EUROPEAN PHARMACOPOEIA

Free access to supportive pharmacopoeial texts during the coronavirus (COVID-19) outbreak

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Free access to supportive pharmacopoeial texts during the coronavirus (COVID-19) outbreak

The British Pharmacopoeia (BP) and European Pharmacopoeia (Ph. Eur.) are committed to supporting users during the coronavirus (COVID-19) outbreak and, as part of the wider healthcare system response to this challenging period, the listed pharmacopoeial texts (monographs, general chapters, appendices and supplementary chapters) are temporarily being made freely available, at no cost, to all professionals involved in public health protection. This is to support those involved in the development, manufacture or testing of these substances and products worldwide. This list is being reviewed regularly and will be updated as required, and ultimately withdrawn when appropriate. For example, when the outbreak is suitably under control.

This in no way affects the existing legal status of the European or British Pharmacopoeias, nor does it imply or confer any demonstrated effectiveness for the treatment of COVID-19 using any of the substances or products in specific monographs. This is confirmed by the inclusion of the following text at the bottom of pharmacopoeial texts reproduced in this document: "Not official text. Please refer to the current legally effective version of the Pharmacopoeia to ensure compliance."

The British and European Pharmacopoeias have coordinated the release of these pharmacopoeial texts to help maximise their distribution to potential users. These texts will be available on the EDQM *freepub* website (https://register.edqm.eu/freepub) and on www.pharmacopoeia.com as two separate PDFs; one which solely includes Ph. Eur. content and another which includes the BP content incorporating Ph. Eur. content. Texts from the British Pharmacopoeia are from the BP 2020 and texts from the European Pharmacopoeia are from the 10th Edition including Supplement 10.1.

If you have any questions or comments please contact bpcom@mhra.gov.uk (for the British Pharmacopoeia) and EDQM, via its Helpdesk (https://helpdesk.edqm.eu/servicedesk), (for the European Pharmacopoeia).

The European Pharmacopoeia is published by the European Directorate for the Quality of Medicines & HealthCare of the Council of Europe (EDQM).

Individual monographs

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1. GENERAL NOTICES

1.1. GENERAL STATEMENTS

The General Notices apply to all monographs and other texts of the European Pharmacopoeia.

The official texts of the European Pharmacopoeia are published in English and French. Translations in other languages may be prepared by the signatory States of the European Pharmacopoeia Convention. In case of doubt or dispute, the English and French versions are alone authoritative.

In the texts of the European Pharmacopoeia, the word 'Pharmacopoeia' without qualification means the European Pharmacopoeia. The official abbreviation Ph. Eur. may be used to indicate the European Pharmacopoeia.

The use of the title or the subtitle of a monograph implies that the article complies with the requirements of the relevant monograph. Such references to monographs in the texts of the Pharmacopoeia are shown using the monograph title and reference number in *italics*.

A preparation must comply throughout its period of validity; a distinct period of validity and/or specifications for opened or broached containers may be decided by the competent authority. The subject of any other monograph must comply throughout its period of use. The period of validity that is assigned to any given article and the time from which that period is to be calculated are decided by the competent authority in light of experimental results of stability studies.

Unless otherwise indicated in the General Notices or in the monographs, statements in monographs constitute mandatory requirements. General chapters become mandatory when referred to in a monograph, unless such reference is made in a way that indicates that it is not the intention to make the text referred to mandatory but rather to cite it for information.

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

Quality systems. The quality standards represented by monographs are valid only where the articles in question are produced within the framework of a suitable quality system. The quality system must assure that the articles consistently meet the requirements of the Pharmacopoeia.

Alternative methods. The tests and assays described are the official methods upon which the standards of the Pharmacopoeia are based. With the agreement of the competent authority, alternative methods of analysis may be used for control purposes, provided that the methods used enable an unequivocal decision to be made as to whether compliance with the standards of the monographs would be achieved if the official methods were used. In the event of doubt or dispute, the methods of analysis of the Pharmacopoeia are alone authoritative.

Demonstration of compliance with the Pharmacopoeia

(1) An article is not of Pharmacopoeia quality unless it complies with all the requirements stated in the monograph. This does not imply that performance of all the tests in a monograph is necessarily a prerequisite for a manufacturer in assessing compliance with the Pharmacopoeia before release of a product. The manufacturer may obtain assurance that a product is of Pharmacopoeia quality on the basis of its design, together with its control strategy and data derived, for example, from validation studies of the manufacturing process.

(2) An enhanced approach to quality control could utilise process analytical technology (PAT) and/or real-time release testing (including parametric release) strategies as alternatives to end-product testing alone. Real-time release testing in circumstances deemed appropriate by the competent authority is thus not precluded by the need to comply with the Pharmacopoeia.

(3) Reduction of animal testing: the European Pharmacopoeia is dedicated to phasing out the use of animals for test purposes, in accordance with the 3Rs (Replacement, Reduction, Refinement) set out in the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes. In demonstrating compliance with the Pharmacopoeia as indicated above (1), manufacturers may consider establishing additional systems to monitor consistency of production. With the agreement of the competent authority, the choice of tests performed to assess compliance with the Pharmacopoeia when animal tests are prescribed is established in such a way that animal usage is minimised as much as possible.

Grade of materials. Certain materials that are the subject of a pharmacopoeial monograph may exist in different grades suitable for different purposes. Unless otherwise indicated in the monograph, the requirements apply to all grades of the material. In some monographs, particularly those on excipients, a list of functionality-related characteristics that are relevant to the use of the substance may be appended to the monograph for information. Test methods for determination of one or more of these characteristics may be given, also for information.

General monographs. Substances and preparations that are the subject of an individual monograph are also required to comply with relevant, applicable general monographs. Cross-references to applicable general monographs are not normally given in individual monographs.

General monographs apply to all substances and preparations within the scope of the Definition section of the general monograph, except where a preamble limits the application, for example to substances and preparations that are the subject of a monograph of the Pharmacopoeia.

General monographs on dosage forms apply to all preparations of the type defined. The requirements are not necessarily comprehensive for a given specific preparation and requirements additional to those prescribed in the general monograph may be imposed by the competent authority.

General monographs and individual monographs are complementary. If the provisions of a general monograph do not apply to a particular product, this is expressly stated in the individual monograph.

Validation of pharmacopoeial methods. The test methods given in monographs and general chapters have been validated in accordance with accepted scientific practice and current recommendations on analytical validation. Unless otherwise stated in the monograph or general chapter, validation of the test methods by the analyst is not required.

Implementation of pharmacopoeial methods. When implementing a pharmacopoeial method, the user must assess whether and to what extent the suitability of the method under the actual conditions of use needs to be demonstrated according to relevant monographs, general chapters and quality systems.

Conventional terms. The term 'competent authority' means the national, supranational or international body or organisation vested with the authority for making decisions concerning the issue in question. It may, for example, be a national pharmacopoeia authority, a licensing authority or an official control laboratory.

The expression 'unless otherwise justified and authorised' means that the requirements have to be met, unless the

competent authority authorises a modification or an exemption where justified in a particular case.

Statements containing the word 'should' are informative or advisory.

In certain monographs or other texts, the terms 'suitable' and 'appropriate' are used to describe a reagent, micro-organism, test method etc.; if criteria for suitability are not described in the monograph, suitability is demonstrated to the satisfaction of the competent authority.

Medicinal product. (a) Any substance or combination of substances presented as having properties for treating or preventing disease in human beings and/or animals; or (b) any substance or combination of substances that may be used in or administered to human beings and/or animals with a view either to restoring, correcting or modifying physiological functions by exerting a pharmacological, immunological or metabolic action, or to making a medical diagnosis.

Herbal medicinal product. Any medicinal product, exclusively containing as active ingredients one or more herbal drugs or one or more herbal drug preparations, or one or more such herbal drugs in combination with one or more such herbal drug preparations.

Active substance. Any substance intended to be used in the manufacture of a medicinal product and that, when so used, becomes an active ingredient of the medicinal product. Such substances are intended to furnish a pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment or prevention of disease, or to affect the structure and function of the body.

Excipient (auxiliary substance). Any constituent of a medicinal product that is not an active substance. Adjuvants, stabilisers, antimicrobial preservatives, diluents, antioxidants, for example, are excipients.

Interchangeable methods. Certain general chapters contain a statement that the text in question is harmonised with the corresponding text of the Japanese Pharmacopoeia and/or the United States Pharmacopeia and that these texts are interchangeable. This implies that if a substance or preparation is found to comply with a requirement using an interchangeable method from one of these pharmacopoeias it complies with the requirements of the European Pharmacopoeia. In the event of doubt or dispute, the text of the European Pharmacopoeia is alone authoritative.

References to regulatory documents. Monographs and general chapters may contain references to documents issued by regulatory authorities for medicines, for example directives and notes for guidance of the European Union. These references are provided for information for users for the Pharmacopoeia. Inclusion of such a reference does not modify the status of the documents referred to, which may be mandatory or for guidance.

1.2. OTHER PROVISIONS APPLYING TO GENERAL CHAPTERS AND MONOGRAPHS

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

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

It is recognised, however, that in certain cases the precision with which quantities are stated does not correspond to the number of significant figures stated in a specified numerical limit. The weighings and measurements are then carried out with a sufficiently improved accuracy.

Apparatus and procedures. Volumetric glassware complies with Class A requirements of the appropriate International Standard issued by the International Organisation for Standardisation.

Unless otherwise prescribed, analytical procedures are carried out at a temperature between 15 °C and 25 °C.

Unless otherwise prescribed, comparative tests are carried out using identical tubes of colourless, transparent, neutral glass with a flat base; the volumes of liquid prescribed are for use with tubes having an internal diameter of 16 mm, but tubes with a larger internal diameter may be used provided the volume of liquid used is adjusted (*2.1.5*). Equal volumes of the liquids to be compared are examined down the vertical axis of the tubes against a white background, or if necessary against a black background. The examination is carried out in diffuse light.

Any solvent required in a test or assay in which an indicator is to be used is previously neutralised to the indicator, unless a blank test is prescribed.

Water-bath. The term 'water-bath' means a bath of boiling water unless water at another temperature is indicated. Other methods of heating may be substituted provided the temperature is near to but not higher than 100 °C or the indicated temperature.

Drying and ignition to constant mass. The terms 'dried to constant mass' and 'ignited to constant mass' mean that 2 consecutive weighings do not differ by more than 0.5 mg, the 2nd weighing following an additional period of drying or of ignition respectively appropriate to the nature and quantity of the residue.

Where drying is prescribed using one of the expressions 'in a desiccator' or '*in vacuo*', it is carried out using the conditions described in chapter *2.2.32. Loss on drying*.

Reagents. The proper conduct of the analytical procedures described in the Pharmacopoeia and the reliability of the results depend, in part, upon the quality of the reagents used. The reagents are described in general chapter *4*. It is assumed that reagents of analytical grade are used; for some reagents, tests to determine suitability are included in the specifications.

Solvents. Where the name of the solvent is not stated, the term 'solution' implies a solution in water.

Where the use of water is specified or implied in the analytical procedures described in the Pharmacopoeia or for the preparation of reagents, water complying with the requirements of the monograph *Purified water (0008)* is used, except that for many purposes the requirements for bacterial endotoxins (*Purified water in bulk*) and microbial contamination (*Purified water in containers)* are not relevant. The term 'distilled water' indicates purified water prepared by distillation.

The term 'ethanol' without qualification means anhydrous ethanol. The term 'alcohol' without qualification means ethanol (96 per cent). Other dilutions of ethanol are indicated by the term 'ethanol' or 'alcohol' followed by a statement of the percentage by volume of ethanol (C_2H_6O) required.

Expression of content. In defining content, the expression 'per cent' is used according to circumstances with one of 2 meanings:

– per cent *m/m* (percentage, mass in mass) expresses the number of grams of substance in 100 g of final product;

– per cent *V/V* (percentage, volume in volume) expresses the number of millilitres of substance in 100 mL of final product.

The expression 'parts per million' (or ppm) refers to mass in mass, unless otherwise specified.

Temperature. Where an analytical procedure describes temperature without a figure, the general terms used have the following meaning:

– in a deep-freeze: below – 15 °C;

– in a refrigerator: 2 °C to 8 °C;

– cold or cool: 8 °C to 15 °C;

– room temperature: 15 °C to 25 °C.

1.3. GENERAL CHAPTERS

Containers. Materials used for containers are described in general chapter *3.1*. General names used for materials, particularly plastic materials, each cover a range of products varying not only in the properties of the principal constituent but also in the additives used. The test methods and limits for materials depend on the formulation and are therefore applicable only for materials whose formulation is covered by the preamble to the specification. The use of materials with different formulations, and the test methods and limits applied to them, are subject to agreement by the competent authority.

The specifications for containers in general chapter *3.2* have been developed for general application to containers of the stated category, but in view of the wide variety of containers available and possible new developments, the publication of a specification does not exclude the use, in justified circumstances, of containers that comply with other specifications, subject to agreement by the competent authority.

Reference may be made within the monographs of the Pharmacopoeia to the definitions and specifications for containers provided in chapter *3.2. Containers*. The general monographs for pharmaceutical dosage forms may, under the heading Definition/Production, require the use of certain types of container; certain other monographs may, under the heading Storage, indicate the type of container that is recommended for use.

1.4. MONOGRAPHS

TITLES

Monograph titles are in English and French in the respective versions and there is a Latin subtitle.

RELATIVE ATOMIC AND MOLECULAR MASSES

The relative atomic mass (A_r) or the relative molecular mass (M_r) is shown, as and where appropriate, at the beginning of each monograph. The relative atomic and molecular masses and the molecular and graphic formulae do not constitute analytical standards for the substances described.

CHEMICAL ABSTRACTS SERVICE (CAS) REGISTRY NUMBER

CAS registry numbers are included for information in monographs, where applicable, to provide convenient access to useful information for users. CAS Registry Number® is a registered trademark of the American Chemical Society.

DEFINITION

Statements under the heading Definition constitute an official definition of the substance, preparation or other article that is the subject of the monograph.

Limits of content. Where limits of content are prescribed, they are those determined by the method described under Assay.

Herbal drugs. In monographs on herbal drugs, the definition indicates whether the subject of the monograph is, for example, the whole drug or the drug in powdered form. Where a monograph applies to the drug in several states, for example both to the whole drug and the drug in powdered form, the definition states this.

PRODUCTION

Statements under the heading Production draw attention to particular aspects of the manufacturing process but are not necessarily comprehensive. They constitute mandatory requirements for manufacturers, unless otherwise stated. They may relate, for example, to source materials; to the manufacturing process itself and its validation and control; to in-process testing; or to testing that is to be carried out by the manufacturer on the final article, either on selected batches or on each batch prior to release. These statements cannot necessarily be verified on a sample of the final article by an independent analyst. The competent authority may establish that the instructions have been followed, for example, by examination of data received from the manufacturer, by inspection of manufacture or by testing appropriate samples.

The absence of a Production section does not imply that attention to features such as those referred to above is not required.

Choice of vaccine strain, Choice of vaccine composition. The Production section of a monograph may define the characteristics of a vaccine strain or vaccine composition. Unless otherwise stated, test methods given for verification of these characteristics are provided for information as examples of suitable methods. Subject to approval by the competent authority, other test methods may be used without validation against the method shown in the monograph.

POTENTIAL ADULTERATION

Due to the increasing number of fraudulent activities and cases of adulteration, information may be made available to Ph. Eur. users to help detect adulterated materials (i.e. active substances, excipients, intermediate products, bulk products and finished products).

To this purpose, a method for the detection of potential adulterants and relevant limits, together with a reminder that all stages of production and sourcing are subjected to a suitable quality system, may be included in this section of monographs on substances for which an incident has occurred or that present a risk of deliberate contamination. The frequency of testing by manufacturers or by users (e.g. manufacturers of intermediate products, bulk products and finished products, where relevant) depends on a risk assessment, taking into account the level of knowledge of the whole supply chain and national requirements.

This section constitutes requirements for the whole supply chain, from manufacturers to users (e.g. manufacturers of intermediate products, bulk products and finished products, where relevant). The absence of this section does not imply that attention to features such as those referred to above is not required.

CHARACTERS

The statements under the heading Characters are not to be interpreted in a strict sense and are not requirements.

Solubility. In statements of solubility in the Characters section, the terms used have the following significance, referred to a temperature between 15 °C and 25 °C.

Descriptive term	Approximate volume of solvent in millilitres per gram of solute			
Very soluble	less than	1		
Freely soluble	from	1	to	10
Soluble	from	10	to	30
Sparingly soluble	from	30	to	100
Slightly soluble	from	100	to	1000
Very slightly soluble	from	1000	to	10 000
Practically insoluble	more than			10 000

The term 'partly soluble' is used to describe a mixture where only some of the components dissolve. The term 'miscible' is used to describe a liquid that is miscible in all proportions with the stated solvent.

IDENTIFICATION

Scope. The tests given in the Identification section are not designed to give a full confirmation of the chemical structure or composition of the product; they are intended to give confirmation, with an acceptable degree of assurance, that the article conforms to the description on the label.

First and second identifications. Certain monographs have subdivisions entitled 'First identification' and 'Second identification'. The test or tests that constitute the 'First identification' may be used in all circumstances. The test or tests that constitute the 'Second identification' may be used in pharmacies provided it can be demonstrated that the substance or preparation is fully traceable to a batch certified to comply with all the other requirements of the monograph.

Certain monographs give two or more sets of tests for the purpose of the first identification, which are equivalent and may be used independently. One or more of these sets usually contain a cross-reference to a test prescribed in the Tests section of the monograph. It may be used to simplify the work of the analyst carrying out the identification and the prescribed tests. For example, one identification set cross-refers to a test for enantiomeric purity while the other set gives a test for specific optical rotation: the intended purpose of the two is the same, that is, verification that the correct enantiomer is present.

Powdered herbal drugs. Monographs on herbal drugs may contain schematic drawings of the powdered drug. These drawings complement the description given in the relevant identification test.

TESTS AND ASSAYS

Scope. The requirements are not framed to take account of all possible impurities. It is not to be presumed, for example, that an impurity that is not detectable by means of the prescribed tests is tolerated if common sense and good pharmaceutical practice require that it be absent. See also below under Impurities.

Calculation. Where the result of a test or assay is required to be calculated with reference to the dried or anhydrous substance or on some other specified basis, the determination of loss on drying, water content or other property is carried out by the method prescribed in the relevant test in the monograph. The words 'dried substance' or 'anhydrous substance' etc. appear in parentheses after the result. Where a quantitative determination of a residual solvent is carried out and a test for loss on drying is not carried out, the content of residual solvent is taken into account for the calculation of the assay content of the substance, the specific optical rotation and the specific absorbance. No further indication is given in the specific monograph.

Limits. The limits prescribed are based on data obtained in normal analytical practice; they take account of normal analytical errors, of acceptable variations in manufacture and compounding and of deterioration to an extent considered acceptable. No further tolerances are to be applied to the limits prescribed to determine whether the article being examined complies with the requirements of the monograph.

In determining compliance with a numerical limit, the calculated result of a test or assay is first rounded to the number of significant figures stated, unless otherwise prescribed. The limits, regardless of whether the values are expressed as percentages or as absolute values, are considered significant to the last digit shown (for example 140 indicates 3 significant figures). The last figure of the result is increased by one when the part rejected is equal to or exceeds one half-unit, whereas it is not modified when the part rejected is less than a half-unit.

Indication of permitted limit of impurities. The acceptance criteria for related substances are expressed in monographs either in terms of comparison of peak areas (comparative tests) or as numerical values. For comparative tests, the approximate content of impurity tolerated, or the sum of impurities, may be indicated in brackets for information only. Acceptance or rejection is determined on the basis of compliance or non-compliance with the stated test. If the use of a reference substance for the named impurity is not prescribed, this content may be expressed as a nominal concentration of the substance used to prepare the reference solution specified in the monograph, unless otherwise described.

Herbal drugs. For herbal drugs, the sulfated ash, total ash, water-soluble matter, alcohol-soluble matter, water content, content of essential oil and content of active principle are calculated with reference to the drug that has not been specially dried, unless otherwise prescribed in the monograph.

Equivalents. Where an equivalent is given, for the purposes of the Pharmacopoeia only the figures shown are to be used in applying the requirements of the monograph.

Culture media. The culture media described in monographs and general chapters have been found to be satisfactory for the intended purpose. However, the components of media, particularly those of biological origin, are of variable quality, and it may be necessary for optimal performance to modulate the concentration of some ingredients, notably:

- peptones and meat or yeast extracts, with respect to their nutritive properties;
- buffering substances;
- bile salts, bile extract, deoxycholate, and colouring matter, depending on their selective properties;
- antibiotics, with respect to their activity.

STORAGE

The information and recommendations given under the heading Storage do not constitute a pharmacopoeial requirement but the competent authority may specify particular storage conditions that must be met.

The articles described in the Pharmacopoeia are stored in such a way as to prevent contamination and, as far as possible, deterioration. Where special conditions of storage are recommended, including the type of container (see section 1.3. General chapters) and limits of temperature, they are stated in the monograph.

The following expressions are used in monographs under Storage with the meaning shown.

In an airtight container means that the product is stored in an airtight container (*3.2*). Care is to be taken when the container is opened in a damp atmosphere. A low moisture content may be maintained, if necessary, by the use of a desiccant in the container provided that direct contact with the product is avoided.

Protected from light means that the product is stored either in a container made of a material that absorbs actinic light sufficiently to protect the contents from change induced by such light, or in a container enclosed in an outer cover that provides such protection, or is stored in a place from which all such light is excluded.

LABELLING

In general, labelling of medicines is subject to supranational and national regulation and to international agreements. The statements under the heading Labelling are not therefore comprehensive and, moreover, for the purposes of the Pharmacopoeia only those statements that are necessary to demonstrate compliance or non-compliance with the monograph are mandatory. Any other labelling statements are included as recommendations. When the term 'label' is used in the Pharmacopoeia, the labelling statements may appear on the container, the package, a leaflet accompanying the package, or a certificate of analysis accompanying the article, as decided by the competent authority.

WARNINGS

Materials described in monographs and reagents specified for use in the Pharmacopoeia may be injurious to health unless adequate precautions are taken. The principles of good quality control laboratory practice and the provisions of any appropriate regulations are to be observed at all times. Attention is drawn to particular hazards in certain monographs by means of a warning statement; absence of such a statement is not to be taken to mean that no hazard exists.

IMPURITIES

A list of all known and potential impurities that have been shown to be detected by the tests in a monograph may be given. See also chapter *5.10. Control of impurities in substances for pharmaceutical use*. The impurities are designated by a letter or letters of the alphabet. Where a letter appears to be missing, the impurity designated by this letter has been deleted from the list during monograph development prior to publication or during monograph revision.

FUNCTIONALITY-RELATED CHARACTERISTICS OF EXCIPIENTS

Monographs on excipients may have a section on functionality-related characteristics. The characteristics, any test methods for determination and any tolerances are not mandatory requirements; they may nevertheless be relevant for use of the excipient and are given for information (see also section 1.1. General statements).

REFERENCE STANDARDS

Certain monographs require the use of reference standards (chemical reference substances, herbal reference standards, biological reference preparations, reference spectra). See also chapter *5.12. Reference standards*. The European Pharmacopoeia Commission establishes the official reference standards, which are alone authoritative in case of arbitration. These reference standards are available from the European Directorate for the Quality of Medicines & HealthCare (EDQM). Information on the available reference standards and a batch validity statement can be obtained via the EDQM website.

1.5. ABBREVIATIONS AND SYMBOLS

A	Absorbance
$A_{1\ \mathrm{cm}}^{1\ \mathrm{per\ cent}}$	Specific absorbance
A_r	Relative atomic mass
$[\alpha]_D^{20}$	Specific optical rotation
bp	Boiling point
BRP	Biological reference preparation
CRS	Chemical reference substance
d_{20}^{20}	Relative density
λ	Wavelength
HRS	Herbal reference standard
IU	International Unit
M	Molarity
M_r	Relative molecular mass
mp	Melting point
n_D^{20}	Refractive index
Ph. Eur. U.	European Pharmacopoeia Unit
ppb	Parts per billion (micrograms per kilogram)
ppm	Parts per million (milligrams per kilogram)
R	Substance or solution defined under *4. Reagents*
R_F	Retardation factor (see chapter *2.2.46*)
R_{st}	Used in chromatography to indicate the ratio of the distance travelled by a substance to the distance travelled by a reference substance
RV	Substance used as a primary standard in volumetric analysis (chapter *4.2.1*)

Abbreviations used in the monographs on immunoglobulins, immunosera and vaccines

CFU	Colony-forming units
LD_{50}	The statistically determined quantity of a substance that, when administered by the specified route, may be expected to cause the death of 50 per cent of the test animals within a given period
MLD	Minimum lethal dose
L+/10 dose	The smallest quantity of a toxin that, in the conditions of the test, when mixed with 0.1 IU of antitoxin and administered by the specified route, causes the death of the test animals within a given period
L+ dose	The smallest quantity of a toxin that, in the conditions of the test, when mixed with 1 IU of antitoxin and administered by the specified route, causes the death of the test animals within a given period
lr/100 dose	The smallest quantity of a toxin that, in the conditions of the test, when mixed with 0.01 IU of antitoxin and injected intracutaneously causes a characteristic reaction at the site of injection within a given period
Lp/10 dose	The smallest quantity of toxin that, in the conditions of the test, when mixed with 0.1 IU of antitoxin and administered by the specified route, causes paralysis in the test animals within a given period
Lo/10 dose	The largest quantity of a toxin that, in the conditions of the test, when mixed with 0.1 IU of antitoxin and administered by the specified route, does not cause symptoms of toxicity in the test animals within a given period
Lf dose	The quantity of toxin or toxoid that flocculates in the shortest time with 1 IU of antitoxin

$CCID_{50}$	The statistically determined quantity of virus that may be expected to infect 50 per cent of the cell cultures to which it is added
EID_{50}	The statistically determined quantity of virus that may be expected to infect 50 per cent of the fertilised eggs into which it is inoculated
ID_{50}	The statistically determined quantity of a virus that may be expected to infect 50 per cent of the animals into which it is inoculated
PD_{50}	The statistically determined dose of a vaccine that, in the conditions of the test, may be expected to protect 50 per cent of the animals against a challenge dose of the micro-organisms or toxins against which it is active
ED_{50}	The statistically determined dose of a vaccine that, in the conditions of the test, may be expected to induce specific antibodies in 50 per cent of the animals for the relevant vaccine antigens
PFU	Pock-forming units or plaque-forming units
SPF	Specified-pathogen-free

Collections of micro-organisms

ATCC	American Type Culture Collection 10801 University Boulevard Manassas, Virginia 20110-2209, USA
C.I.P.	Collection de Bactéries de l'Institut Pasteur B.P. 52, 25 rue du Docteur Roux 75724 Paris Cedex 15, France
IMI	International Mycological Institute Bakeham Lane Surrey TW20 9TY, Great Britain
I.P.	Collection Nationale de Culture de Microorganismes (C.N.C.M.) Institut Pasteur 25, rue du Docteur Roux 75724 Paris Cedex 15, France
NCIMB	National Collection of Industrial and Marine Bacteria Ltd 23 St Machar Drive Aberdeen AB2 1RY, Great Britain
NCPF	National Collection of Pathogenic Fungi London School of Hygiene and Tropical Medicine Keppel Street London WC1E 7HT, Great Britain
NCTC	National Collection of Type Cultures Central Public Health Laboratory Colindale Avenue London NW9 5HT, Great Britain
NCYC	National Collection of Yeast Cultures AFRC Food Research Institute Colney Lane Norwich NR4 7UA, Great Britain
NITE	Biological Resource Center Department of Biotechnology National Institute of Technology and Evaluation 2-5-8 Kazusakamatari, Kisarazu-shi, Chiba, 292-0818 Japan
S.S.I.	Statens Serum Institut 80 Amager Boulevard, Copenhagen, Denmark

1.6. UNITS OF THE INTERNATIONAL SYSTEM (SI) USED IN THE PHARMACOPOEIA AND EQUIVALENCE WITH OTHER UNITS

INTERNATIONAL SYSTEM OF UNITS (SI)

The International System of Units comprises 2 main classes of units, namely base units and derived units[(1)]. The base units are the metre, the kilogram, the second, the ampere, the kelvin, the mole and the candela.

The derived units are formed as products of powers of the base units according to the algebraic relationships linking the corresponding quantities. Some of these derived units have special names and symbols. The derived units used in the Pharmacopoeia are shown in Table 1.6.-1.

Some important and widely used units outside the International System are shown in Table 1.6.-2.

The prefixes shown in Table 1.6.-3 are used to form the names and symbols of the decimal multiples and submultiples of SI units.

Table 1.6.-1. – *Derived units used in the European Pharmacopoeia and equivalence with other units*

Quantity		Unit				Conversion of other units into SI units
Name	Symbol	Name	Symbol	Expression in SI base units	Expression in other SI units	
Wave number	ν	one per metre	1/m	m^{-1}		
Wavelength	λ	micrometre nanometre	µm nm	10^{-6}m 10^{-9}m		
Area	A, S	square metre	m^2	m^2		
Volume	V	cubic metre	m^3	m^3		1 mL = 1 cm^3 = 10^{-6} m^3
Frequency	ν	hertz	Hz	s^{-1}		
Density	ρ	kilogram per cubic metre	kg/m^3	$kg \cdot m^{-3}$		1 g/mL = 1 g/cm^3 = 10^3 $kg \cdot m^{-3}$
Velocity, speed	v	metre per second	m/s	$m \cdot s^{-1}$		

(1) The definitions of the units used in the International System are given in the booklet 'Le Système International d'Unités (SI)', published by the Bureau International des Poids et Mesures, Pavillon de Breteuil, F-92310 Sèvres.

Quantity		Unit				Conversion of other units into SI units
Name	**Symbol**	**Name**	**Symbol**	**Expression in SI base units**	**Expression in other SI units**	
Force	*F*	newton	N	$m \cdot kg \cdot s^{-2}$		1 dyne = 1 $g \cdot cm \cdot s^{-2} = 10^{-5}$ N 1 kp = 9.806 65 N
Pressure, stress	*p*	pascal	Pa	$m^{-1} \cdot kg \cdot s^{-2}$	$N \cdot m^{-2}$	1 dyne/$cm^2 = 10^{-1}$ Pa = 10^{-1} $N \cdot m^{-2}$ 1 atm = 101 325 Pa = 101.325 kPa 1 bar = 10^5 Pa = 0.1 MPa 1 mm Hg = 133.322 387 Pa 1 Torr = 133.322 368 Pa 1 psi = 6.894 757 kPa
Dynamic viscosity	η	pascal second	Pa·s	$m^{-1} \cdot kg \cdot s^{-1}$	$N \cdot s \cdot m^{-2}$	1 P = 10^{-1} Pa·s = 10^{-1} $N \cdot s \cdot m^{-2}$ 1 cP = 1 mPa·s
Kinematic viscosity	ν	square metre per second	m^2/s	$m^2 \cdot s^{-1}$	$Pa \cdot s \cdot m^3 \cdot kg^{-1}$ $N \cdot m \cdot s \cdot kg^{-1}$	1 St = 1 $cm^2 \cdot s^{-1} = 10^{-4}$ $m^2 \cdot s^{-1}$
Energy	*W*	joule	J	$m^2 \cdot kg \cdot s^{-2}$	N·m	1 erg = 1 $cm^2 \cdot g \cdot s^{-2}$ = 1 dyne·cm = 10^{-7} J 1 cal = 4.1868 J
Power, radiant flux	*P*	watt	W	$m^2 \cdot kg \cdot s^{-3}$	$N \cdot m \cdot s^{-1}$ $J \cdot s^{-1}$	1 erg/s = 1 dyne·cm·s^{-1} = 10^{-7} W = 10^{-7} $N \cdot m \cdot s^{-1} = 10^{-7}$ $J \cdot s^{-1}$
Absorbed dose (of radiant energy)	*D*	gray	Gy	$m^2 \cdot s^{-2}$	$J \cdot kg^{-1}$	1 rad = 10^{-2} Gy
Electric potential difference, voltage	*U*	volt	V	$m^2 \cdot kg \cdot s^{-3} \cdot A^{-1}$	$W \cdot A^{-1}$	
Electric resistance	*R*	ohm	Ω	$m^2 \cdot kg \cdot s^{-3} \cdot A^{-2}$	$V \cdot A^{-1}$	
Electric charge	*Q*	coulomb	C	A·s		
Activity referred to a radionuclide	*A*	becquerel	Bq	s^{-1}		1 Ci = $37 \cdot 10^9$ Bq = $37 \cdot 10^9$ s^{-1}
Concentration (of amount of substance), molar concentration	*c*	mole per cubic metre	mol/m^3	$mol \cdot m^{-3}$		1 mol/L = 1 M = 1 $mol/dm^3 = 10^3$ $mol \cdot m^{-3}$
Mass concentration	ρ	kilogram per cubic metre	kg/m^3	$kg \cdot m^{-3}$		1 g/L = 1 g/dm^3 = 1 $kg \cdot m^{-3}$
Catalytic activity	*Z*	katal	kat	$mol \cdot s^{-1}$		

NOTES

1. In the Pharmacopoeia, the Celsius temperature is used (symbol *t*). This is defined by the following equation:

$$t = T - T_0$$

where T_0 = 273.15 K by definition. The Celsius or centigrade temperature is expressed in degrees Celsius (symbol °C). The unit 'degree Celsius' is equal to the unit 'kelvin'.

2. The practical expressions of concentrations used in the Pharmacopoeia are defined in the General Notices.

3. The radian is the plane angle between two radii of a circle that cut off on the circumference an arc equal in length to the radius.

4. In the Pharmacopoeia, conditions of centrifugation are defined by reference to the acceleration due to gravity (*g*):

$$g = 9.806\ 65\ m \cdot s^{-2}$$

5. Certain quantities without dimensions are used in the Pharmacopoeia: relative density (*2.2.5*), absorbance (*2.2.25*), specific absorbance (*2.2.25*) and refractive index (*2.2.6*).

6. The microkatal is defined as the enzymic activity that, under defined conditions, produces the transformation (e.g. hydrolysis) of 1 micromole of the substrate per second.

Table 1.6.-2. – *Non-SI units accepted for use with the SI units*

Quantity	Unit		Value in SI units
	Name	Symbol	
Time	minute	min	1 min = 60 s
	hour	h	1 h = 60 min = 3600 s
	day	d	1 d = 24 h = 86 400 s
Plane angle	degree	°	1° = (π/180) rad
Volume	litre	L	1 L = 1 dm^3 = 10^{-3} m^3
Mass	tonne	t	1 t = 10^3 kg
	dalton	Da	1 Da = 1.660539040(20) × 10^{-27} kg
Rotational frequency	revolution per minute	r/min	1 r/min = (1/60) s^{-1}
Energy	electronvolt	eV	1eV=1.602176634 × 10^{-19}J

Table 1.6.-3. – *Decimal multiples and sub-multiples of SI units*

Factor	Prefix	Symbol	Factor	Prefix	Symbol
10^{18}	exa	E	10^{-1}	deci	d
10^{15}	peta	P	10^{-2}	centi	c
10^{12}	tera	T	10^{-3}	milli	m
10^{9}	giga	G	10^{-6}	micro	µ
10^{6}	mega	M	10^{-9}	nano	n
10^{3}	kilo	k	10^{-12}	pico	p
10^{2}	hecto	h	10^{-15}	femto	f
10^{1}	deca	da	10^{-18}	atto	a

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2.2.1. CLARITY AND DEGREE OF OPALESCENCE OF LIQUIDS

Opalescence is the effect of light being absorbed or scattered by submicroscopic particles or optical density inhomogeneities. The absence of any particles or inhomogeneities in a solution results in a clear solution.

A liquid is considered *clear* if its clarity is the same as that of *water R* or of the solvent used, or if its opalescence is not more pronounced than that of reference suspension I (see Table 2.2.1.-1), when examined under the conditions described below.

Requirements in monographs are expressed in terms of the visual method by comparing with the defined reference suspensions (see Table 2.2.1.-1). However, instrumental methods may also be used for determining compliance with monograph requirements once the suitability of the instrument has been established as described below and calibration with reference suspensions I-IV and with *water R* or the solvent used has been performed.

VISUAL METHOD

Using identical test-tubes of colourless, transparent, neutral glass with a flat base and an internal diameter of 15-25 mm, compare the liquid to be examined with a reference suspension freshly prepared as described below. Ensure that the depths of the layers in the 2 test-tubes are the same (about 40 mm).

Compare the liquids in diffused daylight 5 min after preparation of the reference suspension, viewing vertically against a black background.

System suitability. The diffusion of light must be such that reference suspension I can readily be distinguished from *water R*, and that reference suspension II can readily be distinguished from reference suspension I (see Table 2.2.1.-1).

INSTRUMENTAL METHOD

The instrumental assessment of clarity and opalescence provides a more discriminatory test that does not depend on the visual acuity of the analyst. Numerical results are more useful for process control and quality monitoring, especially in stability studies. For example, previous numerical data on stability can be extrapolated to determine whether a given batch of a preparation will exceed shelf-life limits prior to the expiry date.

TURBIDIMETRY AND NEPHELOMETRY

When a suspension is viewed at right angles to the direction of the incident light, the system appears opalescent due to the scattering of light by the particles of the suspension (Tyndall effect). A certain portion of the light beam entering a turbid liquid is transmitted, another portion is absorbed and the remaining portion is scattered by the suspended particles. The light-scattering effect of suspended particles can be measured either indirectly by observation of the transmitted light (turbidimetry) or directly by measuring the scattered light (nephelometry). Turbidimetry and nephelometry are more reliable in low turbidity ranges, where there is a linear relationship between turbidity values and detector signals. As the degree of turbidity increases, not all the particles are exposed to the incident light and the scattered or the transmitted radiation of other particles is hindered on its way to the detector.

For quantitative measurements, the construction of calibration curves is essential. Linearity must be based on at least 4 levels of concentrations. Reference suspensions must show a sufficiently stable degree of turbidity and must be produced under well-defined conditions.

MEASUREMENTS IN RATIO MODE

The determination of opalescence of coloured liquids is done using instruments with ratio mode, since colour provides a negative interference, attenuating both incident and scattered light and lowering the turbidity value. The effect is so great, even for moderately coloured samples, that conventional nephelometers cannot be used.

In turbidimetry or nephelometry with ratio mode, the ratio of the transmission measurement to the 90° scattered light measurement is determined. This procedure compensates for the light that is diminished by the colour of the sample. Instruments with ratio mode use as light source a tungsten lamp with spectral sensitivity at about 550 nm operating at a filament colour temperature of 2700 K. Other suitable light sources may also be used. Silicon photodiodes and photomultipliers are commonly used as detectors and record changes in light scattered or transmitted by the sample. The light scattered at 90 ± 2.5° is measured by the primary detector. Other detectors measure back and forward scatter (reflected light) as well as transmitted light. The results are obtained by calculating the ratio of the 90° scattered light measured to the sum of the components of forward scattered and transmitted light values.

The instruments used are calibrated against standards of known turbidity and are capable of automatic measurement of turbidity. The test results are obtained directly from the instrument and compared to the specifications in the individual monograph.

Alternatively, the influence of the colour of the sample may also be eliminated by using an infrared light-emitting diode (IR LED) having an emission maximum at 860 nm with a 60 nm spectral bandwidth as the light source of the instrument.

INSTRUMENT REQUIREMENTS

Instruments complying with the following characteristics and verified using reference suspensions as described below may be used instead of visual examination for determination of compliance with monograph requirements.

- *Measuring unit*: NTU (nephelometric turbidity units). NTU is based on the turbidity of a primary standard of formazin. FTU (formazin turbidity units) or FNU (formazin nephelometric units) are also used, and are equivalent to NTU in regions of low turbidity (up to 40 NTU). These units are used in all 3 instrumental methods (nephelometry, turbidimetry and in ratio mode).
- *Measuring range*: 0.01-1100 NTU.
- *Resolution*: 0.01 NTU within the range 0-9.99 NTU; 0.1 NTU within the range 10.0-99.9 NTU; and 1 NTU for the range > 100 NTU.
- *Accuracy*: ± (10 per cent of reading + 0.01 NTU) within the range 0-20 NTU; ± 7.5 per cent within the range 20-1100 NTU.
- *Repeatability*: ± 0.05 NTU within the range 0-20 NTU; ± 2 per cent of the reading within the range 20-1100 NTU.

Instruments with measuring range or resolution, accuracy and repeatability capabilities other than those mentioned above may be used provided they are sufficiently validated and are capable for the intended use.

CONTROL OF INSTRUMENT PERFORMANCE

- *Calibration*: performed with at least 4 reference suspensions of formazin covering the measuring range of interest. Reference suspensions described in this chapter or suitable reference standards calibrated against the primary reference suspensions may be used.
- *Stray light*: < 0.15 NTU within the range 0-10 NTU; < 0.5 NTU within the range 10-1100 NTU. Stray light is defined as that light that reaches the nephelometric detector without being a result of scatter from the sample. Stray light is always a positive interference and is a significant source

of error in low-range turbidity measurements. Sources of stray light include: imperfections in and scratches on sample cells, internal reflections of the optical system, contamination of the optics or sample cell chamber with dust, and electronic noise. Instrument design can also affect stray light. The influence of stray light becomes negligible in ratio mode measurements.

The test methodology for the specific substance/product to be analysed must also be verified to demonstrate its analytical capability. The instrument and methodology shall be consistent with the attributes of the substance to be examined.

Measurements of standards and samples should be carried out under the same temperature conditions, preferably between 20 °C and 25 °C.

REFERENCE SUSPENSIONS

Formazin has several desirable characteristics that make it an excellent turbidity standard. It can be reproducibly prepared from assayed raw materials. The physical characteristics make it a desirable light-scatter calibration standard. The formazin polymer consists of chains of different lengths, which fold into random configurations. This results in a wide variety of particle shapes and sizes, which allows the analysis of different particle sizes and shapes that are found in real samples. Stabilised formazin suspensions that can be used to prepare stable, diluted turbidity standards are commercially available and may be used after comparison with the standards prepared as described.

All steps of the preparation of reference suspensions as described below are carried out at 25 ± 3 °C.

Hydrazine sulfate solution. Dissolve 1.0 g of *hydrazine sulfate R* in *water R* and dilute to 100.0 mL with the same solvent. Allow to stand for 4-6 h.

Primary opalescent suspension (formazin suspension). In a 100 mL ground-glass-stoppered flask, dissolve 2.5 g of *hexamethylenetetramine R* in 25.0 mL of *water R*. Add 25.0 mL of the hydrazine sulfate solution. Mix and allow to stand for 24 h. This suspension is stable for 2 months, provided it is stored in a glass container free from surface defects. The suspension must not adhere to the glass and must be mixed thoroughly before use.

Standard of opalescence. Dilute 15.0 mL of the primary opalescent suspension to 1000.0 mL with *water R*. This suspension is freshly prepared and may be stored for up to 24 h.

Reference suspensions. Prepare the reference suspensions according to Table 2.2.1.-1. Mix and shake before use.

Table 2.2.1.-1

	I	II	III	IV
Standard of opalescence	5.0 mL	10.0 mL	30.0 mL	50.0 mL
Water R	95.0 mL	90.0 mL	70.0 mL	50.0 mL

Measurements of reference suspensions I-IV in ratio mode show a linear relationship between the concentrations and measured NTU values (see Table 2.2.1.-2).

Table 2.2.1.-2

Formazin suspensions	Opalescent values (NTU)
Reference suspension I	3
Reference suspension II	6
Reference suspension III	18
Reference suspension IV	30
Standard of opalescence	60
Primary opalescent suspension	4000

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2.2.2. DEGREE OF COLORATION OF LIQUIDS

The examination of the degree of coloration of liquids in the range brown-yellow-red is carried out by one of the 2 methods below, as prescribed in the monograph.

A solution is *colourless* if it has the appearance of *water R* or the solvent or is not more intensely coloured than reference solution B_9.

METHOD I

Using identical tubes of colourless, transparent, neutral glass of 12 mm external diameter, compare 2.0 mL of the liquid to be examined with 2.0 mL of *water R* or of the solvent or of the reference solution (see Tables of reference solutions) prescribed in the monograph. Compare the colours in diffused daylight, viewing horizontally against a white background.

METHOD II

Using identical tubes of colourless, transparent, neutral glass with a flat base and an internal diameter of 15 mm to 25 mm, compare the liquid to be examined with *water R* or the solvent or the reference solution (see Tables of reference solutions) prescribed in the monograph, the depth of the layer being 40 mm. Compare the colours in diffused daylight, viewing vertically against a white background.

REAGENTS

Primary solutions

Yellow solution. Dissolve 46 g of *ferric chloride R* in about 900 mL of a mixture of 25 mL of *hydrochloric acid R* and 975 mL of *water R* and dilute to 1000.0 mL with the same mixture. Titrate and adjust the solution to contain 45.0 mg of $FeCl_3,6H_2O$ per millilitre by adding the same acidic mixture. Protect the solution from light.

Titration. Place in a 250 mL conical flask fitted with a ground-glass stopper, 10.0 mL of the solution, 15 mL of *water R*, 5 mL of *hydrochloric acid R* and 4 g of *potassium iodide R*, close the flask, allow to stand in the dark for 15 min and add 100 mL of *water R*. Titrate the liberated iodine with *0.1 M sodium thiosulfate*, using 0.5 mL of *starch solution R*, added towards the end of the titration, as indicator.

1 mL of *0.1 M sodium thiosulfate* is equivalent to 27.03 mg of $FeCl_3,6H_2O$.

Red solution. Dissolve 60 g of *cobalt chloride R* in about 900 mL of a mixture of 25 mL of *hydrochloric acid R* and 975 mL of *water R* and dilute to 1000.0 mL with the same mixture. Titrate and adjust the solution to contain 59.5 mg of $CoCl_2,6H_2O$ per millilitre by adding the same acidic mixture.

Titration. Place in a 250 mL conical flask fitted with a ground-glass stopper, 5.0 mL of the solution, 5 mL of *dilute hydrogen peroxide solution R* and 10 mL of a 300 g/L solution of *sodium hydroxide R*. Boil gently for 10 min, allow to cool and add 60 mL of *dilute sulfuric acid R* and 2 g of *potassium iodide R*. Close the flask and dissolve the precipitate by shaking gently. Titrate the liberated iodine with *0.1 M sodium thiosulfate*, using 0.5 mL of *starch solution R*, added towards the end of the titration, as indicator. The end-point is reached when the solution turns pink.

1 mL of *0.1 M sodium thiosulfate* is equivalent to 23.79 mg of $CoCl_2,6H_2O$.

Blue primary solution. Dissolve 63 g of *copper sulfate pentahydrate R* in about 900 mL of a mixture of 25 mL of *hydrochloric acid R* and 975 mL of *water R* and dilute to 1000.0 mL with the same mixture. Titrate and adjust the solution to contain 62.4 mg of $CuSO_4,5H_2O$ per millilitre by adding the same acidic mixture.

Titration. Place in a 250 mL conical flask fitted with a ground-glass stopper, 10.0 mL of the solution, 50 mL of *water R*, 12 mL of *dilute acetic acid R* and 3 g of *potassium iodide R*. Titrate the liberated iodine with *0.1 M sodium thiosulfate*, using 0.5 mL of *starch solution R*, added towards the end of the titration, as indicator. The end-point is reached when the solution shows a slight pale brown colour.

1 mL of *0.1 M sodium thiosulfate* is equivalent to 24.97 mg of $CuSO_4,5H_2O$.

Standard solutions

Using the 3 primary solutions, prepare the 5 standard solutions as follows (Table 2.2.2.-1):

Table 2.2.2.-1

Standard solution	Volume in millilitres			
	Yellow solution	Red solution	Blue solution	Hydrochloric acid (10 g/L HCl)
B (brown)	3.0	3.0	2.4	1.6
BY (brownish-yellow)	2.4	1.0	0.4	6.2
Y (yellow)	2.4	0.6	0.0	7.0
GY (greenish-yellow)	9.6	0.2	0.2	0.0
R (red)	1.0	2.0	0.0	7.0

Reference solutions for Methods I and II

Using the 5 standard solutions, prepare the following reference solutions.

Table 2.2.2.-2. - *Reference solutions B*

Reference solution	Volumes in millilitres	
	Standard solution B	Hydrochloric acid (10 g/L HCl)
B_1	75.0	25.0
B_2	50.0	50.0
B_3	37.5	62.5
B_4	25.0	75.0
B_5	12.5	87.5
B_6	5.0	95.0
B_7	2.5	97.5
B_8	1.5	98.5
B_9	1.0	99.0

Table 2.2.2.-3. - *Reference solutions BY*

Reference solution	Volumes in millilitres	
	Standard solution BY	Hydrochloric acid (10 g/L HCl)
BY_1	100.0	0.0
BY_2	75.0	25.0
BY_3	50.0	50.0
BY_4	25.0	75.0
BY_5	12.5	87.5
BY_6	5.0	95.0
BY_7	2.5	97.5

Table 2.2.2.-4. - *Reference solutions Y*

	Volumes in millilitres	
Reference solution	**Standard solution Y**	**Hydrochloric acid (10 g/L HCl)**
Y_1	100.0	0.0
Y_2	75.0	25.0
Y_3	50.0	50.0
Y_4	25.0	75.0
Y_5	12.5	87.5
Y_6	5.0	95.0
Y_7	2.5	97.5

Table 2.2.2.-5. - *Reference solutions GY*

	Volumes in millilitres	
Reference solution	**Standard solution GY**	**Hydrochloric acid (10 g/L HCl)**
GY_1	25.0	75.0
GY_2	15.0	85.0
GY_3	8.5	91.5
GY_4	5.0	95.0
GY_5	3.0	97.0
GY_6	1.5	98.5
GY_7	0.75	99.25

Table 2.2.2.-6. - *Reference solutions R*

	Volumes in millilitres	
Reference solution	**Standard solution R**	**Hydrochloric acid (10 g/L HCl)**
R_1	100.0	0.0
R_2	75.0	25.0
R_3	50.0	50.0
R_4	37.5	62.5
R_5	25.0	75.0
R_6	12.5	87.5
R_7	5.0	95.0

Storage

For Method I, the reference solutions may be stored in sealed tubes of colourless, transparent, neutral glass of 12 mm external diameter, protected from light.

For Method II, prepare the reference solutions immediately before use from the standard solutions.

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2.2.3. POTENTIOMETRIC DETERMINATION OF pH

The pH of an aqueous solution is defined as the negative logarithm of the activity of its hydrogen ions, expressed conventionally as the hydrogen ion concentration of the solution. For practical purposes, its definition is an experimental one. The pH of a solution to be examined is related to that of a reference solution (pH_s) by the following equation:

$$pH = pH_s - \frac{E - E_s}{k}$$

in which E is the potential, expressed in volts, of the cell containing the solution to be examined and E_s is the potential, expressed in volts, of the cell containing the solution of known pH (pH_s), k is the change in potential per unit change in pH, expressed in volts and calculated from the Nernst equation.

Table 2.2.3.-1. – *Values of k at different temperatures*

Temperature (°C)	*k* (V)
15	0.0572
20	0.0582
25	0.0592
30	0.0601
35	0.0611

The potentiometric determination of pH is made by measuring the potential difference between 2 appropriate electrodes immersed in the solution to be examined; 1 of these electrodes is sensitive to hydrogen ions (usually a glass electrode) and the other is the reference electrode (e.g. a silver-silver chloride electrode). They are often combined as 1 compact electrode, together with a temperature probe.

Apparatus. The measuring apparatus is usually a voltmeter with an input resistance at least 100 times that of the electrodes used. It is normally graduated in pH units and has a sensitivity such that discrimination of at least 0.05 pH unit or at least 0.003 V may be achieved.

Recent pH meters are microprocessor-controlled and are operated using the manufacturer's firmware or software, according to given instructions.

Management of electrodes. The electrodes are stored appropriately and according to the manufacturer's recommendations (e.g. in an electrolyte solution or a suitable storage solution). Before measurement, the electrodes are visually checked. Refillable electrodes are checked for the absence of air bubbles in the glass bulb and to ensure that the inner electrolyte solution level is satisfactory. The filling orifice has to remain open during the measurement. It is also recommended that the diaphragm of the reference electrode is checked. Before first use, or if the electrode has been stored out of electrolyte solution, it is usually necessary to condition it, according to the recommendations of the manufacturer. If pH stabilisation is too slow (i.e. a long response time), or a zero point shift, reduced slope or any other difficulties in calibration are observed, the electrode will probably need to be cleaned or replaced. The cleaning is performed depending on the type of sample and as prescribed in the manufacturer's manual. Regular cleaning is recommended.

Calibration and measurement conditions. Unless otherwise prescribed in the monograph, all measurements are carried out at the same temperature as that used for calibration (± 2.5 °C), usually between 20 °C and 25 °C. Table 2.2.3.-2 shows the variation of pH with respect to temperature of a number of reference buffer solutions used for calibration. Follow the manufacturer's instructions for temperature correction.

The calibration consists of the determination of the slope (e.g. 95-105 per cent) and the offset of the measuring system. Most commercial pH meters offer a "self test" or "start-up test" where, for example, the slope and asymmetry potential are tested and compared to the manufacturer's specifications. The apparatus is calibrated using at least 2 buffer solutions chosen so that the expected pH value of the solution to be examined lies between the pH values of the buffer solutions. The range must be at least 2 pH units. The pH of another buffer solution of intermediate pH, read from the scale, must not differ by more than 0.05 pH units from the value corresponding to that solution.

Reference buffer solutions are preferably commercially available certified reference materials. Alternatively, buffer solutions can be prepared in-house according to Table 2.2.3.-2. These solutions must be traceable to primary standards. Calibration has to be performed on a regular basis, preferably each day of use or before each series of measurements.

Immerse the electrodes in the solution to be examined and take the reading in the same conditions as those applied for the reference buffer solutions.

If suspensions, emulsions or solutions of non-aqueous or partially non-aqueous character are measured on a system calibrated as described above, the pH reading can only be considered to be an approximation of the true value. Suitable electrodes have to be used for pH measurements of such mixtures.

Table 2.2.3.-2. – *pH of reference buffer solutions at various temperatures*

Temperature (°C)	Potassium tetraoxalate 0.05 M	Potassium hydrogen tartrate saturated at 25 °C	Potassium dihydrogen citrate 0.05 M	Potassium hydrogen phthalate 0.05 M	Potassium dihydrogen phosphate 0.025 M + Disodium hydrogen phosphate 0.025 M	Potassium dihydrogen phosphate 0.0087 M + Disodium hydrogen phosphate 0.0303 M	Disodium tetraborate 0.01 M	Sodium carbonate 0.025 M + Sodium bicarbonate 0.025 M	Calcium hydroxide, saturated at 25°C
	$C_4H_3KO_8,2H_2O$	$C_4H_5KO_6$	$C_6H_7KO_7$	$C_8H_5KO_4$	KH_2PO_4+ Na_2HPO_4	KH_2PO_4+ Na_2HPO_4	$Na_2B_4O_7$, $10H_2O$	Na_2CO_3+ $NaHCO_3$	$Ca(OH)_2$
15	1.67		3.80	4.00	6.90	7.45	9.28	10.12	12.81
20	1.68		3.79	4.00	6.88	7.43	9.23	10.06	12.63
25	1.68	3.56	3.78	4.01	6.87	7.41	9.18	10.01	12.45
30	1.68	3.55	3.77	4.02	6.85	7.40	9.14	9.97	12.29

Temperature (°C)	Potassium tetraoxalate 0.05 M	Potassium hydrogen tartrate saturated at 25 °C	Potassium dihydrogen citrate 0.05 M	Potassium hydrogen phthalate 0.05 M	Potassium dihydrogen phosphate 0.025 M + Disodium hydrogen phosphate 0.025 M	Potassium dihydrogen phosphate 0.0087 M + Disodium hydrogen phosphate 0.0303 M	Disodium tetraborate 0.01 M	Sodium carbonate 0.025 M + Sodium bicarbonate 0.025 M	Calcium hydroxide, saturated at 25°C
	$C_4H_3KO_8,2H_2O$	$C_4H_5KO_6$	$C_6H_7KO_7$	$C_8H_5KO_4$	KH_2PO_4+ Na_2HPO_4	KH_2PO_4+ Na_2HPO_4	$Na_2B_4O_7$, $10H_2O$	Na_2CO_3+ $NaHCO_3$	$Ca(OH)_2$
35	1.69	3.55	3.76	4.02	6.84	7.39	9.10	9.93	12.13
$\frac{\Delta pH^{(1)}}{\Delta t}$	+ 0.001	− 0.0014	− 0.0022	+ 0.0012	− 0.0028	− 0.0028	− 0.0082	− 0.0096	− 0.034

(1) pH variation per degree Celsius.

PREPARATION OF REFERENCE BUFFER SOLUTIONS

Potassium tetraoxalate 0.05 M. Dissolve 12.61 g of $C_4H_3KO_8,2H_2O$ in *carbon dioxide-free water R* and dilute to 1000.0 mL with the same solvent.

Potassium hydrogen tartrate, saturated at 25 °C. Shake an excess of $C_4H_5KO_6$ vigorously with *carbon dioxide-free water R* at 25 °C. Filter or decant. Prepare immediately before use.

Potassium dihydrogen citrate 0.05 M. Dissolve 11.41 g of $C_6H_7KO_7$ in *carbon dioxide-free water R* and dilute to 1000.0 mL with the same solvent. Prepare immediately before use.

Potassium hydrogen phthalate 0.05 M. Dissolve 10.13 g of $C_8H_5KO_4$, previously dried for 1 h at 110 ± 2 °C, in *carbon dioxide-free water R* and dilute to 1000.0 mL with the same solvent.

Potassium dihydrogen phosphate 0.025 M + Disodium hydrogen phosphate 0.025 M. Dissolve 3.39 g of KH_2PO_4 and 3.53 g of Na_2HPO_4, both previously dried for 2 h at 120 ± 2 °C, in *carbon dioxide-free water R* and dilute to 1000.0 mL with the same solvent.

Potassium dihydrogen phosphate 0.0087 M + Disodium hydrogen phosphate 0.0303 M. Dissolve 1.18 g of KH_2PO_4 and 4.30 g of Na_2HPO_4, both previously dried for 2 h at 120 ± 2 °C, in *carbon dioxide-free water R* and dilute to 1000.0 mL with the same solvent.

Disodium tetraborate 0.01 M. Dissolve 3.80 g of $Na_2B_4O_7,10H_2O$ in *carbon dioxide-free water R* and dilute to 1000.0 mL with the same solvent. Store protected from atmospheric carbon dioxide.

Sodium carbonate 0.025 M + Sodium hydrogen carbonate 0.025 M. Dissolve 2.64 g of Na_2CO_3 and 2.09 g of $NaHCO_3$ in *carbon dioxide-free water R* and dilute to 1000.0 mL with the same solvent. Store protected from atmospheric carbon dioxide.

Calcium hydroxide, saturated at 25 °C. Shake an excess of *calcium hydroxide R* with *carbon dioxide-free water R* and decant at 25 °C. Store protected from atmospheric carbon dioxide.

STORAGE OF BUFFER SOLUTIONS

Store buffer solutions in suitable chemically-resistant, airtight containers, such as type I glass bottles or plastic containers suitable for aqueous solutions.

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corrected 10.0

2.2.5. RELATIVE DENSITY

The relative density $d_{t_2}^{t_1}$ of a substance is the ratio of the mass of a certain volume of a substance at temperature t_1 to the mass of an equal volume of water at temperature t_2.

Unless otherwise indicated, the relative density d_{20}^{20} is used. Relative density is also commonly expressed as d_4^{20}. Density ρ_{20}, defined as the mass of a unit volume of the substance at 20 °C may also be used, expressed in kilograms per cubic metre or grams per cubic centimetre ($1\ kg{\cdot}m^{-3} = 10^{-3}\ g{\cdot}cm^{-3}$). These quantities are related by the following equations where density is expressed in grams per cubic centimetre:

$$\rho_{20} = 0.998203 \times d_{20}^{20} \text{ or } d_{20}^{20} = 1.00180 \times \rho_{20}$$

$$\rho_{20} = 0.999972 \times d_4^{20} \text{ or } d_4^{20} = 1.00003 \times \rho_{20}$$

$$d_4^{20} = 0.998230 \times d_{20}^{20}$$

Relative density or density is measured according to the number of decimals prescribed in the monograph using a density bottle (solids or liquids), a hydrostatic balance (solids), a hydrometer (liquids) or a digital density meter with an oscillating transducer (liquids and gases). When the determination is made by weighing, the buoyancy of air is disregarded, which may introduce an error of 1 unit in the 3rd decimal place. When using a density meter, the buoyancy of air has no influence.

Oscillating transducer density meter. The apparatus consists of:

- a U-shaped tube, usually of borosilicate glass, which contains the liquid to be examined;
- a magneto-electrical or piezo-electrical excitation system that causes the tube to oscillate as a cantilever oscillator at a characteristic frequency depending on the density of the liquid to be examined;
- a means of measuring the oscillation period (T), which may be converted by the apparatus to give a direct reading of density, or used to calculate density using the constants A and B described below.

The resonant frequency (f) is a function of the spring constant (c) and the mass (m) of the system:

$$f^2 = \frac{1}{T^2} = \frac{c}{m} \times \frac{1}{4\pi^2}$$

Hence:

$$T^2 = \left(\frac{M}{c} + \frac{\rho \times V}{c}\right) \times 4\pi^2$$

M = mass of the tube;

V = inner volume of the tube.

Introduction of 2 constants $A = c / (4\pi^2 \times V)$ and $B = M / V$, leads to the classical equation for the oscillating transducer:

$$\rho = A \times T^2 - B$$

The constants A and B are determined by operating the instrument with the U-tube filled with 2 different samples of known density, for example, degassed *water R* and air. Control measurements are made daily using degassed *water R*. The results displayed for the control measurement using degassed *water R* shall not deviate from the reference value ($\rho_{20} = 0.998203\ g{\cdot}cm^{-3}$, $d_{20}^{20} = 1.000000$) by more than its specified error. For example, an instrument specified to $\pm 0.0001\ g{\cdot}cm^{-3}$ shall display $0.9982 \pm 0.0001\ g{\cdot}cm^{-3}$ in order to be suitable for further measurement. Otherwise a re-adjustment is necessary. Calibration with certified reference materials is carried out regularly. Measurements are made using the same procedure as for calibration. The liquid to be examined is equilibrated in a thermostat at 20 °C before introduction into the tube, if necessary, to avoid the formation of bubbles and to reduce the time required for measurement.

Factors affecting accuracy include:

- temperature uniformity throughout the tube;
- non-linearity over a range of density;
- parasitic resonant effects;
- viscosity, whereby solutions with a higher viscosity than the calibrant have a density that is apparently higher than the true value.

