water. (If the ethereal solutions contain solid suspended matter, filter them into the separator through a fat-free filter-paper and wash the filter-paper with ether R.) Gently rotate the separator for a few minutes without violent shaking, allow the liquids to separate, and run off the aqueous layer. Wash the ethereal solution by shaking vigorously with 2 successive quantities, each of 20 mL, of water; then treat 3 times with 20 mL of potassium hydroxide (0.5 mol/l) VS (NOTE: aqueous reagent), shaking vigorously on each occasion and washing with 20 mL of water after each treatment. Finally wash with successive quantities, each of 20 mL, of water until the aqueous layer is no longer alkaline to phenolphthalein/ethanol TS. Transfer the ethereal extract to a weighed flask, washing out the separator with ether R; distil off the ether with the necessary precautions and add 3 mL of acetone R.

By the aid of a gentle current of air remove the solvent completely from the flask, which is preferably held obliquely and rotated, almost entirely immersed, in a water-bath at about 60 °C. Dry to constant weight at a temperature not above 80 °C and dissolve the contents of the flask in 10 mL of ethanol (~750 g/l) TS, previously neutralized to phenolphthalein/ethanol TS. Titrate with carbonate-free sodium hydroxide (0.1 mol/l) VS, using phenolphthalein/ethanol TS as indicator. If the amount of carbonate-free sodium hydroxide (0.1 mol/l) required does not exceed 0.2 mL, the amount weighed is to be taken as the unsaponifiable matter. Calculate the unsaponifiable matter as a percentage of the oil or fat. If the amount of carbonate-free sodium hydroxide (0.1 mol/l) VS required exceeds 0.2 mL, the amount weighed cannot be taken as the unsaponifiable matter and the test must be repeated.

4.4 Determination of saponification value

The saponification value is the number of mg of potassium hydroxide required to neutralize the fatty acids resulting from the complete hydrolysis of 1 g of the substance.

In the procedure described, a 50-mL burette should preferably be used for titration, as in the blank titration the volume of hydrochloric acid (0.5 mol/l) VS used is exactly 35.5 mL when the concentration of ethanolic potassium hydroxide is exactly 40 g/l.

Recommended procedure

Place about 2 g of the test substance, accurately weighed, or the quantity specified in the monograph, in a flask with a capacity of about 200 mL, add 25 mL of potassium hydroxide/ethanol, TS1 attach a reflux condenser, and heat in a boiling water-bath for 30 minutes, or the time specified in the monograph, frequently rotating the contents of the flask; immediately add 1 mL of phenolphthalein/ethanol TS and titrate the excess of alkali with hydrochloric acid (0.5 mol/l) VS. Note the number of mL of hydrochloric acid (0.5 mol/l) VS required to titrate the sample (*a*). Repeat the operation without the substance being tested and note the number of mL of hydrochloric acid (0.5 mol/l) VS required for neutralization (*b*). Calculate the saponification value from the following formula:

$$\text{Saponification value} = \frac{(b - a) \times 0.02805 \times 1000}{\text{weight (in g) of substance}}$$

4.3 Determination of peroxides in fixed oils

Recommended procedure

Dissolve the quantity of test substance as specified in the monograph, usually about 3 g, accurately weighed, in 15 mL of chloroform R and 30 mL of glacial acetic acid R in a 250-mL glass-stoppered flask. Add 1 mL of a freshly prepared solution of 1.3 g of potassium iodide R in 1 mL of water, stopper the flask, mix by gentle swirling and set aside in the dark for 3 minutes. Add 100 mL of water, shake, and titrate with sodium thiosulfate (0.01 mol/l) VS, using starch TS as indicator. Repeat the operation without the substance being tested and calculate the difference between the titrations, the limit value being specified in the monograph.