The effects of non-linearity and viscosity may be avoided by using calibrants that have density and viscosity close to those of the liquid to be examined (± 5 per cent for density, ± 50 per cent for viscosity). The density meter may have functions for automatic viscosity correction and for correction of errors arising from temperature changes and non-linearity.

Precision is a function of the repeatability and stability of the oscillator frequency, which is dependent on the stability of the volume, mass and spring constant of the cell.

Density meters are able to achieve measurements with an error of the order of $1 \times 10^{-3}\ g{\cdot}cm^{-3}$ to $1 \times 10^{-5}\ g{\cdot}cm^{-3}$ and a repeatability of $1 \times 10^{-4}\ g{\cdot}cm^{-3}$ to $1 \times 10^{-6}\ g{\cdot}cm^{-3}$.

07/2018:20207

2.2.7. OPTICAL ROTATION

PRINCIPLE

Optical rotation (also known as optical activity) is the property displayed by chiral substances of rotating the plane of polarisation of linearly polarised light.

Optical rotation is considered to be positive (+) for dextrorotatory substances (i.e. those that rotate the plane of polarisation in a clockwise direction when viewed in the direction facing the oncoming light beam) and negative (–) for laevorotatory substances (i.e. anticlockwise rotation).

The angle of optical rotation α of a liquid is the angle of rotation of the plane of polarisation, expressed in degrees (°), at the wavelength of the D-line of sodium (λ = 589.3 nm) measured at 20 °C through the liquid when using a path length of 1.00 dm.

The specific optical rotation $[\alpha]_D^{20}$ of a substance in solution is calculated from the angle of optical rotation, as defined above, with reference to a path length of 1.00 dm and a concentration of the substance to be examined of 1 g/mL. The specific optical rotation of a substance in solution is always expressed with reference to a given solvent and concentration.

As some equipment may not use sodium lamps, the wavelength of measurement is given as 589 nm instead of 589.3 nm.

In certain cases specified in the monograph, the angle of optical rotation is measured at other temperatures, other wavelengths and/or in cells with a path length other than 1.00 dm.

In the conventional system adopted by the Pharmacopoeia, the specific optical rotation is expressed by its value without units; the actual units, degree millilitres per decimetre gram [(°)·ml·dm^{-1}·g^{-1}] are understood.

EQUIPMENT

The polarimeter typically consists of:

- a light source, for example a sodium discharge lamp, a light-emitting diode (LED) or another light source capable of providing radiation at the desired wavelength (589 nm unless otherwise prescribed in the monograph); if the light source is polychromatic, a means of isolating the required wavelength is necessary, e.g. an optical filter;
- a polariser and an analyser;
- a sample cell with a path length of 1.00 dm, unless otherwise specified in the monograph;
- a detection system to measure the angle of optical rotation, which must be capable of giving readings to at least the nearest 0.01°, unless otherwise specified in the monograph;
- a temperature control system that indicates the temperature with a readability of 0.1 °C; it may be embedded in the polarimeter (e.g. a Peltier system) or be an external unit (e.g. a cycle-cryostat), and must be able to maintain the temperature of the liquid to within ± 0.5 °C of that prescribed.

EQUIPMENT PERFORMANCE

The accuracy of the scale is checked near the value to be measured or over an appropriate range, usually by means of certified quartz plates. Other certified reference materials may also be suitable (e.g. sucrose solutions).

Optical rotation measurements may be used to quantify the amount of an enantiomer or the ratio of enantiomers present in a sample. For that purpose, the linearity must be checked, for example using sucrose solutions.

PROCEDURE

Determine the zero of the polarimeter and the angle of rotation of the liquid at a wavelength of 589 nm and a temperature of 20 ± 0.5 °C, unless otherwise prescribed. The zero of the polarimeter is determined with the sample cell closed.

For neat liquids, the zero is determined with an empty sample cell.

For solutions, the zero is determined with the sample cell filled with the same solvent as that used for the solution to be examined and measured at the same temperature. The sample preparation procedure is prescribed in the monograph.

Calculate the specific optical rotation at temperature t and wavelength λ using the following formulae.

For neat liquids, the density of the liquid is taken into account:

$$[\alpha]_\lambda^t = \frac{\alpha}{l \cdot \rho_t}$$

For solutions:

$$[\alpha]_\lambda^t = \frac{1000\alpha}{l \cdot c}$$

α = angle of rotation measured at temperature t and wavelength λ, in degrees;

l = path length of the polarimeter sample cell, in decimetres;

ρ_t = density determined at the temperature of measurement t, in grams per cubic centimetre; for the purposes of the Pharmacopoeia, density is replaced by relative density (*2.2.5*);

c = concentration of the solution, in grams per litre.

When the limits for optical rotation or specific optical rotation are expressed as the dried substance, the anhydrous substance or the solvent-free substance, the result must be corrected for loss on drying (*2.2.32*), water content (*2.5.12* or *2.5.32*) or content of solvent as appropriate.

01/2008:20213

2.2.13. DETERMINATION OF WATER BY DISTILLATION

The apparatus (see Figure 2.2.13.-1) consists of a glass flask (*A*) connected by a tube (*D*) to a cylindrical tube (*B*) fitted with a graduated receiving tube (*E*) and reflux condenser (*C*). The receiving tube (*E*) is graduated in 0.1 mL. The source of heat is preferably an electric heater with rheostat control or an oil bath. The upper portion of the flask and the connecting tube may be insulated.

Method. Clean the receiving tube and the condenser of the apparatus, thoroughly rinse with water, and dry.

Introduce 200 mL of *toluene R* and about 2 mL of *water R* into the dry flask. Distil for 2 h, then allow to cool for about 30 min and read the water volume to the nearest 0.05 mL. Place in the flask a quantity of the substance, weighed with an accuracy of 1 per cent, expected to give about 2 mL to 3 mL of water. If the substance has a pasty consistency, weigh it in a boat of metal foil. Add a few pieces of porous material and heat the flask gently for 15 min. When the toluene begins to boil, distil at the rate of about two drops per second until most of the water has distilled over, then increase the rate of distillation to about four drops per second. When the water has all distilled over, rinse the inside of the condenser tube with *toluene R*. Continue the distillation for 5 min, remove the heat, allow the receiving tube to cool to room temperature and dislodge any droplets of water which adhere to the walls of the receiving tube. When the water and toluene have completely separated, read the volume of water and calculate the content present in the substance as millilitres per kilogram, using the formula:

$$\frac{1000(n_2 - n_1)}{m}$$

m = the mass in grams of the substance to be examined,

n_1 = the number of millilitres of water obtained in the first distillation,

n_2 = the total number of millilitres of water obtained in the 2 distillations.

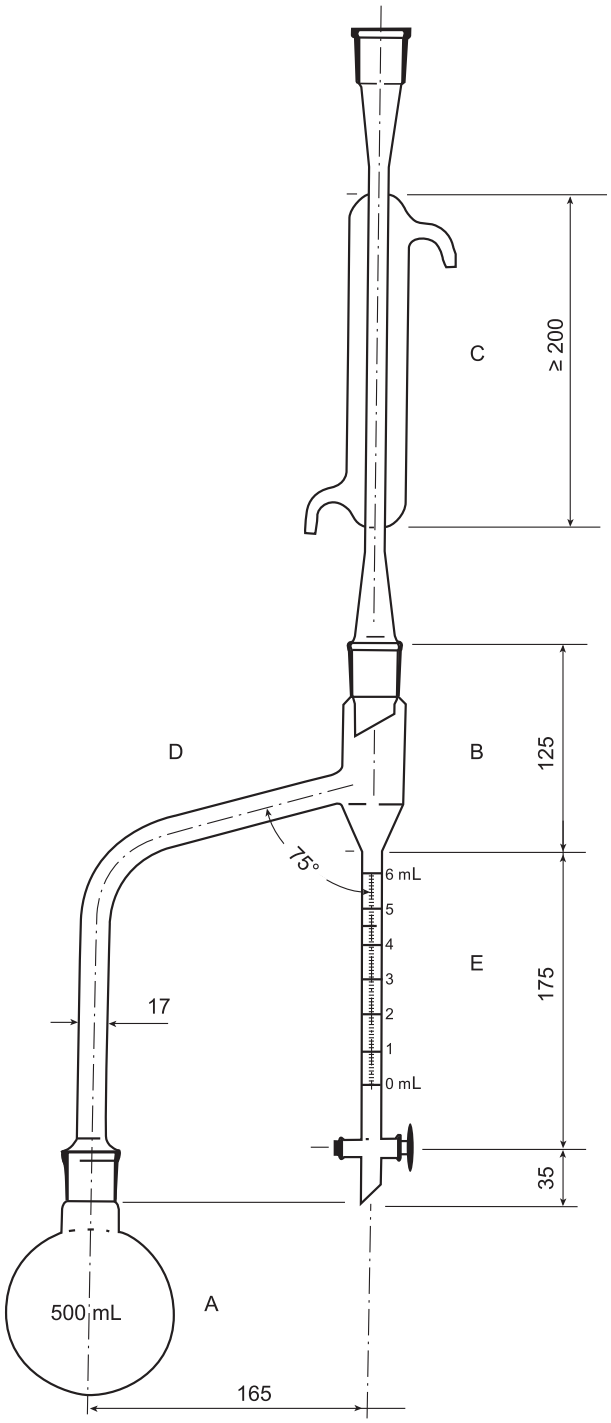

Figure 2.2.13.-1. – *Apparatus for the determination of water by distillation*
Dimensions in millimetres

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2.2.14. MELTING POINT - CAPILLARY METHOD

The melting point determined by the capillary method is the temperature at which the last solid particle of a compact column of a substance in a tube passes into the liquid phase (i.e. clear point). The melting point determined by this method is specific to the methodology (e.g. heating rate) described in this chapter. Similarly, whenever the use of certified reference materials is required, their certified values refer to the described analytical procedure.

When prescribed in the monograph, the same apparatus and method are used for the determination of other factors, such as meniscus formation or melting range, that characterise the melting behaviour of a substance.

Equipment. The equipment consists of a metal heating block with 1 or more compartments for capillary tubes, or of a suitable glass vessel containing a liquid bath (e.g. water, liquid paraffin or silicone oil) and fitted with a suitable means of heating and stirring. The equipment is equipped with a temperature sensor or a suitable certified thermometer allowing readings at least to the nearest 0.1 °C.

Samples are introduced into the equipment in glass capillary tubes. The dimensions are chosen according to the manufacturer's requirements, typically with an external diameter of 1.3-1.5 mm and a wall thickness of 0.1-0.3 mm. In some equipment glass slides are used instead of capillary tubes.

The equipment is capable of heating samples at a rate of 1 °C/min or less. The accuracy of the equipment is at most ± 0.5 °C.

Detection can be performed either visually or instrumentally. In the case of instrumental detection, this is generally performed by image recording and subsequent analysis or by a photodetector that measures the transmitted or reflected light from the sample.

Method. The substance is previously treated as described in the monograph. Coarse crystals are to be avoided as they might lead to false results. If necessary, samples are crushed into a fine powder. Unless otherwise prescribed, dry the finely powdered substance *in vacuo* over *anhydrous silica gel R* for 24 h. Introduce a sufficient quantity into a capillary tube to give a compact column as described by the instrument manufacturer (e.g. 4-6 mm in height). Raise the temperature of the apparatus to about 5 °C below the presumed melting point. Allow the temperature to stabilise and then introduce the capillary tube into the instrument. Finally, adjust the rate of heating to about 1 °C/min unless otherwise prescribed.

In the case of instrumental detection, follow the instrument manufacturer's requirements for the determination of the melting point. For visual detection, record the temperature at which the last particle of the substance to be examined passes into the liquid phase.

Samples can be measured in parallel if the instrument allows multiple sample processing.

System suitability. Carry out a system suitability test before the measurements for example by choosing a suitable reference material with a melting point close to that expected for the substance to be examined.

Qualification / Calibration of the equipment. The qualification / calibration is carried out periodically according to the instrument manufacturer's requirements, using at least 2 certified reference materials. These are selected to cover the temperature range that is used on the equipment. Use capillary tubes with the same dimensions as those used for sample measurement.

Guidance on how to compare results obtained from certified reference materials with values from the certificates can be found on the European Reference Materials (ERM) website (Application note 1).

01/2008:20215

2.2.15. MELTING POINT - OPEN CAPILLARY METHOD

For certain substances, the following method is used to determine the melting point (also referred to as slip point and rising melting point when determined by this method).

Use glass capillary tubes open at both ends, about 80 mm long, having an external diameter of 1.4 mm to 1.5 mm and an internal diameter of 1.0 mm to 1.2 mm.

Introduce into each of 5 capillary tubes a sufficient amount of the substance, previously treated as described, to form in each tube a column about 10 mm high and allow the tubes to stand for the appropriate time and at the prescribed temperature.

Unless otherwise prescribed, substances with a waxy consistency are carefully and completely melted on a water-bath before introduction into the capillary tubes. Allow the tubes to stand at 2-8 °C for 2 h.

Attach one of the tubes to a thermometer graduated in 0.5 °C so that the substance is close to the bulb of the thermometer. Introduce the thermometer with the attached tube into a beaker so that the distance between the bottom of the beaker and the lower part of the bulb of the thermometer is 1 cm. Fill the beaker with water to a depth of 5 cm. Increase the temperature of the water gradually at a rate of 1 °C/min.

The temperature at which the substance begins to rise in the capillary tube is regarded as the melting point.

Repeat the operation with the other 4 capillary tubes and calculate the result as the mean of the 5 readings.

07/2015:20216

2.2.16. MELTING POINT - INSTANTANEOUS METHOD

The instantaneous melting point is calculated using the following expression:

$$\frac{t_1 + t_2}{2}$$

in which t_1 is the first temperature and t_2 the second temperature read under the conditions stated below.

Apparatus. The apparatus consists of a metal block resistant to the substance to be examined, of good heat-conducting capacity, such as brass, with a carefully polished plane upper surface. The block is uniformly heated throughout its mass by means of a micro-adjustable gas heater or an electric heating device with fine adjustment. The block has a cylindrical cavity, which is wide enough to accomodate a thermometer that is maintained in the same position during the calibration of the apparatus and the determination of the melting point of the substance to be examined. The cylindrical cavity is parallel to the upper polished surface of the block and about 3 mm from it. The apparatus is calibrated using appropriate substances of known melting point.

Method. Heat the block at a suitably rapid rate to a temperature about 10 °C below the presumed melting temperature, then adjust the heating rate to about 1 °C/min. At regular intervals drop a few particles of powdered and, where appropriate, dried substance, prepared as for the capillary tube method, onto the block in the vicinity of the thermometer bulb, cleaning the surface after each test. Record the temperature t_1 at which the substance melts instantaneously for the first time in contact with the metal. Stop the heating. During cooling drop a few particles of the substance at regular intervals on the block, cleaning the surface after each test. Record the temperature t_2 at which the substance ceases to melt instantaneously when it comes in contact with the metal.

Calibration of the apparatus. The apparatus may be calibrated using melting point reference substances such as those of the World Health Organization or other appropriate substances.

01/2016:20219

2.2.19. AMPEROMETRIC TITRATION

In an amperometric titration, the end-point is determined by following the variation of the current measured between 2 electrodes (either one indicator electrode and one reference electrode or 2 indicator electrodes) immersed in the solution to be examined and maintained at a constant potential difference as a function of the quantity of titrant added.

The potential of the measuring electrode is sufficient to ensure a diffusion current for the electroactive substance.

Apparatus. The apparatus comprises an adjustable voltage source and a sensitive microammeter; the detection system generally consists of an indicator electrode (for example, a platinum electrode, a rotating-disc electrode or a carbon electrode) and a reference electrode (for example, a silver-silver chloride electrode).

A three-electrode apparatus is sometimes used, consisting of an indicator electrode, a reference electrode and a polarised auxiliary electrode.

Method. Set the potential of the indicator electrode as prescribed and plot a graph of the initial current and the values obtained during the titration as functions of the quantity of titrant added. Add the titrant in not fewer than 3 successive quantities equal to a total of about 80 per cent of the theoretical volume corresponding to the presumed equivalence point. The 3 values must fall on a straight line. Continue adding the titrant beyond the presumed equivalence point in not fewer than 3 successive quantities. The values obtained must fall on a straight line. The point of intersection of the 2 lines represents the end-point of the titration.

For amperometric titrations with 2 indicator electrodes, the whole titration curve is recorded and used to determine the end-point.

01/2016:20220

2.2.20. POTENTIOMETRIC TITRATION

In a potentiometric titration (volumetric titration with potentiometric end-point determination) the end-point is determined by recording the variation of the potential difference between 2 electrodes (either 1 indicator electrode and 1 reference electrode, or a combined electrode) immersed in the solution to be examined as a function of the volume of titrant added.

Apparatus. The apparatus used comprises a millivoltmeter. Commercial autotitrator instruments may be used and are operated in accordance with the manufacturer's instructions, using electrodes recommended for the type of titration described.

The indicator electrode to be used depends on the substance to be determined and may be a glass or metal electrode (e.g. platinum, gold or silver).

For acid-base titrations, a glass-silver-silver chloride electrode combination is generally used.

Method. Prepare the sample solution as described. Add the titrant in suitable aliquots paying particular attention to the rate of addition and the volume increments near the end-point. Continue the titration beyond this point to allow a clear detection of the end-point.

The end-point of the titration is reached when the maximum change in potential occurs in a plot of potential versus volume of titrant, and is expressed as the corresponding volume of titrant. Recording the first or second derivative curve can facilitate the determination of the end-point. In potentiometric titrations of weak acids or bases using non-aqueous solvents, if necessary, either carry out a blank determination or pre-neutralise the solvent mixture. Where it is impracticable to use potentiometric detection for this purpose, the solvent mixture can be pre-neutralised by titration using a suitable indicator. Some examples are given below:

Titrant	Indicator
Perchloric acid	*Crystal violet solution R*
Tetrabutylammonium hydroxide	3 g/L solution of *thymol blue R* in *methanol R*
Ethanolic sodium hydroxide	*Thymolphthalein solution R*

01/2008:20221

2.2.21. FLUORIMETRY

Fluorimetry is a procedure which uses the measurement of the intensity of the fluorescent light emitted by the substance to be examined in relation to that emitted by a given standard.

Method. Dissolve the substance to be examined in the solvent or mixture of solvents prescribed in the monograph, transfer the solution to the cell or the tube of the fluorimeter and illuminate it with an excitant light beam of the wavelength prescribed in the monograph and as near as possible monochromatic.

Measure the intensity of the emitted light at an angle of 90° to the excitant beam, after passing it through a filter which transmits predominantly light of the wavelength of the fluorescence. Other types of apparatus may be used provided that the results obtained are identical.

For quantitative determinations, first introduce into the apparatus the solvent or mixture of solvents used to dissolve the substance to be examined and set the instrument to zero. Introduce the standard solution and adjust the sensitivity of the instrument so that the reading is greater than 50. If the second adjustment is made by altering the width of the slits, a new zero setting must be made and the intensity of the standard must be measured again. Finally introduce the solution of unknown concentration and read the result on the instrument. Calculate the concentration c_x of the substance in the solution to be examined, using the formula:

$$c_x = \frac{I_x c_s}{I_s}$$

c_x = concentration of the solution to be examined,

c_s = concentration of the standard solution,

I_x = intensity of the light emitted by the solution to be examined,

I_s = intensity of the light emitted by the standard solution.

If the intensity of the fluorescence is not strictly proportional to the concentration, the measurement may be effected using a calibration curve.

In some cases, measurement can be made with reference to a fixed standard (for example a fluorescent glass or a solution of another fluorescent substance). In such cases, the concentration of the substance to be examined must be determined using a previously drawn calibration curve under the same conditions.

04/2019:20224

2.2.24. ABSORPTION SPECTROPHOTOMETRY, INFRARED

PRINCIPLE

Infrared absorption spectrophotometry (also known as infrared (IR) spectroscopy) is based on the interaction of infrared radiation with matter, which affects the vibrational energy of molecules and induces intermolecular and intramolecular vibrations at specific frequencies. This results in an infrared absorption spectrum with characteristic bands that correspond to the functional groups of the molecule.

The infrared wavelength region can be further divided into 3 subregions, namely near-infrared, mid-infrared and far-infrared. These subregions have wavelength ranges that are generally accepted by convention to be 0.8-2.5 µm, 2.5-25 µm and 25-1000 µm respectively. However, in IR spectroscopy, wavenumber is more commonly used than wavelength, and can be calculated using the following equation:

$$\tilde{\nu} = \frac{1}{\lambda} \cdot 10^4$$

where $\tilde{\nu}$ is the wavenumber in reciprocal centimetres (cm^{-1}) and λ is the wavelength in micrometres. Thus 12 500-4000 cm^{-1} is near-infrared, 4000-400 cm^{-1} is mid-infrared and 400-10 cm^{-1} is far-infrared.

This chapter concerns only spectroscopy in the mid-infrared region, i.e. 4000-400 cm^{-1} (2.5-25 µm), which hereafter is referred to as infrared for simplicity. This region is where the fundamental molecular vibrations of functional groups appear in the spectrum as absorption bands. The region below 1500 cm^{-1} is known as the 'fingerprint region', a very complex and informative part of the spectrum which characterises the molecule being investigated.

The mid-infrared region is flanked by the near-infrared region, where overtones and combinations of fundamental vibrations, mainly C-H, N-H and O-H functional groups, are detected (*2.2.40*) and the far-infrared region, where absorption bands associated with crystal lattice modes, hydrogen bonds, angle deformation vibrations of heavy atoms and molecular rotations are observed.

APPLICATIONS

As the absorption bands in IR spectra are characteristic of the constituent functional groups of a compound, IR spectroscopy is widely used to identify substances and provide information on their structure. It can also be used for quantitative applications, which requires establishing a mathematical relationship between the intensity of the radiation absorbed by the sample and the concentration of the investigated component in the sample.

IR spectroscopy is widely used in the pharmaceutical field for chemical and physical analysis in the laboratory, and has a wide variety of applications during the manufacturing process as outlined below. IR spectroscopy thereby enables the application of Process Analytical Technology (PAT) as part of an advanced control strategy.

Chemical analysis:

- identification of active substances, excipients, dosage forms, manufacturing intermediates, chemicals and packaging materials;
- quality assessment of active substances, excipients, dosage forms, manufacturing intermediates and packaging materials, including batch-to-batch spectral comparison and supplier change assessment;
- quantification of active substances in a sample matrix, determination of water and solvent content;
- quantification of impurities, e.g. in gases, inorganic materials;
- reaction monitoring, e.g. chemical synthesis.

Physical analysis:

- determination of solid-state properties such as polymorphism.

LIMITATIONS

Notable limitations to the use of IR spectroscopy include the following:

- it may be necessary to use additional techniques to unambiguously identify a substance;
- pure enantiomers of a substance cannot be discriminated;
- it may not be a suitable method for trace analysis;
- sample preparation conditions (e.g. pressure, solvent) may change the crystalline form of a substance that exhibits polymorphism;
- for heterogeneous samples, the limited sampling volume may be problematic.

MEASUREMENT MODES

IR measurements are based on passing radiation through or into a sample and measuring the attenuation of the emerging beam at various wavelengths. This corresponds to 2 main measurement modes, i.e. transmission and attenuated total reflection (ATR). However, other modes also exist for specific applications (e.g. diffuse and specular reflection).

TRANSMISSION MODE

This mode is based on determination of the transmittance (*T*), namely the ability of the sample to transmit IR radiation at a given wavelength (wavenumber). It is defined by the following ratio:

$$T = \frac{I}{I_0}$$

I_0 = intensity of incident radiation;
I = intensity of transmitted radiation.

The resulting spectrum is presented in terms of transmittance (*T*) on the y-axis versus wavelength or wavenumber on the x-axis. It can also be presented in terms of absorbance (*A*) on the y-axis, which is related to transmittance (*T*) by the following equation:

$$A = \log_{10}\left(\frac{1}{T}\right) = \log_{10}\left(\frac{I_0}{I}\right) = a \cdot b \cdot c$$

a = molar absorption coefficient of the sample, in square centimetres per mole ($cm^2 \cdot mol^{-1}$);
b = sample thickness, in centimetres;
c = sample concentration, in moles per cubic centimetre ($mol \cdot cm^{-3}$).

ATTENUATED TOTAL REFLECTION MODE

ATR mode is based on the phenomenon of total internal reflection. The sample, with a refractive index n_2, is brought into close contact with a crystal (diamond, germanium, zinc selenide or any other suitable material), having a refractive index n_1 which is greater than n_2. A beam of IR light is then passed through the crystal. When the angle α between the incident beam and the sample-crystal interface exceeds a critical value α_c, theoretically all of the radiation is reflected (total internal reflection). However, an evanescent wave is produced which slightly penetrates the sample and part of the energy is absorbed. The total reflection is attenuated, which makes it possible to generate an absorption spectrum. In

practice, multiple internal reflections are often used to amplify the absorption intensity, although some accessories allow absorption measurements with a single reflection.
The penetration depth d_p is usually of the order of a few micrometres and is given for a wavelength λ by the following equation:

$$d_p = \frac{\lambda / n_1}{2\pi\sqrt{sin^2\,\alpha - (n_2 / n_1)^2}}$$

where d_p is the penetration depth, λ is the wavelength, α is the angle of incidence and n_1, n_2 are the refractive indices of the reflection element and the sample, respectively.

Due to the relationship between these parameters, the absorption intensity in ATR is greater at higher wavelengths (i.e. smaller wavenumbers) and slight band shifts occur compared to the corresponding transmission spectrum.
It is therefore not advisable to compare ATR spectra with transmission spectra when identifying compounds.

EQUIPMENT

The most commonly used IR spectrometers are Fourier-transform (FT-IR) spectrometers which typically consist of:

- a suitable polychromatic light source, e.g. a conducting ceramic rod;
- an interferometer;
- a sample presentation accessory, e.g. a sample holder;
- a detector;
- appropriate software for controlling the spectrometer, and for spectral evaluation and data processing.

Other spectrometers based on alternative principles may also be used if the requirements described under Control of equipment performance are fulfilled.

IR spectrometers can also be used in association with a microscope for the study of a small part of the sample or for chemical imaging.

IR spectroscopy can be coupled to other analytical techniques such as thermal analysis or chromatography.

CONTROL OF EQUIPMENT PERFORMANCE

Accuracy of wavenumber scale and spectral resolution are critical parameters and must be verified. The tests described below can be used for the control of instrument performance and for qualification. They can also be used as system suitability tests.

These parameters are checked using suitable reference materials which are selected and presented depending on the measurement mode (e.g. transmission or ATR).

For quantitative analysis, appropriate assessment criteria for the control of absorption intensity must also be defined.

WAVENUMBER SCALE

The wavenumber scale is typically verified using a polystyrene film that exhibits IR absorption bands at the wavenumbers shown in Table 2.2.24.-1.

Table 2.2.24.-.1 - *Band positions and associated acceptable tolerances of the polystyrene film used to verify wavenumber accuracy*

Band position (cm⁻¹)		Tolerance (cm⁻¹)
Transmission	ATR	
906.6	906.1	± 1.0
1028.3	1027.7	± 1.0
1601.2	1601.0	± 1.0
3060.0	3059.7	± 1.0

For measurement modes other than transmission or ATR, reference materials must be defined by the user.

SPECTRAL RESOLUTION

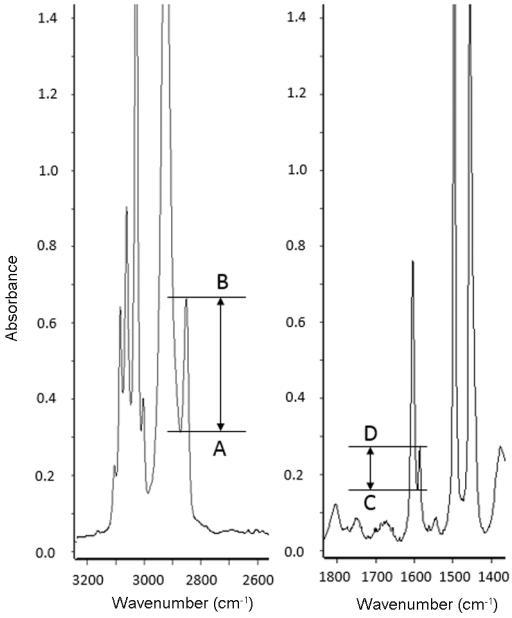

Figure 2.2.24.-1. – *Typical IR absorbance spectrum of polystyrene used to verify spectral resolution*

Spectra recorded in transmission mode. The spectral resolution is typically verified using a polystyrene film approximately 35 µm thick.

Acceptance criteria (see Figure 2.2.24.-1): the difference between the absorbance values at the absorption minimum at 2870 cm^{-1} (*A*) and the absorption maximum at 2849.5 cm^{-1} (*B*) is greater than 0.33; the difference between the absorbance values at the absorption minimum at 1589 cm^{-1} (*C*) and the absorption maximum at 1583 cm^{-1} (*D*) is greater than 0.08.

Spectra recorded in ATR mode. Appropriate assessment criteria for the control of spectral resolution according to the specifications of each instrument need to be defined.

For measurement modes other than transmission or ATR, reference materials have to be defined by the user.

PROCEDURE

SAMPLE PREPARATION AND PRESENTATION

Sample preparation and presentation vary according to the physical state of the sample and the measurement mode. Transmission mode is applied to transparent samples, such as neat liquids, solutions, gases or suitably prepared mulls and alkali halide discs. For liquids and gases, cells with fixed or variable pathlength and IR transparent windows can be used. For alkali-halide disks, specific sample holders are used. Reflection mode, e.g. ATR, is appropriate for the measurement of a wide range of samples in the solid and liquid state.

Some preparation modes (e.g. for discs and mulls in transmission mode or for solids in ATR mode) involve grinding and/or the application of pressure, which may induce unexpected crystal modifications.

Transmission mode

Prepare the substance by one of the following methods depending on the sample state (solid, liquid or gas). If sample bands in a spectrum have a minimum transmission lower than 5 per cent, this spectrum is not used for further data analysis.

Liquids. Examine liquids either in the form of a film between 2 plates transparent to infrared radiation or in a cell of suitable pathlength with windows that are transparent to infrared radiation.

Liquids or solids in solution. Prepare a solution of the substance to be examined in a suitable solvent. Choose a concentration and a pathlength that give a satisfactory spectrum. Generally, good results are obtained with concentrations of 10-100 g/L

for a pathlength of 0.5-0.1 mm. The absorption due to the solvent is usually compensated by successively recording the spectra of the solvent and the sample solution and subtracting the solvent absorption bands from the spectrum of the sample solution.

Solids dispersed in a solid (disc). Grind the substance to be examined taking into consideration any possible changes (e.g. crystalline form) and mix with a suitable amount of finely powdered and dried *potassium bromide R* or *potassium chloride R*, unless otherwise specified. A mixture of a few milligrams (e.g. 1-2 mg) of the substance to be examined in a few hundred milligrams (e.g. 300-400 mg) of halide is normally sufficient to give a disc of 10-15 mm diameter and a spectrum of suitable intensity. If the substance is a hydrochloride salt, it is recommended to use *potassium chloride R*. Carefully grind the mixture, spread it uniformly in a suitable die and apply a suitable pressure. A compacting force of about 800 MPa is generally sufficient to prepare a disc. For substances that are unstable under normal atmospheric conditions or are hygroscopic, the disc may be pressed under vacuum. Several factors may cause the formation of faulty discs, such as insufficient or excessive grinding, humidity or impurities in the dispersion medium. For example, any water in either the sample or the potassium bromide will cause clouding of the disc and produce a low transmission spectrum. A disc is rejected if visual examination shows a lack of uniform transparency or when, in the absence of a specific absorption band, the transmittance is less than 60 per cent or the absorbance is more than 0.22 at about 2000 cm^{-1} (5 µm) and without compensation, unless otherwise prescribed.

Solids dispersed in a liquid (mull). Triturate a small quantity of the substance to be examined with the minimum quantity of *liquid paraffin R* or other suitable liquid. A mixture of a few milligrams (e.g. 5-10 mg) of the substance to be examined in 1 drop of *liquid paraffin R* is generally sufficient to make an adequate mull. Compress the mull between 2 plates transparent to infrared radiation. A mull is rejected if a visual examination shows lack of uniform transparency or where the spectrum shows features such as:

- low transmission at 4000 cm^{-1};
- a strongly sloping baseline between 4000 and about 2500 cm^{-1};
- a ratio of relative intensities of some absorption bands that is less than expected.

Molten solids. If prescribed in the monograph, make a film of a molten mass and fix it on a suitable mount.

Evaporated solution. If prescribed in the monograph, dissolve the substance to be examined in a suitable solvent. Prepare a film by evaporating the solvent on a suitable carrier and fix it on a suitable mount.

Gases. Use a suitable cell transparent to infrared radiation. Evacuate the air from the cell and fill to the desired pressure through a stopcock or needle valve using a suitable gas transfer line between the cell and the container of the gas to be examined. If necessary, adjust the pressure in the cell to atmospheric pressure using a gas transparent to infrared radiation (e.g. *nitrogen R* or *argon R*), or purge with carbon dioxide-free air. An appropriate measurement protocol must be followed to compensate for water, carbon dioxide or other atmospheric gases.

ATR mode

ATR is suitable for liquid and solid samples, and requires no preparation apart from simple treatments such as the grinding of large crystals and coarse material. Proceed as follows depending on the sample state (liquid or solid).

Liquids. Place the sample in contact with the crystal.

Solids. Ensure close and uniform contact between the substance to be examined and the whole crystal surface, either by applying pressure or by dissolving the substance in an appropriate solvent, then covering the crystal with the resulting solution and evaporating to dryness.

METHODS

Infrared spectroscopy is mostly used to identify substances, but it may also be carried out for quantitative applications. Quantitative analysis (based on the Beer-Lambert law, which relates the absorbance of a sample to its concentration) will not be described in this chapter.

The measurement is performed on an appropriately prepared sample. The data is then processed and evaluated, either to identify substances or quantify them (e.g. based on integration of IR-absorption bands).

Spectral quality may be enhanced by mathematical pretreatments. In practice, these are limited to spectral normalisation and subtraction of bands caused by carbon-dioxide and water vapour. The same pretreatments are performed on both the sample and the reference spectra.

Identification

Prepare the substance to be examined appropriately and record the spectra between 4000 and 650 cm^{-1}, unless otherwise prescribed.

Identification testing is performed by comparing the spectrum of the substance to be examined with the spectrum obtained from a Ph. Eur. chemical reference substance (CRS) or with a Ph. Eur. reference spectrum.

The spectrum of the current batch of the Ph. Eur. CRS may be recorded for immediate use or stored, for example, in a spectral library for future consultation. A stored spectrum may be used, provided traceability to the current batch of CRS is ensured.

In the case of substances that are not covered by individual monographs, a suitable reference standard may be used.

In all cases, spectra must be recorded using the same operating conditions and procedure, and especially the same measurement mode.

When comparison of the spectra recorded in the solid state show differences (see below), treat the substance to be examined and the reference substance in the same manner so that they recrystallise or are produced in the same crystalline form, or proceed as prescribed in the monograph, then record the spectra again. However, this procedure must only be done for substances where the monograph does not cover a particular form of a substance that exhibits polymorphism.

Several comparison procedures may be used, and the analyst must document and justify the method used and the specific acceptance criteria that allow a conclusion for identification. The spectra can be compared either by overlaying the spectra (in the whole spectral range or in the region of interest specified in the monograph) or by using mathematical calculations from the software. It is possible for example to perform:

- visual comparison based on band positions and relative intensities unless otherwise specified - the transmission minima (or absorption maxima) in the spectrum obtained with the substance to be examined correspond in position and relative size to those of the reference;
- calculation of the correlation coefficient between the 2 spectra - this value is calculated by the software and the identification threshold is defined by the user;
- evaluation by chemometric methods (e.g. Euclidean distance, Mahalanobis distance, classification methods); these methods involve the set-up, assessment and validation of the chemometric model by the analyst (see *5.21. Chemometric methods applied to analytical data*).

Impurities in gases

For the analysis of impurities, use a cell transparent to infrared radiation and of suitable optical pathlength (e.g. 1-20 m). Fill the cell as prescribed under Gases. For detection and quantification of the impurities, proceed as prescribed in the monograph.

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2.2.25. ABSORPTION SPECTROPHOTOMETRY, ULTRAVIOLET AND VISIBLE

PRINCIPLE

Ultraviolet and visible (UV-Vis) spectroscopy (or spectrophotometry) is based on the ability of atoms, molecules and ions to absorb light at specific wavelengths in the ultraviolet (approximately 180-400 nm) and visible (approximately 400-800 nm) range. This absorption is associated with changes in electronic energy in the form of temporary transitions of electrons to an excited state at a higher energy orbital. As each energy level of a molecule or molecular ion also has associated vibrational and rotational sub-levels, this results in many permitted transitions, which are generally impossible to separate, thereby producing absorption bands rather than sharp lines. These bands are characteristic of the functional groups and bonds in a molecule.

UV-Vis spectroscopy measurements involve exposing a sample to light and measuring the attenuation and/or scattering of the emerging (transmitted or reflected) light either at a single wavelength or over a specified wavelength range.

APPLICATIONS

UV-Vis spectroscopy is traditionally used for the quantitative and qualitative analysis of liquid samples, but is also suitable for solid and gaseous analytes and has other applications such as the determination of physical or chemical properties.

UV-Vis spectroscopy as described in this chapter can be applied in various ways:

- when a monograph or general chapter refers to this chapter, the requirements described in the relevant paragraphs of this chapter are mandatory;
- when used as the detection method in chromatographic systems as described in general chapter *2.2.46*, the requirements listed in the relevant paragraphs of this chapter are mandatory;
- when used as a process analytical technology (PAT) tool for PAT applications similar to the applications described in this chapter, the provisions herein apply; for other PAT applications, the principles are the same, however the criteria are established bearing in mind the intended purpose of the analysis, using a risk-based approach.

EQUIPMENT

Spectrophotometers used for carrying out measurements in the UV-Vis region typically consist of:

- a suitable light source (such as a deuterium lamp for the UV region, a tungsten-halogen lamp for the visible region or a xenon lamp to cover the entire UV-Vis range); UV-Vis spectrophotometers often have 2 sources;
- a monochromator such as a grating system;
- other optical components, such as lenses or mirrors, that relay light through the instrument and that may also be used to generate more than one beam of light, i.e. in double-beam spectrophotometers, as opposed to single-beam spectrophotometers;
- a sample container, holder or sampling device; examples include conventional cuvettes, fibre-optic probes and immersed transmission cells (e.g. high-purity quartz or sapphire transparent to UV-Vis radiation); the choice depends on the intended application, paying particular attention to its suitability for the type of sample to be analysed;
- a single-channel (e.g. photomultiplier, photodiode) or multi-channel detector (e.g. photodiode array (PDA) or charge-coupled device (CCD));
- suitable computerised data processing and evaluation systems.

Control of cuvettes. For benchtop instruments, cuvettes or cells with a defined path length are used. These can be made of different materials such as quartz or glass. The tolerance for the path length of quartz and glass cuvettes is ± 0.5 per cent (e.g. ± 0.005 cm for a 1 cm cuvette). Plastic cuvettes may also be used but the tolerance interval is wider; therefore, their use must be thoroughly justified and based on a risk assessment.

The following method may be applied to check the cleanliness of optical cuvette windows and any significant differences in their thickness or parallelism: fill the cuvette with *water R* and measure its apparent absorbance against air at 240 nm for quartz cuvettes and 650 nm for glass cuvettes; rotate the cuvette 180° in its holder and measure the apparent absorbance again at the same wavelength.

When using scanning instruments, it is recommended to scan over the optical region of interest.

When using double-beam spectrophotometers, measures should be taken (e.g. matching the cuvettes) to ensure that any difference between the absorbance of the cuvettes will not have a significant impact on the analysis to be performed.

Acceptance criteria:

- the apparent absorbance is not greater than 0.093 for 1 cm quartz cuvettes (UV region) and 0.035 for 1 cm glass cuvettes (visible region);
- the absorbance measured after rotation (180°) does not differ by more than 0.005 from the value previously obtained.

MEASUREMENT

Transmission mode. Transmission mode provides a measure of the transmittance (T), at a given UV-Vis wavelength, of a sample placed between the light source and the detector. Transmittance is the ratio of the intensity of the transmitted light to the intensity of the incident light and is given by the following equation:

$$T = \frac{I}{I_0}$$

I = intensity of transmitted radiation;

I_0 = intensity of incident radiation.

A spectrum may be obtained by plotting the variation in transmittance (T) or absorbance (A) as a function of wavelength.

The absorbance is defined as the logarithm to base 10 of the reciprocal of the transmittance for monochromatic radiation. It is a dimensionless quantity expressed in absorbance units (AU), given by the following equation:

$$A = \log_{10}\left(\frac{1}{T}\right) = \log_{10}\left(\frac{I_0}{I}\right)$$

According to the Beer-Lambert law, which applies to clear diluted solutions, in the absence of interfering physico-chemical factors, the absorbance (A) is proportional to the path length (l) of the radiation through the sample, and to the concentration (c) of the substance in solution in accordance with the following equation:

$$A = \varepsilon c l$$

ε = molar absorption coefficient, in litres per mole per centimetre;

c = molar concentration of the substance in solution, in moles per litre;

l = absorption path length, in centimetres.

The specific absorbance ($A_{1\,\mathrm{cm}}^{1\,\mathrm{per\,cent}}$) of the substance is generally used in monographs and is related to absorbance (A) as follows:

$$A = A_{1\,\mathrm{cm}}^{1\,\mathrm{per\,cent}} \times c_m \times l$$

c_m = mass concentration of the substance in solution, in grams per 100 millilitres.

$A_{1\,\mathrm{cm}}^{1\,\mathrm{per\,cent}}$ represents the specific absorbance of a dissolved substance and refers to the absorbance of a 1 g/100 mL (or 1 per cent *m/V*) solution in a 1 cm cuvette or cell and is measured at a defined wavelength. The relationship between $A_{1\,\mathrm{cm}}^{1\,\mathrm{per\,cent}}$ and ε is:

$$A_{1\,\mathrm{cm}}^{1\,\mathrm{per\,cent}} = \frac{10\varepsilon}{M_\mathrm{r}}$$

M_r = relative molecular mass.

Transmittance or absorbance measurements are generally used for liquids (dispersions and solutions), but can also be used for solids (including tablets and capsules). For measurements of solids, a suitable sample accessory is used. Liquid samples are examined using a cell or cuvette with a suitable path length (typically 0.01-1 cm) and made of a material that is transparent to UV-Vis radiation, or by using a fibre-optic probe of a suitable configuration immersed in the liquid.

Diffuse reflection mode. Diffuse reflection mode provides a measure of reflectance (R), which is given by the following equation:

$$R = \frac{I}{I_0}$$

I = intensity of light reflected and/or scattered from the sample;

I_0 = intensity of light reflected and/or scattered from a blank or reference reflective surface.

Depending on the chemical composition and physical characteristics of the sample, the UV-Vis radiation may be absorbed as it passes through the sample. In diffuse reflection mode, it is the non-absorbed radiation which is partially reflected and/or scattered back from the sample that is measured by the detector. UV-Vis reflectance spectra are typically obtained by calculating and plotting $\log_{10}(1/R)$ as a function of the wavelength.

This measurement mode is generally selected for solids. The sample is examined either in a suitable device (e.g. a sample holder) or in direct contact with a probe. For process monitoring, the material can be analysed through a polished window interface (e.g. quartz or sapphire), or in-line using a probe. Care must be taken to ensure that the measurement conditions are as reproducible as possible from one sample to another.

Operation of the equipment. The factors below affect the spectral response and must always be taken into account.

Choose a measurement mode that is appropriate for the intended application and the sample type.

Define the measuring conditions taking into account the sample size and sample probe in such a way as to obtain a satisfactory signal-to-noise ratio (e.g. beam size, measurement time and number of measurements). For scanning spectrophotometers, also select the scan range, scan rate and slit-width that provide the necessary optical resolution for the intended application without losing the required signal-to-noise ratio or the linearity of the analytical method. When using spectrophotometers with array sensors, there is no need to adjust the beam size, scan range, scan rate or slit-width since the optical resolution is typically fixed and the full spectrum is always recorded.

Before an absorbance measurement is carried out, the zero position of the absorption (baseline correction) should be set or determined for the wavelengths of interest or over the appropriate range of wavelengths.

For PAT applications, when measuring moving materials or samples, ensure that there is no fouling of the sensor (e.g. no contamination or build-up of material).

Unless otherwise prescribed in the monograph, measure the absorbance using a path length of 1 cm at the prescribed wavelength. If a single value for the position of an absorption maximum or minimum is given in a monograph, the user must determine the wavelength position. The value obtained may differ by not more than ± 2 nm, unless otherwise prescribed.

Quantitative measurements relying on absorption values above 2.0 should be avoided.

Background correction. Select a suitable spectroscopic blank (e.g. air, blank solvent, solid material). Unless otherwise prescribed, all measurements are carried out with reference to the same solvent or the same mixture of solvents (blank).

Measure the blank and the sample within a short time-frame either in parallel in double-beam spectrophotometers or sequentially in single-beam spectrophotometers. The absorbance values of both blank and sample must be in the working range of the equipment as specified by the manufacturer.

For benchtop instruments, the absorbance of the solvent measured against air and at the prescribed wavelength must not exceed 0.4 and is preferably less than 0.2.

For chromatographic systems, the transmittance of the mobile phase may be used as the blank.

In some PAT applications, it may be impossible to remove the probe for background data collection. Various options are therefore to be considered, including the use of internal references, measurement of a blank using a second detector, etc. Only spectra measured against a blank possessing the same optical properties can be directly compared with one another.

For reflectance measurements, common reflectance blank samples include ceramics, fluoropolymers such as polytetrafluoroethylene (PTFE) and powders such as barium sulfate ($BaSO_4$) and magnesium oxide (MgO), but other suitable materials may also be used.

MATHEMATICAL TREATMENT OF SPECTRAL DATA

In the case of single wavelength analysis used to determine the concentration of an unknown sample (e.g. as prescribed in monographs), mathematical treatment consists in determining the regression of the photometric reading (absorbance) on the concentration of the standard samples.

In the case of full range spectra, data for both diffuse reflection and transmission modes may have to be treated before a classification or calibration model can be developed. The aim can be, for example, to reduce baseline drift or to correct for scatter caused by particle size changes in solid samples. For example, first-, second- or higher-order derivative spectra can typically be used to improve resolution or sensitivity. This pretreatment may be a useful means of simplifying the data and thereby reducing the variations that may cause interference in subsequently applied mathematical models.

A wide range of treatment methods, such as scaling, smoothing, normalisation and derivatisation, can be applied either singly or in combination. More information is available in general chapter *5.21. Chemometric methods applied to analytical data*.

CONTROL OF EQUIPMENT PERFORMANCE

Spectrophotometer performance is controlled (automatically or manually) at regular intervals as defined in the quality management system and dictated by the use of the equipment

and the application. For example, equipment exposed to variations in temperature and humidity may need more frequent performance testing.

Requirements for control of equipment performance for the various measurement modes are summarised in Table 2.2.25.-1. Further such tests may be performed if appropriate.

Table 2.2.25.-1. – *Minimum tests to be carried out for the control of equipment performance*

Purpose	Method	Wavelength accuracy	Absorbance accuracy	Photometric linearity	Stray light	Resolution/spectral bandwidth
Quantitative or limit test	Based on measurement of the absorbance at one or more identified wavelengths (e.g. assay or impurities test)	X	X	X	X	If required in the monograph
Identification test	Based on wavelength of absorption maxima and minima	X	-	-	X	-
	Based on absorption measurement and wavelength of absorption maxima	X	X	-	X	-
	Based on comparison of spectrum with that of reference substance	X	X	-	-	-

Control of wavelength accuracy. Control the wavelength accuracy of an appropriate number of bands in the intended spectral range using one or more reference materials; for example, use solid or liquid filters (e.g. *holmium perchlorate solution R*) to verify the position of absorption bands, or measure the emission from a light source to check emission-line position. Table 2.2.25.-2 shows examples of wavelengths used to check wavelength accuracy. When certified reference materials are used, the reference wavelength is that stated on the corresponding certificate.

Some instruments may have an automatic or inbuilt wavelength accuracy control feature.

Table 2.2.25.-2. – *Examples of wavelengths used for the control of wavelength accuracy*
*Note: the wavelength varies with the resolution of the instrument

Material		Wavelengths (nm)*
Solutions	Cerium in sulfuric acid	201.1; 211.4; 222.6; 240.4; 253.7
	Didymium in perchloric acid	511.8; 731.6; 794.2
	Holmium in perchloric acid	241.1; 287.2; 361.3; 451.4; 485.2; 536.6; 640.5
Solid filters	Didymium glass	513.5
	Holmium glass	279.3; 360.9; 453.4; 637.5
Lamps	Deuterium	486.0; 656.1
	Mercury (low pressure)	184.9; 253.7; 312.5; 365.0; 404.7; 435.8; 546.1; 577.0; 579.1
	Neon	717.4
	Xenon	541.9; 688.2; 764.2

For chromatographic systems, it is also possible to control wavelength accuracy by measuring the absorbance of a 0.05 mg/mL solution of *caffeine R* in *methanol R*; the absorption maximum is obtained at 272 nm and the minimum at 244 nm.

Wavelength accuracy, absorbance accuracy and linearity are controlled using either certified reference materials such as solid filters or liquid filters in appropriate sealed cells, or solutions prepared in the laboratory as described below.

Acceptance criteria

It is recommended to test at least 2 wavelengths that bracket the intended spectral range.

For benchtop instruments, the tolerance for wavelength accuracy of UV-Vis spectroscopy in cuvettes is ± 1 nm at wavelengths below 400 nm and ± 3 nm at wavelengths of 400 nm and above.

For chromatographic systems, the tolerance for wavelength accuracy is ± 2 nm for the whole UV-Vis range.

For PAT applications, a tolerance of ± 2 nm for the UV-Vis range is recommended. However, wider tolerance intervals may be needed for some PAT applications, in which case the requisite wavelength accuracy must be defined by the user depending on the intended purpose, and using a risk-based approach.

The instrument parameters (especially the entrance optics such as slit-width or optical fibre diameter) influence the resolution and must be the same as those intended for the actual measurements.

Control of absorbance accuracy. Control the absorbance accuracy at an appropriate number of wavelengths in the intended spectral range, using suitable solid or liquid filters to check that the absorbance measured at the test wavelength matches the certified absorbance of the filter or the absorbance value that is calculated from a certified specific absorbance. *Nicotinic acid for equipment qualification CRS* may be used.

It is recommended to test absorbance accuracy at selected wavelengths using one or more solid or liquid filters with different absorbance levels; as a minimum, values at approximately the 2 limits of the expected absorbance range should be verified.

For chromatographic systems and PAT applications, the testing of absolute absorbance accuracy may not be necessary, providing that a standard curve is measured as required.

For measurements using *nicotinic acid for equipment qualification CRS*, the certified specific absorbance is given in the corresponding leaflet.

The solution of nicotinic acid can be prepared as follows: dissolve 57.0-63.0 mg of *nicotinic acid for equipment qualification CRS* in a 0.1 M hydrochloric acid solution prepared from *hydrochloric acid R* and dilute to 200.0 mL with the same acid solution; dilute 2.0 mL of the solution

to 50.0 mL with the same acid solution to obtain a final concentration of 12 mg/L. These volumes can be adjusted to obtain nicotinic acid solutions with other concentrations (up to about 40 mg/L), for the purposes of testing different absorbance levels. The absorbance is measured at 213 nm and 261 nm.

Acceptance criteria

The difference between the measured absorbance and the absorbance of the certified material is ± 0.010 or ± 1 per cent, whichever is greater, for each combination of wavelength and absorbance assessed (applies to absorbance values not greater than 2). Tolerances for higher absorbance values should be defined on the basis of a risk assessment.

Control of photometric linearity. Control the photometric linearity in the intended spectral range. In the ultraviolet range, the filters used to control absorbance accuracy may be used, as can solutions of nicotinic acid or caffeine. In the visible range, neutral glass filters may be used. Prior to performing the test, ensure that the absorbance of the standards is compatible with the intended linear range.

Solutions with increasing concentrations (e.g. 5-40 mg/L) of *nicotinic acid for equipment qualification CRS* in a 0.1 M hydrochloric acid solution prepared from *hydrochloric acid R* may be used. The absorbance is measured at 213 nm and 261 nm.

For chromatographic systems, it is also possible to check photometric linearity using 0.5-50 mg/L solutions of *caffeine R* in *water for chromatography R*. The absorbance is measured at 273 nm.

Acceptance criterion

The coefficient of determination (R^2) is not less than 0.999.

Limit of stray light. Stray light is determined at an appropriate wavelength using suitable solid or liquid filters or solutions prepared in-house. The instrument parameters used for the test, such as slit-width and type of light source (e.g. deuterium or tungsten lamp), must be the same as those intended for the actual measurements.

Acceptance criterion

The acceptance criterion depends on the filters or solutions used, for example:

- the absorbance is not less than 3.0 when using a 10 g/L solution of *sodium iodide R* at 220 nm, a 10 g/L solution of *potassium iodide R* at 250 nm or a 50 g/L solution of *sodium nitrite R* at 340 nm and 370 nm;
- the absorbance is not less than 2.0 when using a 12 g/L solution of *potassium chloride R* at 198 nm.

These values apply when using a 1 cm cell and *water R* as the compensation liquid.

Control of resolution. Where prescribed in a monograph, measure the resolution of the equipment either using suitable certified reference materials, or by recording the spectrum of a 0.02 per cent *V/V* solution of *toluene R* in *hexane R* or *heptane R*, with respectively *hexane R* or *heptane R* as the compensation liquid.

Acceptance criterion

For measurements taken with a solution prepared as described above, the minimum ratio of the absorbance at the maximum (269 nm) to that at the minimum (266 nm) is stated in the monograph.

SYSTEM SUITABILITY

System suitability tests may be required prior to sample measurement to verify critical parameters that may have an impact on the result.

These tests may cover wavelength accuracy, absorbance accuracy, stray light and photometric linearity. System functionality tests, for example those performed as part of equipment autotesting, may be considered part of the system suitability tests.

In the case of UV-Vis detection for chromatographic systems, additional system suitability tests are applicable if prescribed in the monograph and/or in general chapter *2.2.46. Chromatographic separation techniques*.

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2.2.27. THIN-LAYER CHROMATOGRAPHY

Thin-layer chromatography is a separation technique in which a stationary phase consisting of an appropriate material is spread in a uniform thin layer on a support (plate) of glass, metal or plastic. Solutions of analytes are deposited on the plate prior to development. The separation is based on adsorption, partition, ion-exchange or on combinations of these mechanisms and is carried out by migration (development) of solutes (solutions of analytes) in a solvent or a suitable mixture of solvents (mobile phase) through the thin-layer (stationary phase).

APPARATUS

Plates. The chromatography is carried out using pre-coated plates as described under *Reagents* (*4.1.1*). The particle size of the silica gel is indicated after the name of the reagent in the tests where it is used.

Pre-treatment of the plates. It may be necessary to wash the plates prior to separation. This can be done by migration of an appropriate solvent. The plates may also be impregnated by procedures such as development, immersion or spraying. At the time of use, the plates may be activated, if necessary, by heating in an oven at 120 °C for 20 min.

Chromatographic tank with a flat bottom or twin trough, of inert, transparent material, of a size suitable for the plates used and provided with a tightly fitting lid. For horizontal development the tank is provided with a trough for the mobile phase and it additionally contains a device for directing the mobile phase to the stationary phase.

Micropipettes, microsyringes, calibrated disposable capillaries or other application devices suitable for the proper application of the solutions.

Fluorescence detection device to measure direct fluorescence or the inhibition of fluorescence.

Visualisation devices and reagents. Suitable devices are used for derivatisation to transfer to the plate reagents by spraying, immersion or exposure to vapour and, where applicable, to facilitate heating for visualisation of separated components.

Documentation. A device may be used to provide documentation of the visualised chromatogram, for example a photograph or a computer file.

METHOD

Sample application. Apply the prescribed volume of the solutions at a suitable distance from the lower edge and from the sides of the plate and on a line parallel to the lower edge; allow an interval of at least 10 mm (5 mm on high-performance plates) between the centres of circular spots and 5 mm (2 mm on high-performance plates) between the edges of bands. Apply the solutions in sufficiently small portions to obtain circular spots 2-5 mm in diameter (1-2 mm on high-performance plates) or bands 10-20 mm (5-10 mm on high-performance plates) by 1-2 mm.

In a monograph, where both normal and high-performance plates may be used, the working conditions for high-performance plates are given in the brackets [ ] after those for normal plates.

Vertical development. Line the walls of the chromatographic tank with filter paper. Pour into the chromatographic tank a sufficient quantity of the mobile phase for the size of the tank to give after impregnation of the filter paper a layer of appropriate depth related to the dimension of the plate to be used. For saturation of the chromatographic tank, replace the lid and allow to stand at 20-25 °C for 1 h. Unless otherwise indicated in the monograph, the chromatographic separation is performed in a saturated tank. Apply the prescribed volume of solutions as described above. When the solvent has evaporated from the applied solutions, place the plate in the chromatographic tank, ensuring that the plate is as vertical as possible and that the spots or bands are above the surface of the mobile phase. Close the chromatographic tank, maintain it at 20-25 °C and protect from sunlight. Remove the plate when the mobile phase has moved over the prescribed distance, measured between the points of application and the solvent front. Dry the plate and visualise the chromatograms as prescribed.

For two-dimensional chromatography, dry the plates after the first development and carry out a second development in a direction perpendicular to that of the first development.

Horizontal development. Apply the prescribed volume of the solutions as described above. When the solvent has evaporated from the applied solutions, introduce a sufficient quantity of the mobile phase into the trough of the chamber using a syringe or pipette, place the plate in the chamber after verifying that the latter is horizontal and connect the mobile phase direction device according to the manufacturer's instructions. If prescribed, develop the plate starting simultaneously at both ends. Close the chamber and maintain it at 20-25 °C. Remove the plate when the mobile phase has moved over the distance prescribed in the monograph. Dry the plate and visualise the chromatograms as prescribed.

For two-dimensional chromatography, dry the plates after the first development and carry out a second development in a direction perpendicular to that of the first development.

VISUAL EVALUATION

Identification. The principal spot in the chromatogram obtained with the test solution is visually compared to the corresponding spot in the chromatogram obtained with the reference solution by comparing the colour, the size and the retardation factor (R_F) of both spots.

The retardation factor (R_F) is defined as the ratio of the distance from the point of application to the centre of the spot and the distance travelled by the solvent front from the point of application.

Verification of the separating power for identification. Normally the performance given by the suitability test described in *Reagents* (*4.1.1*) is sufficient. Only in special cases an additional performance criterion is prescribed in the monograph.

Related substances test. The secondary spot(s) in the chromatogram obtained with the test solution is (are) visually compared to either the corresponding spot(s) in the chromatogram obtained with the reference solution containing the impurity(ies) or the spot in the chromatogram obtained with the reference solution prepared from a dilution of the test solution.

Verification of the separating power. The requirements for the verification of the separating power are prescribed in the monographs concerned.

Verification of the detecting power. The detecting power is satisfactory if a spot or band is clearly visible in the chromatogram obtained with the most dilute reference solution.

QUANTITATIVE MEASUREMENT

The requirements for resolution and separation are prescribed in the monographs concerned.

Substances separated by thin-layer chromatography and responding to UV-Vis irradiation can be determined directly on the plate, using appropriate instrumentation. While moving the plate or the measuring device, examine the plate by measuring the reflectance of the incident light. Similarly,

fluorescence may be measured using an appropriate optical system. Substances containing radionuclides can be quantified in 3 ways: either directly by moving the plate alongside a suitable counter or vice versa (see *Radiopharmaceutical preparations (0125)*), by cutting the plates into strips and measuring the radioactivity on each individual strip using a suitable counter or by scraping off the stationary phase, dissolving it in a suitable scintillation cocktail and measuring the radioactivity using a liquid scintillation counter.

Apparatus. The apparatus for direct measurement on the plate consists of:

- a device for exact positioning and reproducible dispensing of the amount of substances onto the plate;
- a mechanical device to move the plate or the measuring device along the *x*-axis or the *y*-axis;
- a recorder and a suitable integrator or a computer;
- *for substances responding to UV-Vis irradiation*: a photometer with a source of light, an optical device able to generate monochromatic light and a photo cell of adequate sensitivity are used for the measurement of reflectance or transmittance; if fluorescence is measured, a suitable filter is required to prevent light used for excitation from reaching the detector while permitting emitted light or a specific portion thereof to pass;
- *for substances containing radionuclides*: a suitable counter for radioactivity. The linearity range of the counting device is to be verified.

Method. Prepare the solution of the substance to be examined (test solution) as prescribed in the monograph and, if necessary, prepare the reference solutions of the substance to be determined using the same solvent as in the test solution. Apply the same volume of each solution to the plate and develop.

Substances responding to UV-Vis irradiation. Prepare and apply not fewer than 3 reference solutions of the substance to be examined, the concentrations of which span the expected value in the test solution (about 80 per cent, 100 per cent and 120 per cent). Treat with the prescribed reagent, if necessary, and record the reflectance, the transmittance or fluorescence in the chromatograms obtained with the test and reference solutions. Use the measured results for the calculation of the amount of substance in the test solution.

Substances containing radionuclides. Prepare and apply a test solution containing about 100 per cent of the expected value. Determine the radioactivity as a function of the path length and report the radioactivity in each resulting peak as a percentage of the total amount of radioactivity.

Criteria for assessing the suitability of the system are described in the chapter on *Chromatographic separation techniques* (*2.2.46*). The extent to which adjustments of parameters of the chromatographic system can be made to satisfy the criteria of system suitability are also given in this chapter.

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2.2.28. GAS CHROMATOGRAPHY

PRINCIPLE

Gas chromatography (GC) is a chromatographic separation technique based on the difference in the distribution of species between 2 non-miscible phases in which the mobile phase is a carrier gas moving through or passing the stationary phase contained in a column. It is applicable to substances or their derivatives which are volatilised under the temperatures employed.

GC is mainly based on mechanisms of adsorption or mass distribution.

EQUIPMENT

The equipment typically consists of:

- an injector;
- a chromatographic column contained in an oven;
- one or more detector(s);
- a data acquisition system.

The carrier gas flows through the column and then through the detector at a controlled rate or pressure.

The chromatography is carried out either at a constant temperature or according to a given temperature programme.

INJECTORS

Injection may be carried out either into a vaporisation chamber which may be equipped with a stream splitter, or directly at the head of the column using a syringe or an injection valve.

Injections of vapour phase may be effected by static or dynamic head-space injection systems.

Dynamic head-space (purge and trap) injection systems include a sparging device by which volatile substances in solution are swept into an absorbent column maintained at a low temperature. Retained substances are then desorbed into the mobile phase by rapid heating of the absorbent column.

Static head-space injection systems include a thermostatically controlled sample heating chamber in which closed vials containing solid or liquid samples are placed for a fixed period of time to allow equilibration of the volatile components of the sample between the non-gaseous phase and the vapour phase. After equilibration, a predetermined amount of the head-space of the vial is flushed into the gas chromatograph.

STATIONARY PHASES

Stationary phases are contained in columns which may be:

- a capillary column whose stationary phase may be a solid coating the inner surface of the column (e.g. macrogol 20 000), or a liquid deposited on the inner surface (e.g. dimethylpolysiloxane); in the latter case it may be chemically bonded to the inner surface;
- a column packed with the stationary phase which may be a solid phase (e.g. alumina, silica) or an inert solid support (usually a porous polymer) impregnated or coated with a liquid.

Capillary columns, made of fused silica, are 0.1 mm to 0.53 mm in internal diameter (Ø) and at least 5 m in length. The stationary phase is a film 0.1 µm to 5.0 µm thick.

Packed columns, made of glass or metal, are usually 1 m to 3 m in length with an internal diameter (Ø) of 2 mm to 4 mm.

MOBILE PHASES

Retention time and peak efficiency depend on the carrier gas flow rate; retention time is directly proportional to column length and resolution is proportional to the square root of the column length.

The carrier gas flow rate is usually expressed in millilitres per minute at atmospheric pressure and at the stated temperature. Flow rate is measured at the detector outlet, either with a calibrated mechanical device or with a bubble tube, while the column is at operating temperature.

The linear velocity of the carrier gas through a column is inversely proportional to the square of the internal diameter of the column for a given flow volume.

Helium, nitrogen and hydrogen are commonly used carrier gases.

DETECTORS

Flame-ionisation detectors are usually employed but other detectors such as electron-capture, nitrogen-phosphorus, mass spectrometric, thermal conductivity or infrared spectrophotometric detectors may also be used.

PROCEDURE

Equilibrate the column, the injector and the detector at the temperatures and the gas flow rates/pressures specified in the monograph until a stable baseline is achieved. Prepare the test solution(s) and the reference solution(s) as prescribed. The solutions injected must be free from solid particles.

Criteria for assessing the suitability of the system are described in general chapter *2.2.46 Chromatographic separation techniques*. The extent to which adjustments of parameters of the chromatographic system can be made to satisfy the criteria of system suitability are also given in this general chapter.

Static head-space gas chromatography

Static head-space gas chromatography is a technique particularly suitable for separating and determining volatile compounds present in solid or liquid samples. The method is based on the analysis of the vapour phase in equilibrium with the solid or liquid phase.

EQUIPMENT

The equipment consists of a gas chromatograph provided with a sample-introduction device that may be connected to a module that automatically controls the pressure and the temperature. If necessary, a device for eliminating solvents can be added.

The sample to be analysed is introduced into a container fitted with a suitable stopper and a valve-system which permits the passage of the carrier gas. The container is placed in a thermostatically controlled chamber at a temperature set according to the substance to be examined.

The sample is held at this temperature long enough to allow equilibration between the solid or liquid phase and the vapour phase.

The carrier gas is introduced into the container and, after the prescribed time, a suitable valve is opened so that the gas expands towards the chromatographic column taking the volatilised compounds with it.

Instead of using a chromatograph specifically equipped for the introduction of samples, it is also possible to use airtight syringes and a conventional chromatograph. Equilibration is then carried out in a separate chamber and the vapour phase is carried onto the column, while necessary precautions are taken to avoid any changes in the equilibrium.

PROCEDURE

Using the reference preparations, determine suitable instrument settings to produce an adequate response.

DIRECT CALIBRATION

Introduce into separate, identical containers the preparation to be examined and each of the reference preparations, as prescribed in the monograph, avoiding contact between the sampling device and the samples.

Close the containers hermetically and place in the thermostatically controlled chamber set to the temperature and pressure prescribed in the monograph; after equilibration, carry out the chromatography under the prescribed conditions.

STANDARD ADDITIONS

Add to a set of identical suitable containers equal volumes of the preparation to be examined. Add to all but one of the containers, suitable quantities of a reference preparation containing a known concentration of the substance to be determined so as to produce a series of preparations containing steadily increasing concentrations of the substance.

Close the containers hermetically and place in the thermostatically controlled chamber set to the temperature and pressure prescribed in the monograph; after equilibration, carry out the chromatography under the prescribed conditions.

Calculate the linear equation of the graph using a least-squares fit, and derive from it the concentration of the substance to be determined in the preparation to be examined.

Alternatively, plot on a graph the mean of readings against the added quantity of the substance to be determined. Extrapolate the line joining the points on the graph until it meets the concentration axis. The distance between this point and the intersection of the axes represents the concentration of the substance to be determined in the preparation to be examined.

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2.2.29. LIQUID CHROMATOGRAPHY

PRINCIPLE

Liquid chromatography (LC) is a method of chromatographic separation based on the difference in the distribution of species between 2 non-miscible phases, in which the mobile phase is a liquid which percolates through a stationary phase contained in a column.

LC is mainly based on mechanisms of adsorption, mass distribution, ion exchange, size exclusion or stereochemical interaction.

Unless otherwise specified, all the information below is valid for both standard LC and LC using reduced particle-size columns (e.g. sub-2 µm).

The latter requires instrumentation that is able to withstand higher pressures (typically up to 100 MPa, i.e. about 15 000 psi), generates lower extra-column band broadening, provides improved gradient mixing and allows a higher sampling rate in the detection system.

EQUIPMENT

The equipment typically consists of:

- a pumping system;
- an injector;
- a chromatographic column (a column temperature controller may be used);
- 1 or more detector(s);
- a data acquisition system.

The mobile phase is supplied from 1 or more reservoirs and is pumped to the injector, then through the column, usually at a constant rate, and then through the detector(s).

PUMPING SYSTEMS

LC pumping systems deliver the mobile phase at a controlled flow rate. Pressure fluctuations are to be minimised, for example by passing the pressurised solvent through a pulse-dampening device. Tubing and connections are capable of withstanding the pressures developed by the pumping system. LC pumps may be fitted with a facility for 'bleeding' the system of entrapped air bubbles.

Microprocessor-controlled pumping systems are capable of accurately delivering a mobile phase of either constant (isocratic elution) or varying composition (gradient elution), according to a defined programme. In the case of gradient elution, pumping systems which deliver solvent(s) from several reservoirs are available and solvent mixing can be achieved on either the low or high-pressure side of the pump(s).

INJECTORS

The sample solution is introduced into the flowing mobile phase at or near the head of the column using an injection system which can operate at high pressure. Fixed-loop and variable volume devices operated manually or by an autosampler are used. Partial filling of loops during manual injection may adversely affect injection volume precision.

STATIONARY PHASES

There are many types of stationary phases employed in LC, including:

- silica or alumina, commonly used in normal-phase LC (polar stationary phase and non-polar mobile phase), where the separation is based on differences in adsorption on the stationary phase and/or mass distribution between the mobile phase and the stationary phase (partition chromatography);
- a variety of chemically modified supports prepared from polymers, silica or porous graphite, used in normal-phase and reversed-phase LC (non-polar stationary phase and polar mobile phase), where the separation is based principally on partition of the molecules;
- resins or polymers with acidic or basic groups, used in ion-exchange chromatography, where separation is based on competition between the ions to be separated and those in the mobile phase;
- porous silica or polymers, used in size-exclusion chromatography (*2.2.30*), where separation is based on differences between the volumes of the molecules, corresponding to steric exclusion;
- specially modified stationary phases, e.g. cellulose or amylose derivatives, proteins or peptides, cyclodextrins etc., for the separation of enantiomers (chiral chromatography).

Most separations are based on reversed-phase LC utilising chemically modified silica as the stationary phase. The surface of the support, i.e. the silanol groups of silica, is reacted with various silane reagents to produce covalently bound silyl derivatives covering a varying number of active sites on the surface of the support. The nature of the bonded phase is an important parameter for determining the separation properties of the chromatographic system.

Unless otherwise stated by the manufacturer, silica-based reversed-phase columns are considered to be stable in mobile phases having an apparent pH in the range 2.0 to 8.0. Columns containing porous graphite or particles of polymeric materials such as styrene-divinylbenzene copolymer are stable over a wider pH range.

Analysis using normal-phase LC with unmodified silica or polar chemically modified silica (e.g. cyanopropyl or diol) as the stationary phase, with a non-polar mobile phase is applicable in certain cases.

For analytical separations, the particle size of the most commonly used stationary phases varies between 2 and 10 µm. The particles may be spherical or irregular, and of varying porosity and specific surface area. These properties contribute to the chromatographic behaviour of a particular stationary phase. In the case of reversed phases, the nature of the stationary phase, the extent of bonding, e.g. expressed as the carbon loading, and whether the stationary phase is end-capped (i.e. part of the residual silanol groups are silylated) are additional determining factors. Tailing of peaks, particularly of basic substances, can occur when residual silanol groups are present.

In addition to porous particles, superficially porous or monolithic materials may be used.

Unless otherwise prescribed in the monograph, columns made of stainless steel of varying length and internal diameter (Ø) are used for analytical chromatography. Columns with internal diameters of less than 2 mm are often referred to as microbore columns.

The temperature of the mobile phase and the column must be kept constant during the analysis. Most separations are performed at room temperature, but some require a different temperature for optimal performance.

MOBILE PHASES

For normal-phase LC, low-polarity organic solvents are generally employed. The residual water content of the solvents used in the mobile phase is to be strictly controlled to obtain reproducible results.

In reversed-phase LC, aqueous mobile phases, usually with organic solvents and/or modifiers, are employed.

The components of the mobile phase are usually filtered to remove particles greater than 0.45 µm in size (or greater than 0.2 µm when the stationary phase is made of sub-2 µm particles, and when special detectors, e.g. light scattering detectors, are used). Multicomponent mobile phases are prepared by measuring the required volumes (unless masses

are specified) of the individual components, followed by mixing. Alternatively, the solvents may be delivered by individual pumps controlled by proportioning valves, by which mixing is performed according to the desired proportion. Solvents are normally degassed before pumping by sparging with helium, sonication and/or using in-line membrane/vacuum modules to avoid the creation of gas bubbles in the detector cell.

Solvents for the preparation of the mobile phase are normally free of stabilisers and, if an ultraviolet detector is employed, are transparent at the wavelength of detection. Solvents and other components employed are to be of appropriate quality. In particular, *water for chromatography R* is used for the preparation of mobile phases when water, or an aqueous solution, is 1 of the components. Any necessary adjustments of the pH are made to the aqueous component of the mobile phase and not the mixture. If buffer solutions or saline solutions are used, adequate rinsing of the system is carried out with a mixture of water and a small proportion of the organic part of the mobile phase (5 per cent *V/V*) to prevent crystallisation of salts after completion of the analysis.

Mobile phases may contain other components, for example a counter-ion for ion-pair chromatography or a chiral selector for chiral chromatography using an achiral stationary phase.

DETECTORS

Ultraviolet/visible (UV/Vis) spectrophotometers (including diode array detectors) (*2.2.25*), are the most commonly employed detectors. Fluorescence spectrophotometers, differential refractometers (RI), electrochemical detectors (ECD), light scattering detectors, charged aerosol detectors (CAD), mass spectrometers (MS) (*2.2.43*), radioactivity detectors, multi-angle light scattering (MALS) detectors or other detectors may be used.

PROCEDURE

Equilibrate the column with the prescribed mobile phase and flow rate, at room temperature or at the temperature specified in the monograph, until a stable baseline is achieved. Prepare the solution(s) of the substance to be examined and the reference solution(s) required. The solutions must be free from solid particles.

Criteria for assessing the suitability of the system are described in general chapter *2.2.46. Chromatographic separation techniques*. The extent to which adjustments of parameters of the chromatographic system can be made to satisfy the criteria of system suitability are also given in this chapter.

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2.2.32. LOSS ON DRYING

PRINCIPLE

Loss on drying is the loss of mass after drying under specified conditions, calculated as a percentage (*m/m*).

Drying to constant mass means that 2 consecutive weighings do not differ by more than 0.5 mg, the 2nd weighing following an additional period of at least 30 min of drying under the conditions prescribed for the substance to be examined.

EQUIPMENT

The equipment typically consists of:

- weighing bottles that are made of suitable inert material and can easily be dried to constant mass; their diameter is large enough so that the layer of the substance to be examined does not exceed about 5 mm;
- an analytical balance by which it is possible to determine a change in mass of 0.1 mg;
- depending on the procedure to be applied, a desiccator, a vacuum cabinet, a vacuum oven or an ordinary laboratory oven; in any case, the temperature of ovens is adjustable to the specified temperature ± 2 °C; vacuum ovens in which the pressure can at least be reduced to about 2 kPa are suitable; ovens are qualified according to established quality system procedures, for example by using a suitable certified reference material (*sodium aminosalicylate dihydrate for equipment qualification CRS* may be used).

Equipment using other means of drying such as microwaves, halogen lamps, infrared lamps or mixed technologies may be used provided they are demonstrated to be fit for purpose.

PROCEDURE

It is recommended to perform the test in an environment that has minimal impact on sample measurement (e.g. humidity).

Weigh an empty weighing bottle that has been previously dried under the conditions prescribed for the substance to be examined for at least 30 min, then weigh the weighing bottle filled with the prescribed quantity of substance to be examined. Dry to constant mass or for the prescribed time. Where the drying temperature is indicated by a single value rather than a range, drying is carried out at the prescribed temperature ± 2 °C. Use one of the following procedures, unless otherwise prescribed in the monograph.

- In a desiccator: the drying is carried out over about 100 g of *molecular sieve R* at atmospheric pressure and at room temperature.
- *In vacuo*: the drying is carried out over about 100 g of *molecular sieve R* at a pressure not exceeding 2.5 kPa, at room temperature or at the temperature prescribed in the monograph.
- In an oven at a specified temperature: the drying is carried out at atmospheric pressure in an oven at the temperature prescribed in the monograph.

After drying in an oven, allow the weighing bottle and the sample to cool to room temperature in a desiccator and weigh the weighing bottle containing the dried sample.

The mass of the sample is the difference between the mass of the filled weighing bottle and the mass of the dried empty weighing bottle.

The loss on drying is the difference in the mass of the sample before and after drying, expressed as a percentage, *m/m* being implicit.

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2.2.43. MASS SPECTROMETRY

Mass spectrometry is based on the direct measurement of the ratio of the mass to the number of positive or negative elementary charges of ions (*m*/*z*) in the gas phase obtained from the substance to be analysed. This ratio is expressed in atomic mass units (1 a.m.u. = one twelfth the mass of ^{12}C) or in daltons (1 Da = the mass of the hydrogen atom).

The ions, produced in the ion *source* of the apparatus, are accelerated and then separated by the *analyser* before reaching the *detector*. All of these operations take place in a chamber where a pumping system maintains a vacuum of 10^{-3} to 10^{-6} Pa.

The resulting spectrum shows the relative abundance of the various ionic species present as a function of *m*/*z*. The signal corresponding to an ion will be represented by several peaks corresponding to the statistical distribution of the various isotopes of that ion. This pattern is called the *isotopic profile* and (at least for small molecules) the peak representing the most abundant isotopes for each atom is called the *monoisotopic peak*.

Information obtained in mass spectrometry is essentially qualitative (determination of the molecular mass, information on the structure from the fragments observed) or quantitative (using internal or external standards) with limits of detection ranging from the picomole to the femtomole.

INTRODUCTION OF THE SAMPLE

The very first step of an analysis is the introduction of the sample into the apparatus without overly disturbing the vacuum. In a common method, called *direct liquid introduction*, the sample is placed on the end of a cylindrical rod (in a quartz crucible, on a filament or on a metal surface). This rod is introduced into the spectrometer after passing through a vacuum lock where a primary intermediate vacuum is maintained between atmospheric pressure and the secondary vacuum of the apparatus.

Other introduction systems allow the components of a mixture to be analysed as they are separated by an appropriate apparatus connected to the mass spectrometer.

Gas chromatography/mass spectrometry. The use of suitable columns (capillary or semi-capillary) allows the end of the column to be introduced directly into the source of the apparatus without using a separator.

Liquid chromatography/mass spectrometry. This combination is particularly useful for the analysis of polar compounds, which are insufficiently volatile or too heat-labile to be analysed by gas chromatography coupled with mass spectrometry. This method is complicated by the difficulty of obtaining ions in the gas phase from a liquid phase, which requires very special interfaces such as:

- *direct liquid introduction*: the mobile phase is nebulised, and the solvent is evaporated in front of the ion source of the apparatus,
- *particle-beam interface*: the mobile phase, which may flow at a rate of up to 0.6 mL/min, is nebulised in a desolvation chamber such that only the analytes, in neutral form, reach the ion source of the apparatus; this technique is used for compounds of relatively low polarity with molecular masses of less than 1000 Da,
- *moving-belt interface*: the mobile phase, which may flow at a rate of up to 1 mL/min, is applied to the surface of a moving belt; after the solvent evaporates, the components to be analysed are successively carried to the ion source of the apparatus where they are ionised; this technique is rather poorly suited to very polar or heat-labile compounds.

Other types of coupling (electrospray, thermospray, atmospheric-pressure chemical ionisation) are considered to be ionisation techniques in their own right and are described in the section on modes of ionisation.

Supercritical fluid chromatography/mass spectrometry. The mobile phase, usually consisting of supercritical carbon dioxide enters the gas state after passing a heated restrictor between the column and the ion source.

Capillary electrophoresis/mass spectrometry. The eluent is introduced into the ion source, in some cases after adding another solvent so that flow rates of the order of a few microlitres per minute can be attained. This technique is limited by the small quantities of sample introduced and the need to use volatile buffers.

MODES OF IONISATION

Electron impact. The sample, in the gas state, is ionised by a beam of electrons whose energy (usually 70 eV) is greater than the ionisation energy of the sample. In addition to the molecular ion M^+, fragments characteristic of the molecular structure are observed. This technique is limited mainly by the need to vaporise the sample. This makes it unsuited to polar, heat-labile or high molecular mass compounds. Electron impact is compatible with the coupling of gas chromatography to mass spectrometry and sometimes with the use of liquid chromatography.

Chemical ionisation. This type of ionisation involves a reagent gas such as methane, ammonia, nitrogen oxide, nitrogen dioxide or oxygen. The spectrum is characterised by ions of the $(M + H)^+$ or $(M - H)^-$ types, or adduct ions formed from the analyte and the gas used. Fewer fragments are produced than with electron impact. A variant of this technique is used when the substance is heat-labile: the sample, applied to a filament, is very rapidly vaporised by the Joule-Thomson effect (desorption chemical ionisation).

Fast-atom bombardment (FAB) or fast-ion bombardment ionisation (liquid secondary-ion mass spectrometry LSIMS). The sample, dissolved in a viscous matrix such as glycerol, is applied to a metal surface and ionised by a beam of neutral atoms such as argon or xenon or high-kinetic-energy caesium ions. Ions of the $(M + H)^+$ or $(M - H)^-$ types or adduct ions formed from the matrix or the sample are produced. This type of ionisation, well suited to polar and heat-labile compounds, allows molecular masses of up to 10 000 Da to be obtained. The technique can be combined with liquid chromatography by adding 1 per cent to 2 per cent of glycerol to the mobile phase; however, the flow rates must be very low (a few microlitres per minute). These ionisation techniques also allow thin-layer chromatography plates to be analysed by applying a thin layer of matrix to the surface of these plates.

Field desorption and field ionisation. The sample is vaporised near a tungsten filament covered with microneedles (*field ionisation*) or applied to this filament (*field desorption*). A voltage of about 10 kV, applied between this filament and a counter-electrode, ionises the sample. These two techniques mainly produce molecular ions M^+, and $(M + H)^+$ ions and are used for low polarity and/or heat-labile compounds.

Matrix-assisted laser desorption ionisation (MALDI). The sample, in a suitable matrix and deposited on a metal support, is ionised by a pulsed laser beam whose wavelength may range from UV to IR (impulses lasting from a picosecond to a few nanoseconds). This mode of ionisation plays an essential role in the analysis of very high molecular mass compounds (more than 100 000 Da) but is limited to time-of flight analysers (see below).

Electrospray. This mode of ionisation is carried out at atmospheric pressure. The samples, in solution, are introduced into the source through a capillary tube, the end of which has a potential of the order of 5 kV. A gas can be used to facilitate nebulisation. Desolvation of the resulting microdroplets produces singly or multiply charged ions in the gas phase. The flow rates vary from a few microlitres per minute to 1 mL/min. This technique is suited to polar compounds and to the investigation of biomolecules with molecular masses of up to 100 000 Da. It can be coupled to liquid chromatography or capillary electrophoresis.

Atmospheric-pressure chemical ionisation (APCI). Ionisation is carried out at atmospheric pressure by the action of an electrode maintained at a potential of several kilovolts and placed in the path of the mobile phase, which is nebulised both by thermal effects and by the use of a stream of nitrogen. The resulting ions carry a single charge and are of the $(M + H)^+$ type in the positive mode and of the $(M - H)^-$ type in the negative mode. The high flow rates that can be used with this mode of ionisation (up to 2 mL/min) make this an ideal technique for coupling to liquid chromatography.

Thermospray. The sample, in the mobile phase consisting of water and organic modifiers and containing a volatile electrolyte (generally ammonium acetate) is introduced in nebulised form after having passed through a metal capillary tube at controlled temperature. Acceptable flow rates are of the order of 1 mL/min to 2 mL/min. The ions of the electrolyte ionise the compounds to be analysed. This ionisation process may be replaced or enhanced by an electrical discharge of about 800 volts, notably when the solvents are entirely organic. This technique is compatible with the use of liquid chromatography coupled with mass spectrometry.

ANALYSERS

Differences in the performance of analysers depend mainly on two parameters:

- the range over which m/z ratios can be measured, ie, the *mass range*,
- their *resolving power* characterised by the ability to separate two ions of equal intensity with m/z ratios differing by ΔM, and whose overlap is expressed as a given percentage of valley definition; for example, a resolving power ($M/\Delta M$) of 1000 with 10 per cent valley definition allows the separation of m/z ratios of 1000 and 1001 with the intensity returning to 10 per cent above baseline. However, the resolving power may in some cases (time-of-flight analysers, quadrupoles, ion-trap analysers) be defined as the ratio between the molecular mass and peak width at half height (50 per cent valley definition).

Magnetic and electrostatic analysers. The ions produced in the ion source are accelerated by a voltage V, and focused towards a magnetic analyser (magnetic field B) or an electrostatic analyser (electrostatic field E), depending on the configuration of the instrument. They follow a trajectory of radius r according to Laplace's law:

$$\frac{m}{z} = \frac{B^2 r^2}{2V}$$

Two types of scans can be used to collect and measure the various ions produced by the ion source: a scan of B holding V fixed or a scan of V with constant B. The magnetic analyser is usually followed by an electric sector that acts as a kinetic energy filter and allows the resolving power of the instrument to be increased appreciably. The maximum resolving power of such an instrument (double sector) ranges from 10 000 to 150 000 and in most cases allows the value of m/z ratios to be calculated accurately enough to determine the elemental composition of the corresponding ions. For monocharged ions, the mass range is from 2000 Da to 15 000 Da. Some ions may decompose spontaneously (metastable transitions) or by colliding with a gas (collision-activated dissociation (CAD)) in field-free regions between the ion source and the detector. Examination of these decompositions is very useful for the determination of the structure as well as the characterisation of a specific compound in a mixture and involves tandem mass spectrometry. There are many such techniques depending on the region where these decompositions occur:

- *daughter-ion mode* (determination of the decomposition ions of a given parent ion): B/E = constant, *MIKES (Mass-analysed Ion Kinetic Energy Spectroscopy)*,
- *parent-ion mode* (determination of all ions which by decomposition give an ion with a specific m/z ratio): B^2/E = constant,
- *neutral-loss mode* (detection of all the ions that lose the same fragment):
  $B/E(1 - E/E_0)^{1/2}$ = constant, where E_0 is the basic voltage of the electric sector.

Quadrupoles. The analyser consists of four parallel metal rods, which are cylindrical or hyperbolic in cross-section. They are arranged symmetrically with respect to the trajectory of the ions; the pairs diagonally opposed about the axis of symmetry of rods are connected electrically. The potentials to the two pairs of rods are opposed. They are the resultant of a constant component and an alternating component. The ions produced at the ion source are transmitted and separated by varying the voltages applied to the rods so that the ratio of continuous voltage to alternating voltage remains constant. The quadrupoles usually have a mass range of 1 a.m.u. to 2000 a.m.u., but some may range up to 4000 a.m.u. Although they have a lower resolving power than magnetic sector analysers, they nevertheless allow the monoisotopic profile of single charged ions to be obtained for the entire mass range. It is possible to obtain spectra using three quadrupoles arranged in series, Q_1, Q_2, Q_3 (Q_2 serves as a collision cell and is not really an analyser; the most commonly used collision gas is argon).

The most common types of scans are the following:

- *daughter-ion mode*: Q_1 selects an m/z ion whose fragments obtained by collision in Q_2 are analysed by Q_3,
- *parent-ion mode*: Q_3 filters only a specific m/z ratio, while Q_1 scans a given mass range. Only the ions decomposing to give the ion selected by Q_3 are detected,
- *neutral loss mode*: Q_1 and Q_3 scan a certain mass range but at an offset corresponding to the loss of a fragment characteristic of a product or family of compounds.

It is also possible to obtain spectra by combining quadrupole analysers with magnetic or electrostatic sector instruments; such instruments are called *hybrid mass spectrometers*.

Ion-trap analyser. The principle is the same as for a quadrupole, this time with the electric fields in three dimensions. This type of analyser allows product-ion spectra over several generations (MS^n) to be obtained.

Ion-cyclotron resonance analysers. Ions produced in a cell and subjected to a uniform, intense magnetic field move in circular orbits at frequencies which can be directly correlated to their m/z ratio by applying a Fourier transform algorithm. This phenomenon is called ion-cyclotron resonance. Analysers of this type consist of superconducting magnets and are capable of very high resolving power (up to 1000 000 and more) as well as MS^n spectra. However, very low pressures are required (of the order of 10^{-7} Pa).

Time-of-flight analysers. The ions produced at the ion source are accelerated at a voltage V of 10 kV to 20 kV. They pass through the analyser, consisting of a field-free tube, 25 cm to 1.5 m long, generally called a *flight tube*. The time (t) for an ion to travel to the detector is proportional to the square root of the m/z ratio. Theoretically the mass range of such an analyser is infinite. In practice, it is limited by the ionisation or desorption method. Time-of-flight analysers are mainly used for high molecular mass compounds (up to several hundred thousand daltons). This technique is very sensitive (a

few picomoles of product are sufficient). The accuracy of the measurements and the resolving power of such instruments may be improved considerably by using an electrostatic mirror (reflectron).

SIGNAL ACQUISITION

There are essentially three possible modes.

Complete spectrum mode. The entire signal obtained over a chosen mass range is recorded. The spectrum represents the relative intensity of the different ionic species present as a function of *m*/*z*. The results are essentially qualitative. The use of spectral reference libraries for more rapid identification is possible.

Fragmentometric mode (Selected-ion monitoring). The acquired signal is limited to one (single-ion monitoring (SIM)) or several (multiple-ion monitoring (MIM)) ions characteristic of the substance to be analysed. The limit of detection can be considerably reduced in this mode. Quantitative or semiquantitative tests can be carried out using external or internal standards (for example, deuterated standards). Such tests cannot be carried out with time-of-flight analysers.

Fragmentometric double mass spectrometry mode (multiple reaction monitoring (MRM)). The unimolecular or bimolecular decomposition of a chosen precursor ion characteristic of the substance to be analysed is followed specifically. The selectivity and the highly specific nature of this mode of acquisition provide excellent sensitivity levels and make it the most appropriate for quantitative studies using suitable internal standards (for example, deuterated standards). This type of analysis can be performed only on apparatus fitted with three quadrupoles in series, ion-trap analysers or cyclotron-resonance analysers.

CALIBRATION

Calibration allows the corresponding *m*/*z* value to be attributed to the detected signal. As a general rule, this is done using a reference substance. This calibration may be external (acquisition file separate from the analysis) or internal (the reference substance(s) are mixed with the substance to be examined and appear on the same acquisition file). The number of ions or points required for reliable calibration depends on the type of analyser and on the desired accuracy of the measurement, for example, in the case of a magnetic analyser where the *m*/*z* ratio varies exponentially with the value of the magnetic field, there should be as many points as possible.

SIGNAL DETECTION AND DATA PROCESSING

Ions separated by an analyser are converted into electric signals by a detection system such as a photomultiplier or an electron multiplier. These signals are amplified before being re-converted into digital signals for data processing, allowing various functions such as calibration, reconstruction of spectra, automatic quantification, archiving, creation or use of libraries of mass spectra. The various physical parameters required for the functioning of the apparatus as a whole are controlled by computer.

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2.2.46. CHROMATOGRAPHIC SEPARATION TECHNIQUES

Chromatographic separation techniques are multi-stage separation methods in which the components of a sample are distributed between 2 phases, one of which is stationary, while the other is mobile. The stationary phase may be a solid or a liquid supported on a solid or a gel. The stationary phase may be packed in a column, spread as a layer, or distributed as a film, etc. The mobile phase may be gaseous or liquid or supercritical fluid. The separation may be based on adsorption, mass distribution (partition), ion-exchange, etc., or may be based on differences in the physico-chemical properties of the molecules such as size, mass, volume, etc.

This chapter contains definitions and calculations of common parameters and generally applicable requirements for system suitability. Principles of separation, apparatus and methods are given in the following general methods:

- paper chromatography (*2.2.26*);
- thin-layer chromatography (*2.2.27*);
- gas chromatography (*2.2.28*);
- liquid chromatography (*2.2.29*);
- size-exclusion chromatography (*2.2.30*);
- supercritical fluid chromatography (*2.2.45*).

DEFINITIONS

The system suitability and acceptance criteria in monographs have been set using parameters as defined below. With some equipment, certain parameters, such as the signal-to-noise ratio and resolution, can be calculated using software provided by the manufacturer. It is the responsibility of the user to ensure that the calculation methods used in the software are equivalent to the requirements of the European Pharmacopoeia and to make any necessary corrections if this is not the case.

Chromatogram

A graphical or other representation of detector response, effluent concentration or other quantity used as a measure of effluent concentration, versus time or volume. Idealised chromatograms are represented as a sequence of Gaussian peaks on a baseline (Figure 2.2.46.-1).

Peak

The portion of a chromatogram recording the detector response when a single component (or 2 or more unresolved components) is eluted from the column.

The peak may be defined by the peak area, or the peak height (h) and the peak width at half-height (w_h), or the peak height (h) and the peak width between the points of inflection (w_i). In Gaussian peaks (Figure 2.2.46.-1) there is the following relationship:

$$w_h = 1.18 w_i$$

Retention time (t_R)

Time required for elution of a component (Figure 2.2.46.-1, baseline scale being in minutes).

Retention volume (V_R)

Volume of the mobile phase required for elution of a component. It may be calculated from the retention time and the flow rate (F) in millilitres per minute using the following equation:

$$V_R = t_R \times F$$

Hold-up time (t_M)

Time required for elution of an unretained component (Figure 2.2.46.-1, baseline scale being in minutes). In size-exclusion chromatography, the symbol t_0 (see below) is used.

Hold-up volume (V_M)

Volume of the mobile phase required for elution of an unretained component. It may be calculated from the hold-up time and the flow rate (F) in millilitres per minute using the following equation:

$$V_M = t_M \times F$$

In size-exclusion chromatography, the symbol V_0 (see below) is used.

Retention factor (k)

The retention factor (also known as mass distribution ratio (D_m) or capacity factor (k')) is defined as:

$$k = \frac{\text{amount of component in stationary phase}}{\text{amount of component in mobile phase}} = K_C \frac{V_s}{V_M}$$

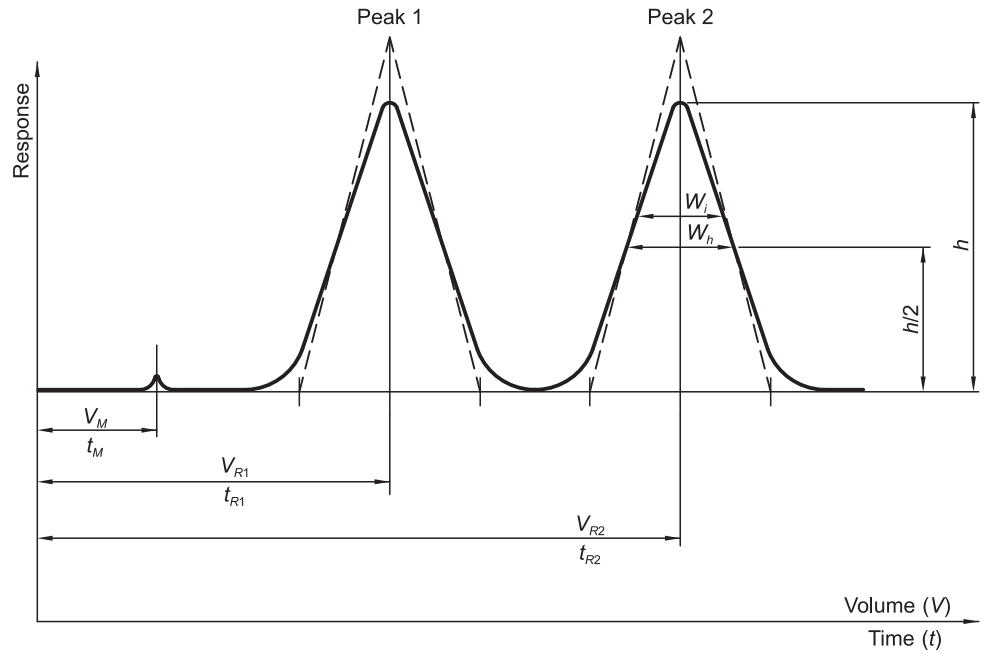


Figure 2.2.46.-1.

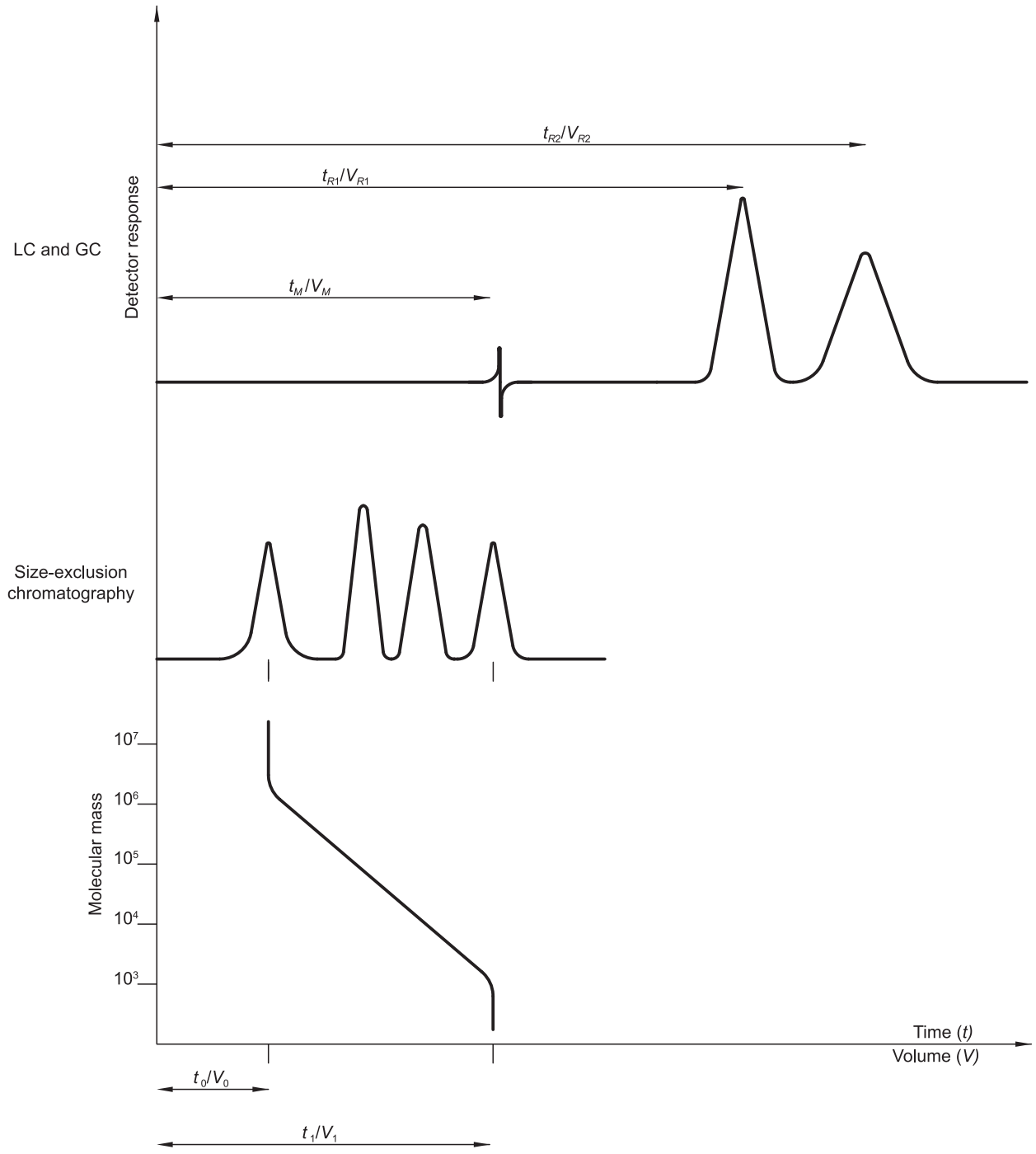


Figure 2.2.46.-2.

K_C = distribution constant (also known as equilibrium distribution coefficient);

V_S = volume of the stationary phase;

V_M = volume of the mobile phase.

The retention factor of a component may be determined from the chromatogram using the following equation:

$$k = \frac{t_R - t_M}{t_M}$$

Total mobile phase time (t_t)

In size-exclusion chromatography, retention time of a component whose molecules are smaller than the smallest gel pores (Figure 2.2.46.-2).

Total mobile phase volume (V_t)

In size-exclusion chromatography, retention volume of a component whose molecules are smaller than the smallest gel pores. It may be calculated from the total mobile phase time and the flow rate (F) in millilitres per minute using the following equation:

$$V_t = t_t \times F$$

Retention time of an unretained compound (t_0)

In size-exclusion chromatography, retention time of a component whose molecules are larger than the largest gel pores (Figure 2.2.46.-2).

Retention volume of an unretained compound (V_0)

In size-exclusion chromatography, retention volume of a component whose molecules are larger than the largest gel pores. It may be calculated from the retention time of an unretained compound and the flow rate (F) in millilitres per minute using the following equation:

$$V_0 = t_0 \times F$$

Distribution constant (K_0)

In size-exclusion chromatography, the elution characteristics of a component in a particular column may be given by the distribution constant (also referred to as distribution coefficient), which is calculated using the following equation:

$$K_0 = \frac{t_R - t_0}{t_t - t_0}$$

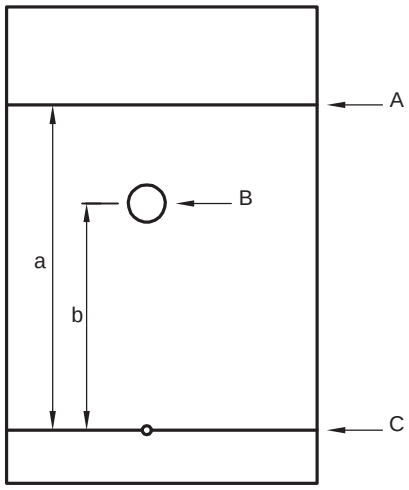
Retardation factor (R_F)

The retardation factor (also known as retention factor (R_f)), used in planar chromatography, is the ratio of the distance from the point of application to the centre of the spot and the distance travelled by the solvent front from the point of application (Figure 2.2.46.-3).

$$R_F = \frac{b}{a}$$

b = migration distance of the component;

a = migration distance of the solvent front.



A. mobile phase front B. spot C. line of application

Figure 2.2.46.-3.

Plate number (N)

The column performance (apparent efficiency) may be calculated from data obtained under either isothermal, isocratic or isodense conditions, depending on the technique, as the plate number (also referred to as number of theoretical plates), using the following equation, the values of t_R and w_h being expressed in the same units:

$$N = 5.54\left(\frac{t_R}{w_h}\right)^2$$

t_R = retention time of the peak corresponding to the component;

w_h = width of the peak at half-height.

The plate number varies with the component as well as with the column, the column temperature, the mobile phase and the retention time.

Dwell volume (D)

The dwell volume (also known as gradient delay volume) is the volume between the point at which the eluents meet and the top of the column. It can be determined using the following procedure.

Column: replace the chromatographic column by an appropriate capillary tubing (e.g. 1 m × 0.12 mm).

Mobile phase:

- *mobile phase A*: *water R*;
- *mobile phase B*: 0.1 per cent *V/V* solution of *acetone R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 20	100 → 0	0 → 100
20 - 30	0	100

Flow rate: set to obtain sufficient back-pressure (e.g. 2 mL/min).

Detection: spectrophotometer at 265 nm.

Determine the time ($t_{0.5}$) in minutes when the absorbance has increased by 50 per cent (Figure 2.2.46.-4).

$$D = t_D \times F$$

t_D = $t_{0.5} - 0.5t_G$ (in minutes);

t_G = pre-defined gradient time (= 20 min);

F = flow rate (in millilitres per minute).

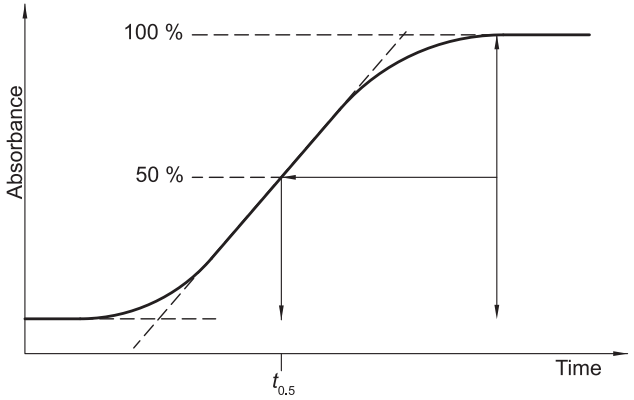


Figure 2.2.46.-4

Symmetry factor (A_s)

The symmetry factor of a peak (Figure 2.2.46.-5) is calculated using the following equation:

$$A_s = \frac{w_{0.05}}{2d}$$

$w_{0.05}$ = width of the peak at one-twentieth of the peak height;

d = distance between the perpendicular dropped from the peak maximum and the leading edge of the peak at one-twentieth of the peak height.

An A_s value of 1.0 signifies symmetry. When $A_s > 1.0$, the peak is tailing. When $A_s < 1.0$, the peak is fronting.

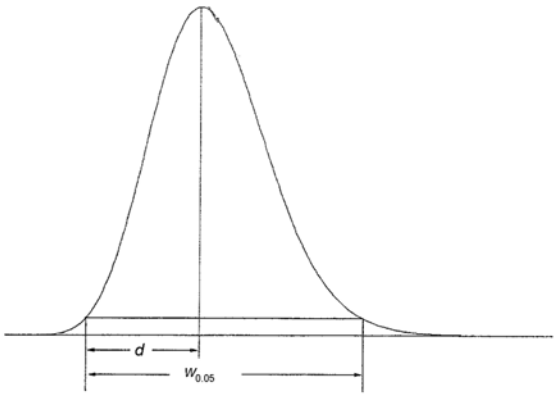


Figure 2.2.46.-5

Resolution (R_s)

The resolution between peaks of 2 components (Figure 2.2.46.-1) may be calculated using the following equation:

$$R_s = \frac{1.18(t_{R2} - t_{R1})}{w_{h1} + w_{h2}}$$

$t_{R2} > t_{R1}$

t_{R1}, t_{R2} = retention times of the peaks;

w_{h1}, w_{h2} = peak widths at half-height.

In quantitative planar chromatography, using densitometry, the migration distances are used instead of retention times and the resolution between peaks of 2 components may be calculated using the following equation:

$$R_s = \frac{1.18a(R_{F2} - R_{F1})}{w_{h1} + w_{h2}}$$

R_{F1}, R_{F2} = retardation factors of the peaks;

w_{h1}, w_{h2} = peak widths at half-height;

a = migration distance of the solvent front.

Peak-to-valley ratio (*p/v*)

The peak-to-valley ratio may be employed as a system suitability criterion in a test for related substances when baseline separation between 2 peaks is not achieved (Figure 2.2.46.-6).

$$p / v = \frac{H_p}{H_v}$$

H_p = height above the extrapolated baseline of the minor peak;

H_v = height above the extrapolated baseline at the lowest point of the curve separating the minor and major peaks.

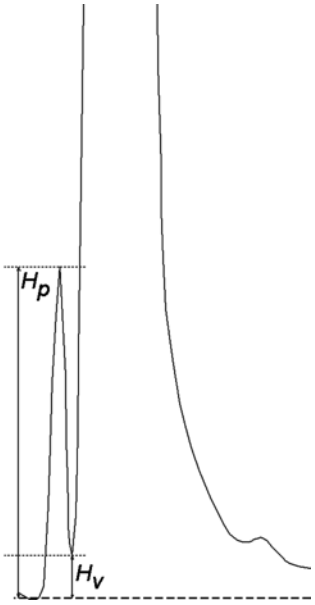


Figure 2.2.46.-6

Relative retention (*r*)

Relative retention is calculated as an estimate using the following equation:

$$r = \frac{t_{Ri} - t_M}{t_{Rst} - t_M}$$

t_{Ri} = retention time of the peak of interest;

t_{Rst} = retention time of the reference peak (usually the peak corresponding to the substance to be examined);

t_M = hold-up time.

The unadjusted relative retention (r_G) is calculated using the following equation:

$$r_G = \frac{t_{Ri}}{t_{Rst}}$$

Unless otherwise indicated, values for relative retention stated in monographs correspond to unadjusted relative retention.

In planar chromatography, the retardation factors R_{Fst} and R_{Fi} are used instead of t_{Rst} and t_{Ri}.

Signal-to-noise ratio (*S/N*)

The short-term noise influences the precision of quantification. The signal-to-noise ratio is calculated using the following equation:

$$S / N = \frac{2H}{h}$$

H = height of the peak (Figure 2.2.46.-7) corresponding to the component concerned, in the chromatogram obtained with the prescribed reference solution, measured from the maximum of the peak to the extrapolated baseline of the signal observed over a distance equal to at least 5 times the width at half-height;

h = range of the noise in a chromatogram obtained after injection or application of a blank, observed over a distance equal to at least 5 times the width at half-height of the peak in the chromatogram obtained with the prescribed reference solution and, if possible, situated equally around the place where this peak would be found.

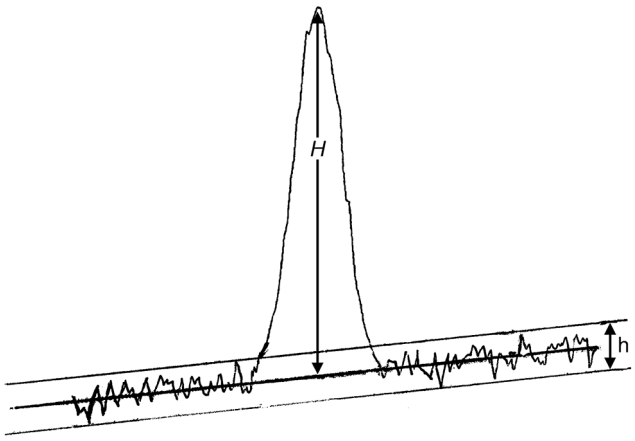


Figure 2.2.46.-7.

System repeatability

The repeatability of response is expressed as an estimated percentage relative standard deviation (s_r(%)) of a consecutive series of measurements for not fewer than 3 injections or applications of a reference solution, and is calculated using the following equation:

$$s_r(\%) = \frac{100}{\overline{y}} \sqrt{\frac{\sum (y_i - \overline{y})^2}{n - 1}}$$

y_i = individual values expressed as peak area, peak height, or ratio of areas by the internal standardisation method;

$\overline{y}$ = mean of individual values;

n = number of individual values.

SYSTEM SUITABILITY

The various components of the equipment employed must be qualified and be capable of achieving the performance required to conduct the test or assay.

The system suitability tests represent an integral part of the method and are used to ensure adequate performance of the chromatographic system. Apparent efficiency, retention factor (mass distribution ratio), resolution and symmetry factor are the parameters that are usually employed in assessing the performance of the column. Factors that may affect the chromatographic behaviour include:

- the composition, ionic strength, temperature and apparent pH of the mobile phase;
- flow rate, column dimensions, column temperature and pressure;
- stationary phase characteristics including type of chromatographic support (particle-based or monolithic), particle or macropore size, porosity, specific surface area;
- reversed-phase and other surface-modification of the stationary phases, the extent of chemical modification (as expressed by end-capping, carbon loading etc.).

The following requirements and any supplementary requirements given in the individual monograph are to be fulfilled unless otherwise prescribed:

- in a related substances test or assay, for a peak in the chromatogram obtained with a reference solution used for quantification, the symmetry factor is 0.8 to 1.5, unless otherwise prescribed;
- in an assay of an active substance where the value is 100 per cent for a pure substance, the maximum permitted relative standard deviation ($s_r(\%)_{max}$) for the defined limits is calculated for a series of injections of the reference solution using the following equation:

$$s_r(\%)_{max} = \frac{KB\sqrt{n}}{t_{90\%,n-1}}$$

K = constant (0.349), obtained from the expression $K = \frac{0.6}{\sqrt{2}} \times \frac{t_{90\%,5}}{\sqrt{6}}$ in which $\frac{0.6}{\sqrt{2}}$ represents the required percentage relative standard deviation after 6 injections for $B = 1.0$;

B = upper limit given in the definition of the individual monograph minus 100 per cent;

n = number of replicate injections of the reference solution ($3 \leq n \leq 6$);

$t_{90\%,n-1}$ = Student's t at the 90 per cent probability level (double sided) with $n-1$ degrees of freedom.

Unless otherwise prescribed, the maximum permitted relative standard deviation does not exceed the appropriate value given in Table 2.2.46.-1. This requirement does not apply to tests for related substances.

Table 2.2.46.-1. – *Repeatability requirements*

	Number of individual injections			
	3	4	5	6
***B* (per cent)**	**Maximum permitted relative standard deviation**			
2.0	0.41	0.59	0.73	0.85
2.5	0.52	0.74	0.92	1.06
3.0	0.62	0.89	1.10	1.27

- in a related substances test, the limit of quantification (corresponding to a signal-to-noise ratio of 10) is equal to or less than the disregard limit.

Compliance with the system suitability criteria is required throughout the chromatographic procedure. Depending on various factors, such as the frequency of use of the procedure and experience with the chromatographic system, the analyst chooses an appropriate verification scheme to monitor this.

ADJUSTMENT OF CHROMATOGRAPHIC CONDITIONS

The extent to which the various parameters of a chromatographic test may be adjusted to satisfy the system suitability criteria without fundamentally modifying the methods are listed below. Adjustment of conditions with gradient elutions is more critical than with isocratic elutions, since it may lead to shifts in peaks to a different step of the gradient, thus leading to the incorrect assignment of peaks, and to the masking of peaks or a shift such that elution occurs beyond the prescribed elution time. Changes other than those indicated require revalidation of the method. The chromatographic conditions described have been validated during the elaboration of the monograph.

The system suitability tests are included to verify that the separation required for satisfactory performance of the test or assay is achieved. Nonetheless, since the stationary phases are described in a general way and there is such a variety available commercially, with differences in chromatographic behaviour, some adjustments of the chromatographic conditions may be necessary to achieve the prescribed system suitability requirements. With reversed-phase liquid chromatographic methods in particular, adjustment of the various parameters will not always result in satisfactory chromatography. In that case, it may be necessary to replace the column with another of the same type (e.g. octadecylsilyl silica gel), which exhibits the desired chromatographic behaviour. The Knowledge database on the EDQM website usually contains information on the column(s) used during monograph elaboration.

For critical parameters the adjustments are defined clearly in the monograph to ensure the system suitability.

Thin-layer chromatography and paper chromatography

Composition of the mobile phase: the amount of the minor solvent component may be adjusted by ± 30 per cent relative or ± 2 per cent absolute, whichever is the larger; for a minor component at 10 per cent of the mobile phase, a 30 per cent relative adjustment allows a range of 7-13 per cent whereas a 2 per cent absolute adjustment allows a range of 8-12 per cent, the relative value therefore being the larger; for a minor component at 5 per cent of the mobile phase, a 30 per cent relative adjustment allows a range of 3.5-6.5 per cent whereas a 2 per cent absolute adjustment allows a range of 3-7 per cent, the absolute value being the larger in this case; no other component is altered by more than 10 per cent absolute.

pH of the aqueous component of the mobile phase: ± 0.2 pH, unless otherwise prescribed, or ± 1.0 pH when non-ionisable substances are to be examined.

Concentration of salts in the buffer component of a mobile phase: ± 10 per cent.

Application volume: 10-20 per cent of the prescribed volume if using fine particle size plates (2-10 µm).

Liquid chromatography: isocratic elution

Composition of the mobile phase: the amount of the minor solvent component may be adjusted by ± 30 per cent relative or ± 2 per cent absolute, whichever is the larger (see example above); no other component is altered by more than 10 per cent absolute.

pH of the aqueous component of the mobile phase: ± 0.2 pH, unless otherwise prescribed, or ± 1.0 pH when non-ionisable substances are to be examined.

Concentration of salts in the buffer component of a mobile phase: ± 10 per cent.

Flow rate: ± 50 per cent; a larger adjustment is acceptable when changing the column dimensions (see the formula below).

Column parameters

Stationary phase:

– no change of the identity of the substituent of the stationary phase permitted (e.g. no replacement of C18 by C8);

– *particle size*: maximum reduction of 50 per cent; no increase permitted.

Column dimensions:

– *length*: ± 70 per cent;

– *internal diameter*: ± 25 per cent.

When column dimensions are changed, the flow rate may be adjusted as necessary using the following equation:

$$F_2 = F_1 \frac{l_2 d_2^2}{l_1 d_1^2}$$

F_1 = flow rate indicated in the monograph, in millilitres per minute;

F_2 = adjusted flow rate, in millilitres per minute;

l_1 = length of the column indicated in the monograph, in millimetres;

l_2 = length of the column used, in millimetres;

d_1 = internal diameter of the column indicated in the monograph, in millimetres;

d_2 = internal diameter of the column used, in millimetres.

Temperature: ± 10 °C, where the operating temperature is specified, unless otherwise prescribed.

Detector wavelength: no adjustment permitted.

Injection volume: may be decreased, provided detection and repeatability of the peak(s) to be determined are satisfactory; no increase permitted.

Liquid chromatography: gradient elution

Adjustment of chromatographic conditions for gradient systems requires greater caution than for isocratic systems.

Composition of the mobile phase/gradient elution: minor adjustments of the composition of the mobile phase and the gradient are acceptable provided that:

– the system suitability requirements are fulfilled;

– the principal peak(s) elute(s) within ± 15 per cent of the indicated retention time(s);

– the final composition of the mobile phase is not weaker in elution power than the prescribed composition.

Where compliance with the system suitability requirements cannot be achieved, it is often preferable to consider the dwell volume or to change the column.

Dwell volume. The configuration of the equipment employed may significantly alter the resolution, retention time and relative retentions described. Should this occur, it may be due to excessive dwell volume. Monographs preferably include an isocratic step before the start of the gradient programme so that an adaptation can be made to the gradient time points to take account of differences in dwell volume between the system used for method development and that actually used. It is the user's responsibility to adapt the length of the isocratic step to the analytical equipment used. If the dwell volume used during the elaboration of the monograph is given in the monograph, the time points (t min) stated in the gradient table may be replaced by adapted time points (t_c min), calculated using the following equation:

$$t_c = t - \frac{(D - D_0)}{F}$$

D = dwell volume, in millilitres;

D_0 = dwell volume used for development of the method, in millilitres;

F = flow rate, in millilitres per minute.

The isocratic step introduced for this purpose may be omitted if validation data for application of the method without this step is available.

pH of the aqueous component of the mobile phase: no adjustment permitted.

Concentration of salts in the buffer component of a mobile phase: no adjustment permitted.

Flow rate: adjustment is acceptable when changing the column dimensions (see the formula below).

Column parameters

Stationary phase:

– no change of the identity of the substituent of the stationary phase permitted (e.g. no replacement of C18 by C8);

– *particle size*: no adjustment permitted.

Column dimensions:

– *length*: ± 70 per cent;

– *internal diameter*: ± 25 per cent.

When column dimensions are changed, the flow rate may be adjusted as necessary using the following equation:

$$F_2 = F_1 \frac{l_2 d_2^2}{l_1 d_1^2}$$

F_1 = flow rate indicated in the monograph, in millilitres per minute;

F_2 = adjusted flow rate, in millilitres per minute;

l_1 = length of the column indicated in the monograph, in millimetres;

l_2 = length of the column used, in millimetres;

d_1 = internal diameter of the column indicated in the monograph, in millimetres;

d_2 = internal diameter of the column used, in millimetres.

Temperature: ± 5 °C, where the operating temperature is specified, unless otherwise prescribed.

Detector wavelength: no adjustment permitted.

Injection volume: may be decreased, provided detection and repeatability of the peak(s) to be determined are satisfactory; no increase permitted.

Gas chromatography

Column parameters

Stationary phase:

– *particle size*: maximum reduction of 50 per cent; no increase permitted (packed columns);

– *film thickness*: – 50 per cent to + 100 per cent (capillary columns).

Column dimensions:

– *length*: ± 70 per cent;

– *internal diameter*: ± 50 per cent.

Flow rate: ± 50 per cent.

Temperature: ± 10 per cent.

Injection volume and split volume: may be adjusted, provided detection and repeatability are satisfactory.

Supercritical fluid chromatography

Composition of the mobile phase: for packed columns, the amount of the minor solvent component may be adjusted by ± 30 per cent relative or ± 2 per cent absolute, whichever is the larger; no adjustment is permitted for a capillary column system.

Detector wavelength: no adjustment permitted.

Column parameters

Stationary phase:

– *particle size*: maximum reduction of 50 per cent; no increase permitted (packed columns).

Column dimensions:

– *length*: ± 70 per cent;

– *internal diameter*:

± 25 per cent (packed columns);

± 50 per cent (capillary columns).

Flow rate: ± 50 per cent.

Temperature: ± 5 °C, where the operating temperature is specified.

Injection volume: may be decreased, provided detection and repeatability are satisfactory; no increase permitted.

QUANTIFICATION

Peaks due to solvents and reagents or arising from the mobile phase or the sample matrix are disregarded during quantification.

– *Detector sensitivity*. The detector sensitivity is the signal output per unit concentration or unit mass of a substance in the mobile phase entering the detector. The relative detector response factor, commonly referred to as *response factor*, expresses the sensitivity of a detector for a given substance relative to a standard substance. The *correction factor* is the reciprocal of the response factor.

– *External standard method*. The concentration of the component(s) to be analysed is determined by comparing the response(s) (peak(s)) obtained with the test solution to the response(s) (peak(s)) obtained with a reference solution.

– *Internal standard method*. Equal amounts of a component that will be resolved from the substance to be examined (the internal standard) are introduced into the test solution and a reference solution. The internal standard is chosen such that it does not react with the substance to be examined, is stable and does not contain impurities with the same retention time as that of the substance to be examined. The concentration of the substance to be examined is determined by comparing the ratio of the peak areas or peak heights due to the substance to be examined and the internal standard in the test solution with the ratio of the peak areas or peak heights due to the substance to be examined and the internal standard in the reference solution.

– *Normalisation procedure*. The percentage content of a component of the substance to be examined is calculated by determining the area of the corresponding peak as a percentage of the total area of all the peaks, excluding those due to solvents or reagents or arising from the mobile phase or the sample matrix, and those at or below the disregard limit.

– *Calibration procedure*. The relationship between the measured or evaluated signal (y) and the quantity (concentration, mass, etc.) of substance (x) is determined and the calibration function is calculated. The analytical results are calculated from the measured signal or evaluated signal of the analyte by means of the inverse function.

In tests for related substances for both the external standard method, when a dilution of the test solution is used for comparison, and the normalisation procedure, any correction factors indicated in the monograph are applied (i.e. when the response factor is outside the range 0.8-1.2).

When the related substances test prescribes the total of impurities or there is a quantitative determination of an impurity, it is important to choose an appropriate threshold setting and appropriate conditions for the integration of the peak areas. In such tests the *disregard limit*, i.e. the limit at or below which a peak is disregarded, is generally 0.05 per cent. Integration of the peak area of any impurity that is not completely separated from the principal peak is preferably performed by valley-to-valley extrapolation (tangential skim).

01/2013:20265

2.2.65. VOLTAMETRIC TITRATION

In voltametric titration the end-point of the titration is determined by following the variation of the voltage measured between 2 electrodes (either 1 indicator electrode and 1 reference electrode or 2 indicator electrodes) immersed in the solution to be examined and maintained at a constant current as a function of the quantity of titrant added.

Apparatus. The apparatus comprises an adjustable current source and a voltmeter; the detection system generally consists of an indicator electrode (for example, a platinum electrode, a rotating-disc electrode or a carbon electrode) and a 2nd electrode (for example, a platinum electrode, a rotating-disc electrode or a carbon electrode).

Method. Set the current to the indicator electrode as prescribed in the monograph and plot a graph of the initial voltage and the values obtained during the titration as functions of the quantity of titrant added. Add the titrant in not fewer than 3 successive quantities equal to a total of about 80 per cent of the theoretical volume corresponding to the presumed equivalence point. The 3 values must fall on a straight line. Continue adding the titrant beyond the presumed equivalence point in not fewer than 3 successive quantities. The values obtained must fall on another straight line. The point of intersection of the 2 lines represents the end-point of the titration.

Using titration systems for voltametric titration with 2 indicator electrodes, the whole titration curve is recorded and used to determine the end-point.

04/2010:20414

2.4.14. SULFATED ASH[(1)]

Ignite a suitable crucible (for example, silica, platinum, porcelain or quartz) at 600 ± 50 °C for 30 min, allow to cool in a desiccator over silica gel or other suitable desiccant and weigh. Place the prescribed amount of the substance to be examined in the crucible and weigh. Moisten the substance to be examined with a small amount of *sulfuric acid R* (usually 1 mL) and heat gently at as low a temperature as practicable until the sample is thoroughly charred. After cooling, moisten the residue with a small amount of *sulfuric acid R* (usually 1 mL), heat gently until white fumes are no longer evolved and ignite at 600 ± 50 °C until the residue is completely incinerated. Ensure that flames are not produced at any time during the procedure. Allow the crucible to cool in a desiccator over silica gel or other suitable desiccant, weigh it again and calculate the percentage of residue.