4.2 Determination of iodine value

The iodine value of a substance is the weight of halogens expressed as iodine absorbed by 100 parts by weight of the substance. The quantity of substance used in the determination should be such that at least 70% of the iodine added, as provided in the recommended procedure, is not absorbed. Unless otherwise specified in the monograph, the quantity of the substance indicated in the following table should be used for the determination, depending on the expected iodine value:

Iodine value	*Quantity of substance in g*
less than 20	1.0
20 - 60	0.5 - 0.25
60 - 100	0.25 - 0.15
more than 100	0.15 - 0.10

Recommended procedure

Place a quantity of the test substance, accurately weighed, as specified in the monograph, in a dry 300-mL to 500-mL stoppered flask, add 15 mL of carbon tetrachloride R and dissolve. Add 25 mL of iodine bromide TS, insert the stopper, previously moistened with potassium iodide (80 g/l) TS, shake the flask gently, and keep in the dark for 30 minutes, unless otherwise specified in the monograph. Add 20 mL of potassium iodide (80 g/l) TS and 150 mL of water, and, whilst shaking the contents of the flask, titrate with sodium thiosulfate (0.1 mol/l) VS, adding starch TS as indicator towards the end of the titration. Note the number of mL required (*a*). At the same time carry out the operation in exactly the same manner, but without the substance being tested, and note the number of mL of sodium thiosulfate (0.1 mol/l) VS required (*b*). Calculate the iodine value from the following formula:

$$\text{Iodine value} = \frac{(b\text{-}a) \times 0.01269 \times 100}{\text{weight (in g) of substance}}$$

4.1 Determination of ash and acid-insoluble ash

Recommended procedures

Determination of ash

Place about 3 g of the ground material, accurately weighed, or the quantity specified in the monograph, in a suitable tared dish (for example, of silica or platinum), previously ignited, cooled and weighed. Incinerate the material by gradually increasing the heat, not exceeding 450 °C, until free from carbon; cool, and weigh. If a carbon-free ash cannot be obtained in this way, exhaust the charred mass with hot water, collect the residue on an ashless filter-paper, incinerate the residue and filter-paper, add the filtrate, evaporate to dryness, and ignite at a temperature not exceeding 450 °C. Calculate the content in mg of ash per g of air-dried material.

Determination of acid-insoluble ash

Boil the ash for 5 minutes with 25 mL of hydrochloric acid (~70 g/l) TS; collect the insoluble matter in a sintered crucible, or on an ashless filter-paper, wash with hot water, and ignite at about 500 °C to constant weight. Calculate the content in mg of acid-insoluble ash per g of air-dried material.

2.3 Sulfated ash

The test Method B is based on the internationally-harmonized texts developed by the Pharmacopoeial Discussion Group (PDG). Some editorial modifications have been made in order to be in line with the style used in The International Pharmacopoeia.

The sulfated ash test uses a procedure to measure the amount of residual substance not volatilized from a sample when the sample is ignited in the presence of sulfuric acid. The test is usually used for determining the content of inorganic impurities in an organic substance. Unless otherwise indicated in the individual monograph, use Method A.

Method A

Accurately weigh about 1 g of the substance, or the quantity specified in the monograph, into a suitable crucible (usually platinum) and moisten with sulfuric acid (~1760 g/l) TS. Heat gently to remove the excess of acid and ignite at about 800 °C until all the black particles have disappeared; again moisten with sulfuric acid (~1760 g/l) TS and reignite. Add a small amount of ammonium carbonate R and ignite to constant weight.

Method B

Ignite a suitable crucible (for example silica, platinum, quartz or porcelain) at 550 °C to 650 °C for 30 minutes, cool the crucible in a desiccator (silica gel or other suitable desiccant) and weigh it accurately. Take the amount of test sample specified in the individual monograph in the crucible and weigh the crucible accurately. Moisten the sample with a small amount (usually 1 mL) of sulfuric acid (~1760 g/l) TS, heat gently at a temperature as low as practicable until the sample is thoroughly charred. After cooling moisten the residue with a small amount (usually 1 mL) of sulfuric acid (~1760 g/l) TS, heat gently until white fumes are no longer evolved and ignite at 550 °C to 650 °C until the residue is completely incinerated. Ensure that flames are not produced at any time during the procedure. Cool the crucible in a desiccator (silica gel or other suitable desiccant), weigh accurately and calculate the percentage of residue.

Unless otherwise specified, if the amount of residue so obtained exceeds the limit specified in the individual monograph, repeat the moistening with sulfuric acid, heating and ignition as before, using a 30-minute ignition period, until two consecutive weighings of the residue do not differ by more than 0.5 mg or until the percentage of residue complies with the limit in the individual monograph.