If the amount of the residue so obtained exceeds the prescribed limit, repeat the moistening with *sulfuric acid R* and ignition, as previously, for 30 min periods until 2 consecutive weighings do not differ by more than 0.5 mg or until the percentage of residue complies with the prescribed limit.

The amount of substance used for the test (usually 1-2 g) is chosen so that at the prescribed limit the mass of the residue (usually about 1 mg) can be measured with sufficient accuracy.

(1) This chapter has undergone pharmacopoeial harmonisation. See chapter *5.8. Pharmacopoeial harmonisation.*

04/2020:20424

2.4.24. IDENTIFICATION AND CONTROL OF RESIDUAL SOLVENTS

The test procedures described in this general method may be used:

i. for the identification of the majority of Class 1 and Class 2 residual solvents in an active substance, excipient or medicinal product when the residual solvents are unknown;

ii. as a limit test for Class 1 and Class 2 solvents when present in an active substance, excipient or medicinal product;

iii. for the quantification of Class 2 solvents when the limits are greater than 1000 ppm (0.1 per cent) or for the quantification of Class 3 solvents when required.

Class 1, Class 2 and Class 3 residual solvents are listed in general chapter *5.4. Residual solvents*.

Three diluents are described for sample preparation and the conditions to be applied for head-space injection of the gaseous sample onto the chromatographic system. Two chromatographic systems are prescribed but System A is preferred whilst System B is employed normally for confirmation of identity. The choice of sample preparation procedure depends on the solubility of the substance to be examined and in certain cases the residual solvents to be controlled.

The following residual solvents are not readily detected by the head-space injection conditions described: formamide, 2-ethoxyethanol, 2-methoxyethanol, ethylene glycol, *N*-methylpyrrolidone and sulfolane. Other appropriate procedures should be employed for the control of these residual solvents.

When the test procedure is applied quantitatively to control residual solvents in a substance, then it must be validated.

PROCEDURE

Examine by gas chromatography with static head-space injection (*2.2.28*).

Sample preparation 1. This is intended for the control of residual solvents in water-soluble substances.

Sample solution (1). Dissolve 0.200 g of the substance to be examined in *water R* and dilute to 20.0 mL with the same solvent.

Sample preparation 2. This is intended for the control of residual solvents in water-insoluble substances.

Sample solution (2). Dissolve 0.200 g of the substance to be examined in *dimethylformamide R* (DMF) and dilute to 20.0 mL with the same solvent.

Sample preparation 3. This is intended for the control of *N,N*-dimethylacetamide and/or *N,N*-dimethylformamide, when it is known or suspected that one or both of these substances are present in the substance to be examined.

Sample solution (3). Dissolve 0.200 g of the substance to be examined in *1,3-dimethyl-2-imidazolidinone R* (DMI) and dilute to 20.0 mL with the same solvent.

In some cases none of the above sample preparation procedures are appropriate, in which case the diluent to be used for the preparation of the sample solution and the static head-space conditions to be employed must be demonstrated to be suitable.

Solvent solution (a). To 1.0 mL of *Class 1 residual solvent solution CRS*, add 9 mL of *dimethyl sulfoxide R* and dilute to 100.0 mL with *water R*. Dilute 1.0 mL of this solution to 100 mL with *water R*. Dilute 1.0 mL of this solution to 10.0 mL with *water R*.

The reference solutions correspond to the following limits:
- benzene: 2 ppm;
- carbon tetrachloride: 4 ppm;
- 1,2-dichloroethane: 5 ppm;
- 1,1-dichloroethene: 8 ppm;
- 1,1,1-trichloroethane: 10 ppm.

Solvent solution (b). Dissolve appropriate quantities of the Class 2 residual solvents in *dimethyl sulfoxide R* and dilute to 100.0 mL with the same solvent. Dilute in *water R* to give a concentration of 1/20 of the limits stated in Table 2 (see *5.4. Residual solvents*).

Solvent solution (c). Dissolve 1.00 g of the solvent or solvents present in the substance to be examined in *dimethyl sulfoxide R* or *water R*, if appropriate, and dilute to 100.0 mL with *water R*. Dilute to give a concentration of 1/20 of the limit(s) stated in Table 1 or 2 (see *5.4. Residual solvents*).

Blank solution. Prepare as described for solvent solution (c) but without the addition of solvent(s) (used to verify the absence of interfering peaks).

Test solution. Introduce 5.0 mL of the sample solution and 1.0 mL of the blank solution into an injection vial.

Reference solution (a) (Class 1). Introduce 1.0 mL of solvent solution (a) and 5.0 mL of the appropriate diluent into an injection vial.

Reference solution (a_1) (Class 1). Introduce 5.0 mL of the sample solution and 1.0 mL of solvent solution (a) into an injection vial.

Reference solution (b) (Class 2). Introduce 1.0 mL of solvent solution (b) and 5.0 mL of the appropriate diluent into an injection vial.

Reference solution (c). Introduce 5.0 mL of the sample solution and 1.0 mL of solvent solution (c) into an injection vial.

Reference solution (d). Introduce 1.0 mL of the blank solution and 5.0 mL of the appropriate diluent into an injection vial.

Close the vials with a tight rubber membrane stopper coated with polytetrafluoroethylene and secure with an aluminium crimp cap. Shake to obtain a homogeneous solution.

The following static head-space injection conditions may be used:

	Sample preparation procedure		
Operating parameters	**1**	**2**	**3**
Equilibration temperature (°C)	80	105	80
Equilibration time (min)	60	45	45
Transfer-line temperature (°C)	85	110	105
Carrier gas: *nitrogen for chromatography R* or *helium for chromatography R* at an appropriate pressure			
Pressurisation time (s)	30	30	30
Injection volume (mL)	1	1	1

The chromatographic procedure may be carried out using:

SYSTEM A

– a fused-silica capillary or wide-bore column 30 m long and 0.32 mm or 0.53 mm in internal diameter coated with *cyanopropyl(3)phenyl(3)methyl(94)polysiloxane R* (film thickness 1.8 µm or 3 µm);

– *nitrogen for chromatography R* or *helium for chromatography R* as the carrier gas, split ratio 1:5 with a linear velocity of about 35 cm/s;

– a flame-ionisation detector (a mass spectrometer may also be used or an electron-capture detector for the chlorinated residual solvents of Class 1);

maintaining the temperature of the column at 40 °C for 20 min, then raising the temperature at a rate of 10 °C per min to 240 °C and maintaining it at 240 °C for 20 min and maintaining the temperature of the injection port at 140 °C and that of the detector at 250 °C; or, where there is interference from the matrix, use:

SYSTEM B

– a fused-silica capillary or wide-bore column 30 m long and 0.32 mm or 0.53 mm in internal diameter coated with *macrogol 20 000 R* (film thickness 0.25 µm);

– *nitrogen for chromatography R* or *helium for chromatography R* as the carrier gas, split ratio 1:5 with a linear velocity of about 35 cm/s;

– a flame-ionisation detector (a mass spectrophotometer may also be used or an electron-capture detector for the chlorinated residual solvents of Class 1);

maintaining the temperature of the column at 50 °C for 20 min, then raising the temperature at a rate of 6 °C per min to 165 °C and maintaining it at 165 °C for 20 min and maintaining the temperature of the injection port at 140 °C and that of the detector at 250 °C.

Inject 1 mL of the gaseous phase of reference solution (a) onto the column described in System A and record the chromatogram under such conditions that the signal-to-noise ratio for 1,1,1-trichloroethane can be measured. The signal-to-noise ratio must be at least 5. A typical chromatogram is shown in Figure 2.4.24.-1.

Inject 1 mL of the gaseous phase of reference solution (a_1) onto the column described in System A. The peaks due to the Class 1 residual solvents are still detectable.

Inject 1 mL of the gaseous phase of reference solution (b) onto the column described in System A and record the chromatogram under such conditions that the resolution between acetonitrile and dichloromethane can be determined. The system is suitable if the chromatogram obtained resembles the chromatogram shown in Figure 2.4.24.-2 and the resolution between acetonitrile and dichloromethane is at least 1.0.

Inject 1 mL of the gaseous phase of the test solution onto the column described in System A. If in the chromatogram obtained, there is no peak which corresponds to one of the residual solvent peaks in the chromatograms obtained with reference solution (a) or (b), then the substance to be examined meets the requirements of the test. If any peak in the chromatogram obtained with the test solution corresponds to any of the residual solvent peaks obtained with reference solution (a) or (b) then System B is to be employed.

Inject 1 mL of the gaseous phase of reference solution (a) onto the column described in System B and record the chromatogram under such conditions that the signal-to-noise ratio for benzene can be measured. The signal-to-noise ratio must be at least 5. A typical chromatogram is shown in Figure 2.4.24.-3.

Inject 1 mL of the gaseous phase of reference solution (a_1) onto the column described in System B. The peaks due to the Class 1 residual solvents are still detectable.

Inject 1 mL of the gaseous phase of reference solution (b) onto the column described in System B and record the chromatogram under such conditions that the resolution between acetonitrile and 1,1,2-trichloroethene can be determined. The system is suitable if the chromatogram obtained resembles the chromatogram shown in Figure 2.4.24.-4 and the resolution between acetonitrile and 1,1,2-trichloroethene is at least 1.0.

Inject 1 mL of the gaseous phase of the test solution onto the column described in System B. If in the chromatogram obtained, there is no peak which corresponds to any of the residual solvent peaks in the chromatogram obtained with the reference solution (a) or (b), then the substance to be examined meets the requirements of the test. If any peak in the chromatogram obtained with the test solution corresponds to any of the residual solvent peaks obtained with reference solution (a) or (b) and confirms the correspondence obtained when using System A, then proceed as follows.

Inject 1 mL of the gaseous phase of reference solution (c) onto the column described for System A or System B. If necessary, adjust the sensitivity of the system so that the height of the peak corresponding to the identified residual solvent(s) is at least 50 per cent of the full scale of the recorder.

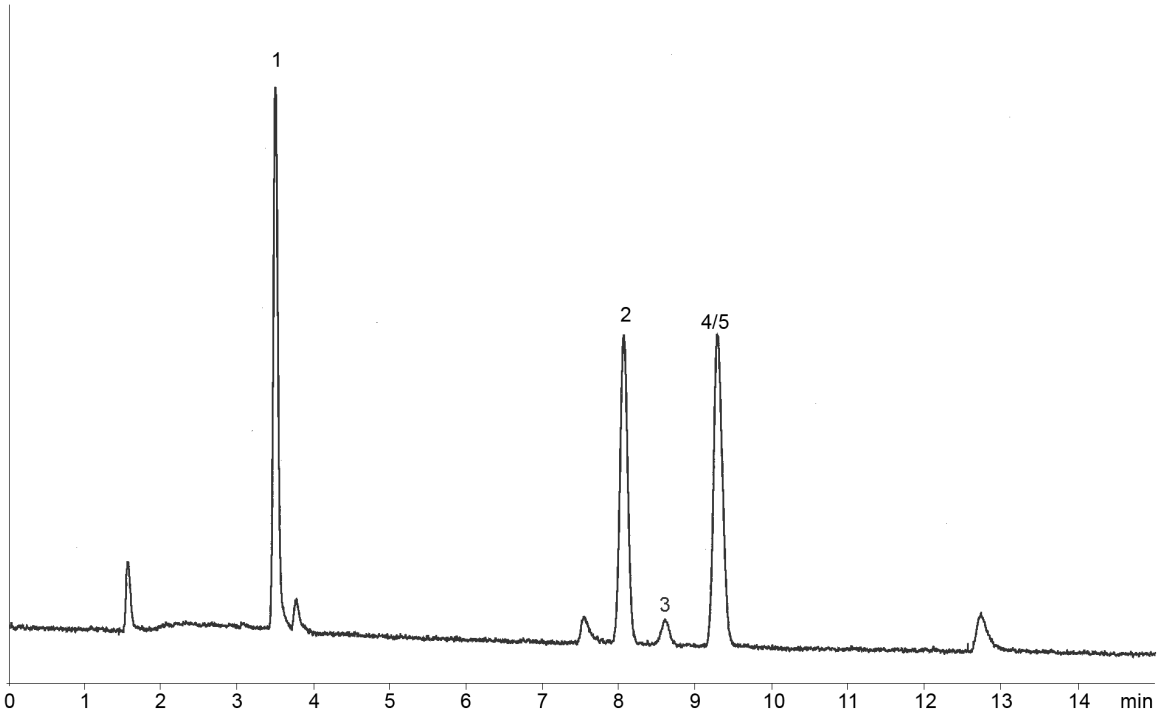
Inject 1 mL of the gaseous phase of reference solution (d) onto the column. No interfering peaks should be observed.

Inject 1 mL of the gaseous phase of the test solution and 1 mL of the gaseous phase of reference solution (c) on to the column. Repeat these injections twice more.

The mean area of the peak of the residual solvent(s) in the chromatograms obtained with the test solution is not greater than half the mean area of the peak of the corresponding residual solvent(s) in the chromatograms obtained with reference solution (c). The test is not valid unless the relative standard deviation of the differences in areas between the analyte peaks obtained from 3 replicate paired injections of reference solution (c) and the test solution, is at most 15 per cent.

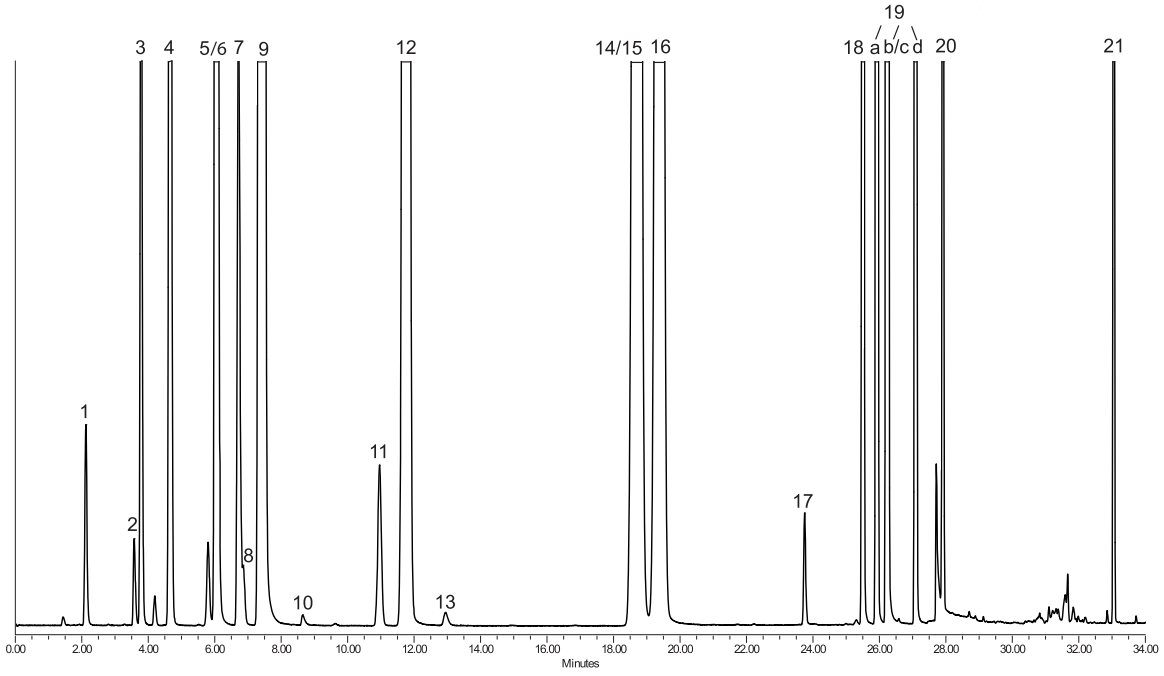
A flow diagram of the procedure is shown in Figure 2.4.24.-5.

When a residual solvent (Class 2 or Class 3) is present at a level of 0.1 per cent or greater then the content may be quantitatively determined by the method of standard additions.



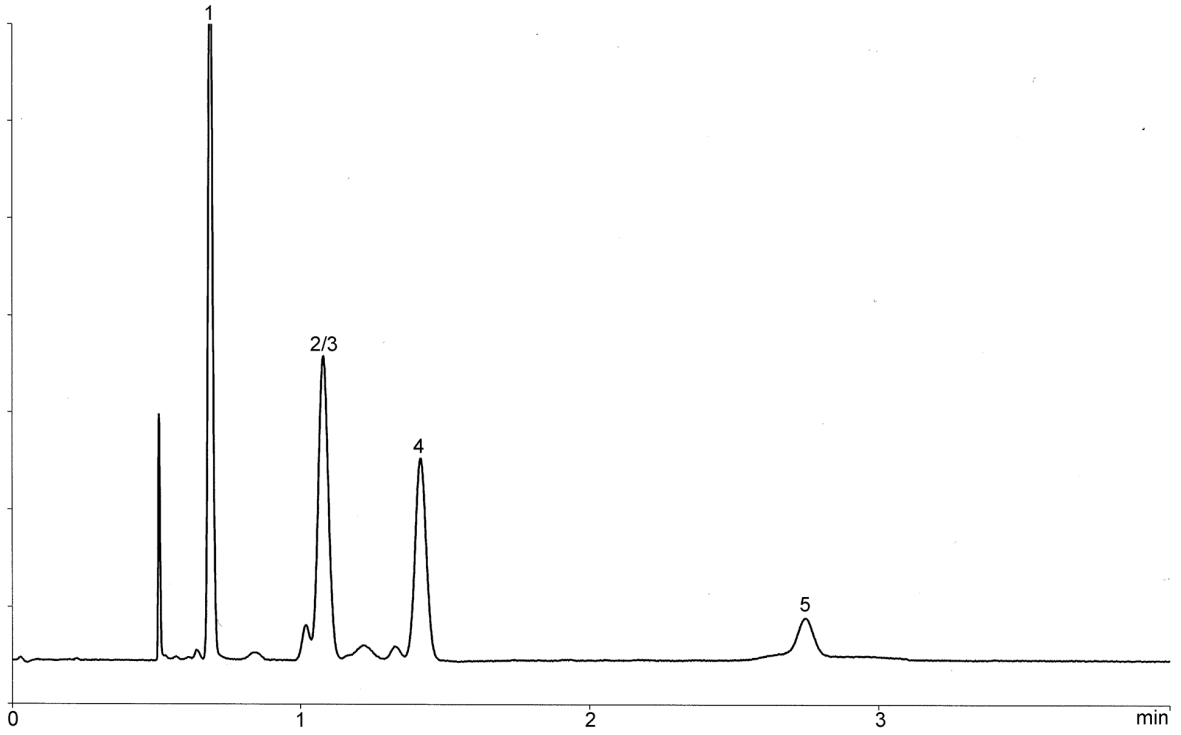
1. 1,1-dichloroethene 2. 1,1,1-trichloroethane 3. carbon tetrachloride 4. benzene 5. 1,2-dichloroethane

Figure 2.4.24.-1. – *Typical chromatogram of Class 1 solvents using the conditions described for System A and Procedure 1. Flame-ionisation detector*



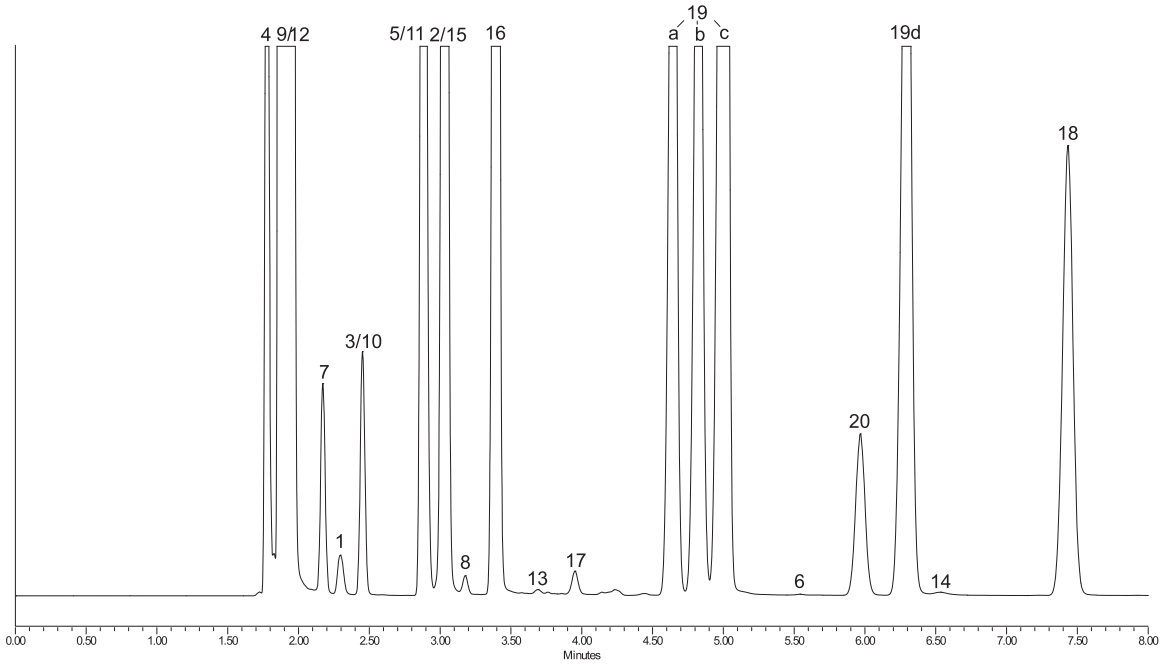
1. methanol
2. acetonitrile
3. dichloromethane
4. hexane
5. 1,2-dichloroethene
6. nitromethane
7. tetrahydrofuran
8. chloroform
9. cyclohexane
10. 1,2-dimethoxyethane
11. 1,1,2-trichloroethene
12. methylcyclohexane
13. 1,4-dioxane
14. pyridine
15. methylisobutylketone
16. toluene
17. methylbutylketone
18. chlorobenzene
19. xylene mix
a. ethylbenzene
b. *p*-xylene
c. *m*-xylene
d. *o*-xylene
20. cumene
21. tetralin

Figure 2.4.24.-2. – *Chromatogram of Class 2 solvents (solvent solution (b)) using the conditions described for System A and Procedure 1. Flame-ionisation detector*



1. 1,1-dichloroethene 2. 1,1,1-trichloroethane 3. carbon tetrachloride 4. benzene 5. 1,2-dichloroethane

Figure 2.4.24.-3. – *Chromatogram of Class 1 residual solvents using the conditions described for System B and Procedure 1. Flame-ionisation detector*



1. methanol	5. 1,2-dichloroethene	9. cyclohexane	13. 1,4-dioxane	17. methylbutylketone	20. cumene
2. acetonitrile	6. nitromethane	10. 1,2-dimethoxyethane	14. pyridine	18. chlorobenzene	21. tetralin (t_R = 27 min)
3. dichloromethane	7. tetrahydrofuran	11. 1,1,2-trichloroethene	15. methylisobutylketone	19. xylene mix a. ethylbenzene b. *p*-xylene c. *m*-xylene d. *o*-xylene	
4. hexane	8. chloroform	12. methylcyclohexane	16. toluene		

Figure 2.4.24.-4. – *Typical chromatogram of Class 2 residual solvents (solvent solution (b)) using the conditions described for System B and Procedure 1. Flame-ionisation detector*

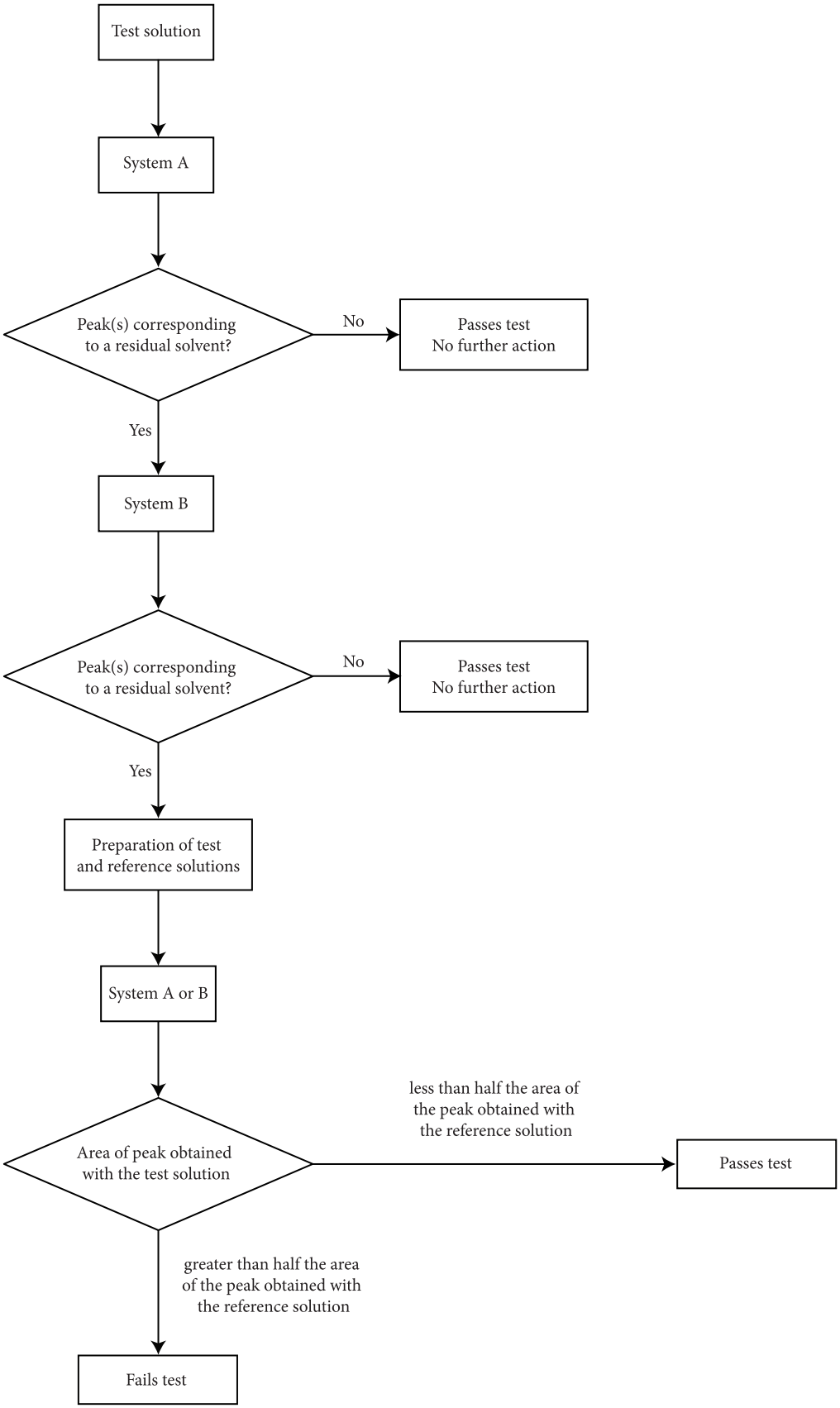


Figure 2.4.24.-5. – *Diagram relating to the identification of residual solvents and the application of limit tests*

2.5.8. DETERMINATION OF PRIMARY AROMATIC AMINO-NITROGEN

Dissolve the prescribed quantity of the substance to be examined in 50 mL of *dilute hydrochloric acid R* or in another prescribed solvent and add 3 g of *potassium bromide R*. Cool in ice-water and titrate by slowly adding *0.1 M sodium nitrite* with constant stirring.

01/2008:20508 Determine the end-point electrometrically or by the use of the prescribed indicator.

04/2011:20537
corrected 10.0

2.5.37. METHYL, ETHYL AND ISOPROPYL METHANESULFONATE IN METHANESULFONIC ACID

The following method has been validated for the methyl, ethyl and isopropyl esters of methanesulfonic acid at concentrations in the range of 0.5 ppm to 100 ppm.

If it is intended to be used to determine levels of methanesulfonic acid esters outside this validated range, for example in early steps of the synthesis prior to their removal, the concentration of the test solution has to be adjusted accordingly.

Gas chromatography (*2.2.28*) coupled with mass spectrometry (*2.2.43*).

Internal standard solution. Dilute 7 µL of *butyl methanesulfonate CRS* (BMS) to 10.0 mL with *methylene chloride R*. Dilute 10 µL of the solution to 100.0 mL with *methylene chloride R*.

Test solution. Add 0.74 g of the substance to be examined to 10.0 mL of *water R* and extract with 10.0 mL of the internal standard solution. Allow to separate and transfer the organic layer to a vial containing *anhydrous sodium sulfate R*. Shake and filter.

Reference solution (a). Dissolve 50 mg each of *methyl methanesulfonate R* (MMS), *ethyl methanesulfonate R* (EMS) and *isopropyl methanesulfonate R* (IMS) in the internal standard solution and dilute to 50.0 mL with the same solution. Dilute 74 µL of the solution to 10.0 mL with the internal standard solution. Dilute 100 µL of this solution to 10.0 mL with the internal standard solution.

Reference solution (b). Dilute 3.0 mL of reference solution (a) to 10.0 mL with the internal standard solution.

Column:

- *material*: fused silica;
- *size*: *l* = 15 m, Ø = 0.25 mm;
- *stationary phase*: *methylpolysiloxane R* (film thickness 1 µm).

Carrier gas: *helium for chromatography R*.

Flow rate: 1 mL/min.

Pulsed splitless: 250 kPa, 0.25 min.

Temperature:

		Time (min)	Temperature (°C)
Column		0 - 1	55
		1 - 9	55 → 135
Injection port			240
Detector:	transfer line		280
	source		230
	analyser		150

Detection: mass spectrometer as described below; adjust the detector settings so as to comply with the system suitability criteria:

- quadrupole mass spectrometer equipped with an electron impact ionisation mode (70 eV);
- mass spectrometer parameters for the fragmentometric mode (single-ion monitoring (SIM)) set as follows:

Substance	*m/z*	Duration of monitoring
Butyl methanesulfonate (BMS)	56	t_R between 7.0 min and 9.0 min
Methyl methanesulfonate (MMS)	80	t_R between 2.0 min and 3.5 min
Ethyl methanesulfonate (EMS)	79	t_R between 4.0 min and 4.7 min
Isopropyl methanesulfonate (IMS)	123	t_R between 4.7 min and 5.5 min

Injection: 2 µL.

Relative retention with reference to the internal standard (BMS) (retention time = about 7.6 min): MMS = about 0.3; EMS = about 0.5; IMS = about 0.6.

System suitability:

- *resolution*: minimum 3.0 between the peaks due to EMS and IMS in the chromatogram obtained with reference solution (a);
- *signal-to-noise ratio*: minimum 10 for the peaks due to MMS, EMS and IMS in the chromatogram obtained with reference solution (b).

Calculate the content of MMS, EMS or IMS in parts per million using the following expression:

$$\frac{A_2 \times I_1 \times W_1 \times C \times 0.148}{A_1 \times I_2 \times W_2}$$

A_1 = area of the peak due to MMS, EMS or IMS in the chromatogram obtained with reference solution (a);

A_2 = area of the peak due to MMS, EMS or IMS in the chromatogram obtained with the test solution;

C = percentage content of MMS, EMS or IMS;

I_1 = area of the peak due to the internal standard in the chromatogram obtained with reference solution (a);

I_2 = area of the peak due to the internal standard in the chromatogram obtained with the test solution;

W_1 = mass of MMS, EMS or IMS used to prepare reference solution (a), in milligrams;

W_2 = mass of the substance to be examined in the test solution, in milligrams;

0.148 = dilution factor.

04/2016:20538
corrected 10.0

2.5.38. METHYL, ETHYL AND ISOPROPYL METHANESULFONATE IN ACTIVE SUBSTANCES

The following general method has been validated for the determination of methyl, ethyl and isopropyl esters of methanesulfonic acid (in concentrations between 0.2 ppm and 5 ppm) in betahistine mesilate.

If it is intended to use the method for other active substances, particularly those that contain different concentrations of the methanesulfonic acid esters, the concentrations of the test solution and reference solutions must be adjusted accordingly and the method must be suitably validated.

METHOD

Head-space gas chromatography (*2.2.28*) coupled with mass spectrometry (*2.2.43*). *Prepare the test solution and reference solutions immediately before use.*

Solvent mixture: *water R*, *acetonitrile R* (20:80 *V/V*). *The use of acetonitrile of appropriate purity is essential.*

Solution A. Dissolve with the aid of ultrasound 30 mg of *anhydrous sodium thiosulfate R* and 60.0 g of *sodium iodide R* in *water R* and dilute to 50.0 mL with the same solvent.

Internal standard solution. Dilute 10 µL of *butyl methanesulfonate CRS* (BMS) to 10.0 mL with the solvent mixture. Dilute 20 µL of the solution to 100.0 mL with the solvent mixture.

Blank solution. Introduce 0.50 mL of solution A and 0.50 mL of the internal standard solution into a headspace vial and seal the vial immediately with a polytetrafluoroethylene-coated silicon membrane and an aluminium cap.

Test solution. Weigh 25.0 mg of the substance to be examined into a 20 mL headspace vial. Add 0.50 mL of solution A and 0.50 mL of the internal standard solution and seal the vial immediately with a polytetrafluoroethylene-coated silicon membrane and an aluminium cap.

Following the derivatisation reaction, a precipitate may be observed, however this does not affect the validity of the quantification.

Reference solution (a). Dissolve 25.0 mg each of *methyl methanesulfonate R* (MMS), *ethyl methanesulfonate R* (EMS) and *isopropyl methanesulfonate R* (IMS) in *toluene R* and dilute to 5.0 mL with the same solvent. Dilute 50 µL of the solution to 25.0 mL with the internal standard solution.

Reference solution (b). Dilute 20 µL of reference solution (a) to 20.0 mL with the internal standard solution. Introduce 0.50 mL of this solution and 0.50 mL of solution A into a 20 mL headspace vial and seal the vial immediately with a polytetrafluoroethylene-coated silicon membrane and an aluminium cap.

Reference solution (c). Dilute 500 µL of reference solution (a) to 20.0 mL with the internal standard solution. Introduce 0.50 mL of this solution and 0.50 mL of solution A into a 20 mL headspace vial and seal the vial immediately with a polytetrafluoroethylene-coated silicon membrane and an aluminium cap.

Column:

- *material*: fused silica;
- *size*: *l* = 30 m, Ø = 0.25 mm;
- *stationary phase*: *polar-deactivated macrogol R* (film thickness 1 µm).

Carrier gas: *helium for chromatography R.*

The use of an inert inlet liner without glass wool significantly reduces the effect of carry-over between the injections.

Flow rate: 0.5 mL/min.

Split ratio: 1:20.

Static head-space conditions that may be used:

- *equilibration temperature*: 60 °C;
- *equilibration time*: 30 min;
- *transfer-line temperature*: 120 °C.

Temperature:

		Time (min)	Temperature (°C)
Column		0 - 1	40
		1 - 10	40 → 130
Injection port			220
Detector	transfer line		280
	source		250
	analyser		200

At the end of analysis, the temperature of the column is raised to 240 °C and maintained at this temperature for 7 min.

Detection: mass spectrometer as described below; adjust the detector settings so as to comply with the system suitability criteria; alternatively a suitable electron-capture detector may be used:

- quadrupole mass spectrometer equipped with an electron impact ionisation mode (70 eV);
- mass spectrometer parameters for the fragmentometric mode (single-ion monitoring (SIM)) set as follows:

Substance	Quantitation ion (*m*/*z*)	Qualification ion (*m*/*z*)
Butyl iodide (BuI)*	184	127
Methyl iodide (MeI)*	142	127
Ethyl iodide (EtI)*	156	127
Isopropyl iodide (iPrI)*	170	127
* formed from BMS, MMS, EMS and IMS in the derivatisation reaction.		

Injection: 1 mL of the gas phase of the test solution, reference solutions (b) and (c) and the blank solution.

Relative retention with reference to the internal standard (BuI) (retention time = about 8.5 min): MeI = about 0.51; EtI = about 0.63; iPrI = about 0.68.

System suitability:

- *resolution*: minimum 1.5 between the peaks due to EtI and iPrI in the chromatogram obtained with reference solution (c);
- *signal-to-noise ratio*: minimum 10 for the peak due to each alkyl iodide in the chromatogram obtained with reference solution (b).

Calculate the content in parts per million of each alkyl methanesulfonate using the following expression:

$$\frac{A_2 \times I_1 \times W_1 \times C \times 0.05}{A_1 \times I_2 \times W_2}$$

A_1 = area of the peak due to each alkyl iodide in the chromatogram obtained with reference solution (c);

A_2 = area of the peak due to each alkyl iodide in the chromatogram obtained with the test solution;

C = percentage content of each ester;

I_1 = area of the peak due to the internal standard in the chromatogram obtained with reference solution (c);

I_2 = area of the peak due to the internal standard in the chromatogram obtained with the test solution;

W_1 = mass of each ester used to prepare reference solution (a), in milligrams;

W_2 = mass of the substance to be examined in the test solution, in milligrams;

0.05 = dilution factor.

04/2013:20539
corrected 10.0

2.5.39. METHANESULFONYL CHLORIDE IN METHANESULFONIC ACID

The following method has been validated for the determination of methanesulfonyl chloride in methanesulfonic acid at concentrations in the range of 0.05 ppm to 50 ppm.

Gas chromatography (*2.2.28*) coupled with mass spectrometry (*2.2.43*).

Internal standard solution. Dissolve 7 µL of *butyl methanesulfonate CRS* (BMS) in *methylene chloride R* and dilute to 10.0 mL with the same solvent. Dilute 5.0 mL of this solution to 50.0 mL with *methylene chloride R*.

Test solution. To 5 mL of *water R*, add 7.4 g of the substance to be examined and mix slowly. After cooling, add 5.0 mL of *methylene chloride R* and 100 µL of the internal standard solution and shake. Allow to separate and transfer the organic layer to a vial containing 1 g of *anhydrous sodium sulfate R*. Repeat the extraction twice with 5.0 mL of *methylene chloride R* each time, combine the organic layers and filter.

Reference solution (a). Dissolve 50.0 mg of *methanesulfonyl chloride R* in *methylene chloride R* and dilute to 10.0 mL with the same solvent. Dilute 1.0 mL of the solution to 10.0 mL with *methylene chloride R*. Dilute 300 µL of this solution to 10.0 mL with *methylene chloride R*.

Reference solution (b). Dilute 500 µL of reference solution (a) and 100 µL of the internal standard solution to 15.0 mL with *methylene chloride R*.

Reference solution (c). Dilute 25 µL of reference solution (a) and 100 µL of the internal standard solution to 15.0 mL with *methylene chloride R*.

Column:

- *material*: fused silica;
- *size*: *l* = 15 m, Ø = 0.25 mm;
- *stationary phase*: *methylpolysiloxane R* (film thickness 1 µm).

Carrier gas: *helium for chromatography R*.

Flow rate: 1 mL/min.

Pulsed splitless: 60 kPa, 0.1 min.

Temperature:

		Time (min)	Temperature (°C)
Column		0 - 4	40
		4 - 8	40 → 200
Injection port			240
Detector:	transfer line		280
	source		230
	analyser		150

At the end of analysis the temperature of the column is raised to 270 °C and maintained at this temperature for 8 min.

Detection: mass spectrometer as described below; adjust the detector settings so as to comply with the system suitability criteria:

- quadrupole mass spectrometer equipped with an electron impact ionisation mode (70 eV);
- mass spectrometer parameters for the fragmentometric mode (single-ion monitoring (SIM)) set as follows:

Substance	*m*/*z*	Duration of monitoring
Methanesulfonyl chloride	79	t_R between 3.3 min and 6.0 min
Butyl methanesulfonate (BMS)	56	t_R between 6.0 min and 8.0 min

Injection: 5 µL of the test solution, reference solutions (b) and (c), the internal standard solution and *methylene chloride R*.

Relative retention with reference to the internal standard (BMS) (retention time = about 7.2 min): methanesulfonyl chloride = about 0.68.

System suitability:

- in the chromatogram obtained with the internal standard solution, there is no peak with the same retention time as methanesulfonyl chloride;
- *resolution*: minimum 5.0 between the peaks due to methanesulfonyl chloride and BMS in the chromatogram obtained with reference solution (b);
- *signal-to-noise ratio*: minimum 10 for the peak due to methanesulfonyl chloride in the chromatogram obtained with reference solution (c).

Calculate the content of methanesulfonyl chloride in parts per million using the following expression:

$$\frac{A_2 \times I_1 \times W_1 \times C \times 1.5}{A_1 \times I_2 \times W_2}$$

A_1 = area of the peak due to methanesulfonyl chloride in the chromatogram obtained with reference solution (b);

A_2 = area of the peak due to methanesulfonyl chloride in the chromatogram obtained with the test solution;

C = percentage content of methanesulfonyl chloride;

I_1 = area of the peak due to BMS in the chromatogram obtained with reference solution (b);

I_2 = area of the peak due to BMS in the chromatogram obtained with the test solution;

W_1 = mass of methanesulfonyl chloride used to prepare reference solution (a), in milligrams;

W_2 = mass of the sample in the test solution, in milligrams;

1.5 = dilution factor.

04/2011:20601
corrected 7.7

2.6.1. STERILITY[(1)]

The test is applied to substances, preparations or articles which, according to the Pharmacopoeia, are required to be sterile. However, a satisfactory result only indicates that no contaminating micro-organism has been found in the sample examined in the conditions of the test.

PRECAUTIONS AGAINST MICROBIAL CONTAMINATION

The test for sterility is carried out under aseptic conditions. In order to achieve such conditions, the test environment has to be adapted to the way in which the sterility test is performed. The precautions taken to avoid contamination are such that they do not affect any micro-organisms which are to be revealed in the test. The working conditions in which the tests are performed are monitored regularly by appropriate sampling of the working area and by carrying out appropriate controls.

CULTURE MEDIA AND INCUBATION TEMPERATURES

Media for the test may be prepared as described below, or equivalent commercial media may be used provided that they comply with the growth promotion test.

The following culture media have been found to be suitable for the test for sterility. Fluid thioglycollate medium is primarily intended for the culture of anaerobic bacteria; however, it will also detect aerobic bacteria. Soya-bean casein digest medium is suitable for the culture of both fungi and aerobic bacteria.

Fluid thioglycollate medium

L-Cystine	0.5 g
Agar	0.75 g
Sodium chloride	2.5 g
Glucose monohydrate/Glucose	5.5 g/5.0 g
Yeast extract (water-soluble)	5.0 g
Pancreatic digest of casein	15.0 g
Sodium thioglycollate or	0.5 g
Thioglycollic acid	0.3 mL
Resazurin sodium solution (1 g/L of resazurin sodium), freshly prepared	1.0 mL
Water R	1000 mL

pH after sterilisation 7.1 ± 0.2

Mix the L-cystine, agar, sodium chloride, glucose, water-soluble yeast extract and pancreatic digest of casein with the *water R* and heat until solution is effected. Dissolve the sodium thioglycollate or thioglycollic acid in the solution and, if necessary, add *1 M sodium hydroxide* so that, after sterilisation, the solution will have a pH of 7.1 ± 0.2. If filtration is necessary, heat the solution again without boiling and filter while hot through moistened filter paper. Add the resazurin sodium solution, mix and place the medium in suitable vessels which provide a ratio of surface to depth of medium such that not more than the upper half of the medium has undergone a colour change indicative of oxygen uptake at the end of the incubation period. Sterilise using a validated process. If the medium is stored, store at a temperature between 2 °C and 25 °C in a sterile, airtight container. If more than the upper one-third of the medium has acquired a pink colour, the medium may be restored once by heating the containers in a water-bath or in free-flowing steam until the pink colour disappears and cooling quickly, taking care to prevent the introduction of non-sterile air into the container. Do not use the medium for a longer storage period than has been validated.

Fluid thioglycollate medium is to be incubated at 30-35 °C.

For products containing a mercurial preservative that cannot be tested by the membrane-filtration method, fluid thioglycollate medium incubated at 20-25 °C may be used instead of soya-bean casein digest medium provided that it has been validated as described in growth promotion test.

Where prescribed or justified and authorised, the following alternative thioglycollate medium may be used. Prepare a mixture having the same composition as that of the fluid thioglycollate medium, but omitting the agar and the resazurin sodium solution, sterilise as directed above. The pH after sterilisation is 7.1 ± 0.2. Heat in a water-bath prior to use and incubate at 30-35 °C under anaerobic conditions.

Soya-bean casein digest medium

Pancreatic digest of casein	17.0 g
Papaic digest of soya-bean meal	3.0 g
Sodium chloride	5.0 g
Dipotassium hydrogen phosphate	2.5 g
Glucose monohydrate/Glucose	2.5 g/2.3 g
Water R	1000 mL

pH after sterilisation 7.3 ± 0.2

Dissolve the solids in *water R*, warming slightly to effect solution. Cool the solution to room temperature. Add *1 M sodium hydroxide*, if necessary, so that after sterilisation the solution will have a pH of 7.3 ± 0.2. Filter, if necessary, to clarify, distribute into suitable vessels and sterilise using a validated process. Store at a temperature between 2 °C and 25 °C in a sterile well-closed container, unless it is intended for immediate use. Do not use the medium for a longer storage period than has been validated.

Soya-bean casein digest medium is to be incubated at 20-25 °C.

The media used comply with the following tests, carried out before or in parallel with the test on the product to be examined.

Sterility. Incubate portions of the media for 14 days. No growth of micro-organisms occurs.

Growth promotion test of aerobes, anaerobes and fungi. Test each batch of ready-prepared medium and each batch of medium prepared either from dehydrated medium or from ingredients. Suitable strains of micro-organisms are indicated in Table 2.6.1.-1.

Inoculate portions of fluid thioglycollate medium with a small number (not more than 100 CFU) of the following micro-organisms, using a separate portion of medium for each of the following species of micro-organism: *Clostridium sporogenes*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*. Inoculate portions of soya-bean casein digest medium with a small number (not more than 100 CFU) of the following micro-organisms, using a separate portion of medium for each of the following species of micro-organism: *Aspergillus brasiliensis*, *Bacillus subtilis*, *Candida albicans*. Incubate for not more than 3 days in the case of bacteria and not more than 5 days in the case of fungi.

Seed lot culture maintenance techniques (seed-lot systems) are used so that the viable micro-organisms used for inoculation are not more than 5 passages removed from the original master seed-lot.

The media are suitable if a clearly visible growth of the micro-organisms occurs.

(1) This chapter has undergone pharmacopoeial harmonisation. See chapter *5.8. Pharmacopoeial harmonisation*.

Table 2.6.1.-1. – *Strains of the test micro-organisms suitable for use in the growth promotion test and the method suitability test*

Aerobic bacteria	
Staphylococcus aureus	ATCC 6538, CIP 4.83, NCTC 10788, NCIMB 9518, NBRC 13276
Bacillus subtilis	ATCC 6633, CIP 52.62, NCIMB 8054, NBRC 3134
Pseudomonas aeruginosa	ATCC 9027, NCIMB 8626, CIP 82.118, NBRC 13275
Anaerobic bacterium	
Clostridium sporogenes	ATCC 19404, CIP 79.3, NCTC 532, ATCC 11437, NBRC 14293
Fungi	
Candida albicans	ATCC 10231, IP 48.72, NCPF 3179, NBRC 1594
Aspergillus brasiliensis	ATCC 16404, IP 1431.83, IMI 149007, NBRC 9455

METHOD SUITABILITY TEST

Carry out a test as described below under Test for sterility of the product to be examined using exactly the same methods except for the following modifications.

Membrane filtration. After transferring the contents of the container or containers to be tested to the membrane add an inoculum of a small number of viable micro-organisms (not more than 100 CFU) to the final portion of sterile diluent used to rinse the filter.

Direct inoculation. After transferring the content of the container or containers to be tested (for catgut and other surgical sutures for veterinary use: strands) to the culture medium add an inoculum of a small number of viable micro-organisms (not more than 100 CFU) to the medium.

In both cases use the same micro-organisms as those described above under Growth promotion test of aerobes, anaerobes and fungi. Perform a growth promotion test as a positive control. Incubate all the containers containing medium for not more than 5 days.

If clearly visible growth of micro-organisms is obtained after the incubation, visually comparable to that in the control vessel without product, either the product possesses no antimicrobial activity under the conditions of the test or such activity has been satisfactorily eliminated. The test for sterility may then be carried out without further modification.

If clearly visible growth is not obtained in the presence of the product to be tested, visually comparable to that in the control vessels without product, the product possesses antimicrobial activity that has not been satisfactorily eliminated under the conditions of the test. Modify the conditions in order to eliminate the antimicrobial activity and repeat the method suitability test.

This method suitability test is performed:

a) when the test for sterility has to be carried out on a new product;

b) whenever there is a change in the experimental conditions of the test.

The method suitability test may be performed simultaneously with the test for sterility of the product to be examined.

TEST FOR STERILITY OF THE PRODUCT TO BE EXAMINED

The test may be carried out using the technique of membrane filtration or by direct inoculation of the culture media with the product to be examined. Appropriate negative controls are included. The technique of membrane filtration is used whenever the nature of the product permits, that is, for filterable aqueous preparations, for alcoholic or oily preparations and for preparations miscible with or soluble in aqueous or oily solvents provided these solvents do not have an antimicrobial effect in the conditions of the test.

Membrane filtration. Use membrane filters having a nominal pore size not greater than 0.45 µm whose effectiveness to retain micro-organisms has been established. Cellulose nitrate filters, for example, are used for aqueous, oily and weakly alcoholic solutions and cellulose acetate filters, for example, for strongly alcoholic solutions. Specially adapted filters may be needed for certain products, e.g. for antibiotics.

The technique described below assumes that membranes about 50 mm in diameter will be used. If filters of a different diameter are used the volumes of the dilutions and the washings should be adjusted accordingly. The filtration apparatus and membrane are sterilised by appropriate means. The apparatus is designed so that the solution to be examined can be introduced and filtered under aseptic conditions; it permits the aseptic removal of the membrane for transfer to the medium or it is suitable for carrying out the incubation after adding the medium to the apparatus itself.

Aqueous solutions. If appropriate, transfer a small quantity of a suitable, sterile diluent such as a 1 g/L neutral solution of meat or casein peptone pH 7.1 ± 0.2 onto the membrane in the apparatus and filter. The diluent may contain suitable neutralising substances and/or appropriate inactivating substances for example in the case of antibiotics.

Transfer the contents of the container or containers to be tested to the membrane or membranes, if necessary after diluting to the volume used in the method suitability test with the chosen sterile diluent but in any case using not less than the quantities of the product to be examined prescribed in Table 2.6.1.-2. Filter immediately. If the product has antimicrobial properties, wash the membrane not less than 3 times by filtering through it each time the volume of the chosen sterile diluent used in the method suitability test. Do not exceed a washing cycle of 5 times 100 mL per filter, even if during the method suitability test it has been demonstrated that such a cycle does not fully eliminate the antimicrobial activity. Transfer the whole membrane to the culture medium or cut it aseptically into 2 equal parts and transfer one half to each of 2 suitable media. Use the same volume of each medium as in the method suitability test. Alternatively, transfer the medium onto the membrane in the apparatus. Incubate the media for not less than 14 days.

Soluble solids. Use for each medium not less than the quantity prescribed in Table 2.6.1.-2 of the product dissolved in a suitable solvent such as the solvent provided with the preparation, water for injections, saline or a 1 g/L neutral solution of meat or casein peptone and proceed with the test as described above for aqueous solutions using a membrane appropriate to the chosen solvent.

Oils and oily solutions. Use for each medium not less than the quantity of the product prescribed in Table 2.6.1.-2. Oils and oily solutions of sufficiently low viscosity may be filtered without dilution through a dry membrane. Viscous oils may be diluted as necessary with a suitable sterile diluent such as isopropyl myristate shown not to have antimicrobial activity in the conditions of the test. Allow the oil to penetrate the membrane by its own weight then filter, applying the pressure or suction gradually. Wash the membrane at least 3 times by filtering through it each time about 100 mL of a suitable sterile solution such as 1 g/L neutral meat or casein peptone containing a suitable emulsifying agent at a concentration

Table 2.6.1.-2. – *Minimum quantity to be used for each medium*

Quantity per container	Minimum quantity to be used for each medium unless otherwise justified and authorised
Liquids	
– less than 1 mL	The whole contents of each container
– 1-40 mL	Half the contents of each container but not less than 1 mL
– greater than 40 mL and not greater than 100 mL	20 mL
– greater than 100 mL	10 per cent of the contents of the container but not less than 20 mL
Antibiotic liquids	1 mL
Insoluble preparations, creams and ointments to be suspended or emulsified	Use the contents of each container to provide not less than 200 mg
Solids	
– less than 50 mg	The whole contents of each container
– 50 mg or more but less than 300 mg	Half the contents of each container but not less than 50 mg
– 300 mg to 5 g	150 mg
– greater than 5 g	500 mg
Catgut and other surgical sutures for veterinary use	3 sections of a strand (each 30 cm long)

shown to be appropriate in the method suitability test, for example polysorbate 80 at a concentration of 10 g/L. Transfer the membrane or membranes to the culture medium or media or vice versa as described above for aqueous solutions, and incubate at the same temperatures and for the same times.

Ointments and creams. Use for each medium not less than the quantities of the product prescribed in Table 2.6.1.-2. Ointments in a fatty base and emulsions of the water-in-oil type may be diluted to 1 per cent in isopropyl myristate as described above, by heating, if necessary, to not more than 40 °C. In exceptional cases it may be necessary to heat to not more than 44 °C. Filter as rapidly as possible and proceed as described above for oils and oily solutions.

Direct inoculation of the culture medium. Transfer the quantity of the preparation to be examined prescribed in Table 2.6.1.-2 directly into the culture medium so that the volume of the product is not more than 10 per cent of the volume of the medium, unless otherwise prescribed.

If the product to be examined has antimicrobial activity, carry out the test after neutralising this with a suitable neutralising substance or by dilution in a sufficient quantity of culture medium. When it is necessary to use a large volume of the product it may be preferable to use a concentrated culture medium prepared in such a way that it takes account of the subsequent dilution. Where appropriate, the concentrated medium may be added directly to the product in its container.

Oily liquids. Use media to which have been added a suitable emulsifying agent at a concentration shown to be appropriate in the method suitability test, for example polysorbate 80 at a concentration of 10 g/L.

Ointments and creams. Prepare by diluting to about 1 in 10 by emulsifying with the chosen emulsifying agent in a suitable sterile diluent such as a 1 g/L neutral solution of meat or casein peptone. Transfer the diluted product to a medium not containing an emulsifying agent.

Incubate the inoculated media for not less than 14 days. Observe the cultures several times during the incubation period. Shake cultures containing oily products gently each day. However when fluid thioglycollate medium is used for the detection of anaerobic micro-organisms keep shaking or mixing to a minimum in order to maintain anaerobic conditions.

Catgut and other surgical sutures for veterinary use. Use for each medium not less than the quantities of the product prescribed in Table 2.6.1.-2. Open the sealed package using aseptic precautions and remove 3 sections of the strand for each culture medium. Carry out the test on 3 sections, each 30 cm long, cut off from the beginning, the centre and the end of the strand. Use whole strands from freshly opened cassette packs. Transfer each section of the strand to the selected medium. Use sufficient medium to cover adequately the material to be tested (20 mL to 150 mL).

OBSERVATION AND INTERPRETATION OF RESULTS

At intervals during the incubation period and at its conclusion, examine the media for macroscopic evidence of microbial growth. If the material being tested renders the medium turbid so that the presence or absence of microbial growth cannot be readily determined by visual examination, 14 days after the beginning of incubation transfer portions (each not less than 1 mL) of the medium to fresh vessels of the same medium and then incubate the original and transfer vessels for not less than 4 days.

If no evidence of microbial growth is found, the product to be examined complies with the test for sterility. If evidence of microbial growth is found the product to be examined does not comply with the test for sterility, unless it can be clearly demonstrated that the test was invalid for causes unrelated to the product to be examined. The test may be considered invalid only if one or more of the following conditions are fulfilled:

a) the data of the microbiological monitoring of the sterility testing facility show a fault;

b) a review of the testing procedure used during the test in question reveals a fault;

c) microbial growth is found in the negative controls;

d) after determination of the identity of the micro-organisms isolated from the test, the growth of this species or these species may be ascribed unequivocally to faults with respect to the material and/or the technique used in conducting the sterility test procedure.

If the test is declared to be invalid it is repeated with the same number of units as in the original test.

If no evidence of microbial growth is found in the repeat test the product examined complies with the test for sterility. If microbial growth is found in the repeat test the product examined does not comply with the test for sterility.

Table 2.6.1.-3. – *Minimum number of items to be tested*

Number of items in the batch*	Minimum number of items to be tested for each medium, unless otherwise justified and authorised**
Parenteral preparations	
– Not more than 100 containers	10 per cent or 4 containers, whichever is the greater
– More than 100 but not more than 500 containers	10 containers
– More than 500 containers	2 per cent or 20 containers (10 containers for large-volume parenterals) whichever is less
Ophthalmic and other non-injectable preparations	
– Not more than 200 containers	5 per cent or 2 containers, whichever is the greater
– More than 200 containers	10 containers
– If the product is presented in the form of single-dose containers, apply the scheme shown above for preparations for parenteral administration	
Catgut and other surgical sutures for veterinary use	2 per cent or 5 packages whichever is the greater, up to a maximum total of 20 packages
Bulk solid products	
– Up to 4 containers	Each container
– More than 4 containers but not more than 50 containers	20 per cent or 4 containers, whichever is the greater
– More than 50 containers	2 per cent or 10 containers, whichever is the greater

* If the batch size is not known, use the maximum number of items prescribed.

**If the contents of one container are enough to inoculate the 2 media, this column gives the number of containers needed for both the media together.

APPLICATION OF THE TEST TO PARENTERAL PREPARATIONS, OPHTHALMIC AND OTHER NON-INJECTABLE PREPARATIONS REQUIRED TO COMPLY WITH THE TEST FOR STERILITY

When using the technique of membrane filtration, use, whenever possible, the whole contents of the container, but not less than the quantities indicated in Table 2.6.1.-2, diluting where necessary to about 100 mL with a suitable sterile solution, such as 1 g/L neutral meat or casein peptone.

When using the technique of direct inoculation of media, use the quantities shown in Table 2.6.1.-2, unless otherwise justified and authorised. The tests for bacterial and fungal sterility are carried out on the same sample of the product to be examined. When the volume or the quantity in a single container is insufficient to carry out the tests, the contents of 2 or more containers are used to inoculate the different media.

MINIMUM NUMBER OF ITEMS TO BE TESTED

The minimum number of items to be tested in relation to the size of the batch is given in Table 2.6.1.-3.

Guidelines on the test for sterility are given in general chapter 5.1.9.

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2.6.14. BACTERIAL ENDOTOXINS[(1)]

The test for bacterial endotoxins (BET) is used to detect or quantify endotoxins from gram-negative bacteria using amoebocyte lysate from the horseshoe crab (*Limulus polyphemus* or *Tachypleus tridentatus*). There are 3 techniques for this test: the gel-clot technique, which is based on gel formation; the turbidimetric technique, based on the development of turbidity after cleavage of an endogenous substrate; and the chromogenic technique, based on the development of colour after cleavage of a synthetic peptide-chromogen complex.

The following 6 methods are described in the present chapter:

Method A. Gel-clot method: limit test

Method B. Gel-clot method: quantitative test

Method C. Turbidimetric kinetic method

Method D. Chromogenic kinetic method

Method E. Chromogenic end-point method

Method F. Turbidimetric end-point method

Proceed by any of the 6 methods for the test. In the event of doubt or dispute, the final decision is made based upon method A unless otherwise indicated in the monograph.

The test is carried out in a manner that avoids endotoxin contamination.

1. APPARATUS

Depyrogenate all glassware and other heat-stable apparatus in a hot-air oven using a validated process. A commonly used minimum time and temperature is 30 min at 250 °C. If employing plastic apparatus, such as microtitre plates and pipette tips for automatic pipetters, use apparatus shown to be free of detectable endotoxin and which does not interfere in the test.

NOTE: in this chapter, the term 'tube' includes all types of receptacles, for example microtitre plate wells.

2. REAGENTS, TEST SOLUTIONS

(1) Amoebocyte lysate

Amoebocyte lysate is a lyophilised product obtained from amoebocyte lysate from the horseshoe crab (*Limulus polyphemus* or *Tachypleus tridentatus*). This reagent refers only to a product manufactured in accordance with the regulations of the competent authority.

NOTE: amoebocyte lysate reacts with some β-glucans in addition to endotoxins. Amoebocyte lysate preparations which do not react with glucans are available; they are prepared by removing from amoebocyte lysate the G factor, which reacts with glucans, or by inhibiting the G factor reacting system of amoebocyte lysate. These preparations may be used for endotoxin testing in the presence of glucans.

(2) Lysate solution

Dissolve amoebocyte lysate in water for BET or in a buffer, as recommended by the lysate manufacturer, by gentle stirring. Store the reconstituted lysate, refrigerated or frozen, as indicated by the manufacturer.

(3) Water for BET (water for bacterial endotoxins test)

Water for injections R or water produced by other procedures that shows no reaction with the lysate employed at the detection limit of the reagent.

3. PREPARATION OF THE STANDARD ENDOTOXIN STOCK SOLUTION

The standard endotoxin stock solution is prepared from an endotoxin reference standard that has been calibrated against the International Standard, for example *endotoxin standard BRP*.

Endotoxin is expressed in International Units (IU). The equivalence in IU of the International Standard is stated by the World Health Organization.

NOTE: one International Unit (IU) of endotoxin is equal to one Endotoxin Unit (E.U.).

Follow the specifications in the package leaflet and on the label for preparation and storage of the standard endotoxin stock solution.

4. PREPARATION OF THE STANDARD ENDOTOXIN SOLUTIONS

After vigorously mixing the standard endotoxin stock solution, prepare appropriate serial dilutions of this solution using water for BET.

Use the solutions as soon as possible to avoid loss of activity by adsorption.

5. PREPARATION OF THE TEST SOLUTIONS

Prepare the test solutions by dissolving or diluting active substances or medicinal products using water for BET. Some substances or preparations may be more appropriately dissolved or diluted in other aqueous solutions. If necessary, adjust the pH of the test solution (or dilution thereof) so that the pH of the mixture of the lysate and test solution falls within the pH range specified by the lysate manufacturer, usually 6.0 to 8.0. The pH may be adjusted by the use of acid, base or a suitable buffer, as recommended by the lysate manufacturer. Acids and bases may be prepared from concentrates or solids with water for BET in containers free of detectable endotoxin. Buffers must be validated to be free of detectable endotoxin and interfering factors.

6. DETERMINATION OF THE MAXIMUM VALID DILUTION

The Maximum Valid Dilution (MVD) is the maximum allowable dilution of a sample at which the endotoxin limit can be determined. Determine the MVD using the following formulae:

$$\mathrm{MVD} = \frac{\text{endotoxin limit} \times \text{concentration of test solution}}{\lambda}$$

Endotoxin limit: the endotoxin limit for active substances administered parenterally, defined on the basis of dose, is equal to:

$$\frac{K}{M}$$

K = threshold pyrogenic dose of endotoxin per kilogram of body mass,

M = maximum recommended bolus dose of product per kilogram of body mass.

When the product is to be injected at frequent intervals or infused continuously, M is the maximum total dose administered in a single hour period.

The endotoxin limit for active substances administered parenterally is specified in units such as IU/mL, IU/mg, IU/Unit of biological activity, etc., in monographs.

(1) This chapter has undergone pharmacopoeial harmonisation. See chapter *5.8. Pharmacopoeial harmonisation*.

Concentration of test solution:

- mg/mL if the endotoxin limit is specified by mass (IU/mg),
- Units/mL if the endotoxin limit is specified by unit of biological activity (IU/Unit),
- mL/mL if the endotoxin limit is specified by volume (IU/mL).

λ = the labelled lysate sensitivity in the gel-clot technique (IU/mL) or the lowest concentration used in the standard curve of the turbidimetric or chromogenic techniques.

7. GEL-CLOT TECHNIQUE (METHODS A AND B)

The gel-clot technique allows detection or quantification of endotoxins and is based on clotting of the lysate in the presence of endotoxins. The minimum concentration of endotoxins required to cause the lysate to clot under standard conditions is the labelled lysate sensitivity. To ensure both the precision and validity of the test, confirm the labelled lysate sensitivity and perform the test for interfering factors as described under 1. Preparatory testing.

1. PREPARATORY TESTING

(i) Confirmation of the labelled lysate sensitivity

Confirm in 4 replicates the labelled sensitivity λ, expressed in IU/mL, of the lysate solution prior to use in the test. Confirmation of the lysate sensitivity is carried out when a new lot of lysate is used or when there is any change in the test conditions which may affect the outcome of the test.

Prepare standard solutions of at least 4 concentrations equivalent to 2λ, λ, 0.5λ and 0.25λ by diluting the standard endotoxin stock solution with water for BET.

Mix a volume of the lysate solution with an equal volume of 1 of the standard solutions (such as 0.1 mL aliquots) in each tube. When single test vials or ampoules containing lyophilised lysate are employed, add solutions of standards directly to the vial or ampoule. Incubate the reaction mixture for a constant period according to the recommendations of the lysate manufacturer (usually at 37 ± 1 °C for 60 ± 2 min), avoiding vibration. Test the integrity of the gel: for tubes, take each tube in turn directly from the incubator and invert it through approximately 180° in one smooth motion. If a firm gel has formed that remains in place upon inversion, record the result as positive. A result is negative if an intact gel is not formed.

The test is considered valid when the lowest concentration of the standard solutions shows a negative result in all replicate tests.

The end-point is the lowest concentration in the series of decreasing concentrations of standard endotoxin that clots the lysate. Determine the geometric mean end-point concentration by calculting the mean of the logarithms of the end-point concentrations of the 4 dilution series, take the antilogarithm of this value, as indicated by the following expression:

$$\text{Geometric mean end-point concentration} = \text{antilog}\frac{\sum e}{f}$$

$\sum e$ = sum of the $\log_{10}$ end-point concentrations of the dilution series used,

f = number of replicates.

The geometric mean end-point concentration is the measured sensitivity of the lysate solution (IU/mL). If this is not less than 0.5λ and not more than 2λ, the labelled sensitivity is confirmed and is used in the tests performed with this lysate.

(ii) Test for interfering factors

Prepare solutions A, B, C and D as shown in Table 2.6.14.-1, and use the test solutions at a dilution less than the MVD, not containing any detectable endotoxins, operating as described under 1. Preparatory testing, (i) Confirmation of the labelled lysate sensitivity.

The geometric mean end-point concentrations of solutions B and C are determined using the expression described in 1. Preparatory testing, (i) Confirmation of the labelled lysate sensitivity.

The test for interfering factors must be repeated when any changes are made to the experimental conditions that are likely to influence the result of the test.

The test is considered valid when all replicates of solutions A and D show no reaction and the result of solution C confirms the labelled lysate sensitivity.

If the sensitivity of the lysate determined with solution B is not less than 0.5λ and not greater than 2λ, the test solution does not contain interfering factors under the experimental conditions used. Otherwise, the test solution interferes with the test.

If the preparation being examined interferes with the test at a dilution less than the MVD, repeat the test for interfering factors using a greater dilution, not exceeding the MVD. The use of a more sensitive lysate permits a greater dilution of the preparation being examined and this may contribute to the elimination of interference.

Interference may be overcome by suitable validated treatment, such as filtration, neutralisation, dialysis or heat treatment. To establish that the treatment chosen effectively eliminates interference without loss of endotoxins, repeat the test for interfering factors using the preparation being examined to which the standard endotoxin has been added and which has then been submitted to the chosen treatment.

Table 2.6.14.-1

Solution	Endotoxin concentration/Solution to which endotoxin is added	Diluent	Dilution factor	Endotoxin concentration	Number of replicates
A	None/Test solution	-	-	-	4
B	2λ/Test solution	Test solution	1	2λ	4
			2	1λ	4
			4	0.5λ	4
			8	0.25λ	4
C	2λ/Water for BET	Water for BET	1	2λ	2
			2	1λ	2
			4	0.5λ	2
			8	0.25λ	2
D	None/Water for BET	-	-	-	2

Solution A = solution of the preparation being examined that is free of detectable endotoxins.
Solution B = test for interference.
Solution C = control of the labelled lysate sensitivity.
Solution D = negative control (water for BET).

2. LIMIT TEST (METHOD A)

(i) Procedure

Prepare solutions A, B, C and D as shown in Table 2.6.14.-2, and perform the test on these solutions following the procedure described under 1. Preparatory testing, (i) Confirmation of the labelled lysate sensitivity.

Table 2.6.14.-2

Solution	Endotoxin concentration/Solution to which endotoxin is added	Number of replicates
A	None/Diluted test solution	2
B	2λ/Diluted test solution	2
C	2λ/Water for BET	2
D	None/Water for BET	2

Prepare solution A and solution B (positive product control) using a dilution not greater than the MVD and treatments as described in 1. Preparatory testing, (ii) Test for interfering factors. Solutions B and C (positive controls) contain the standard endotoxin at a concentration corresponding to twice the labelled lysate sensitivity. Solution D (negative control) consists of water for BET.

(ii) Interpretation

The test is considered valid when both replicates of solution B and C are positive and those of solution D are negative.

When a negative result is found for both replicates of solution A, the preparation being examined complies with the test.

When a positive result is found for both replicates of solution A, the preparation being examined does not comply with the test.

When a positive result is found for one replicate of solution A and a negative result is found for the other, repeat the test. In the repeat test, the preparation being examined complies with the test if a negative result is found for both replicates of solution A. The preparation does not comply with the test if a positive result is found for one or both replicates of solution A.

However, if the preparation does not comply with the test at a dilution less than the MVD, the test may be repeated using a greater dilution, not exceeding the MVD.

3. QUANTITATIVE TEST (METHOD B)

(i) Procedure

The test quantifies bacterial endotoxins in the test solution by titration to an end-point. Prepare solutions A, B, C and D as shown in Table 2.6.14.-3, and test these solutions according to the procedure described under 1. Preparatory testing, (i) Confirmation of the labelled lysate sensitivity.

(ii) Calculation and interpretation

The test is considered valid when the following 3 conditions are met:

(a) both replicates of solution D (negative control) are negative,

(b) both replicates of solution B (positive product control) are positive,

(c) the geometric mean end-point concentration of solution C is in the range of 0.5λ to 2λ.

To determine the endotoxin concentration of solution A, calculate the end-point concentration for each replicate, by multiplying each end-point dilution factor by λ.

The endotoxin concentration in the test solution is the end-point concentration of the replicates. If the test is conducted with a diluted test solution, calculate the concentration of endotoxin in the original solution by multiplying the result by the dilution factor.

If none of the dilutions of the test solution is positive in a valid test, report the endotoxin concentration as less than λ (or, if a diluted sample was tested, report as less than the lowest dilution factor of the sample × λ). If all dilutions are positive, the endotoxin concentration is reported as equal to or greater than the largest dilution factor multiplied by λ (e.g. in Table 2.6.14.-3, the initial dilution factor × 8 × λ).

The preparation being examined meets the requirements of the test if the endotoxin concentration in both replicates is less than that specified in the monograph.

Table 2.6.14.-3

Solution	Endotoxin concentration/Solution to which endotoxin is added	Diluent	Dilution factor	Endotoxin concentration	Number of replicates
A	None/Test solution	Water for BET	1	-	2
			2	-	2
			4	-	2
			8	-	2
B	2λ/Test solution		1	2λ	2
C	2λ/Water for BET	Water for BET	1	2λ	2
			2	1λ	2
			4	0.5λ	2
			8	0.25λ	2
D	None/Water for BET	-	-	-	2

Solution A = test solution at the dilution, not exceeding the MVD, with which the test for interfering factors was carried out. Subsequent dilution of the test solution must not exceed the MVD. Use water for BET to make a dilution series of 4 tubes containing the test solution at concentrations of 1, 1/2, 1/4 and 1/8, relative to the dilution used in the test for interfering factors. Other dilutions up to the MVD may be used as appropriate.

Solution B = solution A containing standard endotoxin at a concentration of 2λ (positive product control).

Solution C = a dilution series of 4 tubes of water for BET containing the standard endotoxin at concentrations of 2λ, λ, 0.5λ and 0.25λ.

Solution D = water for BET (negative control).

8. PHOTOMETRIC QUANTITATIVE TECHNIQUES (METHODS C, D, E AND F)

1. TURBIDIMETRIC TECHNIQUE (METHODS C AND F)

This technique is a photometric test to measure the increase in turbidity. Based on the test principle employed, this technique may be classified as being either the end-point-turbidimetric test or the kinetic-turbidimetric test.

The end-point-turbidimetric test (Method F) is based on the quantitative relationship between the endotoxin concentration and the turbidity (absorbance or transmission) of the reaction mixture at the end of an incubation period.

The kinetic-turbidimetric test (Method C) is a method to measure either the time (onset time) needed for the reaction mixture to reach a predetermined absorbance or transmission, or the rate of turbidity development.

The test is carried out at the incubation temperature recommended by the lysate manufacturer (usually 37 ± 1 °C).

2. CHROMOGENIC TECHNIQUE (METHODS D AND E)

This technique is used to measure the chromophore released from a suitable chromogenic peptide by the reaction of endotoxins with the lysate. Depending on the test principle employed, this technique may be classified as being either the end-point-chromogenic test or the kinetic-chromogenic test.

The end-point-chromogenic test (Method E) is based on the quantitative relationship between the endotoxin concentration and the quantity of chromophore released at the end of an incubation period.

The kinetic-chromogenic test (Method D) measures either the time (onset time) needed for the reaction mixture to reach a predetermined absorbance, or the rate of colour development.

The test is carried out at the incubation temperature recommended by the lysate manufacturer (usually 37 ± 1 °C).

3. PREPARATORY TESTING

To assure the precision or validity of the turbidimetric and chromogenic techniques, preparatory tests are conducted to show that the criteria for the standard curve are satisfied and that the test solution does not interfere with the test.

Validation of the test method is required when any changes are made to the experimental conditions that are likely to influence the result of the test.

(i) Assurance of criteria for the standard curve

The test must be carried out for each lot of lysate reagent.

Using the standard endotoxin solution, prepare at least 3 endotoxin concentrations within the range indicated by the lysate manufacturer to generate the standard curve. Perform the test using at least 3 replicates of each standard endotoxin solution as recommended by the lysate manufacturer (volume ratios, incubation time, temperature, pH, etc.).

If the desired range is greater than 2 $\log_{10}$ in the kinetic methods, additional standards must be included to bracket each $\log_{10}$ increase in the range of the standard curve.

The absolute value of the correlation coefficient, $|r|$, must be greater than or equal to 0.980, for the range of endotoxin concentrations set up.

(ii) Test for interfering factors

Select an endotoxin concentration at or near the middle of the endotoxin standard curve.

Prepare solutions A, B, C and D as shown in Table 2.6.14.-4. Perform the test on at least 2 replicates of these solutions as recommended by the lysate manufacturer (volume of test solution and lysate solution, volume ratio of test solution to lysate solution, incubation time, etc.).

Table 2.6.14.-4

Solution	Endotoxin concentration	Solution to which endotoxin is added	Number of replicates
A	None	Test solution	Not less than 2
B	Middle concentration of the standard curve	Test solution	Not less than 2
C	At least 3 concentrations (lowest concentration is designated λ)	Water for BET	Each concentration not less than 2
D	None	Water for BET	Not less than 2

Solution A = test solution, that may be diluted not to exceed the MVD.

Solution B = preparation to be examined at the same dilution as solution A, containing added endotoxin at a concentration equal to or near the middle of the standard curve.

Solution C = standard endotoxin solution at the concentrations used in the validation of the method as described under 3. Preparatory testing, (i) Assurance of criteria for the standard curve (positive controls).

Solution D = water for BET (negative control).

The test is considered valid when the following conditions are met:

- the absolute value of the correlation coefficient of the standard curve generated using solution C is greater than or equal to 0.980;
- the result with solution D does not exceed the limit of the blank value required in the description of the lysate reagent employed, or it is less than the endotoxin detection limit of the lysate reagent employed.

Calculate the mean recovery of the added endotoxin by subtracting the mean endotoxin concentration in the solution (if any) (solution A, Table 2.6.14.-4) from that in the solution containing the added endotoxin (solution B, Table 2.6.14.-4).

The test solution is considered free of interfering factors if under the conditions of the test, the measured concentration of the endotoxin added to the test solution is within 50-200 per cent of the known added endotoxin concentration, after subtraction of any endotoxin detected in the solution without added endotoxin.

When the endotoxin recovery is out of the specified range, the test solution is considered to contain interfering factors. Repeat the test using a greater dilution, not exceeding the MVD. Furthermore, interference of the test solution or diluted test solution not to exceed the MVD may be eliminated by suitable validated treatment, such as filtration, neutralisation, dialysis or heat treatment. To establish that the treatment chosen effectively eliminates interference without loss of endotoxins, repeat the test for interfering factors using the preparation being examined to which the standard endotoxin has been added and which has then been submitted to the chosen treatment.

4. TEST

(i) Procedure

Follow the procedure described in 3. Preparatory testing, (ii) Test for interfering factors.

(ii) Calculation

Calculate the endotoxin concentration of each replicate of solution A using the standard curve generated by the positive control solution C.

The test is considered valid when the following 3 requirements are met:

(1) the results obtained with solution C comply with the requirements for validation defined under 3. Preparatory testing, (i) Assurance of criteria for the standard curve,

(2) the endotoxin recovery, calculated from the endotoxin concentration found in solution B after subtracting the endotoxin concentration found in solution A, is within the range of 50-200 per cent,

(3) the result obtained with solution D (negative control) does not exceed the limit of the blank value required in the description of the lysate employed, or it is less than the endotoxin detection limit of the lysate reagent employed.

(iii) Interpretation

The preparation being examined complies with the test if the mean endotoxin concentration of the replicates of solution A, after correction for dilution and concentration, is less than the endotoxin limit for the product.

Guidelines on the test for bacterial endotoxins are given in general chapter 5.1.10.

01/2020:20901

2.9.1. DISINTEGRATION OF TABLETS AND CAPSULES[(1)]

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 long. For larger tablets or capsules use apparatus B.◆

TEST A - TABLETS AND CAPSULES OF NORMAL SIZE

Apparatus. The apparatus consists of a basket-rack assembly, a 1 L, low-form beaker, 149 ± 11 mm in height and having an inside diameter of 106 ± 9 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 55 ± 2 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 6 open-ended transparent tubes, each 77.5 ± 2.5 mm long and having an inside diameter of 21.85 ± 1.15 mm and a wall 1.9 ± 0.9 mm thick; the tubes are held in a vertical position by 2 plates, each 90 ± 2 mm in diameter and 6.75 ± 1.75 mm in thickness, with 6 holes, each 24 ± 2 mm in diameter, equidistant from the centre of the plate and equally spaced from one another. Attached to the under surface of the lower plate is a woven stainless steel wire cloth, which has a plain square weave with 2.0 ± 0.2 mm mesh apertures and with a wire diameter of 0.615 ± 0.045 mm. The parts of the apparatus are assembled and rigidly held by means of 3 bolts passing through the 2 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 2.9.1.-1.

Discs. The use of discs is permitted only where specified or allowed. Each tube is provided with a cylindrical disc 9.5 ± 0.15 mm thick and 20.7 ± 0.15 mm in diameter. The disc is made of a suitable, transparent plastic material having a specific gravity of 1.18-1.20. 5 parallel 2 ± 0.1 mm holes extend between the ends of the cylinder. One of the holes is centered on the cylindrical axis. The other holes are parallel to the cylindrical axis and centered 6 ± 0.2 mm from the axis on imaginary lines perpendicular to the axis and to each other, as defined in Figure 2.9.1.-1. 4 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 2 adjacent holes 6 mm from the cylindrical axis. The parallel side of the trapezoid on the bottom of the cylinder has a length of 1.6 ± 0.1 mm and its bottom edges lie at a depth of 1.5 mm to 1.8 mm from the cylinder's circumference. The parallel side of the trapezoid on the top of the cylinder has a length of 9.4 ± 0.2 mm and its centre lies at a depth of 2.6 ± 0.1 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 shown in Figure 2.9.1.-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 1 dosage unit in each of the 6 tubes of the basket and, if prescribed, add a disc. Operate the apparatus using the specified medium, maintained at 37 ± 2 °C, as the immersion fluid. 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 1 or 2 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 have disintegrated.

◆TEST B – LARGE TABLETS AND LARGE CAPSULES

Apparatus. The main part of the apparatus (Figure 2.9.1.-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 under side 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 L 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

(1) This chapter has undergone pharmacopoeial harmonisation. See chapter *5.8. Pharmacopoeial harmonisation*.

beaker containing the specified liquid. Operate the apparatus 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.◆

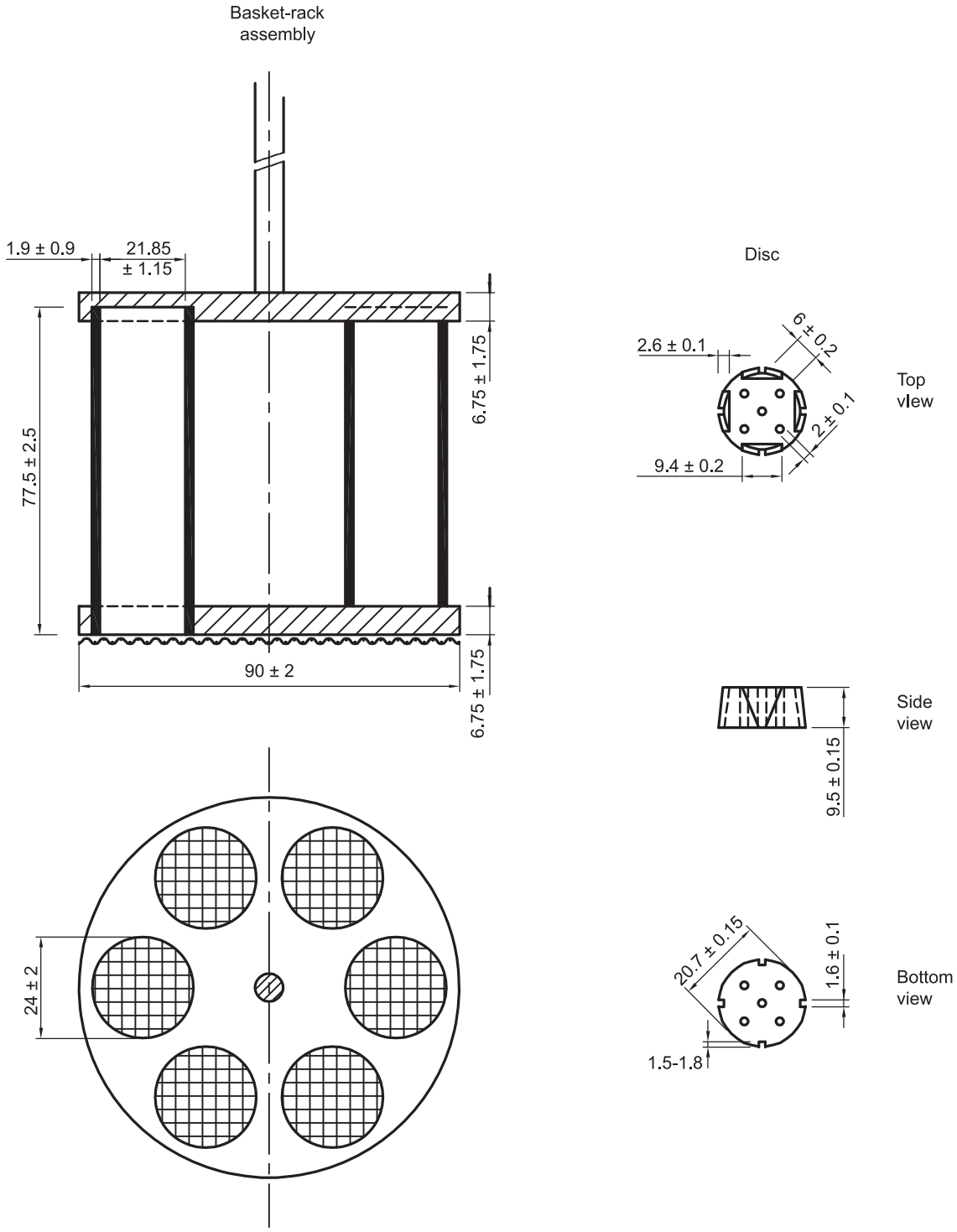

Figure 2.9.1.-1. – *Disintegration apparatus A*
Dimensions in millimetres

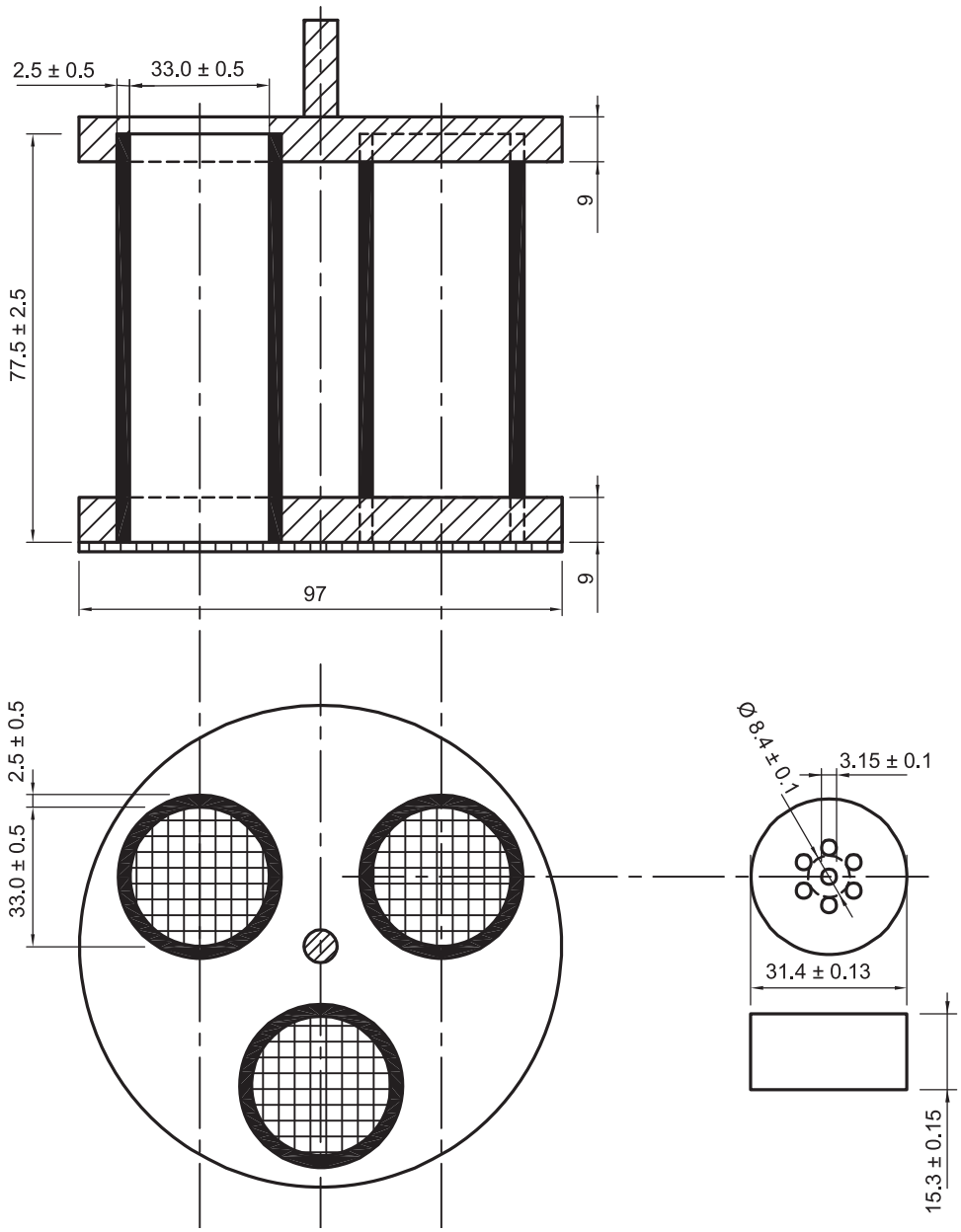

Figure 2.9.1.-2. – *Disintegration apparatus B*
Dimensions in millimetres

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2.9.2. DISINTEGRATION OF SUPPOSITORIES AND PESSARIES

The disintegration test determines whether the suppositories or pessaries soften or disintegrate within the prescribed time when placed in a liquid medium in the experimental conditions described below.

Disintegration is considered to be achieved when:

a) dissolution is complete,

b) the components of the suppository or pessary have separated: melted fatty substances collect on the surface of the liquid, insoluble powders fall to the bottom and soluble components dissolve, depending on the type of preparation, the components may be distributed in one or more of these ways,

c) there is softening of the sample that may be accompanied by appreciable change of shape without complete separation of the components, the softening is such that the suppository or pessary no longer has a solid core offering resistance to pressure of a glass rod,

d) rupture of the gelatin shell of rectal or vaginal capsules occurs allowing release of the contents,

e) no residue remains on the perforated disc or if a residue remains, it consists only of a soft or frothy mass having no solid core offering resistance to pressure of a glass rod (vaginal tablets).

Apparatus. The apparatus (Figure 2.9.2.-1) consists of a sleeve of glass or suitable transparent plastic, of appropriate thickness, to the interior of which is attached by means of three hooks a metal device consisting of two perforated stainless metal discs each containing 39 holes 4 mm in diameter; the diameter of the discs is similar to that of the interior of the sleeve; the discs are about 30 mm apart. The test is carried out using three such apparatuses each containing a single sample. Each apparatus is placed in a beaker with a capacity of at least 4 L filled with water maintained at 36-37 °C, unless otherwise prescribed. The apparatuses may also be placed together in a vessel with a capacity of at least 12 L. The beaker is fitted with a slow stirrer and a device that will hold the cylinders vertically not less than 90 mm below the surface of the water and allow them to be inverted without emerging from the water.

Method. Use three suppositories or pessaries. Place each one on the lower disc of a device, place the latter in the sleeve and secure. Invert the apparatuses every 10 min. Examine the samples after the period prescribed in the monograph. To pass the test all the samples must have disintegrated.

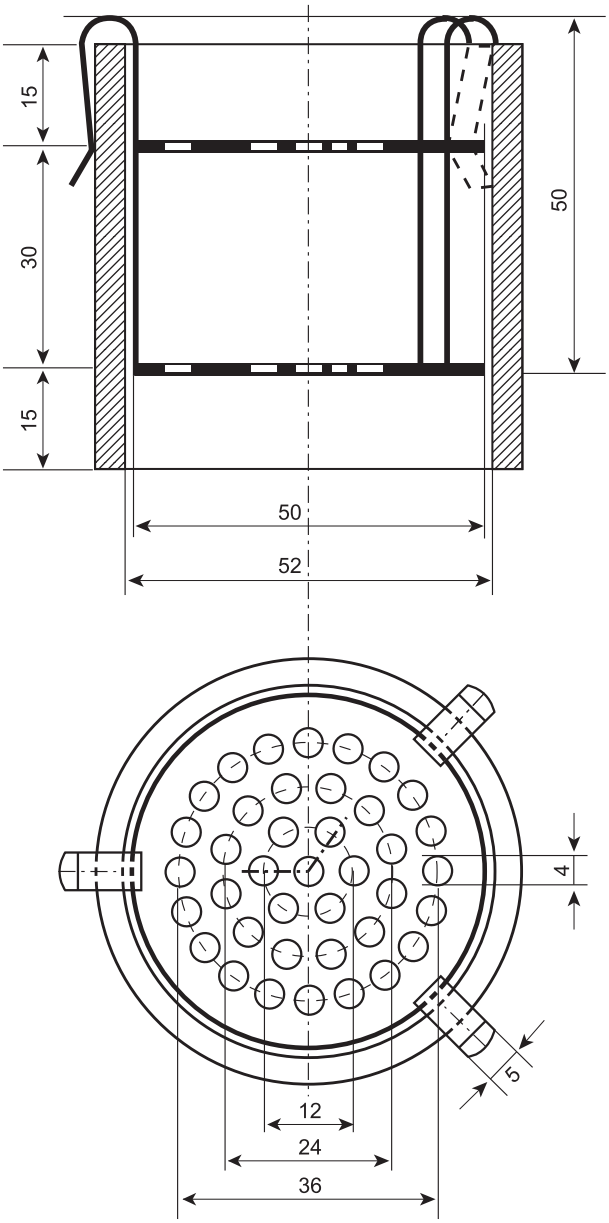
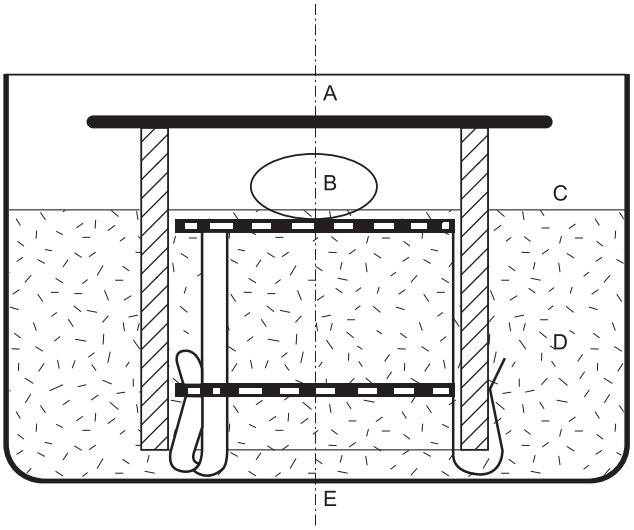


Figure 2.9.2.-1. – *Apparatus for disintegration of suppositories and pessaries*
Dimensions in millimetres

METHOD OF OPERATION FOR VAGINAL TABLETS

Use the apparatus described above, arranged so as to rest on the hooks (see Figure 2.9.2.-2). Place it in a beaker of suitable diameter containing water maintained at 36-37 °C with the level just below the upper perforated disc. Using a pipette, adjust the level with water at 36-37 °C until a uniform film covers the perforations of the disc. Use three vaginal tablets. Place each one on the upper plate of an apparatus and cover the latter with a glass plate to maintain appropriate conditions of humidity. Examine the state of the samples after the period prescribed in the monograph. To pass the test all the samples must have disintegrated.



A. glass plate
B. vaginal tablet
C. water surface
D. water
E. dish, beaker

Figure 2.9.2.-2.

01/2008:20905

2.9.5. UNIFORMITY OF MASS OF SINGLE-DOSE PREPARATIONS

Weigh individually 20 units taken at random or, for single-dose preparations presented in individual containers, the contents of 20 units, and determine the average mass. Not more than 2 of the individual masses deviate from the average mass by more than the percentage deviation shown in Table 2.9.5.-1 and none deviates by more than twice that percentage.

For capsules and powders for parenteral administration, proceed as described below.

Table 2.9.5.-1

Pharmaceutical Form	Average Mass	Percentage deviation
Tablets (uncoated and film-coated)	80 mg or less	10
	More than 80 mg and less than 250 mg	7.5
	250 mg or more	5
Capsules, granules (uncoated, single-dose) and powders (single-dose)	Less than 300 mg	10
	300 mg or more	7.5
Powders for parenteral administration* (single-dose)	More than 40 mg	10
Suppositories and pessaries	All masses	5
Powders for eye-drops and powders for eye lotions (single-dose)	Less than 300 mg	10
	300 mg or more	7.5

* When the average mass is equal to or below 40 mg, the preparation is not submitted to the test for uniformity of mass but to the test for uniformity of content of single-dose preparations (*2.9.6*).

CAPSULES

Weigh an intact capsule. Open the capsule without losing any part of the shell and remove the contents as completely as possible. For soft shell capsules, wash the shell with a suitable solvent and allow to stand until the odour of the solvent is no longer perceptible. Weigh the shell. The mass of the contents is the difference between the weighings. Repeat the procedure with another 19 capsules.

POWDERS FOR PARENTERAL ADMINISTRATION

Remove any paper labels from a container and wash and dry the outside. Open the container and without delay weigh the container and its contents. Empty the container as completely as possible by gentle tapping, rinse it if necessary with *water R* and then with *alcohol R* and dry at 100-105 °C for 1 h, or, if the nature of the container precludes heating at this temperature, dry at a lower temperature to constant mass. Allow to cool in a desiccator and weigh. The mass of the contents is the difference between the weighings. Repeat the procedure with another 19 containers.

01/2017:20906

2.9.6. UNIFORMITY OF CONTENT OF SINGLE-DOSE PREPARATIONS

The test for uniformity of content of single-dose preparations is based on the assay of the individual contents of active substance(s) of a number of single-dose units to determine whether the individual contents are within limits set with reference to the average content of the sample.

The test is not required for multivitamin and trace-element preparations and in other justified and authorised circumstances.

Method. Using a suitable analytical method, determine the individual contents of active substance(s) of 10 dosage units taken at random.

Apply the criteria of test A, test B or test C as specified in the monograph for the dosage form in question.

TEST A

The preparation complies with the test if each individual content is between 85 per cent and 115 per cent of the average content. The preparation fails to comply with the test if more than one individual content is outside these limits or if one individual content is outside the limits of 75 per cent to 125 per cent of the average content.

If one individual content is outside the limits of 85 per cent to 115 per cent but within the limits of 75 per cent to 125 per cent, determine the individual contents of another 20 dosage units taken at random. The preparation complies with the test if not more than one of the individual contents of the 30 units is outside 85 per cent to 115 per cent of the average content and none is outside the limits of 75 per cent to 125 per cent of the average content.

TEST B

The preparation complies with the test if not more than one individual content is outside the limits of 85 per cent to 115 per cent of the average content and none is outside the limits of 75 per cent to 125 per cent of the average content. The preparation fails to comply with the test if more than 3 individual contents are outside the limits of 85 per cent to 115 per cent of the average content or if one or more individual contents are outside the limits of 75 per cent to 125 per cent of the average content.

If 2 or 3 individual contents are outside the limits of 85 per cent to 115 per cent but within the limits of 75 per cent to 125 per cent, determine the individual contents of another 20 dosage units taken at random. The preparation complies with the test if not more than 3 individual contents of the 30 units are outside the limits of 85 per cent to 115 per cent of the average content and none is outside the limits of 75 per cent to 125 per cent of the average content.

TEST C

The preparation complies with the test if the average content of the 10 dosage units is between 90 per cent and 110 per cent of the content stated on the label and if the individual content of each dosage unit is between 75 per cent and 125 per cent of the average content.

01/2008:20912

2.9.12. SIEVE TEST

The degree of fineness of a powder may be expressed by reference to sieves that comply with the specifications for non-analytical sieves (*2.1.4*).

Where the degree of fineness of powders is determined by sieving, it is defined in relation to the sieve number(s) used either by means of the following terms or, where such terms cannot be used, by expressing the fineness of the powder as a percentage *m/m* passing the sieve(s) used.

The following terms are used in the description of powders:

Coarse powder. Not less than 95 per cent by mass passes through a number 1400 sieve and not more than 40 per cent by mass passes through a number 355 sieve.

Moderately fine powder. Not less than 95 per cent by mass passes through a number 355 sieve and not more than 40 per cent by mass passes through a number 180 sieve.

Fine powder. Not less than 95 per cent by mass passes through a number 180 sieve and not more than 40 per cent by mass passes through a number 125 sieve.

Very fine powder. Not less than 95 per cent by mass passes through a number 125 sieve and not more than 40 per cent by mass passes through a number 90 sieve.

If a single sieve number is given, not less than 97 per cent of the powder passes through the sieve of that number, unless otherwise prescribed.

Assemble the sieves and operate in a suitable manner until sifting is practically complete. Weigh the separated fractions of the powder.

04/2010:20917

2.9.17. TEST FOR EXTRACTABLE VOLUME OF PARENTERAL PREPARATIONS[(1)]

Suspensions and emulsions are 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 20-25 °C before measuring the volume.

SINGLE-DOSE CONTAINERS

Select 1 container if the nominal volume is 10 mL or more, 3 containers if the nominal volume is more than 3 mL and less than 10 mL, or 5 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 3 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 standardised 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 per cent 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 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 1 container if the nominal volume is 10 mL or more, 3 containers if the nominal volume is more than 3 mL and less than 10 mL, or 5 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 per cent of the nominal volume of the cylinder. Measure the volume transferred.

The volume is not less than the nominal volume.

(1) This chapter has undergone pharmacopoeial harmonisation. See chapter *5.8. Pharmacopoeial harmonisation.*

01/2008:20918

2.9.18. PREPARATIONS FOR INHALATION: AERODYNAMIC ASSESSMENT OF FINE PARTICLES

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

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

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

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

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

APPARATUS A - GLASS IMPINGER

The apparatus is shown in Figure 2.9.18.-1 (see also Table 2.9.18.-1).

Procedure for nebulisers

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

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

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

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

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

Procedure for pressurised inhalers

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

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

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

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

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

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

Procedure for powder inhalers

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

Connect all the component parts. Ensure that the assembly is vertical and adequately supported and that the jet-spacer peg of the lower jet assembly just touches the bottom of the lower impingement chamber. Without the inhaler in place, connect a suitable pump to the outlet of the apparatus. Adjust the air flow through the apparatus, as measured at the inlet to the throat, to 60 ± 5 L/min.

Prepare the inhaler for use and locate the mouthpiece in the apparatus by means of a suitable adapter. Switch on the pump for 5 s. Switch off the pump and remove the inhaler. Repeat the discharge sequence. The number of discharges should be minimised and typically would not be greater than 10. Dismantle the apparatus.

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

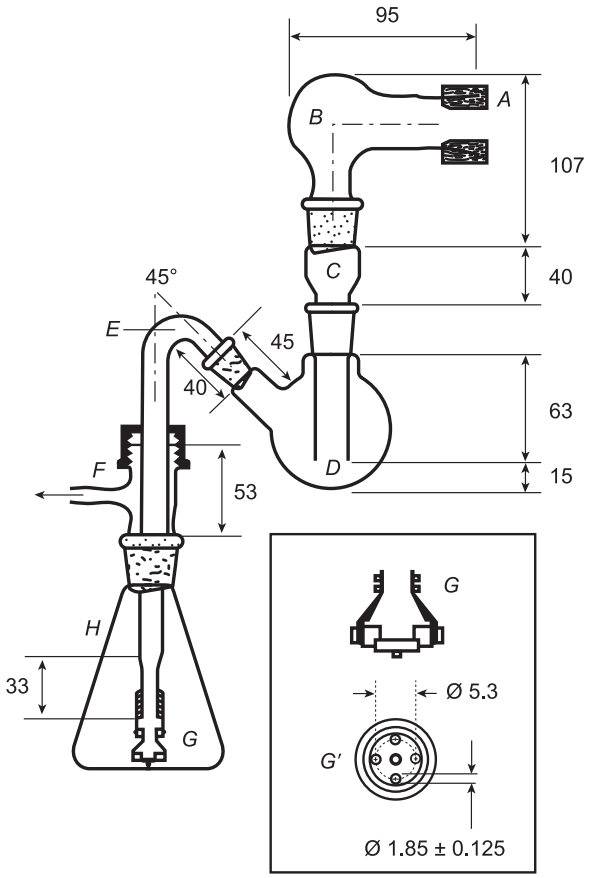


Figure 2.9.18.-1. – *Apparatus A: glass impinger*

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

Table 2.9.18.-1. – *Component specification for apparatus A in Figure 2.9.18.-1*

Code	Item	Description	Dimensions*
A	Mouthpiece adaptor	Moulded rubber adapter for actuator mouthpiece.	
B	Throat	Modified round-bottomed flask:	50 mL
		– *ground-glass inlet socket*	29/32
		– *ground-glass outlet cone*	24/29
C	Neck	Modified glass adapter:	
		– *ground-glass inlet socket*	24/29
		– *ground-glass outlet cone*	24/29
		Lower outlet section of precision-bore glass tubing:	
		– *bore diameter*	14
		Selected bore light-wall glass tubing:	
		– *external diameter*	17
D	Upper impingement chamber	Modified round-bottomed flask	100 mL
		– *ground-glass inlet socket*	24/29
		– *ground-glass outlet cone*	24/29
E	Coupling tube	Medium-wall glass tubing:	
		– *ground-glass cone*	14/23
		Bent section and upper vertical section:	
		– *external diameter*	13
		Lower vertical section:	
		– *external diameter*	8
F	Screwthread, side-arm adaptor	Plastic screw cap	28/13
		Silicone rubber ring	28/11
		PTFE washer	28/11
		Glass screwthread:	
		– *thread size*	28
		Side-arm outlet to vacuum pump:	
		– *minimum bore diameter*	5
G	Lower jet assembly	Modified polypropylene filter holder connected to lower vertical section of coupling tube by PTFE tubing.	see Figure 2.9.18.-1
		Acetal circular disc with the centres of four jets arranged on a projected circle of diameter 5.3 mm with an integral jet spacer peg:	10
		– *peg diameter*	2
		– *peg protrusion*	2
H	Lower impingement chamber	Conical flask	250 mL
		– *ground-glass inlet socket*	24/29

* Dimensions in millimetres, unless otherwise stated.

Fine particle dose and particle size distribution

APPARATUS C - MULTI-STAGE LIQUID IMPINGER

The multi-stage liquid impinger consists of impaction stages 1 (pre-separator), 2, 3 and 4 and an integral filter stage (stage 5), see Figures 2.9.18.-4/6. An impaction stage comprises an upper horizontal metal partition wall (B) through which a metal inlet jet tube (A) with its impaction plate (D) is protruding. A glass cylinder (E) with sampling port (F) forms the vertical wall of the stage, and a lower horizontal metal partition wall (G) through which the tube (H) connects to the next lower stage. The tube into stage 4 (U) ends in a multi-jet arrangement. The impaction plate (D) is secured in a metal frame (J) which is fastened by 2 wires (K) to a sleeve (L) secured on the jet tube. The horizontal face of the collection plate is perpendicular to the axis of the jet tube and centrally aligned. The upper surface of the impaction plate is slightly raised above the edge of the metal frame. A recess around the perimeter of the horizontal partition wall guides the position of the glass cylinder. The glass cylinders are sealed against the horizontal partition walls with gaskets (M) and clamped together by 6 bolts (N). The sampling ports are sealed by stoppers. The bottom-side of the lower partition wall of stage 4 has a concentrical protrusion fitted with a rubber O-ring (P) which seals against the edge of a filter placed in the filter holder. The filter holder (R) is constructed as a basin with a concentrical recess in which a perforated filter support (S) is flush-fitted. The filter holder is dimensioned for 76 mm diameter filters. The assembly of impaction stages is clamped onto the filter holder by 2 snap-locks (T). Connect an induction port (see Figure 2.9.18.-7) onto the stage 1 inlet

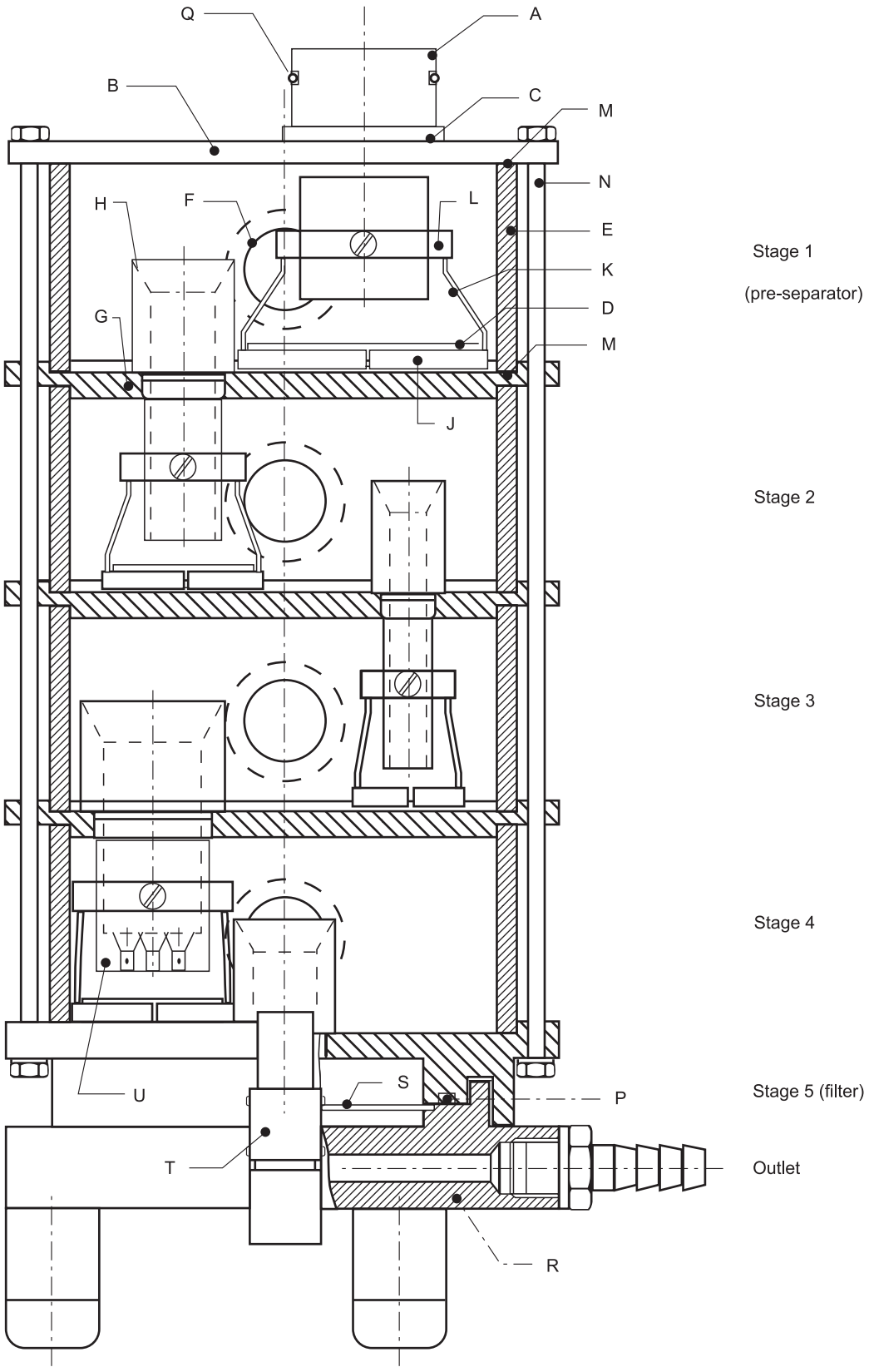


Figure 2.9.18.-4. – *Apparatus C: multi-stage liquid impinger*

jet tube of the impinger. A rubber O-ring on the jet tube provides an airtight connection to the induction port. A suitable mouthpiece adapter is used to provide an airtight seal between the inhaler and the induction port. The front face of the inhaler mouthpiece must be flush with the front face of the induction port.

Procedure for pressurised inhalers

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

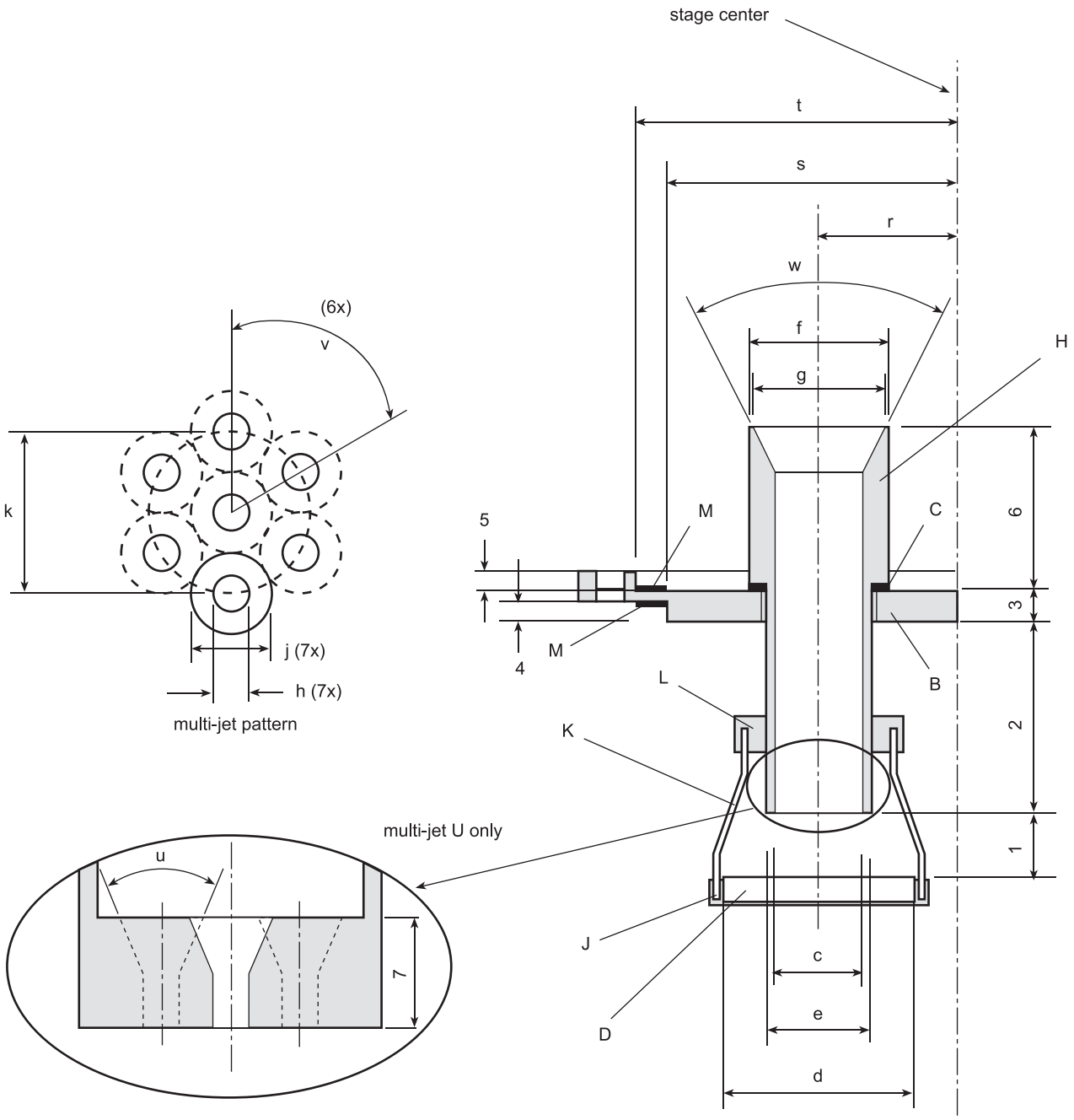


Figure 2.9.18.-5. – *Apparatus C: details of jet tube and impaction plate. Inserts show end of multi-jet tube U leading to stage 4. (Numbers and lowercase letters refer to Table 2.9.18.-3 and uppercase letters refer to Figure 2.9.18.-4).*

and adjust the air flow through the apparatus, as measured at the inlet to the induction port, to 30 L/min (± 5 per cent). Switch off the pump.

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

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

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

Calculate the fine particle dose (see Calculations).

Table 2.9.18.-2. – *Component specification for apparatus C in Figures 2.9.18.-4/6*

Code*	Item	Description	Dimensions**
A,H	Jet tube	Metal tube screwed onto partition wall sealed by gasket (C), polished inner surface	see Figure 2.9.18.-5
B,G	Partition wall	Circular metal plate	
		– *diameter*	120
		– *thickness*	see Figure 2.9.18.-5
C	Gasket	e.g. PTFE	to fit jet tube
D	Impaction plate	Porosity 0 sintered-glass disk	
		– *diameter*	see Figure 2.9.18.-5
E	Glass cylinder	Plane polished cut glass tube	
		– *height, including gaskets*	46
		– *outer diameter*	100
		– *wall thickness*	3.5
		– *sampling port (F) diameter*	18
		– *stopper in sampling port*	ISO 24/25
J	Metal frame	L-profiled circular frame with slit	
		– *inner diameter*	to fit impaction plate
		– *height*	4
		– *thickness of horizontal section*	0.5
		– *thickness of vertical section*	2
K	Wire	Steel wire interconnecting metal frame and sleeve (2 for each frame)	
		– *diameter*	1
L	Sleeve	Metal sleeve secured on jet tube by screw	
		– *inner diameter*	to fit jet tube
		– *height*	6
		– *thickness*	5
M	Gasket	e.g. silicone	to fit glass cylinder
N	Bolt	Metal bolt with nut (6 pairs)	
		– *length*	205
		– *diameter*	4
P	O-ring	Rubber O-ring	
		– *diameter × thickness*	66.34 × 2.62
Q	O-ring	Rubber O-ring	
		– *diameter × thickness*	29.1 × 1.6
R	Filter holder	Metal housing with stand and outlet	see Figure 2.9.18.-6
S	Filter support	Perforated sheet metal	
		– *diameter*	65
		– *hole diameter*	3
		– *distance between holes (centre-points)*	4
T	Snap-locks		
U	Multi-jet tube	Jet tube (H) ending in multi-jet arrangement.	see inserts Figure 2.9.18.-5

* Refers to Figure 2.9.18.-4.

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

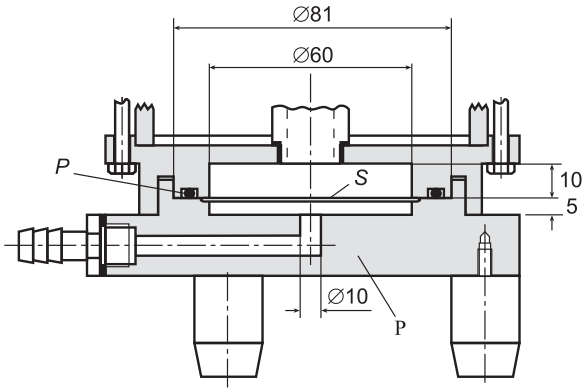


Figure 2.9.18.-6. – *Apparatus C: details of the filter stage (stage 5). Numbers refer to dimensions (Ø = diameter). Uppercase letters refer to Table 2.9.18.-2.*

Dimensions in millimetres unless otherwise stated

Table 2.9.18.-3. – *Dimensions[(1)] of jet tube with impaction plate of apparatus C*

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

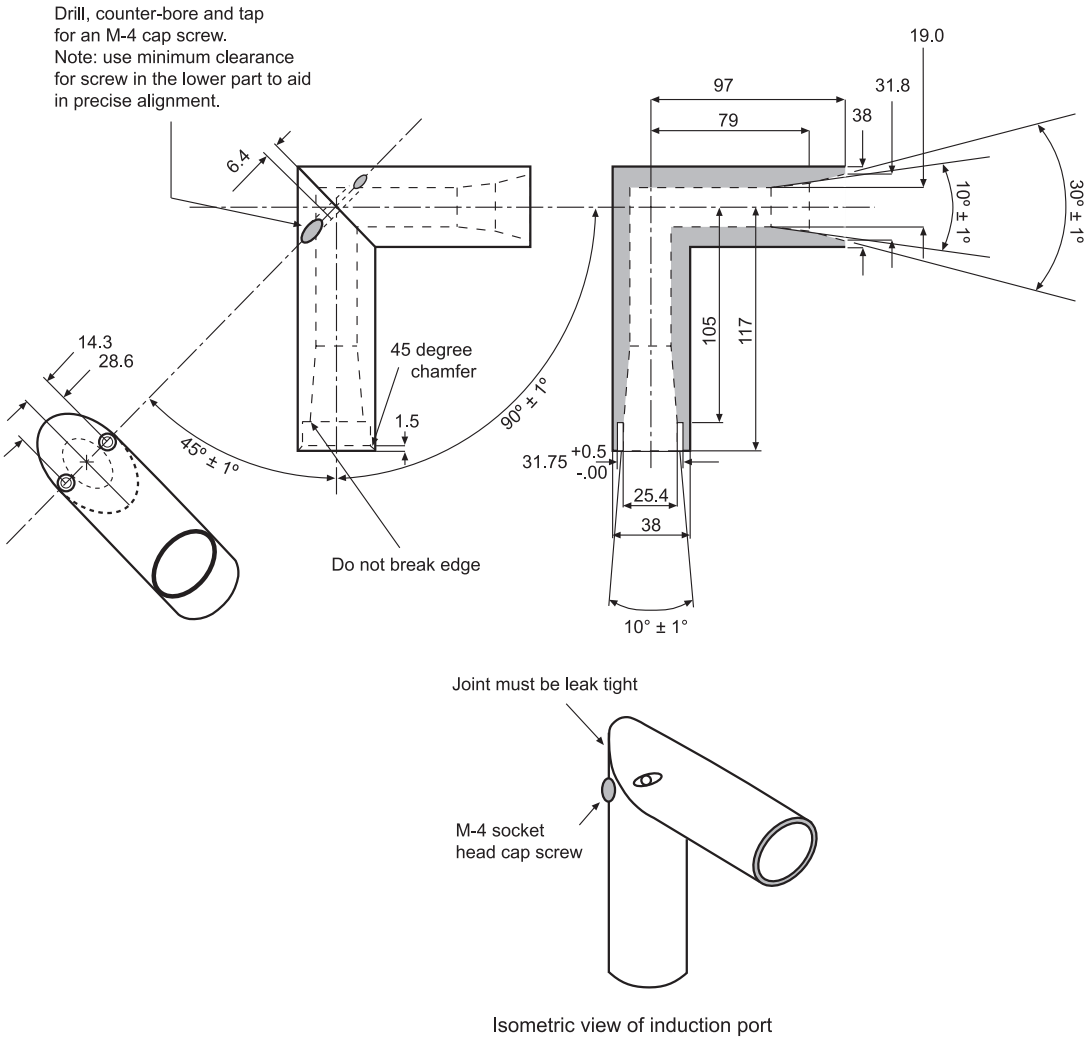
(1) Measures in millimetres with tolerances according to ISO 2768-m unless otherwise stated

(2) Refer to Figure 2.9.18.-5

(3) Including gasket

(4) Relative centreline of stage compartment

n.a. = not applicable



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

Figure 2.9.18.-7. – *Induction port*

Dimensions in millimetres unless otherwise stated

Procedure for powder inhalers

Place a suitable low resistance filter capable of quantitatively collecting the active substance in stage 5 and assemble the apparatus. Connect the apparatus to a flow system according to the scheme specified in Figure 2.9.18.-8 and Table 2.9.18.-4. Unless otherwise defined, conduct the test at the flow rate, Q_{out}, used in the test for uniformity of delivered dose, drawing 4 L of air from the mouthpiece of the inhaler and through the apparatus.

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

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

P_0 = atmospheric pressure,

ΔP = pressure drop over the meter.

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

With the inhaler in place and the test flow rate established, measure the absolute pressure on both sides of the control valve (pressure reading points P2 and P3 in Figure 2.9.18.-8). A ratio P3/P2 of less than or equal to 0.5 indicates critical flow. Switch to a more powerful pump and re-measure the test flow rate if critical flow is not indicated.

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

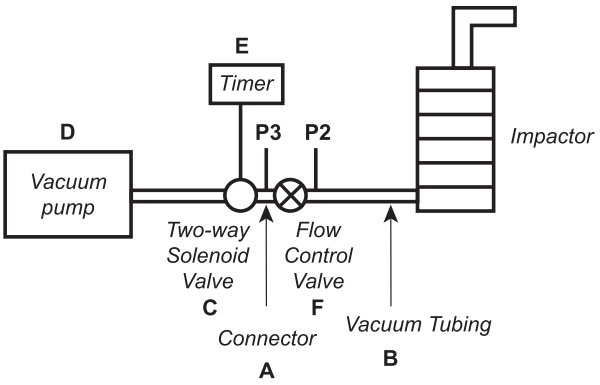


Figure 2.9.18.-8. – *Experimental set-up for testing powder inhalers*

Table 2.9.18.-4. – *Component specification for Figure 2.9.18.-8*

Code	Item	Description
A	Connector	ID ≥ 8 mm, e.g., short metal coupling, with low-diameter branch to P3.
B	Vacuum tubing	A length of suitable tubing having an ID ≥ 8 mm and an internal volume of 25 ± 5 mL.
C	2-way solenoid valve	A 2-way, 2-port solenoid valve having a minimum airflow resistance orifice with ID ≥ 8 mm and an opening time ≤ 100 ms. (e.g. type 256-A08, Bürkert GmbH, D-74653 Ingelfingen), or equivalent.
D	Vacuum pump	Pump must be capable of drawing the required flow rate through the assembled apparatus with the powder inhaler in the mouthpiece adapter (e.g. product type 1023, 1423 or 2565, Gast Manufacturing Inc., Benton Harbor, MI 49022), or equivalent. Connect the pump to the 2-way solenoid valve using short and/or wide (ID ≥ 10 mm) vacuum tubing and connectors to minimise pump capacity requirements.
E	Timer	Timer capable to drive the 2-way solenoid valve for the required duration (e.g. type G814, RS Components International, Corby, NN17 9RS, UK), or equivalent.
P2 P3	Pressure measurements	Determine under steady-state flow condition with an absolute pressure transducer.
F	Flow control valve	Adjustable regulating valve with maximum $C_v \geq 1$, (e.g. type 8FV12LNSS, Parker Hannifin plc., Barnstaple, EX31 1NP, UK), or equivalent.

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

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

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

Calculate the fine particle dose (see Calculations).

APPARATUS D - ANDERSEN CASCADE IMPACTOR

The Andersen 1 ACFM non-viable cascade impactor consists of 8 stages together with a final filter. Material of construction may be aluminium, stainless steel or other suitable material. The stages are clamped together and sealed with O-rings. Critical dimensions applied by the manufacturer of apparatus D are provided in Table 2.9.18.-5. In use, some occlusion and wear of holes will occur. In-use mensuration tolerances need to be justified. In the configuration used for pressurised inhalers (Figure 2.9.18.-9) the entry cone of the impactor is connected to an induction port (see Figure 2.9.18.-7). A suitable mouthpiece adapter is used to provide an airtight seal between the inhaler and the induction port. The front face of the inhaler mouthpiece must be flush with the front face of the induction port.

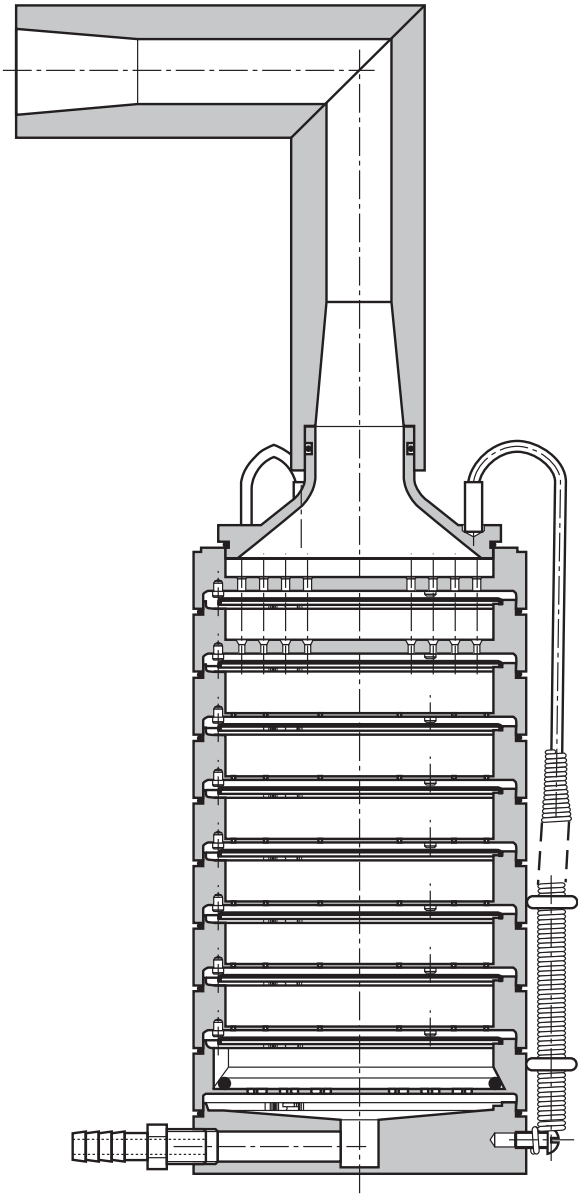


Figure 2.9.18.-9. – *Apparatus D: Andersen cascade impactor used for pressurised inhalers*

In the configuration for powder inhalers, a pre-separator is placed above the top stage to collect large masses of non-respirable powder. It is connected to the induction port as shown in Figure 2.9.18.-10. To accommodate high flow rates through the impactor, the outlet nipple, used to connect the impactor to the vacuum system is enlarged to have an internal diameter of greater than or equal to 8 mm.

Table 2.9.18.-5. – *Critical dimensions for apparatus D*

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

Procedure for pressurised inhalers

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

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

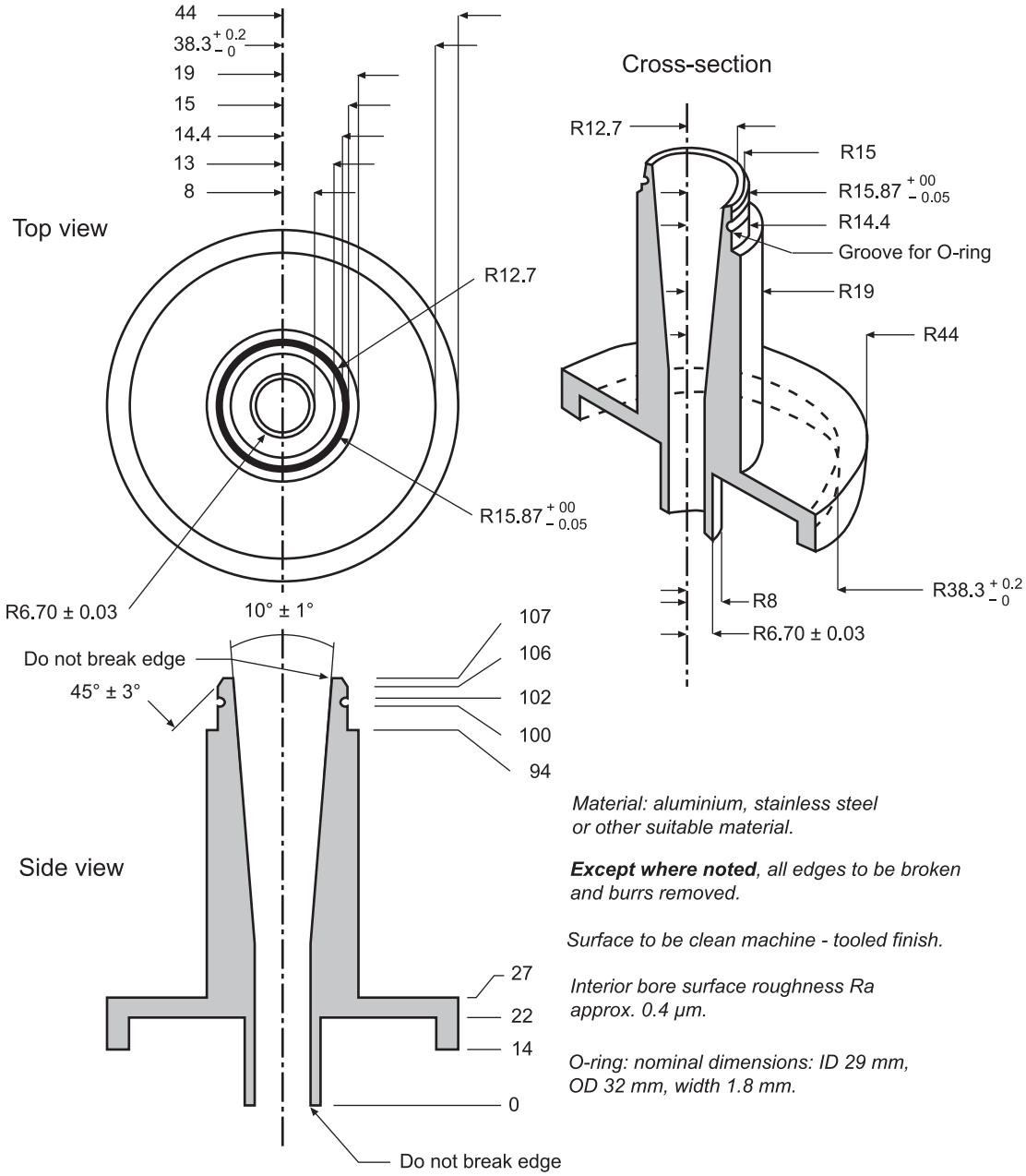


Figure 2.9.18.-10. – *Connection of the induction port to the preseparator of the Andersen cascade impactor*
Dimensions in millimetres unless otherwise stated

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

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

Calculate the fine particle dose (see Calculations).

Procedure for powder inhalers

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

Assemble the Andersen impactor with the pre-separator and a suitable filter in place and ensure that the system is airtight. Depending on the product characteristics, the pre-separator may be omitted, where justified and authorised. Stages 6 and 7 may also be omitted at high flow rates, if justified. The pre-separator may be coated in the same way as the plates or may contain 10 mL of a suitable solvent. Connect the apparatus to a flow system according to the scheme specified in Figure 2.9.18.-8 and Table 2.9.18.-4.

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

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

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

P_0 = atmospheric pressure,

ΔP = pressure drop over the meter.

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

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

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

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

Calculate the fine particle dose (see Calculations).

APPARATUS E

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

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

The impactor configuration has removable impaction cups with all the cups in one plane (Figures 2.9.18.-11/14). There are 3 main sections to the impactor; the bottom frame that holds the impaction cups, the seal body that holds the jets and the lid that contains the interstage passageways (Figures 2.9.18.-11/12). Multiple nozzles are used at all but the first stage (Figure 2.9.18.-13). The flow passes through the impactor in a saw-tooth pattern.

Critical dimensions are provided in Table 2.9.18.-6.

Table 2.9.18.-6. – *Critical dimensions for apparatus E*

Description	Dimension (mm)
Pre-separator (dimension a - see Figure 2.9.18.-15)	12.8 ± 0.05
Stage 1* Nozzle diameter	14.3 ± 0.05
Stage 2* Nozzle diameter	4.88 ± 0.04
Stage 3* Nozzle diameter	2.185 ± 0.02
Stage 4* Nozzle diameter	1.207 ± 0.01
Stage 5* Nozzle diameter	0.608 ± 0.01
Stage 6* Nozzle diameter	0.323 ± 0.01
Stage 7* Nozzle diameter	0.206 ± 0.01
MOC*	approx. 0.070
Cup depth (dimension b - see Figure 2.9.18.-14)	14.625 ± 0.10
Collection cup surface roughness (Ra)	0.5 - 2 µm
Stage 1 nozzle to seal body distance** - dimension c	0 ± 1.18
Stage 2 nozzle to seal body distance** - dimension c	5.236 ± 0.736
Stage 3 nozzle to seal body distance** - dimension c	8.445 ± 0.410
Stage 4 nozzle to seal body distance** - dimension c	11.379 ± 0.237
Stage 5 nozzle to seal body distance** - dimension c	13.176 ± 0.341
Stage 6 nozzle to seal body distance** - dimension c	13.999 ± 0.071
Stage 7 nozzle to seal body distance** - dimension c	14.000 ± 0.071
MOC nozzle to seal body distance** - dimension c	14.429 to 14.571

* See Figure 2.9.18.-13
** See Figure 2.9.18.-14

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

An induction port with internal dimensions (relevant to the airflow path) defined in Figure 2.9.18.-7 connects to the impactor inlet. A pre-separator can be added when required, typically with powder inhalers, and connects between the induction port and the impactor. A suitable mouthpiece adapter is used to provide an airtight seal between the inhaler and the induction port.

Apparatus E contains a terminal Micro-Orifice Collector (MOC) that for most formulations will eliminate the need for a final filter as determined by method validation. The MOC is an impactor plate with nominally 4032 holes, each approximately 70 µm in diameter. Most particles not captured on stage 7 of the impactor will be captured on the cup surface below the MOC. For impactors operated at 60 L/min, the MOC is capable of collecting 80 per cent of 0.14 µm particles. For formulations with a significant fraction of

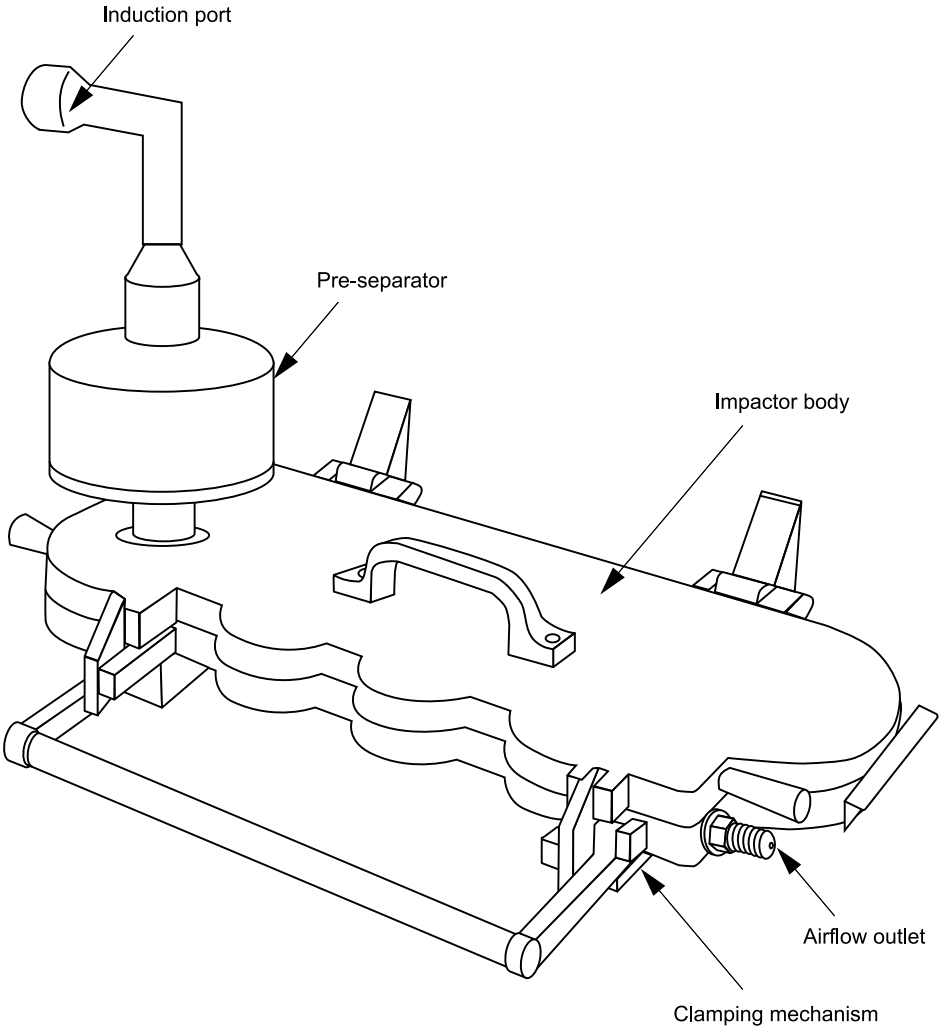


Figure 2.9.18.-11. – *Apparatus E (shown with the pre-separator in place)*

particles not captured by the MOC, there is an optional filter holder that can replace the MOC or be placed downstream of the MOC (a glass fibre filter is suitable).

Procedure for pressurised inhalers

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

Connect an induction port with internal dimensions defined in Figure 2.9.18.-7 to the impactor inlet. Place a suitable mouthpiece adapter in position at the end of the induction port so that the mouthpiece end of the actuator, when inserted, lines up along the horizontal axis of the induction port. The front face of the inhaler mouthpiece must be flush with the front face of the induction port. When attached to the mouthpiece adapter, the inhaler is positioned in the same orientation as intended for use. Connect a suitable pump to the outlet of the apparatus and adjust the air flow through the apparatus, as measured at the inlet to the induction port, to 30 L/min (± 5 per cent). Switch off the pump.

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

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

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

Calculate the fine particle dose (see Calculations).

Procedure for powder inhalers

Assemble the apparatus with the pre-separator (Figure 2.9.18.-15). Depending on the product characteristics, the pre-separator may be omitted, where justified.

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

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

Connect an induction port with internal dimensions defined in Figure 2.9.18.-7 to the impactor inlet or pre-separator inlet. Place a suitable mouthpiece adapter in position at the

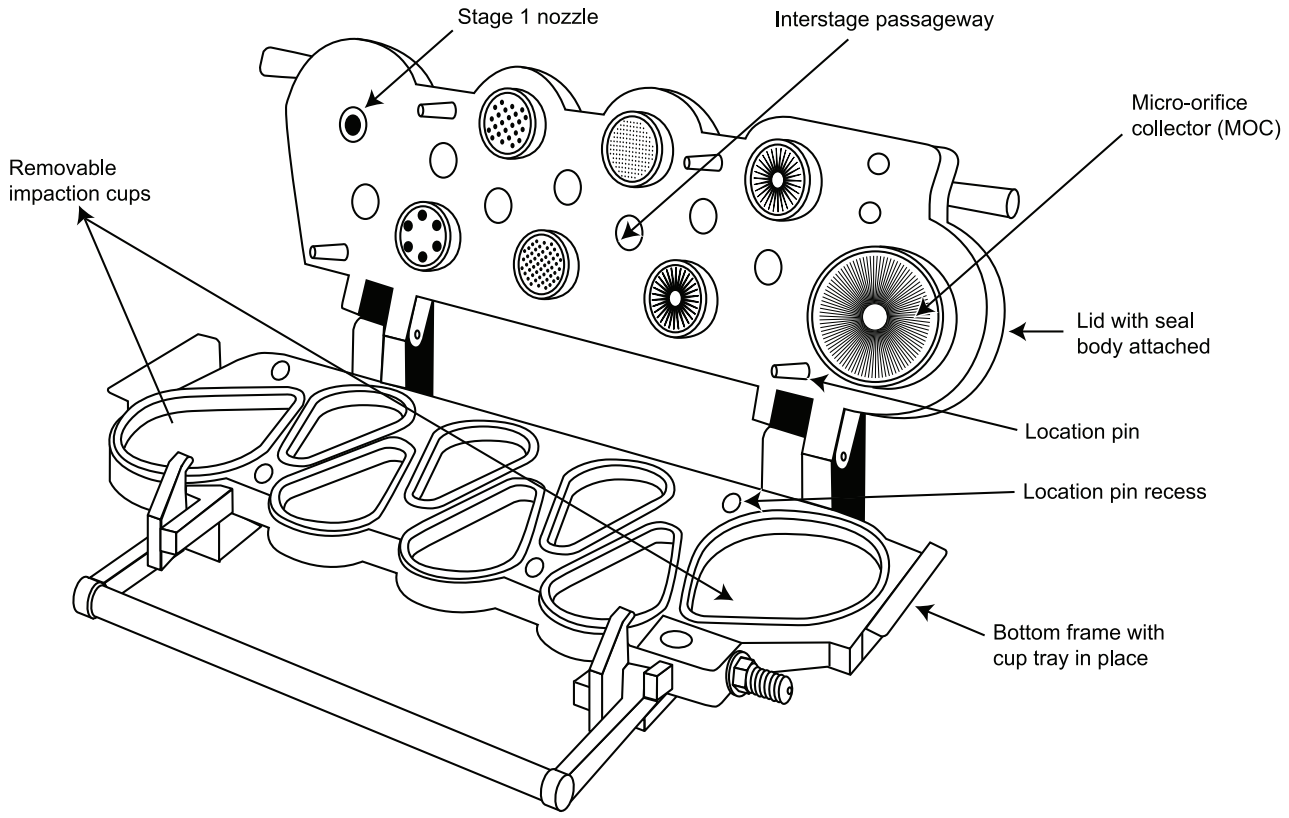


Figure 2.9.18.-12. – *Apparatus E showing component parts*

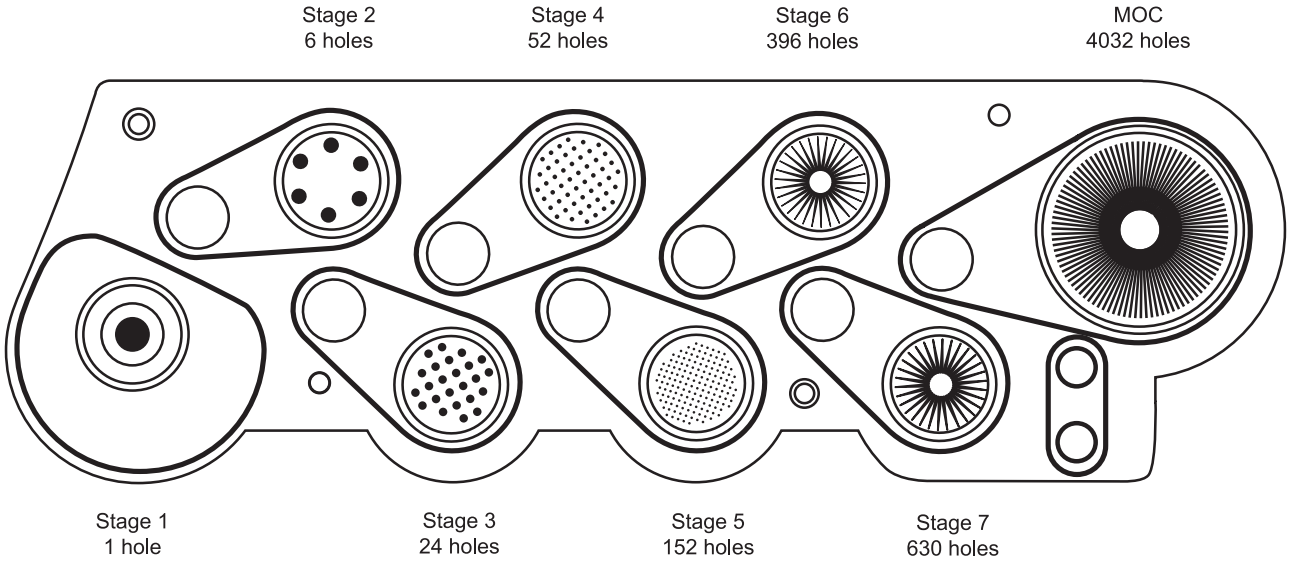


Figure 2.9.18.-13. – *Apparatus E: nozzle configuration*

end of the induction port so that the mouthpiece end of the inhaler, when inserted, lines up along the horizontal axis of the induction port. The front face of the inhaler mouthpiece must be flush with the front face of the induction port. When attached to the mouthpiece adapter, the inhaler is positioned in the same orientation as intended for use. Connect the apparatus to a flow system according to the scheme specified in Figure 2.9.18.-8 and Table 2.9.18.-4.

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

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

P_0 = atmospheric pressure,

ΔP = pressure drop over the meter.

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

Prepare the powder inhaler for use according to the patient instructions. With the pump running and the 2-way solenoid valve closed, locate the mouthpiece of the inhaler in the mouthpiece adapter. Discharge the powder into the apparatus by opening the valve for the required time, T (± 5 per cent). Repeat the discharge sequence. The number of discharges

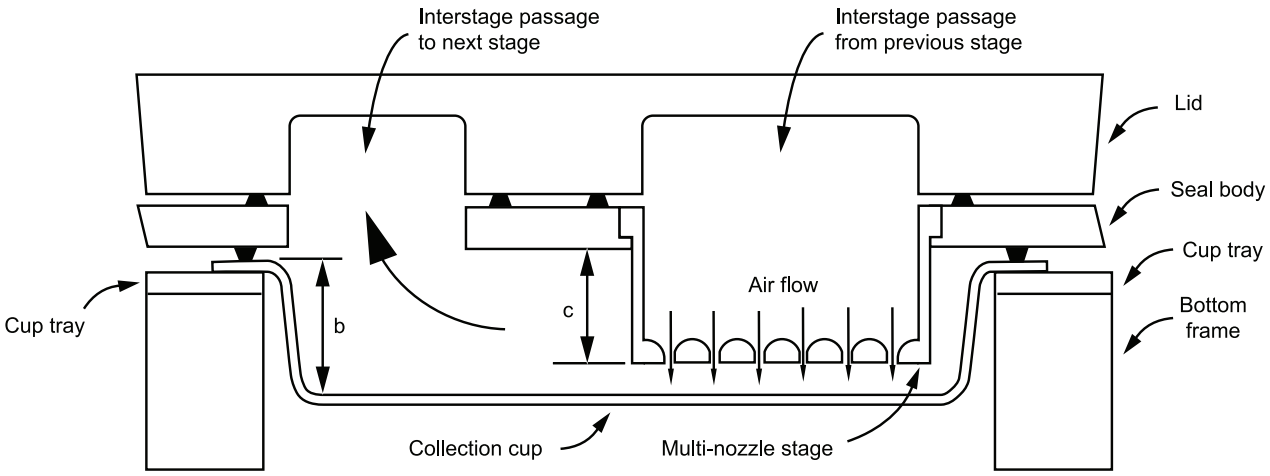


Figure 2.9.18.-14. – *Apparatus E: configuration of interstage passageways*

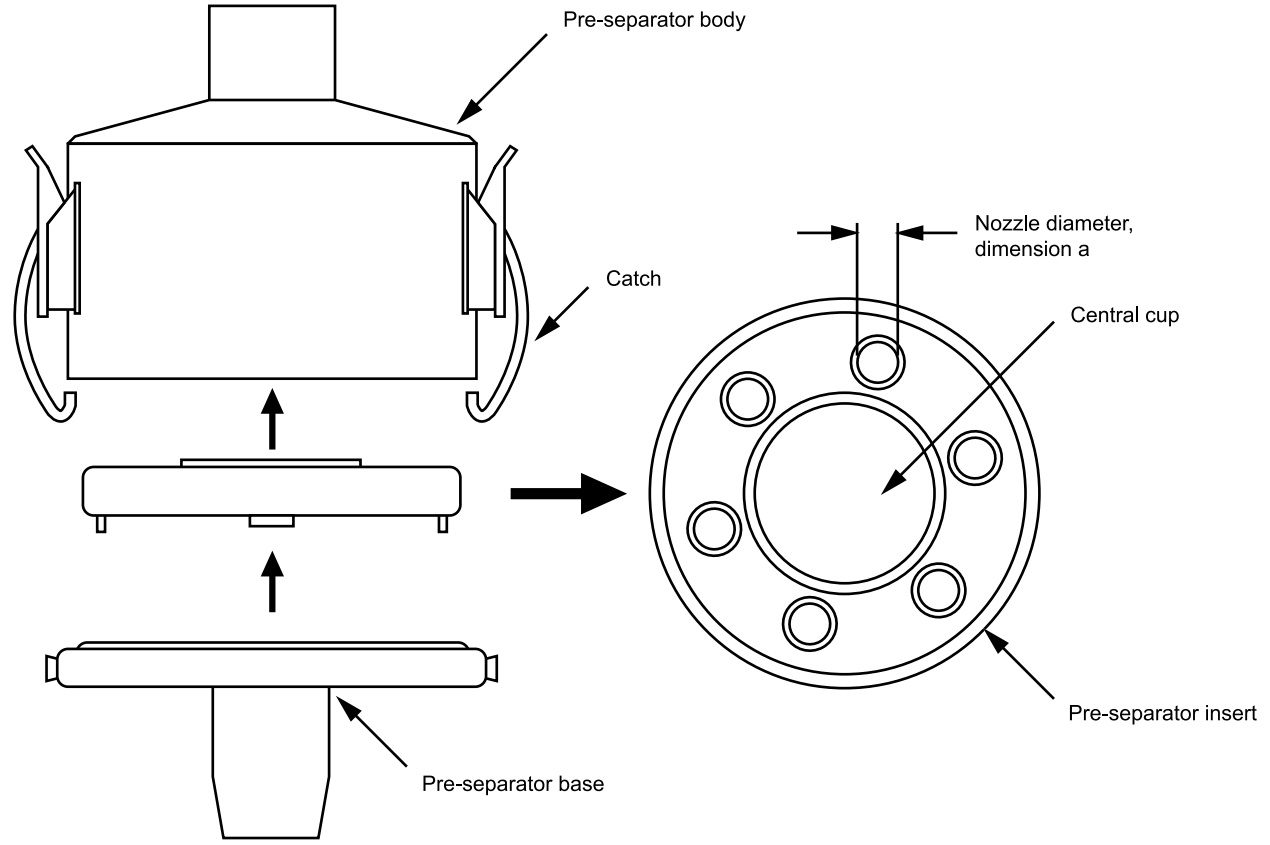


Figure 2.9.18.-15. – *Apparatus E: pre-separator configuration*

should be minimised and typically would not be greater than 10. The number of discharges is sufficient to ensure an accurate and precise determination of fine particle dose.

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

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

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

Calculate the fine particle dose (see Calculations).

CALCULATIONS

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

Starting at the final collection site (filter or MOC), derive a table of cumulative mass versus cut-off diameter of the respective stage (see Tables 2.9.18.-7 for Apparatus C, 2.9.18.-8 for Apparatus D, 2.9.18.-9 for Apparatus E). Calculate by interpolation the mass of the active substance less than 5 µm. This is the Fine Particle Dose (FPD).

If necessary, and where appropriate (e.g., where there is a log-normal distribution), plot the cumulative fraction of active substance versus cut-off diameter (see Tables 2.9.18.-7/9) on log probability paper, and use this plot to determine values

Table 2.9.18.-7. – *Calculations for Apparatus C. Use* $q = \sqrt{(60 / Q)}$*, where Q is the test flow rate in litres per minute (*Q_{out} *for powder inhalers)*

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

Table 2.9.18.-8. – *Calculations for Apparatus D when used at a flow rate of 28.3 L/min*

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

Table 2.9.18.-9. – *Calculations for Apparatus E. Use* $q = (60/Q)^x$*, where Q is the test flow rate in litres per minute, and x is listed in the table*

Cut-off diameter (µm)	x	Mass of active substance deposited per discharge	Cumulative mass of active substance deposited per discharge	Cumulative fraction of active substance (per cent)
$d_7 = 0.34 \times q$	0.67	mass from MOC or terminal filter, m_8	$c_7 = m_8$	$F_7 = (c_7/c) \times 100$
$d_6 = 0.55 \times q$	0.60	mass from stage 7, m_7	$c_6 = c_7 + m_7$	$F_6 = (c_6/c) \times 100$
$d_5 = 0.94 \times q$	0.53	mass from stage 6, m_6	$c_5 = c_6 + m_6$	$F_5 = (c_5/c) \times 100$
$d_4 = 1.66 \times q$	0.47	mass from stage 5, m_5	$c_4 = c_5 + m_5$	$F_4 = (c_4/c) \times 100$
$d_3 = 2.82 \times q$	0.50	mass from stage 4, m_4	$c_3 = c_4 + m_4$	$F_3 = (c_3/c) \times 100$
$d_2 = 4.46 \times q$	0.52	mass from stage 3, m_3	$c_2 = c_3 + m_3$	$F_2 = (c_2/c) \times 100$
$d_1 = 8.06 \times q$	0.54	mass from stage 2, m_2	$c_1 = c_2 + m_2$	$F_1 = (c_1/c) \times 100$
		mass from stage 1, m_1	$c = c_1 + m_1$	100

for the Mass Median Aerodynamic Diameter (MMAD) and Geometric Standard Deviation (GSD) as appropriate. Appropriate computational methods may also be used.

01/2008:20927

2.9.27. UNIFORMITY OF MASS OF DELIVERED DOSES FROM MULTIDOSE CONTAINERS

The following test is intended for oral dosage forms such as granules, powders for oral use and liquids for oral use, which are supplied in multidose containers provided at manufacture with a measuring device.

Weigh individually 20 doses taken at random from one or more containers with the measuring device provided and determine the individual and average masses. Not more than 2 of the individual masses deviate from the average mass by more than 10 per cent and none deviates by more than 20 per cent.

04/2019:20935

2.9.35. POWDER FINENESS(1)

Particle-size distribution is estimated by analytical sieving (*2.9.38*) or by application of other suitable methods where appropriate. A simple descriptive classification of powder fineness is provided in this chapter. For practical reasons, sieves are commonly used to measure powder fineness. Sieving is most suitable where a majority of the particles are larger than about 75 µm, although it can be used for some powders having smaller particle sizes where the method can be validated. Light diffraction is also a widely used technique for measuring the size of a wide range of particles.

Where the cumulative distribution has been determined by analytical sieving or by application of other methods, particle size may be characterised in the following manner:

x_{90} = particle size corresponding to 90 per cent of the cumulative undersize distribution;

x_{50} = median particle size (i.e. 50 per cent of the particles are smaller and 50 per cent of the particles are larger);

x_{10} = particle size corresponding to 10 per cent of the cumulative undersize distribution.

It is recognised that the symbol *d* is also widely used to designate these values. Therefore, the symbols d_{90}, d_{50}, d_{10} may be used.

The following parameters may be defined based on the cumulative distribution.

$Q_r(x)$ = cumulative distribution of particles with a dimension less than or equal to x where the subscript r reflects the distribution type.

r	**Distribution type**
0	Number
1	Length
2	Area
3	Volume

Therefore, by definition:

$Q_r(x) = 0.90$ when $x = x_{90}$

$Q_r(x) = 0.50$ when $x = x_{50}$

$Q_r(x) = 0.10$ when $x = x_{10}$

An alternative but less informative method of classifying powder fineness is by use of the descriptive terms in Table 2.9.35.-1.

Table 2.9.35.-1.

Classification of powders by fineness		
Descriptive term	**x_{50} (µm)**	**Cumulative distribution by volume basis, $Q_3(x)$**
Coarse	> 355	$Q_3(355) < 0.50$
Moderately fine	180 - 355	$Q_3(180) < 0.50$ and $Q_3(355) \geq 0.50$
Fine	125 - 180	$Q_3(125) < 0.50$ and $Q_3(180) \geq 0.50$
Very fine	≤ 125	$Q_3(125) \geq 0.50$

(1) This chapter has undergone pharmacopoeial harmonisation. See chapter *5.8. Pharmacopoeial harmonisation*.

04/2017:20940

2.9.40. UNIFORMITY OF DOSAGE UNITS[(1)]

To ensure the consistency of dosage units, each unit in a batch should have an active substance content within a narrow range around the label claim. Dosage units are defined as dosage forms containing a single dose or a part of a dose of an active substance in each dosage unit. ◊Unless otherwise stated,◊ the uniformity of dosage units specification is not intended to apply to solutions, suspensions, emulsions or gels in single-dose containers intended for local action following cutaneous administration. ◊The test for content uniformity is not required for multivitamin, single-vitamin and trace-element preparations.◊

The term 'uniformity of dosage unit' is defined as the degree of uniformity in the amount of the active substance among dosage units. Therefore, the requirements of this chapter apply to each active substance being comprised in dosage units containing 1 or more active substances, unless otherwise specified elsewere in this Pharmacopoeia.

The uniformity of dosage units can be demonstrated by either of 2 methods: content uniformity or mass variation (see Table 2.9.40.-1).

The test for content uniformity of preparations presented in dosage units is based on the assay of the individual contents of active substance(s) of a number of dosage units to determine whether the individual contents are within the limits set. The content uniformity method may be applied in all cases.

The test for mass variation is applicable for the following dosage forms:

(1) solutions enclosed in single-dose containers and in soft capsules;

(2) solids (including powders, granules and sterile solids) that are packaged in single-dose containers and contain no added active or inactive substances;

(3) solids (including sterile solids) that are packaged in single-dose containers, with or without added active or inactive substances, that have been prepared from true solutions and freeze-dried in the final containers and are labelled to indicate this method of preparation;

(4) hard capsules, uncoated tablets, or film-coated tablets, containing 25 mg or more of an active substance comprising 25 per cent or more, by mass, of the dosage unit or, in the case of hard capsules, the capsule contents, except that uniformity of other active substances present in lesser proportions is demonstrated by meeting content uniformity requirements.

The test for content uniformity is required for all dosage forms not meeting the above conditions for the mass variation test. ◆Alternatively, products that do not meet the 25 mg/25 per cent threshold limit may be tested for uniformity of dosage units by mass variation instead of the content uniformity test on the following condition: the concentration Relative Standard Deviation (RSD) of the active substance in the final dosage units is not more than 2 per cent, based on process validation data and development data, and if there has been regulatory approval of such a change. The concentration RSD is the RSD of the concentration per dosage unit (*m/m* or *m/V*), where concentration per dosage unit equals the assay result per dosage unit divided by the individual dosage unit mass. See the RSD formula in Table 2.9.40.-2.◆

Table 2.9.40.-1. – *Application of Content Uniformity (CU) and Mass Variation (MV) test for dosage forms*

Dosage forms	Type	Sub-Type	Dose and ratio of active substance	
			≥ 25 mg and ≥ 25 per cent	< 25 mg or < 25 per cent
Tablets	uncoated		MV	CU
	coated	film-coated	MV	CU
		others	CU	CU
Capsules	hard		MV	CU
	soft	suspensions, emulsions, gels	CU	CU
		solutions	MV	MV
Solids in single-dose containers	single component		MV	MV
	multiple components	solution freeze-dried in final container	MV	MV
		others	CU	CU
Solutions enclosed in single-dose containers			MV	MV
Others: dosage forms not addressed by the other categories in this table including but not limited to suppositories, transdermal patches and semi-solid preparations applied cutaneously and intended for systemic distribution of the active substance			CU	CU

(1) This chapter has undergone pharmacopoeial harmonisation. See chapter *5.8. Pharmacopoeial harmonisation*.

Table 2.9.40.-2.

Variable	Definition	Conditions	Value
$\overline{X}$	Mean of individual contents (x_1, x_2,..., x_n), expressed as a percentage of the label claim		
x_1, x_2,..., x_n	Individual contents of the dosage units tested, expressed as a percentage of the label claim		
n	Sample size (number of dosage units in a sample)		
k	Acceptability constant	If n = 10, then	2.4
		If n = 30, then	2.0
s	Sample standard deviation		$\left[\frac{\sum_{i=1}^{n}(x_i - \overline{X})^2}{n-1}\right]^{1/2}$
RSD	Relative standard deviation		$\frac{100s}{\overline{X}}$
M (case 1) To be applied when $T \leq 101.5$	Reference value	If 98.5 per cent $\leq \overline{X} \leq$ 101.5 per cent, then	$M = \overline{X}$ $(AV = ks)$
		If $\overline{X}$ < 98.5 per cent, then	M = 98.5 per cent $(AV = 98.5 - \overline{X} + ks)$
		If $\overline{X}$ > 101.5 per cent, then	M = 101.5 per cent $(AV = \overline{X} - 101.5 + ks)$
M (case 2) To be applied when $T > 101.5$	Reference value	If 98.5 per cent $\leq \overline{X} \leq T$, then	$M = \overline{X}$ $(AV = ks)$
		If $\overline{X}$ < 98.5 per cent, then	M = 98.5 per cent $(AV = 98.5 - \overline{X} + ks)$
		If $\overline{X} > T$, then	$M = T$ per cent $(AV = \overline{X} - T + ks)$
Acceptance value (AV)			General formula: $\lvert M - \overline{X} \rvert + ks$ Calculations are specified above for the different cases.
$L1$	Maximum allowed acceptance value		$L1$ = 15.0 unless otherwise specified
$L2$	Maximum allowed range for deviation of each dosage unit tested from the calculated value of M	On the low side, no dosage unit result can be less than 0.75 M while on the high side, no dosage unit result can be greater than 1.25 M (This is based on $L2$ value of 25.0)	$L2$ = 25.0 unless otherwise specified
T	Target content per dosage unit at time of manufacture, expressed as a percentage of the label claim. Unless otherwise stated, T is equal to 100 per cent or T is the manufacturer's approved target content per dosage unit		

CONTENT UNIFORMITY

Select not fewer than 30 units, and proceed as follows for the dosage form designated. Where different procedures are used for assay of the preparation and for the content uniformity test, it may be necessary to establish a correction factor to be applied to the results of the latter.

Solid dosage forms. Assay 10 units individually using an appropriate analytical method. Calculate the acceptance value (see Table 2.9.40.-2).

Liquid or semi-solid dosage forms. Assay 10 units individually using an appropriate analytical method. Carry out the assay on the amount of well-mixed material that is removed from an individual container in conditions of normal use. Express the results as delivered dose. Calculate the acceptance value (see Table 2.9.40.-2).

Calculation of Acceptance Value

Calculate the Acceptance Value (AV) using the formula:

$$\lvert M - \overline{X} \rvert + ks$$

for which the terms are as defined in Table 2.9.40.-2.

MASS VARIATION

Carry out an assay for the active substance(s) on a representative sample of the batch using an appropriate analytical method. This value is result *A*, expressed as percentage of label claim (see Calculation of Acceptance Value). Assume that the concentration (mass of active substance per mass of dosage unit) is uniform. Select not fewer than 30 dosage units, and proceed as follows for the dosage form designated.

Uncoated or film-coated tablets. Accurately weigh 10 tablets individually. Calculate the active substance content, expressed as percentage of label claim, of each tablet from the mass of the individual tablets and the result of the assay. Calculate the acceptance value.

Hard capsules. Accurately weigh 10 capsules individually, taking care to preserve the identity of each capsule. Remove the contents of each capsule by suitable means. Accurately 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 respective gross mass. Calculate the active substance content in each capsule from the mass of product removed from the individual capsules and the result of the assay. Calculate the acceptance value.

Soft capsules. Accurately weigh 10 intact capsules individually to obtain their gross masses, taking care to preserve the identity of each capsule. Then cut open the capsules by means of a suitable clean, dry cutting instrument such as scissors or a sharp open blade, and remove the contents by washing with a suitable solvent. Allow the occluded solvent to evaporate from the shells at room temperature over a period of about 30 min, taking precautions to avoid uptake or loss of moisture. Weigh the individual shells, and calculate the net contents. Calculate the active substance content in each capsule from the mass of product removed from the individual capsules and the result of the assay. Calculate the acceptance value.

Solid dosage forms other than tablets and capsules. Proceed as directed for hard capsules, treating each unit as described therein. Calculate the acceptance value.

Liquid ◊or semi-solid◊ dosage forms. Accurately weigh the amount of liquid or semi-solid that is removed from each of 10 individual containers in conditions of normal use. If necessary, compute the equivalent volume after determining the density. Calculate the active substance content in each container from the mass of product removed from the individual containers and the result of the assay. Calculate the acceptance value.

Calculation of Acceptance Value. Calculate the acceptance value (*AV*) as shown in content uniformity, except that the individual contents of the units are replaced with the individual estimated contents defined below.

$x_1, x_2, \ldots, x_n$ = individual estimated contents of the dosage units tested;

where

$$x_i = w_i \times \frac{A}{\overline{W}}$$

$w_1, w_2, \ldots, w_n$ = individual masses of the dosage units tested;

A = content of active substance (percentage of label claim) obtained using an appropriate analytical method (assay);

$\overline{W}$ = mean of individual masses ($w_1, w_2, \ldots, w_n$).

CRITERIA

Apply the following criteria, unless otherwise specified.

Solid, semi-solid and liquid dosage forms. The requirements for dosage uniformity are met if the acceptance value of the first 10 dosage units is less than or equal to $L1$ per cent. If the acceptance value is greater than $L1$ per cent, test the next 20 dosage units and calculate the acceptance value. The requirements are met if the final acceptance value of the 30 dosage units is less than or equal to $L1$ per cent and no individual content of the dosage unit is less than $(1 - L2 \times 0.01)M$ or more than $(1 + L2 \times 0.01)M$ in calculation of acceptance value under content uniformity or under mass variation. Unless otherwise specified, $L1$ is 15.0 and $L2$ is 25.0.

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2.9.44. PREPARATIONS FOR NEBULISATION: CHARACTERISATION

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

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

These tests standardise the approach given to the assessment of the dose that would be delivered to a patient but are not intended to provide assessment of the nebuliser device itself, which is described in the European standard EN 13544-1:2007+A1:2009, Respiratory therapy equipment - Part 1: Nebulizing systems and their components.

The mass- rather than the number-weighted size distribution is more appropriate to evaluate product performance. Indeed, active substance mass as a function of aerodynamic diameter is more indicative of therapeutic effect within the respiratory tract.

ACTIVE SUBSTANCE DELIVERY RATE AND TOTAL ACTIVE SUBSTANCE DELIVERED

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

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

APPARATUS

Breathing simulator. A commercially available breathing simulator, which is able to generate the breathing profiles specified in Table 2.9.44.-1, is used for the test. The breathing profile indicated for adults is used unless the medicinal product is specifically intended for use in paediatrics, where alternate patterns should be used, as indicated in Table 2.9.44.-1.

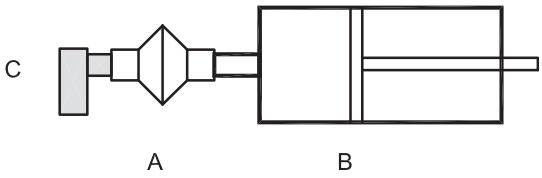
Table 2.9.44.-1. – *Breathing simulator specifications*

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

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

METHOD

Attach the filter (contained in the filter holder) (A) to the breath simulator (B) according to Figure 2.9.44.-1. Fill the nebuliser (C) with the volume of the medicinal product as specified in the patient instructions. Attach the mouthpiece of the nebuliser to the inhalation filter using a mouthpiece adapter if required, ensuring that connections are airtight. Make sure the nebuliser is positioned in the same orientation as intended for use; this may require tilting the breathing simulator and filter holder. Set the breathing simulator to generate the specified breathing pattern.



A. inhalation filter and filter holder B. breathing simulator C. nebuliser

Figure 2.9.44.-1. – *Experimental set-up for breathing simulator testing*

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

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

RESULTS

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

AERODYNAMIC ASSESSMENT OF NEBULISED AEROSOLS

Nebulised products need to be size-characterised at flow rates lower than the range that is normally used for powder inhalers and metered-dose inhalers. A flow rate of 15 L/min is recommended in the European standard because this value represents a good approximation to the mid-inhalation flow rate achievable by a tidally breathing healthy adult (500 mL tidal volume).

Although low-angle laser light scattering instruments (laser diffractometers) can provide rapid size-distribution measurements of nebuliser-generated aerosols, these techniques do not detect the active substance; rather they measure the size distribution of the droplets irrespective of their content. This may not be a problem with homogeneous solutions, but can result in significant error if the product to be nebulised is a suspension, or if droplet evaporation is significant as can be the case with certain nebuliser types. Cascade impactors enable the aerosol to be characterised

unambiguously in terms of the mass of active substance as a function of aerodynamic diameter. Laser diffraction may be used if validated against a cascade impaction method.

Apparatus E (see below under Apparatus), a cascade impactor, has been calibrated at 15 L/min specifically to meet the recommendation of the European standard, and is therefore used for this test. Determining mass balance in the same way as for powder inhalers and metered-dose inhalers is not straightforward, in that the dose is being captured as a continuous output, and hence is not included. As part of method development, recovery experiments must be performed to validate the method.

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

APPARATUS

A detailed description of Apparatus E and the induction port is contained in general chapter *2.9.18*, and includes details of critical dimensions and the qualification process for the impactor (stage mensuration).

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

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

METHOD VALIDATION

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

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

METHOD

Pre-cool the assembled impactor and induction port in a refrigerator (set at about 5 °C) for not less than 90 min and start the determination within about 5 min of removal of the impactor from the refrigerator. Other methods that maintain the impactor at a constant temperature (for example, use of a cooling cabinet) can also be employed when validated.

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

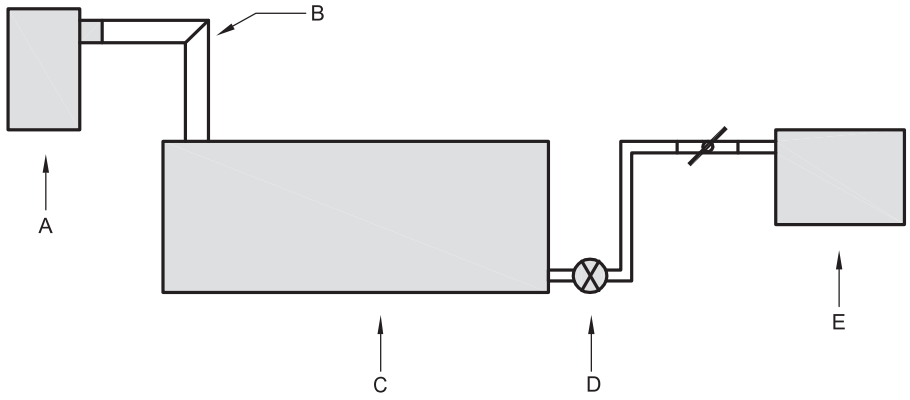
Remove the impactor from the refrigerator. Attach the induction port to the impactor, and connect the outlet of the impactor/external filter to a vacuum source that is capable of drawing air through the system at 15 L/min as specified in Figure 2.9.44.-2. Turn on the flow through the impactor.

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

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

Dismantle the impactor and, using a suitable method of analysis, determine the mass of active substance collected in the induction port, on each stage and on the back-up filter/external filter as described for Apparatus E in general chapter *2.9.18*. Add the mass of active substance collected in the MOC to that deposited on the back-up filter/external filter and treat as a single sample for the purpose of subsequent calculations.

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



A. nebuliser B. induction port C. impactor (apparatus E) D. flow control valve E. vacuum source

Figure 2.9.44.-2. – *Apparatus E for measuring the size distribution of preparations for nebulisation*

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

m_{comp} = mass associated with the component under evaluation ;
M = total mass collected by the system.

Present $F_{m,comp}$ in order of location within the measurement equipment, beginning at the induction port and ending with the back-up filter of the impactor (see Figure 2.9.44.-3). Where appropriate, $F_{m,comp}$ for adjacent stages of the impactor may be combined in order to report the mass fraction collected on a group of stages as a single value.

Determine the cumulative mass-weighted particle-size distribution of the aerosol size-fractionated by the impactor in accordance with the procedure given in general chapter *2.9.18*. Starting at the filter, derive a cumulative mass versus effective cut-off diameter of the respective stages (see Table 2.9.44.-2 for the appropriate cut-off diameters at 15 L/min). Plot the cumulative fraction of active substance versus cut-off diameter in a suitable format, for example logarithmic or log-probability format. Where appropriate, determine by interpolation the fraction either below a given size or between an upper and a lower size limit.

Table 2.9.44.-2. – *Cut-off sizes for Apparatus E at 15 L/min*

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

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

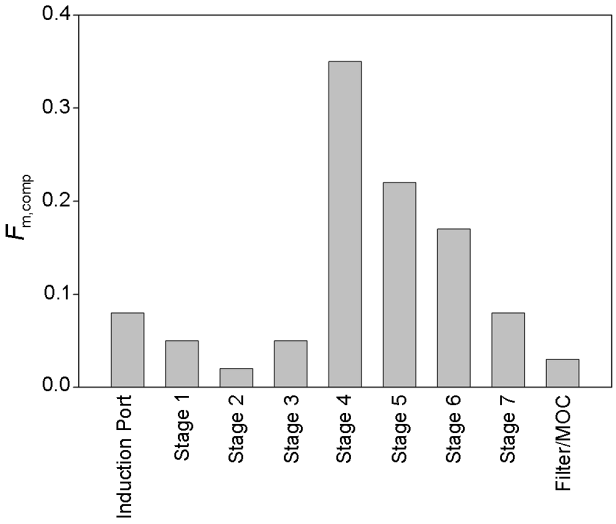


Figure 2.9.44.-3. – *Example of mass fraction of droplets presented in terms of location within the sampling system*

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corrected 8.1

2.9.47. DEMONSTRATION OF UNIFORMITY OF DOSAGE UNITS USING LARGE SAMPLE SIZES

The procedure is intended for, but not limited to, the evaluation of medicinal products that are manufactured using process analytical technology (PAT) methodology.

Compliance with general chapter *2.9.40. Uniformity of dosage units* can be demonstrated by the following procedure, when large samples (sample size $n \geq 100$) are evaluated.

Application of this chapter does not constitute a mandatory requirement. It presents 2 alternative tests (Alternative 1 and Alternative 2). Fulfilling the requirements of either of the 2 alternatives is considered as evidence that the medicinal product tested complies with general chapter *2.9.40*. The 2 alternatives are considered equivalent in their demonstration of compliance with general chapter *2.9.40*.

ALTERNATIVE 1 (PARAMETRIC)

Select not fewer than 100 units according to a predefined sampling plan.

The consistency of dosage units is evaluated by content uniformity or mass variation as prescribed in Table 2.9.40.-1. Calculate the acceptance value (AV) using the following expression:

$$|M - \overline{X}| + ks$$

for which the terms are defined in Table 2.9.40.-2, but using the sample size-dependent value for k defined in Table 2.9.47.-1.

CRITERIA

Apply the following criteria, unless otherwise specified.

The requirements for dosage form uniformity are met if:

1. the acceptance value (AV) is less than or equal to $L1$; and
2. in the calculation of acceptance value (AV) under content uniformity or under mass variation, the number of individual dosage units outside $(1 \pm L2 \times 0.01)M$ is less than or equal to $c2$ as defined for a given sample size n in Table 2.9.47.-1.

Unless otherwise specified, $L1$ is 15.0 and $L2$ is 25.0.

Table 2.9.47.-1. is to be interpreted as follows:

Table 2.9.47.-1. – *Acceptability constant (k) and acceptable number of dosage units with a content outside $(1 \pm L2 \times 0.01)M$ (= c2) for a given sample size n*

n (≥)	k	c2	n (≥)	k	c2	n (≥)	k	c2	n (≥)	k	c2	n (≥)	k	c2	n (≥)	k	c2
100	2.15		**804**	2.26	7	**2480**	2.29	23	**4366**	2.30	41	**6252**	2.31	59	**8243**	2.31	78
105	2.16		**905**	2.27		**2585**	2.29	24	**4471**	2.30	42	**6357**	2.31	60	**8347**	2.31	79
120	2.17	0	**908**	2.27	8	**2690**	2.29	25	**4576**	2.30	43	**6462**	2.31	61	**8452**	2.31	80
139	2.18		**1013**	2.27	9	**2794**	2.29	26	**4680**	2.30	44	**6566**	2.31	62	**8557**	2.31	81
161	2.19		**1118**	2.27	10	**2899**	2.29	27	**4785**	2.30	45	**6671**	2.31	63	**8662**	2.31	82
176	2.19		**1223**	2.27	11	**3004**	2.29	28	**4890**	2.30	46	**6776**	2.31	64	**8767**	2.31	83
189	2.20		**1276**	2.28		**3109**	2.29	29	**4995**	2.30	47	**6881**	2.31	65	**8871**	2.31	84
224	2.21	1	**1328**	2.28	12	**3171**	2.30		**5099**	2.30	48	**6985**	2.31	66	**8976**	2.31	85
270	2.22		**1432**	2.28	13	**3213**	2.30	30	**5204**	2.30	49	**7090**	2.31	67	**9081**	2.31	86
280	2.22		**1537**	2.28	14	**3318**	2.30	31	**5309**	2.30	50	**7195**	2.31	68	**9186**	2.31	87
328	2.23	2	**1642**	2.28	15	**3423**	2.30	32	**5414**	2.30	51	**7300**	2.31	69	**9290**	2.31	88
385	2.23		**1747**	2.28	16	**3528**	2.30	33	**5519**	2.30	52	**7404**	2.31	70	**9395**	2.31	89
407	2.24	3	**1851**	2.28	17	**3633**	2.30	34	**5623**	2.30	53	**7509**	2.31	71	**9500**	2.31	90
490	2.24		**1918**	2.29		**3737**	2.30	35	**5728**	2.30	54	**7614**	2.31	72	**9605**	2.31	91
516	2.25	4	**1956**	2.29	18	**3842**	2.30	36	**5833**	2.30	55	**7719**	2.31	73	**9710**	2.31	92
594	2.25		**2061**	2.29	19	**3947**	2.30	37	**5938**	2.30	56	**7824**	2.31	74	**9814**	2.31	93
672	2.26	5	**2166**	2.29	20	**4052**	2.30	38	**6042**	2.30	57	**7928**	2.31	75	**9919**	2.31	94
699	2.26	6	**2270**	2.29	21	**4156**	2.30	39	**6136**	2.31		**8033**	2.31	76			
			2375	2.29	22	**4261**	2.30	40	**6147**	2.31	58	**8138**	2.31	77			

Table 2.9.47.-2. – *Acceptable number of individual dosage units with a content outside (1 ± L1 × 0.01)T (= c1) and (1 ± L2 × 0.01)T (= c2) respectively, for a given sample size n*

n (≥)	*c*1	*c*2
100	3	
123	4	0
159	5	
176	5	
196	6	1
234	7	
273	8	
280	8	
313	9	2
353	10	
385	10	
394	11	3
434	12	
476	13	
490	13	
517	14	4
559	15	
594	15	
601	16	5
644	17	
686	18	
699	18	
729	19	6
772	20	
804	20	
815	21	7
858	22	
902	23	
908	23	
945	24	8
989	25	
1013	25	
1033	26	9
1077	27	
1118	27	
1121	28	10
1165	29	
1209	30	
1223	30	
1253	31	11
1298	32	
1328	32	
1342	33	12
1387	34	
1432	35	
1476	36	13
1521	37	
1537	37	
1566	38	14
1611	39	
1642	39	
1656	40	15
1701	41	
1746	42	
1747	42	
1791	43	16
1836	44	
1851	44	
1882	45	17
1927	46	
1956	46	
1972	47	18
2018	48	
2061	48	
2063	49	19
2109	50	
2154	51	
2166	51	
2200	52	20
2246	53	
2270	53	
2291	54	21
2337	55	
2375	55	
2383	56	22
2429	57	
2475	58	
2480	58	
2520	59	23
2566	60	
2585	60	
2612	61	24
2658	62	
2690	62	
2704	63	25
2750	64	
2794	64	
2796	65	26
2843	66	
2889	67	
2899	67	
2935	68	27
2981	69	
3004	69	
3027	70	28
3073	71	
3109	71	
3120	72	29
3166	73	
3212	74	
3213	74	
3259	75	30
3305	76	
3318	76	
3351	77	31
3398	78	
3423	78	
3444	79	32
3491	80	
3528	80	
3537	81	33
3584	82	
3630	83	
3633	83	
3677	84	34
3723	85	
3737	85	
3770	86	35
3817	87	
3842	87	
3863	88	36
3910	89	
3947	89	
3956	90	37
4003	91	
4050	92	
4052	92	
4097	93	38
4143	94	
4156	94	
4190	95	39
4237	96	
4261	96	
4284	97	40
4330	98	
4366	98	
4377	99	41
4424	100	
4471	101	
4518	102	42
4565	103	
4576	103	
4612	104	43
4658	105	
4680	105	
4705	106	44
4752	107	
4785	107	
4799	108	45
4846	109	
4890	109	
4893	110	46
4940	111	
4987	112	
4995	112	
5034	113	47
5081	114	
5099	114	
5128	115	48
5175	116	
5204	116	
5222	117	49
5269	118	
5309	118	
5317	119	50
5364	120	
5411	121	
5414	121	
5458	122	51
5505	123	
5519	123	
5552	124	52
5599	125	
5623	125	
5647	126	53
5694	127	
5728	127	
5741	128	54
5788	129	
5833	129	
5835	130	55
5883	131	
5930	132	
5938	132	
5977	133	56
6024	134	
6042	134	
6072	135	57
6119	136	
6147	136	
6166	137	58
6214	138	
6252	138	
6261	139	59
6308	140	
6355	141	
6357	141	
6403	142	60
6450	143	
6462	143	
6498	144	61
6545	145	
6566	145	
6592	146	62
6640	147	
6671	147	
6687	148	63
6734	149	
6776	149	
6782	150	64
6829	151	
6877	152	
6881	152	
6924	153	65
6972	154	
6985	154	
7019	155	66
7067	156	
7090	156	
7114	157	67
7161	158	
7195	158	
7209	159	68
7256	160	
7300	160	
7304	161	69
7351	162	
7399	163	
7404	163	
7447	164	70
7494	165	
7509	165	
7542	166	71
7589	167	
7614	167	
7637	168	72
7684	169	
7719	169	
7732	170	73
7779	171	
7824	171	
7827	172	74
7875	173	
7922	174	
7928	174	
7970	175	75
8017	176	
8033	176	
8065	177	76
8113	178	
8138	178	
8160	179	77
8208	180	
8243	180	
8256	181	78
8303	182	
8347	182	
8351	183	79
8399	184	
8446	185	
8452	185	
8494	186	80
8542	187	
8557	187	
8589	188	81
8637	189	
8662	189	
8685	190	82
8732	191	
8767	191	
8780	192	83
8828	193	
8871	193	
8875	194	84
8923	195	
8971	196	
8976	196	
9019	197	85
9066	198	
9081	198	
9114	199	86
9162	200	
9186	200	
9210	201	87
9257	202	
9290	202	
9305	203	88
9353	204	
9395	204	
9401	205	89
9449	206	
9496	207	
9500	207	
9544	208	90
9592	209	
9605	209	
9640	210	91
9688	211	
9710	211	
9735	212	92
9783	213	
9814	213	
9831	214	93
9879	215	
9919	215	
9927	216	
9975	217	94
10023	218	
10070	219	

– for a sample size of $n = 400$, enter the table at $n \geq 385$: $k = 2.23$ and $c2 = 3$;
– for a sample size of $n = 450$, enter the table at $n \geq 407$: $k = 2.24$ and $c2 = 3$;
– for a sample size of $n = 500$, enter the table at $n \geq 490$: $k = 2.24$ and $c2 = 4$.

ALTERNATIVE 2 (NON-PARAMETRIC)

Select not fewer than 100 units according to a predefined sampling plan.

The consistency of dosage units is evaluated by content uniformity or mass variation as prescribed in Table 2.9.40.-1. Assay individually or weigh the units and calculate individual contents as prescribed in general chapter *2.9.40*. Count the number of individual dosage units with a content outside $(1 \pm L1 \times 0.01)T$ and the number of individual dosage units with a content outside $(1 \pm L2 \times 0.01)T$. Evaluate if the values are within the limits defined in Table 2.9.47.-2.

CRITERIA

Apply the following criteria, unless otherwise specified.

The requirements for dosage form uniformity are met if:

1. the number of individual dosage units outside $(1 \pm L1 \times 0.01)T$ is less than or equal to $c1$; and
2. the number of individual dosage units outside $(1 \pm L2 \times 0.01)T$ is less than or equal to $c2$.

$c1$ and $c2$ for a given sample size n are defined in Table 2.9.47.-2. Unless otherwise specified, $L1$ is 15.0 and $L2$ is 25.0.

Table 2.9.47.-2 is to be interpreted as follows:

– for a sample size of $n = 400$, enter the table at $n \geq 394$: $c1 = 11$ and $c2 = 3$;
– for a sample size of $n = 450$, enter the table at $n \geq 434$: $c1 = 12$ and $c2 = 3$;
– for a sample size of $n = 500$, enter the table at $n \geq 490$: $c1 = 13$ and $c2 = 4$.

01/2019:30201

3.2.1. GLASS CONTAINERS FOR PHARMACEUTICAL USE

Glass containers for pharmaceutical use are glass articles intended to come into direct contact with pharmaceutical preparations.

Colourless glass is highly transparent in the visible spectrum.

Coloured glass is obtained by the addition of small amounts of metal oxides, chosen according to the desired spectral absorbance.

Neutral glass is a borosilicate glass containing significant amounts of boric oxide, aluminium oxide, alkali metal oxides and/or alkaline earth oxides in the glass network. Due to its composition, neutral glass has a high hydrolytic resistance and a high thermal shock resistance.

Soda-lime-silica glass is a silica glass containing alkali metal oxides, mainly sodium oxide, and alkaline earth oxides, mainly calcium oxide, in the glass network. Due to its composition, soda-lime-silica glass has only a moderate hydrolytic resistance.

The hydrolytic stability of glass containers for pharmaceutical use is expressed by the resistance to the release of soluble mineral substances into water under the prescribed conditions of contact between the inner surface of the container or glass grains and water. The hydrolytic resistance is evaluated by titrating released alkali reacting ions. According to their hydrolytic resistance, glass containers are classified as follows:

- type I glass containers: neutral glass, with a high hydrolytic resistance due to the chemical composition of the glass itself;
- type II glass containers: usually of soda-lime-silica glass with a high hydrolytic resistance resulting from suitable treatment of the inner surface;
- type III glass containers: usually of soda-lime-silica glass with only moderate hydrolytic resistance.

The following italicised statements constitute general recommendations concerning the type of glass container that may be used for different types of pharmaceutical preparations. The manufacturer of a pharmaceutical product is responsible for ensuring the suitability of the chosen container.

Type I glass containers are suitable for most preparations whether or not for parenteral administration.

Type II glass containers are suitable for most acidic and neutral, aqueous preparations whether or not for parenteral administration.

Type III glass containers are in general suitable for non-aqueous preparations for parenteral administration, for powders for parenteral administration (except for freeze-dried preparations) and for preparations not for parenteral administration.

Glass containers with a hydrolytic resistance higher than that recommended above for a particular type of preparation may generally also be used.

The container chosen for a given preparation shall be such that the glass material does not release substances in quantities sufficient to affect the stability of the preparation or to present a risk of toxicity. In justified cases, further detailed information may be necessary to assess the impact on chronic use and for vulnerable patient groups.

Preparations for parenteral administration are normally presented in colourless glass, but coloured glass may be used for substances known to be light-sensitive. Colourless or coloured glass is used for the other pharmaceutical preparations. It is recommended that all glass containers for liquid preparations and for powders for parenteral administration permit the visual inspection of the contents.

The inner surface of glass containers may be specially treated to improve hydrolytic resistance, to confer water-repellancy, etc. The outer surface may also be treated, for example to reduce friction and to improve resistance to abrasion. The outer treatment is such that it does not contaminate the inner surface of the container.

Except for type I glass containers, glass containers for pharmaceutical preparations are not to be re-used. Containers for human blood and blood components must not be re-used.

PRODUCTION

When glass containers for pharmaceutical use are manufactured under stressed conditions (e.g. temperature-time profile) and/or are placed in contact with particularly aggressive pharmaceutical preparations, they may undergo delamination, i.e the separation of the inner glass surface into thin layers called lamellae or flakes. Glass delamination may be the result of a chemical attack that occurs according to well-known glass corrosion mechanisms, such as dissolution by hydrolysis and ion exchange (leaching) as a function of the pH. The process of interaction between the glass surface and the pharmaceutical preparation requires incubation time, and flaking may only become visible a number of months after filling.

Several risk factors are known to increase the propensity of a glass to delaminate. The chemical composition of the pharmaceutical preparation, the presence of buffers like citrate or phosphate, which are known to corrode glass, and the ionic strength of the liquid medium may all strongly favour delamination. The manufacturing process of the container, chemical treatments of the inner surface, and terminal sterilisation and processing at the pharmaceutical filling lines are other important risk factors to be considered. It is recommended that the user of the container assesses the compatibility of the glass container and the pharmaceutical preparation on a case-by-case basis, considering for example the dosage form, properties of the formulation and glass quality.

The propensity to delamination of glass containers from different sources can be assessed and ranked by exposing the container to accelerated degradation testing, carried out at specified temperatures for a short time and using the solutions associated with the actual pharmaceutical preparation as extractants. The presence of particles in the extraction solution, the occurrence of phase separation on the inner surface, and the steep increase of silica concentration in the extraction solution are all indicators of a potential propensity for delamination. Accelerated degradation testing can be used as a predictive tool to select the most appropriate container for the intended preparation, but the full compatibility of the active substance with the glass leachate can only be assessed by a stability test under normal conditions of use.

TESTS

Glass containers for pharmaceutical use comply with the relevant test or tests for hydrolytic resistance. When glass containers have non-glass components, the tests apply only to the glass part of the container.

To define the quality of glass containers according to the intended use, one or more of the following tests are necessary.

Tests for hydrolytic resistance are carried out to define the type of glass (I, II or III) and to control its hydrolytic resistance.

In addition, containers for aqueous parenteral preparations are tested for arsenic release and coloured glass containers are tested for spectral transmission.

HYDROLYTIC RESISTANCE

Table 3.2.1.-1. – *Types of glass*

Type of container	Test to be performed
Type I and type II glass containers (to distinguish from type III glass containers)	Test A (surface test)
Type I glass containers (to distinguish from type II and type III glass containers)	Test B (glass grains test) or test C (etching test)
Type I and type II glass containers (if there are doubts whether the high hydrolytic resistance is due to the chemical composition or to the surface treatment)	Tests A and B, or tests A and C

The test is carried out by titration of the extraction solutions obtained under the conditions described for tests A, B and C. Test C is performed if there are uncertainties whether the container is type I or type II.

EQUIPMENT

– An autoclave or steam steriliser capable of withstanding a pressure of 2.5×10^5 N/m^2 (equivalent to 0.25 MPa = 2.5 bar) or more and capable of carrying out the heating cycle described under Autoclaving process. Preferably it is equipped with a constant-pressure regulator or other suitable means in order to maintain the temperature at 121 ± 1 °C. The autoclave vessel is equipped with a heating device, a thermometer integrated in the autoclave, a pressure gauge, a vent cock (for manually operated autoclaves only) and a tray of sufficient capacity to accommodate, above the water level, the number of containers needed to carry out the test. The autoclave has the possibility to connect a calibrated resistance thermometer or a calibrated thermocouple from the inner chamber to an external measuring device to allow a temperature measurement independent from the autoclave system.

The autoclave vessel and all ancillary equipment must be washed thoroughly with water R before use.

– A calibrated resistance thermometer or calibrated thermocouple connected to a suitable temperature measuring device.

– Burettes with a suitable capacity.

– One-mark volumetric flasks, with a capacity of 1000 mL.

– Pipettes and beakers.

– Conical flasks with capacities of 100 mL and 250 mL.

– A water-bath.

– Metal foil (e.g. aluminium, stainless steel).

Flasks and beakers must already have been used for the test or have been filled with *water R* and kept in an autoclave at 121 °C for at least 1 h before being used.

DETERMINATION OF THE FILLING VOLUME

The filling volume is the volume of water to be introduced into the container for the purpose of the test. For vials and bottles the filling volume is 90 per cent of the brimful capacity. For ampoules it is the volume up to the height of the shoulder.

Vials and bottles. Select, at random, 6 containers from the sample lot, or 3 if their capacity exceeds 100 mL, and remove any debris or dust. Weigh the empty containers with an accuracy of 0.1 g. Place the containers on a horizontal surface and fill them with *water R* until about the rim edge, avoiding overflow and introduction of air bubbles. Adjust the liquid levels to the brimful line. Weigh the filled containers to obtain the mass of the water expressed to 2 decimal places for containers having a nominal volume less than or equal to 30 mL, and expressed to 1 decimal place for containers having a nominal volume greater than 30 mL. Calculate the mean value of the brimful capacity in millilitres and multiply it by 0.9. This volume, expressed to 1 decimal place, is the filling volume for the particular container lot.

Ampoules. Place at least 6 dry ampoules on a flat, horizontal surface and fill them with *water R* from a burette, until the water reaches point A, where the body of the ampoule declines to the shoulder (see Figure 3.2.1.-1). Read the capacities (expressed to 2 decimal places) and calculate the mean value. This volume, expressed to 1 decimal place, is the filling volume for the particular ampoule lot. The filling volume may also be determined by weighing.

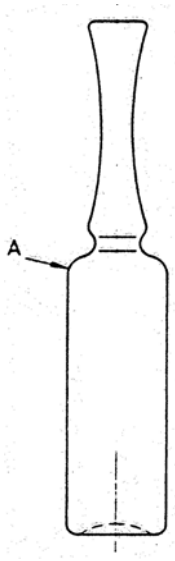

Figure 3.2.1.-1. – *Filling volume of ampoules (up to point A)*

Syringes and cartridges. Select 6 syringes or cartridges. Close the small opening (mouth of cartridges and needle and/or Luer cone of syringes) using an inert material (e.g. a tip cap) or any other suitable means to prevent water leakage. Determine the mean brimful volume in accordance with the procedure described under Vials and bottles and multiply it by 0.9. This volume, expressed to 1 decimal place, is the filling volume for the particular container lot.

TEST A. HYDROLYTIC RESISTANCE OF THE INNER SURFACES OF GLASS CONTAINERS (SURFACE TEST)

The determination is carried out on unused containers. The volumes of the test liquid necessary are indicated in Table 3.2.1.-2.

Table 3.2.1.-2. – *Volume of test liquid and number of titrations*

Filling volume (mL)	Volume of test liquid for 1 titration (mL)	Number of titrations
Up to 3	25.0	1
Above 3 and up to 30	50.0	2
Above 30 and up to 100	100.0	2
Above 100	100.0	3

Cleaning. Remove any debris or dust. Shortly before the test, fill each container to the brim with *water R* and allow to stand, filled with water, for 20 ± 5 min. Empty the containers, carefully rinse twice with *water R* and once with *water R1* and allow to drain.

Closed ampoules are not rinsed before testing. Closed ampoules may be warmed on a water-bath or in an oven at about 40 °C for approximately 2 min before opening to avoid underpressure when opening.

Filling. Fill the containers with *water R1* up to the filling volume.

Loosely cap each container with an inert material, for example with inverted beakers of such a size that the bottoms of the beakers fit snugly down on the rims of the sample. Ampoules and vials capped with clean aluminium foil are further

examples. Place syringes and cartridges in a beaker and cover the beaker with clean aluminium foil.

Containers of a volume of 2 mL or less, in which the water is not sufficiently retained during the autoclaving process, may be closed in a suitable way, e.g. with a stopper or plug of inert material, such as silicone, and fixed using a plunger or a stable fixing or clamping device.

Place the samples, gathered in groups in glass dishes or in beakers or other suitable holders, on the rack in the autoclave containing *water R* at room temperature. Ensure that they are held above the level of the water in the autoclave.

Autoclaving process

Reference thermal cycle

The autoclave is run in such a way that the temperature in the containers to be tested follows a thermal cycle with the following characteristics: temperature raised from room temperature to 100 °C within 20-30 min; temperature maintained at 100 ± 1 °C for 10 ± 1 min; temperature in the containers raised from 100 °C to 121 °C within 20-22 min; temperature maintained at 121 ± 1 °C for 60 ± 1 min; temperature cooled to 100 °C within 40-44 min.

Autoclave calibration

Before being used for the first time, the autoclave and the temperature measuring system are calibrated to ensure that the autoclave settings are suitable to guarantee that the temperature inside the containers is 121 ± 1 °C.

NOTE: significant differences may be observed between the temperature measured in the autoclave chamber and inside the containers.

Take a set of containers of intermediate capacity (10 mL for instance) and fill them with *water R1*. Select a sufficient number of containers to fill completely the tray within the autoclave chamber. Insert the end of the calibrated resistance thermometer or calibrated thermocouple into one of the containers through a hole in the closure having approximately the same diameter as the probe and connect it to the external measuring device. If the container is too small to insert a thermocouple, place the thermocouple in a similar container of suitable size filled with *water R1*. Close the autoclave door or lid securely and run the autoclave to achieve the target thermal cycle in the containers. Where a manual autoclave is run, leave the vent cock open. Heat the autoclave at a regular rate so that steam issues vigorously from the vent cock after 20-30 min, and maintain a vigorous evolution of steam for a further 10 min.

Close the vent cock, follow the temperature increase on the calibrated thermocouple measuring device by comparison with readings taken from the autoclave thermometer and adjust the autoclave settings accordingly in order to match the target thermal cycle. Keep the temperature ramp as smooth as possible.

Using the calibrated thermocouple measuring device, ensure that deviations from the holding temperature of 121 ± 1 °C are within the tolerance. When cooling down, vent to prevent the formation of a vacuum. For safety reasons (boiling retardation) do not open the autoclave before the water in the containers has reached a temperature of 95 °C. Remove the hot samples from the autoclave and cool cautiously to room temperature within 30 min.

Record the autoclave settings used to carry out the thermal cycle and use these settings for routine autoclave runs.

At regular intervals verify the validation of the calibration. Establish a re-calibration plan based on quality assurance criteria, recalibrate as appropriate and keep records.

Routine autoclave runs

Use the autoclave settings established during the calibration stage and follow the same thermal cycle described above. Container sets of different capacity can be tested during the same run. Keep the glass load very similar in size and mass to the load used during the calibration stage. The use of the calibrated thermocouple is no longer necessary provided the calibration is proved to be valid over a defined time span.

At the end of the cycle, remove the hot samples from the autoclave and cool them cautiously to room temperature within 30 min.

NOTE: depending on the type or size of the autoclave the heat transfer and thus the resulting thermal cycle in the containers may vary with the total load of the autoclave. It may therefore be necessary to adjust the autoclave load.

Method. Carry out the titration within 1 h of removal of the containers from the autoclave. Combine the liquids obtained from the containers and mix. Introduce the prescribed volume (Table 3.2.1.-2) into a conical flask (test solution). Place the same volume of *water R1* into a 2nd similar flask as a blank. Add to each flask 0.05 mL of *methyl red solution R* for each 25 mL of liquid. Titrate the blank with *0.01 M hydrochloric acid*. Titrate the test solution with the same acid until the colour of the resulting solution is the same as that obtained for the blank. Subtract the value found for the blank titration from that found for the test solution and express the results in millilitres of *0.01 M hydrochloric acid* per 100 mL. Express titration values of less than 1.0 mL to 2 decimal places and titration values of more than or equal to 1.0 mL to 1 decimal place.

Limits. The results, or the average of the results if more than 1 titration is performed, is not greater than the values stated in Table 3.2.1.-3.

Table 3.2.1.-3. – *Limit values in the test for surface hydrolytic resistance*

	Maximum volume of 0.01 M HCl per 100 mL of test solution (mL)	
Filling volume (mL)	**Types I and II glass containers**	**Type III glass containers**
Up to 0.5	3.0	30.0
Above 0.5 and up to 1	2.0	20.0
Above 1 and up to 2	1.8	17.6
Above 2 and up to 3	1.6	16.1
Above 3 and up to 5	1.3	13.2
Above 5 and up to 10	1.0	10.2
Above 10 and up to 20	0.80	8.1
Above 20 and up to 50	0.60	6.1
Above 50 and up to 100	0.50	4.8
Above 100 and up to 200	0.40	3.8
Above 200 and up to 500	0.30	2.9
Above 500	0.20	2.2

TEST B. HYDROLYTIC RESISTANCE OF GLASS GRAINS (GLASS GRAINS TEST)

Check that the articles as received have been annealed to a commercially acceptable quality.

The test may be performed on the canes used for the manufacture of tubing glass containers or on the containers.

Equipment

- a mortar, pestle (see Figure 3.2.1.-2) and hammer made of tempered, magnetic steel;
- as an alternative to the mortar, pestle and hammer, a ball mill can be used; the ball mill is made of agate, zirconia or stainless steel with a volume of 250 mL; 2 balls with a diameter of 40 mm or 3 balls with a diameter of 30 mm are suitable;
- a set of 3 square-mesh sieves of stainless steel, mounted on frames of the same material and consisting of the following:
  (a) sieve no. 710;

(b) sieve no. 425;

(c) sieve no. 300;

- a mechanical sieve-shaker or a sieving machine may be used to sieve the grains;
- a permanent magnet;
- metal foil (e.g. aluminium, stainless steel);
- a hot-air oven, capable of maintaining a temperature of 140 ± 5 °C;
- a balance, capable of weighing up to 500 g with an accuracy of 0.005 g;
- a desiccator;
- an ultrasonic bath.

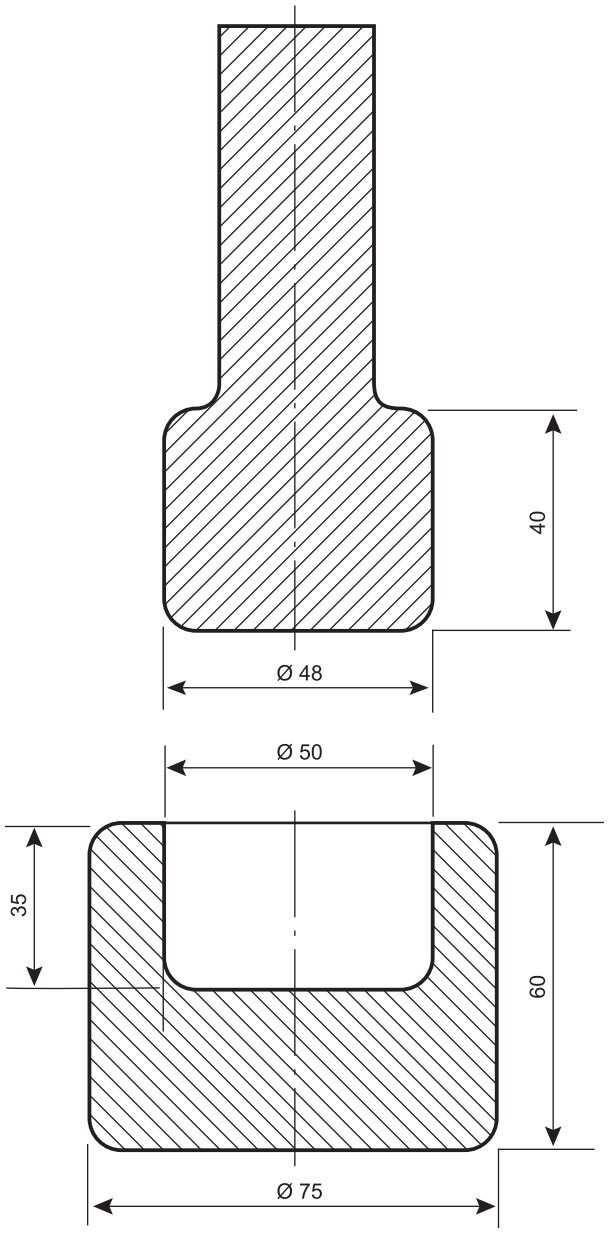

Figure 3.2.1.-2. – *Mortar and pestle apparatus for glass grains method (dimensions in millimetres)*

Method. Rinse the containers to be tested with *water R* and dry in the oven. Wrap at least 3 of the glass articles in clean paper and crush to produce 2 samples of about 100 g each, in pieces not more than 30 mm across.

Where a mortar, pestle and hammer are used, place in the mortar 30-40 g of the pieces 10-30 mm across taken from 1 of the samples, insert the pestle and strike it heavily, once only, with the hammer. Transfer the contents of the mortar to sieve (a), the coarsest of the set. Repeat the operation until all fragments have been transferred to the sieve. Shake the set of sieves for a short time by hand and remove the glass that remains on sieves (a) and (b). Submit these portions to further fracture, repeating the operation until about 10 g of glass remains on sieve (a). Reject this portion and the portion that passes through sieve (c). Reassemble the set of sieves and shake for 5 min. Transfer to a weighing bottle those glass grains that pass through sieve (b) and are retained on sieve (c).

Where a ball mill is used, place in the ball mill beaker about 50 g of the pieces 10-30 mm across taken from 1 of the samples, add the balls and crush thin-walled glass (wall thickness up to 1.5 mm) for up to 2 min and thick-walled glass (wall thickness greater than 1.5 mm) for up to 5 min. Transfer the grains to sieve (a), sieve for about 30 s and collect the grains retained on sieve (c). Transfer the glass from sieves (a) and (b) into the ball mill and crush and sieve again as indicated above. Combine the grains retained on sieve (c).

Repeat the crushing and sieving procedure with the other glass sample and thus 2 samples of grains, each of which shall be in excess of 10 g, are obtained. Spread each sample on a piece of clean glazed paper and remove any iron particles by passing the magnet over them. Transfer each sample into a beaker for cleaning. Add to the grains in each beaker 30 mL of *acetone R* and scour the grains by suitable means, such as a rubber- or plastic-coated glass rod. After scouring the grains, allow to settle and decant as much acetone as possible. Add another 30 mL of *acetone R*, swirl, allow to settle and decant again, and add 30 mL of *acetone R*.

Fill the bath of the ultrasonic vessel with water at room temperature, then place the beaker in the rack and immerse it until the level of the acetone is at the level of the water; apply the ultrasound for 1 min. Swirl the beaker, allow to settle and decant the acetone as completely as possible, add 30 mL of *acetone R* and repeat the ultrasonic cleaning operation. If a fine turbidity persists, repeat the ultrasonic cleaning and acetone washing until the solution remains clear. Swirl and decant the acetone then dry the grains, first by putting the beaker on a warm plate to remove excess acetone and then by heating at 140 °C for 20 min in the drying oven. Transfer the dried grains from each beaker into separate weighing bottles, insert the stoppers and cool in the desiccator. Weigh 10.00 g of the cleaned and dried grains into 2 separate conical flasks. Add 50 mL of *water R1* into each by means of a pipette (test solutions). Pipette 50 mL of *water R1* into a 3rd conical flask as a blank. Distribute the grains evenly over the flat bases of the flasks by gentle shaking. Close the flasks with neutral glass dishes or aluminium foil rinsed with *water R*, or with inverted beakers so that the inner surface of the beakers fit snugly down onto the top rims of the flasks. Place all 3 flasks in the rack in the autoclave containing the water at room temperature, and ensure that they are held above the level of the water in the vessel. Carry out the autoclaving procedure in a similar manner to that described under test A, but maintain the temperature of 121 ± 1 °C only for 30 ± 1 min. Do not open the autoclave until it has cooled to 95 °C. Remove the hot samples from the autoclave and cool the flasks in running tap water as soon as possible, avoiding thermal shock. To each of the 3 flasks add 0.05 mL of *methyl red solution R*. Titrate the blank solution immediately with *0.02 M hydrochloric acid* then titrate the test solutions with the same acid until the colour matches that obtained with the blank solution. Subtract the titration volume for the blank solution from that for the test solution.

NOTE: where necessary to obtain a sharp end-point, the clear solution is to be decanted into a separate 250 mL flask. Rinse the grains with 3 quantities, each of 15 mL, of water R1 by swirling and add the washings to the main solution. Add 0.05 mL of methyl red solution R. Titrate and calculate as described below. In this case also add 45 mL of water R1 and 0.05 mL of methyl red solution R to the blank solution.

Calculate the mean value of the results in millilitres of *0.02 M hydrochloric acid* per gram of the sample and if required its equivalent in alkali extracted, calculated as micrograms of sodium oxide per gram of glass grains.

1 mL of *0.02 M hydrochloric acid* is equivalent to 620 µg of sodium oxide.

Repeat the test if the highest and lowest observed values differ by more than 20 per cent.

Limits. Type I glass containers require not more than 0.1 mL of *0.02 M hydrochloric acid* per gram of glass, type II and type III glass containers require not more than 0.85 mL of *0.02 M hydrochloric acid* per gram of glass.

TEST C. TO DETERMINE WHETHER THE CONTAINERS HAVE BEEN SURFACE-TREATED (ETCHING TEST)

If there are uncertainties whether a container has been surface-treated, and/or to distinguish between type I and type II glass containers, test C is used in addition to test A. Alternatively, tests A and B may be used. Test C may be carried out either on unused samples or on samples previously used in test A.

Vials and bottles. The volumes of test liquid required are shown in Table 3.2.1.-2.

Rinse the containers twice with *water R*, fill to the brimful point with a mixture of 1 volume of *hydrofluoric acid R* and 9 volumes of *hydrochloric acid R* and allow to stand for 10 min. Empty the containers and rinse carefully 5 times with *water R*. Immediately before the test, rinse once again with *water R*. Submit the containers thus prepared to the same autoclaving and determination procedure as described in Test A for surface hydrolytic resistance. If the results are considerably higher than those obtained from the original surfaces (by about a factor of 5 to 10), the samples have been surface-treated.

Ampoules, cartridges and syringes

NOTE: ampoules, cartridges and syringes made from glass tubing are not normally subjected to internal surface treatment because their high chemical resistance is dependent upon the chemical composition of the glass as a material.

Apply the test method as described above for vials and bottles. If the ampoules are not surface-treated, the new values are slightly lower than those obtained in previous tests.

Distinction between type I and type II glass containers

The results obtained in Test C are compared to those obtained in Test A. The interpretation of the result is shown in Table 3.2.1.-4.

Table 3.2.1.-4. – *Distinction between type I and type II glass containers*

Type I	Type II
The values are closely similar to those found in the test for surface hydrolytic resistance for type I glass containers.	The values greatly exceed those found in the test for surface hydrolytic resistance and are similar to but not larger than those for type III glass containers.

ARSENIC

The test applies to glass containers for aqueous parenteral preparations.

Hydride generation atomic absorption spectrometry (*2.2.23, Method I*).

Test solution. Use the extraction solution obtained from containers of types I and II, after autoclaving at 121 °C for 1 h as described under Test A for surface hydrolytic resistance. Transfer 10.0 mL to a 100 mL volumetric flask. Add 10 mL of *hydrochloric acid R* and 5 mL of a 200 g/L solution of *potassium iodide R*. Heat on a water-bath at 80 °C for 20 min, allow to cool and dilute to 100.0 mL with *water R*.

Reference solutions. Prepare the reference solutions using *arsenic standard solution (1 ppm As) R*. Add 10 mL of *hydrochloric acid R* and 5 mL of a 200 g/L solution of *potassium iodide R*. Heat on a water-bath at 80 °C for 20 min, allow to cool and dilute to 100.0 mL with *water R*. The concentration range of the reference solutions is typically 0.005-0.015 ppm of As.

Acid reservoir. Hydrochloric acid R.

Reducing reservoir. Sodium tetrahydroborate reducing solution R.

Use a hydride generation device to introduce the test solution into the cuvette of the spectrometer. Establish and standardise instrumental operating conditions according to the manufacturer's instructions, optimise the uptake rate of the peristaltic pump, then connect it to the acid reservoir, the reducing reservoir and the test solution.

Source: hollow-cathode lamp.

Wavelength: 193.7 nm.

Atomisation device: air-acetylene flame.

Limit: maximum 0.1 ppm of As.

SPECTRAL TRANSMISSION FOR COLOURED GLASS CONTAINERS

Equipment. A UV-Vis spectrophotometer, equipped with a photodiode detector or equipped with a photomultiplier tube coupled with an integrating sphere.

Preparation of the specimen. Break the glass container or cut it with a circular saw fitted with a wet abrasive wheel, such as a carborundum or a bonded-diamond wheel. Select sections representative of the wall thickness and trim them as suitable for mounting in a spectrophotometer. If the specimen is too small to cover the opening in the specimen holder, mask the uncovered portion with opaque paper or tape, provided that the length of the specimen is greater than that of the slit. Before placing in the holder, wash, dry and wipe the specimen with a lens tissue. Mount the specimen with the aid of wax, or by other convenient means, taking care to avoid leaving fingerprints or other marks.

Method. Place the specimen in the spectrophotometer with its cylindrical axis parallel to the slit and in such a way that the light beam is perpendicular to the surface of the section and that the losses due to reflection are at a minimum. Measure the transmission of the specimen with reference to air in the spectral region of 290-450 nm, continuously or at intervals of 20 nm.

Limits. The observed spectral transmission for coloured glass containers for preparations that are not for parenteral administration does not exceed 10 per cent at any wavelength in the range of 290-450 nm, irrespective of the type and the capacity of the glass container. The observed spectral transmission in coloured glass containers for parenteral preparations does not exceed the limits given in Table 3.2.1.-5.

Table 3.2.1.-5. – *Limits of spectral transmission for coloured glass containers for parenteral preparations*

	Maximum percentage of spectral transmission at any wavelength between 290 nm and 450 nm	
Nominal volume (mL)	Flame-sealed containers	Containers with closures
Up to 1	50	25
Above 1 and up to 2	45	20
Above 2 and up to 5	40	15
Above 5 and up to 10	35	13
Above 10 and up to 20	30	12
Above 20	25	10

Annex – test for surface hydrolytic resistance – determination by flame spectrometry

The surface hydrolytic resistance of glass of types I and II may be determined by analysis of the leaching solution by flame spectrometry. A number of elements that, when present as oxides in glass, contribute to the alkalinity of the solution, are determined and used to express an alkali equivalent. The spectrometric method has the advantage of allowing the use of a much smaller sample of extract so that it can be applied to small individual containers. This enables an evaluation of the uniformity of the containers in a given batch where this is critical. The results of this measurement are not equivalent to those of titrimetry and the 2 methods cannot be considered interchangeable. A correlation between the 2 is dependent on the type of glass and the size and shape of the container. The titrimetric method is the reference method of the Pharmacopoeia; the spectrometric method may be used in justified and authorised cases.

A method suitable for this type of analysis is shown below.

The determination is carried out on unused containers. The number of containers to be examined is indicated in Table 3.2.1.-6.

Table 3.2.1.-6. – *Number of containers to be examined for the spectrometric method*

Filling volume (mL)	Number of containers to be measured separately	Additional containers for preliminary measurements
Up to 2	20	2
Above 2 and up to 5	15	2
Above 5 and up to 30	10	2
Above 30 and up to 100	5	1
Above 100	3	1

Instructions on determination of the filling volume, cleaning of the containers, filling and heating are given above under Hydrolytic resistance and Test A.

SOLUTIONS

Spectrochemical buffer solution. Dissolve 80 g of *caesium chloride R* in about 300 mL of *water R1*, add 10mL of *6 M hydrochloric acid R*, dilute to 1.0 L with *water R1* and mix.

Stock solutions:

- sodium oxide, $c(Na_2O)$ = 1 mg/mL;
- potassium oxide, $c(K_2O)$ = 1 mg/mL;
- calcium oxide, $c(CaO)$ = 1 mg/mL.

Commercially available stock solutions may also be used.

Standard solutions. Prepare standard solutions by diluting the stock solutions with *water R1* to obtain concentrations suitable for establishing the reference solutions in an appropriate manner, e.g. with concentrations of 20 µg/mL of sodium oxide, potassium oxide and calcium oxide, respectively. Commercially available standard solutions may also be used.

Reference solutions. Prepare the reference solutions for establishing the calibration graph (set of calibration solutions) by diluting suitable concentrated standard solutions with *water R1*, so that the normal working ranges of the specific elements are covered, taking into account the instrument used for the measurement. Typical concentration ranges of the reference solutions are:

- for determination by atomic emission spectrometry of sodium oxide and potassium oxide: up to 10 µg/mL;
- for determination by atomic absorption spectrometry of sodium oxide and potassium oxide: up to 3 µg/mL;
- for determination by atomic absorption spectrometry of calcium oxide: up to 7 µg/mL.

Use reference solutions containing 5 per cent *V/V* of the spectrochemical buffer solution.

METHOD

Carry out preliminary measurements of the potassium oxide and calcium oxide concentrations on one of the extraction solutions. If, for one container type, the concentration of potassium oxide is less than 0.2 µg/mL and the concentration of calcium oxide is less than 0.1 µg/mL, the remaining extraction solutions of this container type need not be analysed for these ions. Aspirate the extraction solution from each sample directly into the flame of the atomic absorption or atomic emission instrument and determine the approximate concentrations of sodium oxide (and potassium oxide and calcium oxide, if present) by reference to calibration graphs produced from the reference solutions of suitable concentration.

FINAL ANALYSIS

If dilution is unnecessary, add to each container a volume of the spectrochemical buffer solution equivalent to 5 per cent of the filling volume, mix well and determine sodium oxide, calcium oxide and potassium oxide, by reference to calibration graphs. For the determination of the calcium oxide concentration by flame spectrometry, a nitrous oxide/acetylene flame is used.

If dilution is necessary, determine sodium oxide, calcium oxide and potassium oxide, if present, following the procedures as described above. The solutions shall contain 5 per cent *V/V* of the spectrochemical buffer solution. Concentration values less than 1.0 µg/mL are expressed to 2 decimal places, values greater than or equal to 1.0 µg/mL to 1 decimal place. Correct the result for the buffer addition and for any dilution.

DETERMINATION

Determine the mean value of the concentration of individual oxides found in the samples tested, in micrograms of the oxide per millilitre of the extraction solution, and calculate the sum of the individual oxides, expressed as micrograms of sodium oxide per millilitre of the extraction solution, using the following mass conversion factors:

- 1 µg of potassium oxide corresponds to 0.658 µg of sodium oxide;
- 1 µg of calcium oxide corresponds to 1.105 µg of sodium oxide.

Limits. The mean value is not greater than the value given in Table 3.2.1.-7.

Table 3.2.1.-7. – *Limit values in the test for surface hydrolytic resistance by flame spectrometry, for type I and type II glass containers*

Filling volume (mL)	Limit values for the concentration of oxides, expressed as sodium oxide (µg/mL)
Up to 0.5	7.50
Above 0.5 and up to 1	5.00
Above 1 and up to 2	4.50
Above 2 and up to 3	4.10
Above 3 and up to 5	3.20
Above 5 and up to 10	2.50
Above 10 and up to 20	2.00
Above 20 and up to 50	1.50
Above 50 and up to 100	1.20
Above 100 and up to 200	1.00
Above 200 and up to 500	0.75
Above 500	0.50

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3.3.4. STERILE PLASTIC CONTAINERS FOR HUMAN BLOOD AND BLOOD COMPONENTS

Plastic containers for the collection, storage, processing and administration of blood and its components are manufactured from one or more polymers, if necessary with additives. The composition and the conditions of manufacture of the containers are registered by the appropriate competent authorities in accordance with the relevant national legislation and international agreements.

When the composition of the materials of the different parts of the containers corresponds to the appropriate specifications, their quality is controlled by the methods indicated in those specifications (see *3.1. Materials used for the manufacture of containers* and subsections and *3.3. Containers for human blood and blood components, and materials used in their manufacture; transfusion sets and materials used in their manufacture; syringes* and subsections).

Materials other than those described in the Pharmacopoeia may be used provided that their composition is authorised by the competent authority and that the containers manufactured from them comply with the requirements prescribed in this general chapter.

In normal conditions of use the materials do not release monomers, or other substances, in amounts likely to be harmful nor do they lead to any abnormal modifications of the blood.

The containers may contain anticoagulant solutions, depending on their intended use, and are supplied sterile.

Each container is fitted with attachments suitable for the intended use. The container may be in the form of a single unit or the collecting container may be connected by one or more tubes to one or more secondary containers to allow separation of the blood components to be effected within a closed system.

The outlets are of a shape and size allowing for adequate connection of the container with the blood-giving equipment. The protective coverings on the blood-taking needle and on the appendages must be such as to ensure the maintenance of sterility. They must be easily removable but must be tamper-evident.

The capacity of the containers is related to the nominal capacity prescribed by the national authorities and to the appropriate volume of anticoagulant solution. The nominal capacity is the volume of blood to be collected in the container. The containers are of a shape such that when filled they may be centrifuged.

The containers are fitted with a suitable device for suspending or fixing which does not hinder the collection, storage, processing or administration of the blood.

The containers are enclosed in sealed, protective envelopes.

CHARACTERS

The container is sufficiently transparent to allow adequate visual examination of its contents before and after the taking of the blood and is sufficiently flexible to offer minimum resistance during filling and emptying under normal conditions of use. The container contains not more than 5 mL of air.

TESTS

Solution S_1. Fill the container with 100 mL of a sterile, pyrogen-free 9 g/L solution of *sodium chloride R*. Close the container and heat it in an autoclave so that the contents are maintained at 110 °C for 30 min.

If the container to be examined contains an anticoagulant solution, first empty it, rinse the container with 250 mL of *water for injections R* at 20 ± 1 °C and discard the rinsings.

Solution S_2. Introduce into the container a volume of *water for injections R* corresponding to the intended volume of anticoagulant solution. Close the container and heat it in an autoclave so that the contents are maintained at 110 °C for 30 min. After cooling, add sufficient *water for injections R* to fill the container to its nominal capacity.

If the container to be examined contains an anticoagulant solution, first empty it and rinse it as indicated above.

Resistance to centrifugation. Introduce into the container a volume of *water R*, acidified by the addition of 1 mL of *dilute hydrochloric acid R*, sufficient to fill it to its nominal capacity. Envelop the container with absorbent paper impregnated with a 1 in 5 dilution of *bromophenol blue solution R1* or other suitable indicator and then dried. Centrifuge at 5000 *g* for 10 min. No leakage perceptible on the indicator paper and no permanent distortion occur.

Resistance to stretch. Introduce into the container a volume of *water R*, acidified by the addition of 1 mL of *dilute hydrochloric acid R*, sufficient to fill it to its nominal capacity. Suspend the container by the suspending device at the opposite end from the blood-taking tube and apply along the axis of this tube an immediate force of 20 N (2.05 kgf). Maintain the traction for 5 s. Repeat the test with the force applied to each of the parts for filling and emptying. No break and no deterioration occur.

Leakage. Place the container which has been submitted to the stretch test between two plates covered with absorbent paper impregnated with a 1 in 5 dilution of *bromophenol blue solution R1* or other suitable indicator and then dried. Progressively apply force to the plates to press the container so that its internal pressure (i.e. the difference between the applied pressure and atmospheric pressure) reaches 67 kPa within 1 min. Maintain the pressure for 10 min. No signs of leakage are detectable on the indicator paper or at any point of attachment (seals, joints, etc.).

Vapour permeability. For a container containing an anticoagulant solution, fill with a volume of a 9 g/L solution of *sodium chloride R* equal to the volume of blood for which the container is intended.

For an empty container, fill with the same mixture of anticoagulant solution and sodium chloride solution. Close the container, weigh it and store it at 5 ± 1 °C in an atmosphere with a relative humidity of (50 ± 5) per cent for 21 days. At the end of this period the loss in mass is not greater than 1 per cent.

Emptying under pressure. Fill the container with a volume of *water R* at 5 ± 1 °C equal to the nominal capacity. Attach a transfusion set without an intravenous cannula to one of the connectors. Compress the container so as to maintain throughout the emptying an internal pressure (i.e the difference between the applied pressure and atmospheric pressure) of 40 kPa. The container empties in less than 2 min.

Speed of filling. Attach the container by means of the blood-taking tube fitted with the needle to a reservoir containing a suitable solution having a viscosity equal to that of blood, such as a 335 g/L solution of *sucrose R* at 37 °C. Maintain the internal pressure of the reservoir (i.e. the difference between the applied pressure and atmospheric pressure) at 9.3 kPa with the base of the reservoir and the

upper part of the container at the same level. The volume of liquid which flows into the container in 8 min is not less than the nominal capacity of the container.

Resistance to temperature variations. Place the container in a suitable chamber having an initial temperature of 20-23 °C. Cool it rapidly in a deep-freeze to – 80 °C and maintain it at this temperature for 24 h. Raise the temperature to 50 °C and maintain for 12 h. Allow to cool to room temperature. The container complies with the tests for resistance to centrifugation, resistance to stretch, leakage, vapour permeability emptying under pressure and speed of filling prescribed above.

Transparency. Fill the empty container with a volume equal to its nominal capacity of the primary opalescent suspension (*2.2.1*) diluted so as to have an absorbance (*2.2.25*) at 640 nm of 0.37 to 0.43 (dilution factor about 1 in 16). The cloudiness of the suspension must be perceptible when viewed through the bag, as compared with a similar container filled with *water R*.

Extractable matter. Tests are carried out by methods designed to simulate as far as possible the conditions of contact between the container and its contents which occur in conditions of use.

The conditions of contact and the tests to be carried out on the eluates are prescribed, according to the nature of the constituent materials, in the particular requirements for each type of container.

Haemolytic effects in buffered systems

Stock buffer solution. Dissolve 90.0 g of *sodium chloride R*, 34.6 g of *disodium hydrogen phosphate dodecahydrate R* and 2.43 g of *sodium dihydrogen phosphate R* in *water R* and dilute to 1000 mL with the same solvent.

Buffer solution A_0. To 30.0 mL of stock buffer solution add 10.0 mL of *water R*.

Buffer solution B_0. To 30.0 mL of stock buffer solution add 20.0 mL of *water R*.

Buffer solution C_0. To 15.0 mL of stock buffer solution add 85.0 mL of *water R*.

Introduce 1.4 mL of solution S_2 into each of three centrifuge tubes. To tube I add 0.1 mL of buffer solution A_0, to tube II add 0.1 mL of buffer solution B_0 and to tube III add 0.1 mL of buffer solution C_0. To each tube add 0.02 mL of fresh, heparinised human blood, mix well and warm on a water-bath at 30 ± 1 °C for 40 min. Use blood collected less than 3 h previously or blood collected into an anticoagulant citrate-phosphate-dextrose solution (CPD) less than 24 h previously.

Prepare three solutions containing, respectively:

3.0 mL of buffer solution A_0 and 12.0 mL of *water R* (solution A_1),

4.0 mL of buffer solution B_0 and 11.0 mL of *water R* (solution B_1),

4.75 mL of buffer solution B_0 and 10.25 mL of *water R* (solution C_1).

To tubes I, II and III add, respectively, 1.5 mL of solution A_1, 1.5 mL of solution B_1 and 1.5 mL of solution C_1. At the same time and in the same manner, prepare three other tubes, replacing solution S_2 by *water R*. Centrifuge simultaneously the tubes to be examined and the control tubes at exactly 2500 *g* in the same horizontal centrifuge for 5 min. After centrifuging, measure the absorbances (*2.2.25*) of the liquids at 540 nm using the stock buffer solution as compensation liquid. Calculate the haemolytic value as a percentage from the expression:

$$\frac{A_{exp}}{A_{100}} \times 100$$

A_{100} = absorbance of tube III;

A_{exp} = absorbance of tube I or II or of the corresponding control tubes.

The solution in tube I gives a haemolytic value not greater than 10 per cent and the haemolytic value of the solution in tube II does not differ by more than 10 per cent from that of the corresponding control tube.

Sterility (*2.6.1*). The containers comply with the test for sterility. Introduce aseptically into the container 100 mL of a sterile 9 g/L solution of sodium chloride and shake the container to ensure that the internal surfaces have been entirely wetted. Filter the contents of the container through a membrane filter and place the membrane in the appropriate culture medium, as prescribed in the test for sterility.

Pyrogens (*2.6.8*). Solution S_1 complies with the test for pyrogens. Inject 10 mL of the solution per kilogram of the rabbit's mass.

PACKAGING

The containers are packed in protective envelopes.

On removal from its protective envelope the container shows no leakage and no growth of micro-organisms. The protective envelope is sufficiently robust to withstand normal handling.

The protective envelope is sealed in such a manner that it cannot be opened and re-closed without leaving visible traces that the seal has been broken.

LABELLING

The labelling complies with the relevant national legislation and international agreements. The label states:

- the name and address of the manufacturer;
- a batch number which enables the history of the container and of the plastic material of which it is manufactured to be traced.

A part of the label is reserved for:

- the statement of the blood group, the reference number and all other information required by national legislation or international agreements, and an empty space is provided for the insertion of supplementary labelling.

The label of the *protective envelope* or the *label* on the container, visible through the envelope, states:

- the expiry date;
- that, once withdrawn from its protective envelope, the container must be used within 10 days.

The ink or other substance used to print the labels or the writing must not diffuse into the plastic material of the container and must remain legible up to the time of use.

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3.3.5. EMPTY STERILE CONTAINERS OF PLASTICISED POLY(VINYL CHLORIDE) FOR HUMAN BLOOD AND BLOOD COMPONENTS

This general chapter is published for information.

DEFINITION

Unless otherwise authorised as described in general chapter *3.3.4. Sterile plastic containers for human blood and blood components*, the nature and composition of the material from which the containers are made comply with the requirements in general chapter *3.3.2. Materials based on plasticised poly(vinyl chloride) for containers for human blood and blood components*.

TESTS

They comply with the tests prescribed in general chapter *3.3.4. Sterile plastic containers for human blood and blood components* and with the following tests.

Reference solution. Heat *water R* in a borosilicate-glass flask in an autoclave at 110 °C for 30 min.

Acidity or alkalinity. To a volume of solution S_2 (see *3.3.4*) corresponding to 4 per cent of the nominal value of the container add 0.1 mL of *phenolphthalein solution R*. The solution remains colourless. Add 0.4 mL of *0.01 M sodium hydroxide*. The solution is pink. Add 0.8 mL of *0.01 M hydrochloric acid* and 0.1 mL of *methyl red solution R*. The solution is orange-red or red.

Absorbance (*2.2.25*): maximum 0.30, determined between wavelengths of 230 nm and 250 nm on solution S_2 (see *3.3.4*); maximum 0.10, determined between wavelengths of 251 nm and 360 nm on solution S_2. Use the reference solution as the compensation liquid.

Reducing substances. Immediately after preparation of solution S_2 (see *3.3.4*), transfer to a borosilicate-glass flask a volume corresponding to 8 per cent of the nominal value of the container. At the same time, prepare a blank using an equal volume of the freshly prepared reference solution in another borosilicate-glass flask. To each solution add 20.0 mL of *0.002 M potassium permanganate* and 1 mL of *dilute sulfuric acid R*. Allow to stand protected from light for 15 min. To each solution add 0.1 g of *potassium iodide R*. Allow to stand protected from light for 5 min and titrate immediately with *0.01 M sodium thiosulfate*, using 0.25 mL of *starch solution R* as indicator. The difference between the 2 titrations is not greater than 2.0 mL.

Plastic additives 01, 24, 25, 26 and 27. Gas chromatography (*2.2.28*) coupled with mass spectrometry (*2.2.43*).

Internal standard solution S3: 1 mg/mL solution of *di-n-octyl phthalate R* in *tetrahydrofuran for chromatography R*.

Internal standard solution S4. 5 µg/mL solution of *di-n-octyl phthalate R* in *anhydrous ethanol R*.

Test solution. Cut 0.2 g of the material to be examined into pieces about 0.5 cm in length. Dissolve the pieces in 12.5 mL of internal standard solution S3 using a polytetrafluoroethylene magnetic stirring bar. Complete dissolution of the material to be examined is obtained after stirring for about 20-30 min. The poly(vinyl chloride) is precipitated as a white powder by adding dropwise 37.5 mL of *anhydrous ethanol R*. Centrifuge, then dilute 1.0 mL of the supernatant to 50.0 mL with *anhydrous ethanol R*. The final concentration of the internal standard in the test solution is 5 µg/mL.

The stock solutions may be stored at 4 °C for up to 2 weeks.

Stock solution (a). Dissolve 20.0 mg of *plastic additive 01 CRS* in internal standard solution S4 and dilute to 20.0 mL with internal standard solution S4.

Stock solution (b). Dissolve 20.0 mg of *plastic additive 24 CRS* in internal standard solution S4 and dilute to 20.0 mL with internal standard solution S4.

Stock solution (c). Dissolve 20.0 mg of *plastic additive 25 CRS* in internal standard solution S4 and dilute to 20.0 mL with internal standard solution S4.

Stock solution (d). Dissolve 20.0 mg of *plastic additive 26 CRS* in internal standard solution S4 and dilute to 20.0 mL with internal standard solution S4.

Stock solution (e). Dissolve 20.0 mg of *plastic additive 27 CRS* in internal standard solution S4 and dilute to 20.0 mL with internal standard solution S4.

Reference solutions (a1)-(a5). Dilute stock solution (a) with internal standard solution S4 to obtain 5 reference solutions containing 10-40 µg/mL of *plastic additive 01 CRS*.

Reference solutions (b1)-(b5). Dilute stock solution (b) with internal standard solution S4 to obtain 5 reference solutions containing 10-40 µg/mL of *plastic additive 24 CRS*.

Reference solutions (c1)-(c5). Dilute stock solution (c) with internal standard solution S4 to obtain 5 reference solutions containing 10-40 µg/mL of *plastic additive 25 CRS*.

Reference solutions (d1)-(d5). Dilute stock solution (d) with internal standard solution S4 to obtain 5 reference solutions containing 10-40 µg/mL of *plastic additive 26 CRS*.

Reference solutions (e1)-(e5). Dilute stock solution (e) with internal standard solution S4 to obtain 5 reference solutions containing 10-40 µg/mL of *plastic additive 27 CRS*.

Column:

- *material*: fused silica;
- *size*: *l* = 30 m, Ø = 0.25 mm;
- *stationary phase*: *phenyl(5)methyl(95)polysiloxane R* (film thickness 0.25 µm).

Carrier gas: *helium for chromatography R*.

Flow rate: 1 mL/min.

Split ratio: 1:20.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 3.3	100 → 200
	3.3 - 20	200 → 250
	20 - 22.5	250
	22.5 - 23	250 → 270
	23 - 25	270
	25 - 25.6	270 → 320
	25.6 - 30.6	320
Injection port		300

Detection: mass spectrometer as described below; adjust the detector settings so as to comply with the system suitability criteria:

- quadrupole mass spectrometer equipped with an electron impact ionisation mode (70 eV);
- *ion source temperature*: 230 °C;
- *acquisition system*: performed on full-scan (*m/z* = 40-350) and on single-ion monitoring (SIM) modes;
- *solvent delay*: 2.5 min;
- mass spectrometer parameters for the fragmentometric mode (SIM) set as follows:

Substance	Ion 1 [*m/z*]	Ion 2 [*m/z*]	Ion 3 [*m/z*]
Plastic additive 01	149	167	279
Plastic additive 24	155	127	299
Plastic additive 25	71	213	315
Plastic additive 26	305	193	323
Plastic additive 27	261	149	167
DnOP (internal standard)	149	279	167

Injection: 1 µL.

Relative retention with reference to di-*n*-octyl phthalate (retention time = about 22 min): plastic additive 01 = about 0.80; plastic additive 24 = about 0.95-1.09; plastic additive 27 = about 1.02; plastic additive 25 = about 1.14; plastic additive 26 = about 1.34.

The specificity of the detection is checked by monitoring 3 different ions for each substance using a mass spectrometer in SIM mode. Ion ratios are determined from the peak areas after the injection of a standard solution. The ratios in the table below are given for information.

Substance	Ion 1 [*m/z*]	Ion 2 [*m/z*]	Ion 3 [*m/z*]	Ion ratio 2/1 (%)	Ion ratio 3/1 (%)
Plastic additive 01	149	167	279	50	30
Plastic additive 24	155	127	299	30	13
Plastic additive 25	71	213	315	45	20
Plastic additive 26	305	193	323	55	20
Plastic additive 27	261	149	167	130	85
DnOP (internal standard)	149	279	167	/	/

System suitability:

– *resolution*: if plastic additive 27 is tested, minimum 1.5 between the peaks due to the internal standard and plastic additive 27;

– *repeatability*: maximum relative standard deviation of 1.0 per cent for the retention time of the peak due to the plastic additive, determined on 6 injections of a reference solution of each plastic additive tested situated in the middle of the calibration range (e.g. 20 µg/mL); maximum relative standard deviation of 3.0 per cent for the ratio of the area of the peak due to the plastic additive to that due to the internal standard, determined on 6 injections of a reference solution of each plastic additive tested situated in the middle of the calibration range (e.g. 20 µg/mL).

From the calibration curve obtained with the reference solutions, calculate the percentage content of plastic additives in the material to be examined.

Limits:

– *plastic additive 01*: maximum 40 per cent;
– *plastic additive 24*: maximum 45 per cent;
– *plastic additive 25*: maximum 45 per cent;
– *plastic additive 26*: maximum 45 per cent;
– *plastic additive 27*: maximum 45 per cent.

Chlorides (*2.4.4*): maximum 0.4 ppm, determined with solution S_2 (see *3.3.4*).

Prepare the standard using a mixture of 1.2 mL of *chloride standard solution (5 ppm Cl) R* and 13.8 mL of *water R*.

Residue on evaporation. Evaporate to dryness 100 mL of solution S_2 (see *3.3.4*) in a borosilicate-glass beaker of appropriate capacity, previously heated to 105 °C. Evaporate to dryness in the same conditions 100 mL of the reference solution (blank test). Dry to constant mass at 100-105 °C. The residue from solution S_2 weighs a maximum of 3 mg, taking into account the blank test.

STORAGE

See general chapter *3.3.4. Sterile plastic containers for human blood and blood components*.

LABELLING

See general chapter *3.3.4. Sterile plastic containers for human blood and blood components*.

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3.3.6. STERILE CONTAINERS OF PLASTICISED POLY(VINYL CHLORIDE) FOR HUMAN BLOOD CONTAINING ANTICOAGULANT SOLUTION

This general chapter is published for information.

DEFINITION

Sterile plastic containers containing an anticoagulant solution complying with the monograph *Anticoagulant and preservative solutions for human blood (0209)* are used for the collection, storage and administration of blood. Before filling they comply with the description and characters given in general chapter *3.3.5. Empty sterile containers of plasticised poly(vinyl chloride) for human blood and blood components*.

Unless otherwise authorised as described in general chapter *3.3.4. Sterile plastic containers for human blood and blood components*, the nature and composition of the material from which the containers are made comply with the requirements prescribed in general chapter *3.3.2. Materials based on plasticised poly(vinyl chloride) for containers for human blood and blood components*.

TESTS

They comply with the tests prescribed in general chapter *3.3.4. Sterile plastic containers for human blood and blood components* and with the following tests.

Volume of anticoagulant solution. Empty the container, collecting the anticoagulant solution in a graduated cylinder. The volume does not differ by more than ± 10 per cent from the stated volume.

Absorbance (*2.2.25*). Measure the absorbance of the anticoagulant solution removed from the container between 250 nm and 350 nm, using as the compensation liquid an anticoagulant solution of the same composition that has not been in contact with a plastic material. The absorbance at the maximum at 280 nm is not greater than 0.5.

Plastic additives 01, 24, 25, 26 and 27. Gas chromatography (*2.2.28*) coupled with mass spectrometry (*2.2.43*).

Carefully remove the anticoagulant solution by means of the flexible transfer tube. Using a funnel fitted to the tube, completely fill the container with *water R*, leave in contact for 1 min while squeezing the container gently, then empty completely. Repeat the rinsing. The container, emptied and rinsed in this manner, complies with the test for plastic additives 01, 24, 25, 26 and 27 prescribed in general chapter *3.3.5. Empty sterile containers of plasticised poly(vinyl chloride) for human blood and blood components*.

STORAGE

See general chapter *3.3.4. Sterile plastic containers for human blood and blood components*.

LABELLING

See general chapter *3.3.4. Sterile plastic containers for human blood and blood components*.

4. REAGENTS

Additional information for reagents that can only be fully identified by a trademark or whose availability is limited may be found in the Knowledge database on the EDQM website.

04/2013:40000 *This information is given only to make it easier to obtain such reagents and this does not suggest in any way that the mentioned suppliers are especially recommended or certified by the European Pharmacopoeia Commission or the Council of Europe. It is therefore acceptable to use reagents from another source provided that they comply with the standards of the Pharmacopoeia.*

07/2018:40100

4.1. REAGENTS, STANDARD SOLUTIONS, BUFFER SOLUTIONS

Where the name of a substance or a solution is followed by the letter R (the whole in italics), this indicates a reagent included in the following list. The specifications given for reagents do not necessarily guarantee their quality for use in medicines.

Within the description of each reagent there is a 7-digit reference code in italics (for example, *1002501*). This number, which will remain unchanged for a given reagent during subsequent revisions of the list, is used for identification purposes by the Secretariat, and users of the Pharmacopoeia may also find it useful, for example in the management of reagent stocks. The description may also include a CAS number (Chemical Abstract Service Registry Number) recognisable by its typical format, for example 9002-93-1.

Some of the reagents included in the list are toxic and are to be handled in conformity with good quality control laboratory practice.

Reagents in aqueous solution are prepared using *water R*. For liquid chromatography, *water for chromatography R* is used for the preparation of mobile phases when water, or an aqueous solution, is one of the components. Where a reagent solution is described using an expression such as 'hydrochloric acid (10 g/L HCl)', the solution is prepared by an appropriate dilution with *water R* of a more concentrated reagent solution specified in this chapter. Reagent solutions used in the limit tests for barium, calcium and sulfates are prepared using *distilled water R*. Where the name of the solvent is not stated, an aqueous solution is intended.

The reagents and reagent solutions are to be stored in well-closed containers. The labelling should comply with the relevant national legislation and international agreements.

01/2020:40101

4.1.1. REAGENTS

Acacia. *1000100.*
See *Acacia (0307)*.

Acacia solution. *1000101.*
Dissolve 100 g of *acacia R* in 1000 mL of *water R*. Stir with a mechanical stirrer for 2 h. Centrifuge at about 2000 *g* for 30 min to obtain a clear solution.
Storage: in polyethylene containers of about 250 mL capacity at a temperature of 0 °C to − 20 °C.

Acebutolol hydrochloride. *1148900.* [34381-68-5].
See *Acebutolol hydrochloride (0871)*.

Acetal. $C_6H_{14}O_2$. (M_r 118.2). *1112300.* [105-57-7].
Acetaldehyde diethyl acetal. 1,1-Diethoxyethane.
Clear, colourless, volatile liquid, miscible with water and with ethanol (96 per cent).
d_{20}^{20}: about 0.824.
n_D^{20}: about 1.382.
bp: about 103 °C.

Acetaldehyde. C_2H_4O. (M_r 44.1). *1000200.* [75-07-0].
Ethanal.
Clear, colourless flammable liquid, miscible with water and with ethanol (96 per cent).
d_{20}^{20}: about 0.788.
n_D^{20}: about 1.332.
bp: about 21 °C.

Acetaldehyde ammonia trimer trihydrate.
$C_6H_{15}N_3,3H_2O$. (M_r 183.3). *1133500.* [58052-80-5].
2,4,6-Trimethylhexahydro-1,3,5-triazine trihydrate.
Content: minimum 95.0 per cent.
Colourless or white or pale yellow crystals or powder.
mp: 95 °C to 97 °C.
Assay. Dissolve 0.900 g in *water R* and dilute to 50.0 mL with the same solvent. Titrate with *1 M hydrochloric acid*, determining the end-point potentiometrically (*2.2.20*).
1 mL of *1 M hydrochloric acid* is equivalent to 61.08 mg of $C_6H_{15}N_3,3H_2O$.

Acetic acid, anhydrous. $C_2H_4O_2$. (M_r 60.1). *1000300.* [64-19-7].
Content: minimum 99.6 per cent *m/m* of $C_2H_4O_2$.
Colourless liquid or white or almost white, shining, fern-like crystals, miscible with or very soluble in water, in ethanol (96 per cent), in glycerol (85 per cent), and in most fatty and essential oils.
d_{20}^{20}: 1.052 to 1.053.
bp: 117 °C to 119 °C.
A 100 g/L solution is strongly acid (*2.2.4*).
A 5 g/L solution neutralised with *dilute ammonia R2* gives reaction (b) of acetates (*2.3.1*).
Freezing point (*2.2.18*): minimum 15.8 °C.
Water (*2.5.12*): maximum 0.4 per cent. If the water content is more than 0.4 per cent it may be adjusted by adding the calculated amount of *acetic anhydride R*.
Storage: protected from light.

Acetic acid, glacial. $C_2H_4O_2$. (M_r 60.1). *1000400.* [64-19-7].
See *Acetic acid, glacial (0590)*.

Acetic acid. *1000401.*
Content: 290 g/L to 310 g/L of $C_2H_4O_2$ (M_r 60.1).
Dilute 30 g of *glacial acetic acid R* to 100 mL with *water R*.

Acetic acid, dilute. *1000402.*
Content: 115 g/L to 125 g/L of $C_2H_4O_2$ (M_r 60.1).
Dilute 12 g of *glacial acetic acid R* to 100 mL with *water R*.

Acetic acid, dilute R1. *1000403.*
Content: 57.5 g/L to 62.5 g/L (M_r 60.1).
Dilute 6 g of *glacial acetic acid R* to 100 mL with *water R*.

Acetic anhydride. $C_4H_6O_3$. (M_r 102.1). *1000500.* [108-24-7].
Content: minimum 97.0 per cent *m/m* of $C_4H_6O_3$.
Clear, colourless liquid.
bp: 136 °C to 142 °C.
Assay. Dissolve 2.00 g in 50.0 mL of *1 M sodium hydroxide* in a ground-glass-stoppered flask and boil under a reflux condenser for 1 h. Titrate with *1 M hydrochloric acid*, using 0.5 mL of *phenolphthalein solution R* as indicator. Calculate the number of millilitres of *1 M sodium hydroxide* required for 1 g (n_1). Dissolve 2.00 g in 20 mL of *cyclohexane R* in a ground-glass-stoppered flask, cool in ice and add a cold mixture of 10 mL of *aniline R* and 20 mL of *cyclohexane R*. Boil the mixture under a reflux condenser for 1 h, add 50.0 mL of *1 M sodium hydroxide* and shake vigorously. Titrate with *1 M hydrochloric acid*, using 0.5 mL of *phenolphthalein solution R* as indicator. Calculate the number of millilitres of *1 M sodium hydroxide* required for 1 g (n_2). Calculate the percentage of $C_4H_6O_3$ from the following expression:

$$10.2(n_1 - n_2)$$

Acetic anhydride solution R1. *1000501.*
Dissolve 25.0 mL of *acetic anhydride R* in *anhydrous pyridine R* and dilute to 100.0 mL with the same solvent.
Storage: protected from light and air.

Acetic anhydride - sulfuric acid solution. *1000502.*
Carefully mix 5 mL of *acetic anhydride R* with 5 mL of *sulfuric acid R*. Add dropwise and with cooling to 50 mL of *anhydrous ethanol R*.
Prepare immediately before use.

Acetone. *1000600.* [67-64-1].
See *Acetone (0872)*.

Acetonitrile. C_2H_3N. (M_r 41.05). *1000700.* [75-05-8]. Methyl cyanide. Ethanenitrile.
Clear, colourless liquid, miscible with water, with acetone and with methanol.
d_{20}^{20}: about 0.78.
n_D^{20}: about 1.344.
A 100 g/L solution is neutral to litmus paper.
Distillation range (*2.2.11*). Not less than 95 per cent distils between 80 °C and 82 °C.
Acetonitrile used in spectrophotometry complies with the following additional test.
Absorbance (*2.2.25*): maximum 0.01 from 255 nm to 420 nm, determined using *water R* as compensation liquid.

Acetonitrile for chromatography. *1000701.*
See *Acetonitrile R*.
Acetonitrile used in chromatography complies with the following additional tests.
Absorbance (*2.2.25*): maximum 0.01 at 240 nm and higher wavelengths, determined using *water R* as compensation liquid.
Content (*2.2.28*): minimum 99.8 per cent.

Acetonitrile R1. *1000702.*
Complies with the requirements prescribed for *acetonitrile R* and with the following additional requirements.

Content: minimum 99.9 per cent.

Absorbance (*2.2.25*): maximum 0.10, determined at 200 nm using *water R* as the compensation liquid.

Acetoxyvalerenic acid. $C_{17}H_{24}O_4$. (M_r 292.4). *1165800.* [81397-67-3]. (2*E*)-3-[(1*RS*,4*S*,7*R*,7a*R*)-1-(Acetyloxy)-3,7-dimethyl-2,4,5,6,7,7a-hexahydro-1*H*-inden-4-yl]-2-methylprop-2-enoic acid.

Colourless or pale yellow viscous oil.

Absorbance (*2.2.25*). A solution in *methanol R* shows an absorption maximum at about 216 nm.

Acetylacetamide. $C_4H_7NO_2$. (M_r 101.1). *1102600.* [5977-14-0]. 3-Oxobutanamide.

mp: 53 °C to 56 °C.

Acetylacetone. $C_5H_8O_2$. (M_r 100.1). *1000900.* [123-54-6]. 2,4-Pentanedione.

Colourless or slightly yellow, easily flammable liquid, freely soluble in water, miscible with acetone, with ethanol (96 per cent) and with glacial acetic acid.

n_D^{20}: 1.452 to 1.453.

bp: 138 °C to 140 °C.

Acetylacetone reagent R1. *1000901.*

To 100 mL of *ammonium acetate solution R* add 0.2 mL of *acetylacetone R*.

Acetylacetone reagent R2. *1000902.*

Dissolve 0.2 mL of *acetylacetone R*, 3 mL of *glacial acetic acid R* and 25 g of *ammonium acetate R* in *water R* and dilute to 100 mL with the same solvent.

***N*-Acetyl-ε-caprolactam.** $C_8H_{13}NO_2$. (M_r 155.2). *1102700.* [1888-91-1]. *N*-Acetylhexane-6-lactam.

Colourless liquid, miscible with anhydrous ethanol.

d_{20}^{20}: about 1.100.

n_D^{20}: about 1.489.

bp: about 135 °C.

Acetyl chloride. C_2H_3ClO. (M_r 78.5). *1000800.* [75-36-5].

Clear, colourless liquid, flammable, decomposes in contact with water and with ethanol (96 per cent), miscible with ethylene chloride.

d_{20}^{20}: about 1.10.

Distillation range (*2.2.11*). Not less than 95 per cent distils between 49 °C and 53 °C.

Acetylcholine chloride. $C_7H_{16}ClNO_2$. (M_r 181.7). *1001000.* [60-31-1].

Crystalline powder, very soluble in cold water and in ethanol (96 per cent). It decomposes in hot water and in alkalis.

Storage: at − 20 °C.

Acetylene. C_2H_2. (M_r 26.04). *1199800.* [74-86-2]. Ethyne.

Content: minimum 99.0 per cent *V/V*.

Acetyleugenol. $C_{12}H_{14}O_3$. (M_r 206.2). *1100700.* [93-28-7]. 2-Methoxy-4-(2-propenyl)phenylacetate.

Yellow coloured, oily liquid, practically insoluble in water, freely soluble in ethanol (96 per cent).

n_D^{20}: about 1.521.

bp: 281 °C to 282 °C.

Acetyleugenol used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Clove oil (1091)*.

Test solution. The substance to be examined.

Content: minimum 98.0 per cent, calculated by the normalisation procedure.

***N*-Acetylglucosamine.** $C_8H_{15}NO_6$. (M_r 221.2). *1133600.* [7512-17-6]. 2-(Acetylamino)-2-deoxy-D-glucopyranose.

mp: about 202 °C.

Acetyl-11-keto-β-boswellic acid. $C_{32}H_{48}O_5$. (M_r 512.7). *1167700.* [67416-61-9]. 3α-(Acetyloxy)-11-oxours-12-en-24-oic acid. (4β)-3α-(Acetyloxy)-11-oxours-12-en-23-oic acid.

White or almost white powder, insoluble in water, soluble in acetone, in anhydrous ethanol and in methanol.

mp: 271 °C to 274 °C.

Acetyl-11-keto-β-boswellic acid used in liquid chromatography complies with the following additional test.

Assay. Liquid chromatography (*2.2.29*) as prescribed in the monograph on *Indian frankincense (2310)*.

Content: minimum 90 per cent, calculated by the normalisation procedure.

***N*-Acetylneuraminic acid.** $C_{11}H_{19}NO_9$. (M_r 309.3). *1001100.* [131-48-6]. *O*-Sialic acid.

White or almost white acicular crystals, soluble in water and in methanol, slightly soluble in anhydrous ethanol, practically insoluble in acetone.

$[\alpha]_D^{20}$: about − 36, determined on a 10 g/L solution.

mp: about 186 °C, with decomposition.

***N*-Acetyltryptophan.** $C_{13}H_{14}N_2O_3$. (M_r 246.3). *1102800.* [1218-34-4]. 2-Acetylamino-3-(indol-3-yl)propanoic acid.

White or almost white powder or colourless crystals, slightly soluble in water. It dissolves in dilute solutions of alkali hydroxides.

mp: about 205 °C.

Assay. Liquid chromatography (*2.2.29*) as prescribed in the monograph *Tryptophan (1272)*.

Test solution. Dissolve 10.0 mg in a mixture of 10 volumes of *acetonitrile R* and 90 volumes of *water R* and dilute to 100.0 mL with the same mixture of solvents.

Content: minimum 99.0 per cent, calculated by the normalisation procedure.

Acetyltyrosine ethyl ester. $C_{13}H_{17}NO_4,H_2O$. (M_r 269.3). *1001200.* [36546-50-6]. *N*-Acetyl-L-tyrosine ethyl ester monohydrate. Ethyl (*S*)-2-acetamido-3-(4-hydroxyphenyl)propionate monohydrate.

White or almost white, crystalline powder suitable for the assay of chymotrypsin.

$[\alpha]_D^{20}$: + 21 to + 25, determined on a 10 g/L solution in *ethanol (96 per cent) R*.

$A_{1\,cm}^{1\%}$: 60 to 68, determined at 278 nm in *ethanol (96 per cent) R*.

Acetyltyrosine ethyl ester, 0.2 M. *1001201.*

Dissolve 0.54 g of *acetyltyrosine ethyl ester R* in *ethanol (96 per cent) R* and dilute to 10.0 mL with the same solvent.

Acid blue 83. $C_{45}H_{44}N_3NaO_7S_2$. (M_r 826). *1012200.* [6104-59-2].

Colour Index No. 42660.
Brilliant blue. Coomassie brilliant blue R 250.

Brown powder insoluble in cold water, slightly soluble in boiling water and in anhydrous ethanol, soluble in sulfuric acid, glacial acetic acid and in dilute solutions of alkali hydroxides.

Acid blue 90. $C_{47}H_{48}N_3NaO_7S_2$. (M_r 854). *1001300.* [6104-58-1].

Colour Index No. 42655.

Sodium [4-[[4-[(4-ethoxyphenyl)amino]phenyl][[4-(ethyl)(3-sulfonatobenzyl)amino]phenyl]methylene]cyclo-hexa-2,5-dien-1-ylidene](ethyl)-(3-sulfonatobenzyl)ammonium.

A dark brown powder, with a violet sheen and some particles having a metallic lustre, soluble in water and in anhydrous ethanol.

$A_{1\,cm}^{1\%}$: greater than 500, determined at 577 nm in a 0.01 g/L solution in buffer solution pH 7.0 and calculated with reference to the dried substance.

Loss on drying (*2.2.32*): maximum 5.0 per cent, determined on 0.500 g by drying in an oven at 105 °C.

Acid blue 92. $C_{26}H_{16}N_3Na_3O_{10}S_3$. ($M_r$ 696). *1001400.* [3861-73-2].

Colour Index No. 13390.

Coomassie blue. Anazolene sodium. Trisodium 8-hydroxy-4′-(phenylamino)azonaphthalene-3,5′,6-trisulfonate.

Dark blue crystals, soluble in water, in acetone and in ethylene glycol monoethylether, slightly soluble in ethanol (96 per cent).

Acid blue 92 solution. *1001401.*

Dissolve 0.5 g of *acid blue 92 R* in a mixture of 10 mL of *glacial acetic acid R*, 45 mL of *ethanol (96 per cent) R* and 45 mL of *water R*.

Acid blue 93. $C_{37}H_{27}N_3Na_2O_9S_3$. ($M_r$ 800). *1134200.* [28983-56-4].

Colour Index No. 42780.

Methyl blue. Poirrier blue.

Mixture of triphenylrosaniline di- and trisulfonate and of triphenylpararosaniline.

Dark blue powder.

Colour change: pH 9.4 to pH 14.0.

Acid blue 93 solution. *1134201.*

Dissolve 0.2 g of *acid blue 93 R* in *water R* and dilute to 100 mL with the same solvent.

α1-Acid-glycoprotein silica gel for chiral separation. *1148700.*

A very finely divided silica gel for chromatography consisting of spherical particles coated with α1-acid glycoprotein.

Acrylamide. C_3H_5NO. (M_r 71.1). *1001500.* [79-06-1].

Propenamide.

Colourless or white flakes or a white or almost white, crystalline powder, very soluble in water and in methanol, freely soluble in anhydrous ethanol.

mp: about 84 °C.

30 per cent acrylamide/bisacrylamide (29:1) solution. *1001501.*

Prepare a solution containing 290 g of *acrylamide R* and 10 g of *methylenebisacrylamide R* per litre of *water R*. Filter.

30 per cent acrylamide/bisacrylamide (36.5:1) solution. *1001502.*

Prepare a solution containing 292 g of *acrylamide R* and 8 g of *methylenebisacrylamide R* per litre of *water R*. Filter.

Acrylic acid. $C_3H_4O_2$. (M_r 72.1). *1133700.* [79-10-7].

Prop-2-enoic acid. Vinylformic acid.

Content: minimum 99 per cent.

It is stabilised with 0.02 per cent of hydroquinone monomethyl ether.

Corrosive liquid, miscible with water and ethanol (96 per cent). It polymerises readily in the presence of oxygen.

d_{20}^{20}: about 1.05.

n_D^{20}: about 1.421.

bp: about 141 °C.

mp: 12 °C to 15 °C.

Actein. $C_{37}H_{56}O_{11}$. (M_r 677). *1181500.* [18642-44-9].

(23*R*,24*R*,25*S*,26*S*)-3β-(β-D-Xylopyranosyloxy)-16β,23:23,26:24,25-triepoxy-26-hydroxy-9,19-cyclolanostan-12β-yl acetate.

Acteoside. $C_{29}H_{36}O_{15}$. (M_r 624.6). *1145100.* [61276-17-3]. 2-(3,4-Dihydroxyphenyl)ethyl 3-*O*-(6-deoxy-α-L-mannopyranosyl)-4-*O*-[(2*E*)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]-β-D-glucopyranoside. Verbascoside.

Light yellowish powder, freely soluble in water and in methanol.

mp: about 140 °C, with decomposition.

Adamantane. $C_{10}H_{16}$. (M_r 136.2). *1181600.* [281-23-2]. Tricyclo[3.3.1.1^{3,7}]decane.

mp: about 270 °C.

Adenine. *1172800.* [73-24-5].

See *Adenine (0800)*.

Adenosine. $C_{10}H_{13}N_5O_4$. (M_r 267.2). *1001600.* [58-61-7]. 6-Amino-9-β-D-ribofuranosyl-9*H*-purine.

White or almost white, crystalline powder, slightly soluble in water, practically insoluble in acetone and in ethanol (96 per cent). It dissolves in dilute solutions of acids.

mp: about 234 °C.

Adipic acid. $C_6H_{10}O_4$. (M_r 146.1). *1095600.* [124-04-9].

Prisms, freely soluble in methanol, soluble in acetone, practically insoluble in light petroleum.

mp: about 152 °C.

Adrenaline. $C_9H_{13}NO_3$. (M_r 183.2). *1155000.* [51-43-4]. (1*R*)-1-(3,4-Dihydroxyphenyl)-2-(methylamino)ethanol. 4-[(1*R*)-1-hydroxy-2-(methylamino)ethyl]benzene-1,2-diol.

White or almost white powder, gradually becoming brown on exposure to light and air, very slightly soluble in water and in ethanol (96 per cent), insoluble in acetone. It dissolves in dilute solutions of mineral acids and alkali hydroxides.

mp: about 215 °C.

Adrenalone hydrochloride. $C_9H_{12}ClNO_3$. (M_r 217.7). *1155100.* [62-13-5]. 1-(3,4-Dihydroxyphenyl)-2-(methylamino)ethanone hydrochloride. 3′,4′-Dihydroxy-2-(methylamino)acetophenone hydrochloride.

Pale yellow crystals, freely soluble in water, soluble in ethanol (96 per cent).

mp: about 244 °C.

Aescin. *1001700.* [6805-41-0].

A mixture of related saponins obtained from the seeds of *Aesculus hippocastanum* L.

Fine, almost white or slightly reddish or yellowish, amorphous powder.

Chromatography. Thin-layer chromatography (*2.2.27*).

Test solution. Dissolve 10 mg of *aescin R* in *ethanol (70 per cent V/V) R* and dilute to 10 mL with the same solvent.

Plate: *TLC silica gel plate R*.

Mobile phase: the upper layer of a mixture of 10 volumes of *glacial acetic acid R*, 40 volumes of *water R* and 50 volumes of *butanol R*.

Application: 20 µL of the test solution as bands of 20 mm by 3 mm.

Development: over a path of 12 cm.

Drying: at 100-105 °C.

Detection: spray with about 10 mL of *anisaldehyde solution R* for a plate 200 mm square and heat again at 100-105 °C.

Results: the chromatogram shows a principal band with an R_F of about 0.4.

Aflatoxin B_1. $C_{17}H_{12}O_6$. (M_r 312.3). *1166000.* [1162-65-8]. (6a*R*,9a*S*)-4-Methoxy-2,3,6a,9a-tetrahydrocyclopenta[*c*]furo[3′,2′:4,5]furo[2,3-*h*][1]benzopyran-1,11-dione.

White or faint yellow crystals.

Agarose/cross-linked polyacrylamide. *1002200.*

Agarose trapped within a cross-linked polyacrylamide network; it is used for the separation of globular proteins with relative molecular masses of 2×10^4 to 35×10^4.

Agarose-DEAE for ion-exchange chromatography. *1002100.* [57407-08-6].

Cross-linked agarose substituted with diethylaminoethyl groups, presented as beads.

Agarose for chromatography. *1001800.* [9012-36-6].

Swollen beads 60-140 µm in diameter presented as a 4 per cent suspension in *water R*.

Used in size-exclusion chromatography for the separation of proteins with relative molecular masses of 6×10^4 to 20×10^6 and of polysaccharides with relative molecular masses of 3×10^3 to 5×10^6.

Agarose for chromatography, cross-linked. *1001900.* [61970-08-9].

Prepared from agarose by reaction with 2,3-dibromopropanol in strongly alkaline conditions.

It occurs as swollen beads 60-140 µm in diameter and is presented as a 4 per cent suspension in *water R*.

Used in size-exclusion chromatography for the separation of proteins with relative molecular masses of 6×10^4 to 20×10^6 and of polysaccharides with relative molecular masses of 3×10^3 to 5×10^6.

Agarose for chromatography, cross-linked R1. *1001901.* [65099-79-8].

Prepared for agarose by reaction with 2,3-dibromopropanol in strongly alkaline conditions.

It occurs as swollen beads 60-140 µm in diameter and is presented as a 4 per cent suspension in *water R*.

Used in size-exclusion chromatography for the separation of proteins with relative molecular masses of 7×10^4 to 40×10^6 and of polysaccharides with relative molecular masses of 1×10^5 to 2×10^7.

Agarose for electrophoresis. *1002000.* [9012-36-6].

A neutral, linear polysaccharide, the main component of which is derived from agar.

White or almost white powder, practically insoluble in cold water, very slightly soluble in hot water.

Agnuside. $C_{22}H_{26}O_{11}$. (M_r 466.4). *1162000.* [11027-63-7]. (1*RS*,4a*SR*,5*RS*,7a*RS*)-5-Hydroxy-7-[[(4-hydroxybenzoyl)oxy]methyl]-1,4a,5,7a-tetrahydrocyclopenta[*c*]pyran-1-yl β-D-glucopyranoside.

White or almost white crystals.

Air, hydrocarbon-free. *1188700.*

Complies with the requirements prescribed for the monograph *Medicinal air (1238)* with the following additional requirement.

Hydrocarbons: maximum 5 ppm *V/V*, calculated as CH_4.

Alanine. *1102900.* [56-41-7].

See *Alanine (0752)*.

β-Alanine. *1004500.* [107-95-9].

See *3-aminopropionic acid R*.

Albumin, bovine. *1002300.* [9048-46-8].

Bovine serum albumin containing about 96 per cent of protein.

White to light brownish-yellow powder.

Water (*2.5.12*): maximum 3.0 per cent, determined on 0.800 g.

Albumin, bovine R1. *1183500.* [9048-46-8].

Bovine serum albumin containing about 96 per cent of protein.

White or light brownish-yellow powder.

Albumin, human. *1133800.*

Human serum albumin containing not less than 96 per cent of albumin.

Albumin solution, human. *1002400.* [9048-46-8].

See *Human albumin solution (0255)*.

Albumin solution, human R1. *1002401.*

Dilute *human albumin solution R* with a 9 g/L solution of *sodium chloride R* to a concentration of 1 g/L of protein. Adjust the pH to 3.5-4.5 with *glacial acetic acid R*.

Alcohol. *1002500.* [64-17-5].

See *Ethanol (96 per cent) R*.

Alcohol (*x* per cent *V/V*). *1002502.*

See *Ethanol (x per cent V/V) R*.

Alcohol, aldehyde-free. *1002501.*

Mix 1200 mL of *ethanol (96 per cent) R* with 5 mL of a 400 g/L solution of *silver nitrate R* and 10 mL of a cooled 500 g/L solution of *potassium hydroxide R*. Shake, allow to stand for a few days and filter. Distil the filtrate immediately before use.

Aldehyde dehydrogenase. *1103000.*

Enzyme obtained from baker's yeast which oxidises acetaldehyde to acetic acid in the presence of nicotinamide-adenine dinucleotide, potassium salts and thiols, at pH 8.0.

Aldehyde dehydrogenase solution. *1103001.*

Dissolve in *water R* a quantity of *aldehyde dehydrogenase R*, equivalent to 70 units and dilute to 10 mL with the same solvent. This solution is stable for 8 h at 4 °C.

Aldrin. $C_{12}H_8Cl_6$. (M_r 364.9). *1123100.* [309-00-2].

bp: about 145 °C.

mp: about 104 °C.

A suitable certified reference solution (10 ng/µL in cyclohexane) may be used.

Aleuritic acid. $C_{16}H_{32}O_5$. (M_r 304.4). *1095700.* [533-87-9]. (9*RS*,10*SR*)-9,10,16-Trihydroxyhexadecanoic acid.

White or almost white powder, greasy to the touch, soluble in methanol.

mp: about 101 °C.

Alizarin S. $C_{14}H_7NaO_7S,H_2O$. (M_r 360.3). *1002600.* [130-22-3].

Schultz No. 1145.

Colour Index No. 58005.
Sodium 1,2-dihydroxyanthraquinone-3-sulfonate monohydrate. Sodium 3,4-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-sulfonate monohydrate.

Orange-yellow powder, freely soluble in water and in ethanol (96 per cent).

Alizarin S solution. *1002601.*

A 1 g/L solution of *alizarin S R*.

Test for sensitivity. If alizarin S solution is used for the standardisation of *0.05 M barium perchlorate*, it shows a colour change from yellow to orange-red when it is tested according to the standardisation of *0.05 M barium perchlorate*.

Colour change: pH 3.7 (yellow) to pH 5.2 (violet).

Aloe emodin. $C_{15}H_{10}O_5$. (M_r 270.2). *1188800.* [481-72-1].
1,8-Dihydroxy-3-(hydroxymethyl)anthracene-9,10-dione.
1,8-Dihydroxy-3-(hydroxymethyl)anthraquinone.

Alovudine. $C_{10}H_{13}FN_2O_4$. (M_r 244.2). *1185400.* [25526-93-6]. 1-[(2*R*,4*S*,5*R*)-4-Fluoro-5-(hydroxymethyl)tetrahydrofuran-2-yl]-5-methylpyrimidine-2,4(1*H*,3*H*)-dione. Fluorodeoxythymidine. 3′-Deoxy-3′-fluorothymidine.
Content: minimum 95 per cent.
Colourless crystals.

Aluminium. Al. (A_r 26.98). *1118200.* [7429-90-5].
White or almost white, malleable, flexible, bluish metal, available as bars, sheets, powder, strips or wire. In moist air an oxide film forms which protects the metal from corrosion.
Analytical grade.

Aluminium chloride. $AlCl_3,6H_2O$. (M_r 241.4). *1002700.* [7784-13-6]. Aluminium chloride hexahydrate.
Content: minimum 98.0 per cent of $AlCl_3,6H_2O$.
White or slightly yellowish, crystalline powder, hygroscopic, freely soluble in water and in ethanol (96 per cent).
Storage: in an airtight container.

Aluminium chloride reagent. *1002702.*
Dissolve 2.0 g of *aluminium chloride R* in 100 mL of a 5 per cent *V/V* solution of *glacial acetic acid R* in *methanol R*.

Aluminium chloride solution. *1002701.*
Dissolve 65.0 g of *aluminium chloride R* in *water R* and dilute to 100 mL with the same solvent. Add 0.5 g of *activated charcoal R*, stir for 10 min, filter and add to the filtrate, with continuous stirring, sufficient of a 10 g/L solution of *sodium hydroxide R* (about 60 mL) to adjust the pH to about 1.5.

Aluminium nitrate. $Al(NO_3)_3,9H_2O$. (M_r 375.1). *1002800.* [7784-27-2]. Aluminium nitrate nonahydrate.
Crystals, deliquescent, very soluble in water and ethanol (96 per cent), very slightly soluble in acetone.
Storage: in an airtight container.

Aluminium oxide, anhydrous. *1002900.* [1344-28-1].
Aluminium oxide, consisting of γ-Al_2O_3, dehydrated and activated by heat treatment.
Particle size: 75 µm to 150 µm.

Aluminium oxide, basic. *1118300.*
A basic grade of *anhydrous aluminium oxide R* suitable for column chromatography.
pH (*2.2.3*). Shake 1 g with 10 mL of *carbon dioxide-free water R* for 5 min. The pH of the suspension is 9 to 10.

Aluminium oxide for chromatography, deactivated. *1188900.*
Aluminium oxide suitably deactivated for the separation and detection of traces of polar hydrocarbons, with porous layer open tubular (PLOT) design.

Aluminium oxide, neutral. *1118400.*
See *Aluminium oxide, hydrated (0311)*.

Aluminium potassium sulfate. *1003000.* [7784-24-9].
See *Alum (0006)*.

Aluminium test strip. *1199900.*
Commercially available test strip for the determination of aluminium in aqueous solvents at a level below 5 ppm.

Americium-243 spiking solution. *1167500.*
Contains 50 Bq/L ^{243}Am and a 134 mg/L solution of *lanthanum chloride heptahydrate R* in a 103 g/L solution of *hydrochloric acid R*.

Amido black 10B. $C_{22}H_{14}N_6Na_2O_9S_2$. ($M_r$ 617). *1003100.* [1064-48-8].
Schultz No. 299.
Colour Index No. 20470.
Disodium 5-amino-4-hydroxy-6-[(4-nitrophenyl)azo]-3-(phenylazo)naphthalene-2,7-disulfonate.
Dark-brown to black powder, sparingly soluble in water, soluble in ethanol (96 per cent).

Amido black 10B solution. *1003101.*
A 5 g/L solution of *amido black 10B R* in a mixture of 10 volumes of *acetic acid R* and 90 volumes of *methanol R*.

Aminoazobenzene. $C_{12}H_{11}N_3$. (M_r 197.2). *1003200.* [60-09-3].
Colour Index No. 11000.
4-(Phenylazo)aniline.
Brownish-yellow needles with a bluish tinge, slightly soluble in water, freely soluble in ethanol (96 per cent).
mp: about 128 °C.

2-Aminobenzoic acid. $C_7H_7NO_2$. (M_r 137.1). *1003400.* [118-92-3]. Anthranilic acid.
A white or pale-yellow, crystalline powder, sparingly soluble in cold water, freely soluble in hot water, in ethanol (96 per cent) and in glycerol. Solutions in ethanol (96 per cent) or in ether and, particularly, in glycerol show a violet fluorescence.
mp: about 145 °C.

3-Aminobenzoic acid. $C_7H_7NO_2$. (M_r 137.1). *1147400.* [99-05-8].
White or almost white crystals. An aqueous solution turns brown on standing in air.
mp: about 174 °C.
Storage: in an airtight container, protected from light.

4-Aminobenzoic acid. $C_7H_7NO_2$. (M_r 137.1). *1003300.* [150-13-0].
White or almost white, crystalline powder, slightly soluble in water, freely soluble in ethanol (96 per cent), practically insoluble in light petroleum.
mp: about 187 °C.
Chromatography. Thin-layer chromatography (*2.2.27*) as prescribed in the monograph *Procaine hydrochloride (0050)*; the chromatogram shows only one principal spot.
Storage: protected from light.

4-Aminobenzoic acid solution. *1003301.*
Dissolve 1 g of *4-aminobenzoic acid R* in a mixture of 18 mL of *anhydrous acetic acid R*, 20 mL of *water R* and 1 mL of *phosphoric acid R*. Immediately before use, mix 2 volumes of the solution with 3 volumes of *acetone R*.

***N*-(4-Aminobenzoyl)-L-glutamic acid.** $C_{12}H_{14}N_2O_5$. (M_r 266.3). *1141700.* [4271-30-1]. ABGA. (2*S*)-2-[(4-Aminobenzoyl)amino]pentanedioic acid.
White or almost white, crystalline powder.
mp: about 175 °C, with decomposition.

4-Aminobutanoic acid. $C_4H_9NO_2$. (M_r 103.1). *1123200.* [56-12-2]. γ-Aminobutyric acid. GABA.
Leaflets from methanol and ether, needles from water and ethanol (96 per cent). Freely soluble in water, practically insoluble or slightly soluble in other solvents.
mp: about 202 °C (decreases on rapid heating).

Aminobutanol. $C_4H_{11}NO$. (M_r 89.1). *1003500.* [5856-63-3]. 2-Aminobutanol.
Oily liquid, miscible with water, soluble in ethanol (96 per cent).
d_{20}^{20}: about 0.94.

n_D^{20}: about 1.453.

bp: about 180 °C.

Aminochlorobenzophenone. $C_{13}H_{10}ClNO$. (M_r 231.7). *1003600.* [719-59-5]. 2-Amino-5-chlorobenzophenone.

Yellow, crystalline powder, practically insoluble in water, freely soluble in acetone, soluble in ethanol (96 per cent).

mp: about 97 °C.

Content: minimum 95.0 per cent.

Storage: protected from light.

4-Aminofolic acid. $C_{19}H_{20}N_8O_5$. (M_r 440.4). *1163700.* [54-62-6]. (2*S*)-2-[[4-[[(2,4-Diaminopteridin-6-yl)methyl]amino]benzoyl]amino]pentanedioic acid. *N*-[4-[[(2,4-Diaminopteridin-6-yl)methyl]amino]benzoyl]-L-glutamic acid. Aminopterine.

Yellowish powder.

mp: about 230 °C.

6-Aminohexanoic acid. $C_6H_{13}NO_2$. (M_r 131.2). *1103100.* [60-32-2].

Colourless crystals, freely soluble in water, sparingly soluble in methanol, practically insoluble in anhydrous ethanol.

mp: about 205 °C.

Aminohippuric acid. $C_9H_{10}N_2O_3$. (M_r 194.2). *1003700.* [61-78-9]. (4-Aminobenzamido)acetic acid.

White or almost white powder, sparingly soluble in water, soluble in ethanol (96 per cent).

mp: about 200 °C.

Aminohippuric acid reagent. *1003701.*

Dissolve 3 g of *phthalic acid R* and 0.3 g of *aminohippuric acid R* in *ethanol (96 per cent) R* and dilute to 100 mL with the same solvent.

Aminohydroxynaphthalenesulfonic acid. $C_{10}H_9NO_4S$. (M_r 239.3). *1112400.* [116-63-2]. 4-Amino-3-hydroxynaphthalene-1-sulfonic acid.

White or grey needles, turning pink on exposure to light, especially when moist, practically insoluble in water and in ethanol (96 per cent), soluble in solutions of alkali hydroxides and in hot solutions of sodium metabisulfite.

Storage: protected from light.

Aminohydroxynaphthalenesulfonic acid solution. *1112401.*

Mix 5.0 g of *anhydrous sodium sulfite R* with 94.3 g of *sodium hydrogensulfite R* and 0.7 g of *aminohydroxynaphthalenesulfonic acid R*. Dissolve 1.5 g of the mixture in *water R* and dilute to 10.0 mL with the same solvent. Prepare the solution daily.

***cis*-Aminoindanol.** $C_9H_{11}NO$. (M_r 149.2). *1168300.* [126456-43-7]. (1*S*,2*R*)-1-Amino-2,3-dihydro-1*H*-inden-2-ol. (−)-*cis*-1-Aminoindan-2-ol.

Content: minimum 98.0 per cent (sum of enantiomers, determined by gas chromatography).

$[\alpha]_D^{20}$: − 69 to − 59, determined on a 2 g/L solution in *chloroform R*.

mp: 118 °C to 122 °C.

Aminomethylalizarindiacetic acid. $C_{19}H_{15}NO_8,2H_2O$. (M_r 421.4). *1003900.* [3952-78-1]. 2,2′-[(3,4-dihydroxy-anthraquinon-3-yl)methylenenitrilo]diacetic acid dihydrate. Alizarin complexone dihydrate.

Fine, pale brownish-yellow or orange-brown powder, practically insoluble in water, soluble in solutions of alkali hydroxides.

mp: about 185 °C.

Loss on drying (*2.2.32*): maximum 10.0 per cent, determined on 1.000 g.

Aminomethylalizarindiacetic acid reagent. *1003901.*

Solution A. Dissolve 0.36 g of *cerous nitrate R* in *water R* and dilute to 50 mL with the same solvent.

Solution B. Suspend 0.7 g of *aminomethylalizarindiacetic acid R* in 50 mL of *water R*. Dissolve with the aid of about 0.25 mL of *concentrated ammonia R*, add 0.25 mL of *glacial acetic acid R* and dilute to 100 mL with *water R*.

Solution C. Dissolve 6 g of *sodium acetate R* in 50 mL of *water R*, add 11.5 mL of *glacial acetic acid R* and dilute to 100 mL with *water R*.

To 33 mL of *acetone R* add 6.8 mL of solution C, 1.0 mL of solution B and 1.0 mL of solution A and dilute to 50 mL with *water R*.

Test for sensitivity. To 1.0 mL of *fluoride standard solution (10 ppm F) R* add 19.0 mL of *water R* and 5.0 mL of the aminomethylalizarindiacetic acid reagent. After 20 min, the solution assumes a blue colour.

Storage: use within 5 days.

Aminomethylalizarindiacetic acid solution. *1003902.*

Dissolve 0.192 g of *aminomethylalizarindiacetic acid R* in 6 mL of freshly prepared *1 M sodium hydroxide*. Add 750 mL of *water R*, 25 mL of *succinate buffer solution pH 4.6 R* and, dropwise, *0.5 M hydrochloric acid* until the colour changes from violet-red to yellow (pH 4.5 to 5). Add 100 mL of *acetone R* and dilute to 1000 mL with *water R*.

4-Aminomethylbenzoic acid. $C_8H_9NO_2$. (M_r 151.2). *1167800.* [56-91-7].

Aminonitrobenzophenone. $C_{13}H_{10}N_2O_3$. (M_r 242.2). *1004000.* [1775-95-7]. 2-Amino-5-nitrobenzophenone.

Yellow, crystalline powder, practically insoluble in water, soluble in tetrahydrofuran, slightly soluble in methanol.

mp: about 160 °C.

$A_{1\,cm}^{1\%}$: 690 to 720, determined at 233 nm using a 0.01 g/L solution in *methanol R*.

6-Aminopenicillanic acid. $C_8H_{12}N_2O_3S$. (M_r 216.3). *1162100.* [551-16-6]. (2*S*,5*R*,6*R*)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

Appearance: white or almost white powder.

mp: about 205 °C, with decomposition.

Aminophenazone. $C_{13}H_{17}N_3O$. (231.3). *1133900.* [58-15-1]. 4-(Dimethylamino)-1,5-dimethyl-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-one.

White or almost white, crystalline powder or colourless crystals, soluble in water, freely soluble in ethanol (96 per cent).

mp: about 108 °C.

2-Aminophenol. C_6H_7NO. (M_r 109.1). *1147500.* [95-55-6].

Pale yellowish-brown crystals which rapidly become brown, sparingly soluble in water, soluble in ethanol (96 per cent).

mp: about 172 °C.

Storage: in an airtight container, protected from light.

3-Aminophenol. C_6H_7NO. (M_r 109.1). *1147600.* [591-27-5].

Pale yellowish-brown crystals, sparingly soluble in water.

mp: about 122 °C.

4-Aminophenol. C_6H_7NO. (M_r 109.1). *1004300.* [123-30-8].

Content: minimum 95 per cent.

White or slightly coloured, crystalline powder, becoming coloured on exposure to air and light, sparingly soluble in water, soluble in anhydrous ethanol.

mp: about 186 °C, with decomposition.

Storage: protected from light.

Aminopolyether. $C_{18}H_{36}N_2O_6$. (M_r 376.5). *1112500.* [23978-09-8]. 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8,8,8]hexacosane.

mp: 70 °C to 73 °C.

3-Aminopropanol. C_3H_9NO. (M_r 75.1). *1004400.* [156-87-6]. 3-Aminopropan-1-ol. Propanolamine.

Clear, colourless, viscous liquid.

d_{20}^{20}: about 0.99.

n_D^{20}: about 1.461.

mp: about 11 °C.

3-Aminopropionic acid. $C_3H_7NO_2$. (M_r 89.1). *1004500.* [107-95-9]. β-Alanine.

Content: minimum 99 per cent.

White or almost white, crystalline powder, freely soluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in acetone.

mp: about 200 °C, with decomposition.

Aminopyrazolone. $C_{11}H_{13}N_3O$. (M_r 203.2). *1004600.* [83-07-8]. 4-Amino-2,3-dimethyl-1-phenylpyrazolin-5-one.

Light-yellow needles or powder, sparingly soluble in water, freely soluble in ethanol (96 per cent).

mp: about 108 °C.

Aminopyrazolone solution. *1004601.*

A 1 g/L solution of *aminopyrazolone R* in *buffer solution pH 9.0 R*.

3-Aminosalicylic acid. $C_7H_7NO_3$. (M_r 153.1). *1183600.* [570-23-0]. 3-Amino-2-hydroxybenzoic acid.

mp: about 240 °C.

Slightly soluble in water.

4-Aminosalicylic acid. $C_7H_7NO_3$. (M_r 153.1). *1183700.* [65-49-6]. 4-Amino-2-hydroxybenzoic acid.

White or almost white, bulky powder, slightly soluble in water, soluble in ethanol (96 per cent), in dilute nitric acid and in sodium hydroxide. It darkens on exposure to air and light.

mp: 135 °C to 145 °C.

Storage: at a temperature not exceeding 30 °C, in an airtight container, protected from light.

Ammonia, concentrated. *1004700.*

See *Concentrated ammonia solution (0877)*.

Ammonia. *1004701.*

Content: 170 g/L to 180 g/L of NH_3 (M_r 17.03).

Dilute 67 g of *concentrated ammonia R* to 100 mL with *water R*.

d_{20}^{20}: 0.931 to 0.934.

When used in the test for iron, *ammonia R* complies with the following additional requirement. Evaporate 5 mL of ammonia to dryness on a water-bath, add 10 mL of *water R*, 2 mL of a 200 g/L solution of *citric acid monohydrate R* and 0.1 mL of *thioglycollic acid R*. Make alkaline by adding *ammonia R* and dilute to 20 mL with *water R*. No pink colour develops.

Storage: protected from atmospheric carbon dioxide, at a temperature below 20 °C.

Ammonia, dilute R1. *1004702.*

Content: 100 g/L to 104 g/L of NH_3 (M_r 17.03).

Dilute 41 g of *concentrated ammonia R* to 100 mL with *water R*.

Ammonia, dilute R2. *1004703.*

Content: 33 g/L to 35 g/L of NH_3 (M_r 17.03).

Dilute 14 g of *concentrated ammonia R* to 100 mL with *water R*.

Ammonia, dilute R3. *1004704.*

Content: 1.6 g/L to 1.8 g/L of NH_3 (M_r 17.03).

Dilute 0.7 g of *concentrated ammonia R* to 100 mL with *water R*.

Ammonia, dilute R4. *1004706.*

Content: 8.4 g/L to 8.6 g/L of NH_3 (M_r 17.03).

Dilute 3.5 g of *concentrated ammonia R* to 100 mL with *water R*.

Ammonia, lead-free. *1004705.*

Complies with the requirements prescribed for *dilute ammonia R1* with the following additional test: to 20 mL of lead-free ammonia, add 1 mL of *lead-free potassium cyanide solution R*, dilute to 50 mL with *water R* and add 0.10 mL of *sodium sulfide solution R*. The solution is not more intensely coloured than a reference solution prepared without sodium sulfide.

Ammonia, concentrated R1. *1004800.*

Content: minimum 30.0 per cent *m/m* of NH_3 (M_r 17.03).

A clear, colourless liquid.

d_{20}^{20}: less than 0.892.

Assay. Weigh accurately a ground-glass-stoppered flask containing 50.0 mL of *1 M hydrochloric acid*. Introduce 2 mL of *concentrated ammonia R1* and weigh again. Titrate the solution with *1 M sodium hydroxide*, using 0.5 mL of *methyl red mixed solution R* as indicator.

1 mL of *1 M hydrochloric acid* is equivalent to 17.03 mg of NH_3.

Storage: protected from atmospheric carbon dioxide, at a temperature below 20 °C.

Ammonium acetate. $C_2H_7NO_2$. (M_r 77.1). *1004900.* [631-61-8].

Colourless crystals, very deliquescent, very soluble in water and in ethanol (96 per cent).

Storage: in an airtight container.

Ammonium acetate solution. *1004901.*

Dissolve 150 g of *ammonium acetate R* in *water R*. Add 3 mL of *glacial acetic acid R* and dilute to 1000 mL with *water R*.

Storage: use within 1 week.

Ammonium and cerium nitrate. $(NH_4)_2Ce(NO_3)_6$. (M_r 548.2). *1005000.* [16774-21-3].

Orange-yellow, crystalline powder, or orange transparent crystals, soluble in water.

Ammonium and cerium sulfate. $(NH_4)_4Ce(SO_4)_4,2H_2O$. (M_r 633). *1005100.* [10378-47-9].

Orange-yellow, crystalline powder or crystals, slowly soluble in water.

(1*R*)-(–)-Ammonium 10-camphorsulfonate. $C_{10}H_{19}NO_4S$. (M_r 249.3). *1103200.*

Content: minimum 97.0 per cent of (1*R*)-(–)-ammonium 10-camphorsulfonate.

$[\alpha]_D^{20}$: – 18 ± 2, determined on a 50 g/L solution.

Ammonium carbamate. $CH_6N_2O_2$. (M_r 78.1). *1168400.* [1111-78-0]. Carbamic acid ammonium salt.

Ammonium carbonate. *1005200.* [506-87-6]. A mixture of varying proportions of ammonium hydrogen carbonate (NH_4HCO_3, M_r 79.1) and ammonium carbamate (NH_2COONH_4, M_r 78.1).

White or almost white translucent mass, slowly soluble in about 4 parts of water. It is decomposed by boiling water. Ammonium carbonate liberates not less than 30 per cent *m/m* of NH_3 (M_r 17.03).

Assay. Dissolve 2.00 g in 25 mL of *water R*. Slowly add 50.0 mL of *1 M hydrochloric acid*, titrate with *1 M sodium hydroxide*, using 0.1 mL of *methyl orange solution R* as indicator.

1 mL of *1 M hydrochloric acid* is equivalent to 17.03 mg of NH_3.

Storage: at a temperature below 20 °C.

Ammonium carbonate solution. *1005201.*

A 158 g/L solution of *ammonium carbonate R*.

Ammonium carbonate solution R1. *1005202.*

Dissolve 20 g of *ammonium carbonate R* in 20 mL of *dilute ammonia R1* and dilute to 100 mL with *water R*.

Ammonium chloride. *1005300.* [12125-02-9].

See *Ammonium chloride (0007)*.

Ammonium chloride solution. *1005301.*

A 107 g/L solution of *ammonium chloride R*.

Ammonium citrate. $C_6H_{14}N_2O_7$. (M_r 226.2). *1103300.* [3012-65-5]. Diammonium hydrogen citrate.

White or almost white, crystalline powder or colourless crystals, freely soluble in water, slightly soluble in ethanol (96 per cent).

pH (*2.2.3*): about 4.3 for a 22.6 g/L solution.

Ammonium dihydrogen phosphate. $(NH_4)H_2PO_4$. (M_r 115.0). *1005400.* [7722-76-1]. Monobasic ammonium phosphate.

White or almost white, crystalline powder or colourless crystals, freely soluble in water.

pH (*2.2.3*): about 4.2 for a 23 g/L solution.

Ammonium formate. CH_5NO_2. (M_r 63.1). *1112600.* [540-69-2].

Deliquescent crystals or granules, very soluble in water, soluble in ethanol (96 per cent).

mp: 119 °C to 121 °C.

Storage: in an airtight container.

Ammonium hexafluorogermanate(IV). $(NH_4)_2GeF_6$. (M_r 222.7). *1134000.* [16962-47-3].

White or almost white crystals, freely soluble in water.

Ammonium hydrogen carbonate. NH_4HCO_3. (M_r 79.1). *1005500.* [1066-33-7].

Content: minimum 99 per cent.

Ammonium molybdate. $(NH_4)_6Mo_7O_{24},4H_2O$. (M_r 1236). *1005700.* [12054-85-2].

Colourless or slightly yellow or greenish crystals, soluble in water, practically insoluble in ethanol (96 per cent).

Ammonium molybdate reagent. *1005701.*

Mix, in the given order, 1 volume of a 25 g/L solution of *ammonium molybdate R*, 1 volume of a 100 g/L solution of *ascorbic acid R* and 1 volume of *sulfuric acid R* (294.5 g/L H_2SO_4). Add 2 volumes of *water R*.

Storage: use within 1 day.

Ammonium molybdate reagent R1. *1005706.*

Mix 10 mL of a 60 g/L solution of *disodium arsenate R*, 50 mL of *ammonium molybdate solution R*, 90 mL of *dilute sulfuric acid R* and dilute to 200 mL in *water R*.

Storage: in amber flasks at 37 °C for 24 h.

Ammonium molybdate reagent R2. *1005708.*

Dissolve 50 g of *ammonium molybdate R* in 600 mL of *water R*. To 250 mL of cold *water R* add 150 mL of *sulfuric acid R* and cool. Mix the 2 solutions together. *Storage*: use within 1 day.

Ammonium molybdate solution. *1005702.*

A 100 g/L solution of *ammonium molybdate R*.

Ammonium molybdate solution R2. *1005703.*

Dissolve 5.0 g of *ammonium molybdate R* with heating in 30 mL of *water R*. Cool, adjust the pH to 7.0 with *dilute ammonia R2* and dilute to 50 mL with *water R*.

Ammonium molybdate solution R3. *1005704.*

Solution A. Dissolve 5 g of *ammonium molybdate R* in 20 mL of *water R* with heating.

Solution B. Mix 150 mL of *ethanol (96 per cent) R* with 150 mL of *water R*. Add with cooling 100 mL of *sulfuric acid R*.

Immediately before use add 80 volumes of solution B to 20 volumes of solution A.

Ammonium molybdate solution R4. *1005705.*

Dissolve 1.0 g of *ammonium molybdate R* in *water R* and dilute to 40 mL with the same solvent. Add 3 mL of *hydrochloric acid R* and 5 mL of *perchloric acid R* and dilute to 100 mL with *acetone R*.

Storage: protected from light; use within 1 month.

Ammonium molybdate solution R5. *1005707.*

Dissolve 1.0 g of *ammonium molybdate R* in 40.0 mL of a 15 per cent *V/V* solution of *sulfuric acid R*. Prepare the solution daily.

Ammonium molybdate solution R6. *1005709.*

Slowly add 10 mL of *sulfuric acid R* to about 40 mL of *water R*. Mix and allow to cool. Dilute to 100 mL with *water R* and mix. Add 2.5 g of *ammonium molybdate R* and 1 g of *cerium sulfate R*, and shake for 15 min to dissolve.

Ammonium nitrate. NH_4NO_3. (M_r 80.0). *1005800.* [6484-52-2].

White or almost white, crystalline powder or colourless crystals, hygroscopic, very soluble in water, freely soluble in methanol, soluble in ethanol (96 per cent).

Storage: in an airtight container.

Ammonium nitrate R1. *1005801.*

Complies with the requirements prescribed for *ammonium nitrate R* with the following additional requirements.

Acidity. The solution of the substance is slightly acid (*2.2.4*).

Chlorides (*2.4.4*): maximum 100 ppm, determined on 0.50 g.

Sulfates (*2.4.13*): maximum 150 ppm, determined on 1.0 g.

Sulfated ash (*2.4.14*): maximum 0.05 per cent, determined on 1.0 g.

Ammonium oxalate. $C_2H_8N_2O_4,H_2O$. (M_r 142.1). *1005900.* [6009-70-7].

Colourless crystals, soluble in water.

Ammonium oxalate solution. *1005901.*

A 40 g/L solution of *ammonium oxalate R*.

Ammonium persulfate. $(NH_4)_2S_2O_8$. (M_r 228.2). *1006000.* [7727-54-0].

White or almost white, crystalline powder or granular crystals, freely soluble in water.

Ammonium phosphate. $(NH_4)_2HPO_4$. (M_r 132.1). *1006100.* [7783-28-0]. Diammonium hydrogen phosphate.

White or almost white crystals or granules, hygroscopic, very soluble in water, practically insoluble in ethanol (96 per cent).

pH (*2.2.3*): about 8 for a 200 g/L solution.

Storage: in an airtight container.

Ammonium pyrrolidinedithiocarbamate. $C_5H_{12}N_2S_2$. (M_r 164.3). *1006200.* [5108-96-3]. Ammonium 1-pyrrolidinyl-dithioformate.

White or pale yellow, crystalline powder, sparingly soluble in water, very slightly soluble in ethanol (96 per cent).

Storage: in a bottle containing a piece of ammonium carbonate in a muslin bag.

Ammonium reineckate. $NH_4[Cr(NCS)_4(NH_3)_2],H_2O$. ($M_r$ 354.4). *1006300.* [13573-16-5]. Ammonium diamine-tetrakis(isothiocyanato)chromate(III) monohydrate.

Red powder or crystals, sparingly soluble in cold water, soluble in hot water and in ethanol (96 per cent).

Ammonium reineckate solution. *1006301.*

A 10 g/L solution of *ammonium reineckate R*. Prepare immediately before use.

Ammonium sulfamate. $NH_2SO_3NH_4$. (M_r 114.1). *1006400.* [7773-06-0].

White or almost white, crystalline powder or colourless crystals, hygroscopic, very soluble in water, slightly soluble in ethanol (96 per cent).

mp: about 130 °C.

Storage: in an airtight container.

Ammonium sulfate. $(NH_4)_2SO_4$. (M_r 132.1). *1006500.* [7783-20-2].

Colourless crystals or white or almost white granules, very soluble in water, practically insoluble in acetone and in ethanol (96 per cent).

pH (*2.2.3*): 4.5 to 6.0 for a 50 g/L solution in *carbon dioxide-free water R*.

Sulfated ash (*2.4.14*): maximum 0.1 per cent.

Ammonium sulfide solution. *1123300.*

Saturate 120 mL of *dilute ammonia R1* with *hydrogen sulfide R* and add 80 mL of *dilute ammonia R1*. Prepare immediately before use.

Ammonium thiocyanate. NH_4SCN. (M_r 76.1). *1006700.* [1762-95-4].

Colourless crystals, deliquescent, very soluble in water, soluble in ethanol (96 per cent).

Storage: in an airtight container.

Ammonium thiocyanate solution. *1006701.*

A 76 g/L solution of *ammonium thiocyanate R*.

Ammonium vanadate. NH_4VO_3. (M_r 117.0). *1006800.* [7803-55-6]. Ammonium trioxovanadate(V).

White or slightly yellowish, crystalline powder, slightly soluble in water, soluble in *dilute ammonia R1*.

Ammonium vanadate solution. *1006801.*

Dissolve 1.2 g of *ammonium vanadate R* in 95 mL of *water R* and dilute to 100 mL with *sulfuric acid R*.

Amoxicillin trihydrate. *1103400.*

See *Amoxicillin trihydrate (0260)*.

α-Amylase. *1100800.* 1,4-α-D-glucane-glucanohydrolase (EC *3.2.1.1*).

White or light brown powder.

α-Amylase solution. *1100801.*

A solution of *α-amylase R* with an activity of 800 FAU/g.

β-Amyrin. $C_{30}H_{50}O$. (M_r 426.7). *1141800.* [559-70-6]. Olean-12-en-3β-ol.

White or almost white powder.

mp: 187 °C to 190 °C.

Andrographolide. $C_{20}H_{30}O_5$. (M_r 350.4). *1198100.* [5508-58-7]. (3*E*,4*S*)-3-[2-[(1*R*,4a*S*,5*R*,6*R*,8a*S*)-6-Hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylenedecahydronaphthalen-1-yl]ethylidene]-4-hydroxydihydrofuran-2(3*H*)-one.

Anethole. $C_{10}H_{12}O$. (M_r 148.2). *1006900.* [4180-23-8]. 1-Methoxy-4-(propen-1-yl)benzene.

White or almost white, crystalline mass up to 20 °C to 21 °C, liquid above 23 °C, practically insoluble in water, freely soluble in anhydrous ethanol, soluble in ethyl acetate and in light petroleum.

n_D^{25}: about 1.56.

bp: about 230 °C.

Anethole used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Anise oil (0804)*.

Test solution. The substance to be examined.

Content: minimum 99.0 per cent of *trans*-anethole (retention time: about 41 min), calculated by the normalisation procedure.

Anhydrous colloidal silica. *1202000.* [7631-86-9].

See *Anhydrous colloidal silica (0434)*.

Aniline. C_6H_7N. (M_r 93.1). *1007100.* [62-53-3]. Benzeneamine.

Colourless or slightly yellowish liquid, soluble in water, miscible with ethanol (96 per cent).

d_{20}^{20}: about 1.02.

bp: 183 °C to 186 °C.

Storage: protected from light.

Aniline hydrochloride. C_6H_8ClN. (M_r 129.6). *1147700.* [142-04-1]. Benzenamine hydrochloride.

Crystals.

It darkens on exposure to air and light.

mp: about 198 °C.

Storage: protected from light.

Content: minimum 97.0 per cent.

Anion-exchange resin. *1007200.*

Resin in chlorinated form containing quaternary ammonium groups $[CH_2N^+(CH_3)_3]$ attached to a polymer lattice consisting of polystyrene cross-linked with 2 per cent of divinylbenzene. It is available as spherical beads.

Wash the resin with *1 M sodium hydroxide* on a sintered-glass filter (40) (*2.1.2*) until the washings are free from chloride, then wash with *water R* until the washings are neutral. Suspend in freshly prepared *ammonium-free water R* and protect from atmospheric carbon dioxide.

Anion-exchange resin R1. *1123400.*

Resin containing quaternary ammonium groups $[CH_2N^+(CH_3)_3]$ attached to a lattice consisting of methacrylate.

Anion-exchange resin R2. *1141900.*

Conjugate of homogeneous 10 µm hydrophilic polyether particles, and a quaternary ammonium salt, providing a matrix suitable for strong anion-exchange chromatography of proteins.

Anion-exchange resin R3. *1180900.*

Resin with quaternary ammonium groups attached to a lattice of ethylvinylbenzene crosslinked with 55 per cent of divinylbenzene.

Anion-exchange resin for chromatography, strongly basic. *1112700.*

Resin with quaternary amine groups attached to a lattice of latex cross linked with divinylbenzene.

Anion-exchange resin for chromatography, strongly basic R1. *1187400.*

Non-porous resin agglomerated with a 100 nm alkyl quaternary ammonium functionalised latex.

Anion-exchange resin for chromatography, strongly basic R2. *1203000.*

Non-porous resin agglomerated with a 43 nm quaternary amine functionalised latex, cross-linked with ethylvinylbenzene/divinylbenzene.

Anion-exchange resin, strongly basic. *1026600.*

Gel-type resin in hydroxide form containing quaternary ammonium groups [$CH_2N^+(CH_3)_3$, type 1] attached to a polymer lattice consisting of polystyrene cross-linked with 8 per cent of divinylbenzene.

Brown transparent beads.

Particle size: 0.2 mm to 1.0 mm.

Moisture content: about 50 per cent.

Total exchange capacity: minimum 1.2 meq/mL.

Anion-exchange resin, weak. *1146700.*

Resin with diethylaminoethyl groups attached to a lattice consisting of poly(methyl methacrylate).

Anisaldehyde. $C_8H_8O_2$. (M_r 136.1). *1007300.* [123-11-5]. 4-Methoxybenzaldehyde.

Oily liquid, very slightly soluble in water, miscible with ethanol (96 per cent).

bp: about 248 °C.

Anisaldehyde used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Anise oil (0804)*.

Test solution. The substance to be examined.

Content: minimum 99.0 per cent, calculated by the normalisation procedure.

Anisaldehyde solution. *1007301.*

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 R*.

Anisaldehyde solution R1. *1007302.*

To 10 mL of *anisaldehyde R* add 90 mL of *ethanol (96 per cent) R*, mix, add 10 mL of *sulfuric acid R* and mix again.

Anise ketone. $C_{10}H_{12}O_2$. (M_r 164.2). *1174700.* [122-84-9]. 1-(4-Methoxyphenyl)propan-2-one.

***p*-Anisidine.** C_7H_9NO. (M_r 123.2). *1103500.* [104-94-9]. 4-Methoxyaniline.

White or almost white crystals, sparingly soluble in water, soluble in anhydrous ethanol.

Content: minimum 97.0 per cent.

Caution: *skin irritant, sensitiser.*

Storage: protected from light, at 0 °C to 4 °C.

On storage, *p*-anisidine tends to darken as a result of oxidation. A discoloured reagent can be reduced and decolorised in the following way: dissolve 20 g of *p-anisidine R* in 500 mL of *water R* at 75 °C. Add 1 g of *sodium sulfite heptahydrate R* and 10 g of *activated charcoal R* and stir for 5 min. Filter, cool the filtrate to about 0 °C and allow to stand at this temperature for at least 4 h. Filter, wash the crystals with a small quantity of *water R* at about 0 °C and dry the crystals *in vacuo* (*2.2.32*).

Anthracene. $C_{14}H_{10}$. (M_r 178.2). *1007400.* [120-12-7].

White or almost white, crystalline powder, practically insoluble in water, slightly soluble in chloroform.

mp: about 218 °C.

Anthrone. $C_{14}H_{10}O$. (M_r 194.2). *1007500.* [90-44-8]. 9(10*H*)-Anthracenone.

Pale yellow, crystalline powder.

mp: about 155 °C.

Antimony potassium tartrate. $C_8H_4K_2O_{12}Sb_2,3H_2O$. ($M_r$ 668). *1007600.* [28300-74-5]. Dipotassium di[tartrato(4-)O^1,O^2,O^3,O^4]bis[antimonate(III)] trihydrate.

White or almost white, granular powder or colourless, transparent crystals, soluble in water and in glycerol, freely soluble in boiling water, practically insoluble in ethanol (96 per cent). The aqueous solution is slightly acid.

Antimony trichloride. $SbCl_3$. (M_r 228.1). *1007700.* [10025-91-9].

Colourless crystals or a transparent crystalline mass, hygroscopic, freely soluble in anhydrous ethanol. Antimony trichloride is hydrolysed by water.

Storage: in an airtight container, protected from moisture.

Antimony trichloride solution. *1007701.*

Rapidly wash 30 g of *antimony trichloride R* with two quantities, each of 15 mL, of *ethanol-free chloroform R*, drain off the washings, and dissolve the washed crystals immediately in 100 mL of *ethanol-free chloroform R*, warming slightly.

Storage: over a few grams of *anhydrous sodium sulfate R*.

Antithrombin III. *1007800.* [90170-80-2].

Antithrombin III is purified from human plasma by heparin agarose chromatography and should have a specific activity of at least 6 IU/mg.

Antithrombin III solution R1. *1007801.*

Reconstitute *antithrombin III R* as directed by the manufacturer and dilute with *tris(hydroxymethyl)aminomethane sodium chloride buffer solution pH 7.4 R* to 1 IU/mL.

Antithrombin III solution R2. *1007802.*

Reconstitute *antithrombin III R* as directed by the manufacturer and dilute with *tris(hydroxymethyl)aminomethane sodium chloride buffer solution pH 7.4 R* to 0.5 IU/mL.

Antithrombin III solution R3. *1007803.*

Reconstitute *antithrombin III R* as directed by the manufacturer and dilute to 0.3 IU/mL with *phosphate buffer solution pH 6.5 R*.

Antithrombin III solution R4. *1007804.*

Reconstitute *antithrombin III R* as directed by the manufacturer and dilute to 0.1 IU/mL with *tris(hydroxymethyl)aminomethane-EDTA buffer solution pH 8.4 R*.

Antithrombin III solution R5. *1007805.*

Reconstitute *antithrombin III R* as directed by the manufacturer and dilute to 0.125 IU/mL with *tris(hydroxymethyl)aminomethane-EDTA buffer solution pH 8.4 R1*.

Antithrombin III solution R6. *1007806.*

Reconstitute *antithrombin III R* as directed by the manufacturer and dilute to 1.0 IU/mL with *tris(hydroxymethyl)aminomethane-EDTA buffer solution pH 8.4 R1*.

Apigenin. $C_{15}H_{10}O_5$. (M_r 270.2). *1095800.* [520-36-5]. 4′,5,7-Trihydroxyflavone.

Light yellowish powder, practically insoluble in water, sparingly soluble in ethanol (96 per cent).

mp: about 310 °C, with decomposition.

Chromatography. Thin-layer chromatography (*2.2.27*) as prescribed in the monograph *Roman chamomile flower (0380)*: apply 10 µL of a 0.25 g/L solution in *methanol R*; the chromatogram shows in the upper third a principal zone of yellowish-green fluorescence.

Apigenin 7-glucoside. $C_{21}H_{20}O_{10}$. (M_r 432.4). *1095900.* [578-74-5]. Apigetrin. 7-(β-D-Glucopyranosyloxy)-5-hydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one.

Light yellowish powder, practically insoluble in water, sparingly soluble in ethanol (96 per cent).

mp: 198 °C to 201 °C.

Chromatography. Thin-layer chromatography (*2.2.27*) as prescribed in the monograph *Roman chamomile flower (0380)*: apply 10 µL of a 0.25 g/L solution in *methanol R*; the chromatogram shows in the middle third a principal zone of yellowish fluorescence.

Apigenin-7-glucoside used in liquid chromatography complies with the following additional test.

Assay. Liquid chromatography (*2.2.29*) as prescribed in the monograph *Matricaria flower (0404)*.

Test solution. Dissolve 10.0 mg in *methanol R* and dilute to 100.0 mL with the same solvent.

Content: minimum 95.0 per cent, calculated by the normalisation procedure.

Aprotinin. *1007900.* [9087-70-1].

See *Aprotinin (0580)*.

Arabinose. $C_5H_{10}O_5$. (M_r 150.1). *1008000.* [87-72-9]. (3*R*,4*S*,5*S*)-Tetrahydro-2*H*-pyran-2,3,4,5-tetrol. L-Arabinopyranose. L-(+)-Arabinose.

White or almost white, crystalline powder, freely soluble in water.

$[\alpha]_D^{20}$: + 103 to + 105, determined on a 50 g/L solution in *water R* containing about 0.05 per cent of NH_3.

Arachidyl alcohol. $C_{20}H_{42}O$. (M_r 298.5). *1156300.* [629-96-9]. 1-Eicosanol.

mp: about 65 °C.

Content: minimum 96 per cent of $C_{20}H_{42}O$.

Arbutin. $C_{12}H_{16}O_7$. (M_r 272.3). *1008100.* [497-76-7]. Arbutoside. 4-Hydroxyphenyl-β-D-glucopyranoside.

Fine, white or almost white, shiny needles, freely soluble in water, very soluble in hot water, soluble in ethanol (96 per cent).

Chromatography. Thin-layer chromatography (*2.2.27*) as prescribed in the monograph *Bearberry leaf (1054)*; the chromatogram shows only one principal spot.

Arginine. *1103600.* [74-79-3].

See *Arginine (0806)*.

Argon. Ar. (A_r 39.95). *1008200.* [7440-37-1].

Content: minimum 99.995 per cent *V/V*.

Carbon monoxide (*2.5.25, Method I*): maximum 0.6 ppm *V/V*; after passage of 10 L of *argon R* at a flow rate of 4 L/h, not more than 0.05 mL of *0.002 M sodium thiosulfate* is required for the titration.

Argon R1. Ar. (A_r 39.95). *1176000.* [7440-37-1].

Content: minimum 99.99990 per cent *V/V*.

Argon for chromatography. Ar. (A_r 39.95). *1166200.* [7440-37-1].

Content: minimum 99.95 per cent *V/V*.

Aromadendrene. $C_{15}H_{24}$. (M_r 204.4). *1139100.* [489-39-4]. (1*R*,2*S*,4*R*,8*R*,11*R*)-3,3,11-Trimethyl-7-methylenetricyclo-[6.3.0.0^{2,4}]undecane.

Clear, almost colourless liquid.

d_4^{20}: about 0.911.

n_D^{20}: about 1.497.

$[\alpha]_D^{20}$: about + 12.

bp: about 263 °C.

Aromadendrene used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph on *Tea tree oil (1837)*.

Content: minimum 92 per cent, calculated by the normalisation procedure.

Arsenazo III. $C_{22}H_{18}As_2N_4O_{14}S_2$. ($M_r$ 776). *1198200.* [1668-00-4]. 3,6-Bis[(2-arsonophenyl)diazenyl]-4,5-dihydroxynaphthalene-2,7-disulfonic acid.

Brown powder.

Arsenious trioxide. As_2O_3. (M_r 197.8). *1008300.* [1327-53-3]. Arsenious anhydride. Diarsenic trioxide.

Crystalline powder or a white or almost white mass, slightly soluble in water, soluble in boiling water.

Ascorbic acid. *1008400.* [50-81-7].

See *Ascorbic acid (0253)*.

Ascorbic acid solution. *1008401.*

Dissolve 50 mg in 0.5 mL of *water R* and dilute to 50 mL with *dimethylformamide R*.

Asiaticoside. $C_{48}H_{78}O_{19}$. (M_r 959). *1123500.* [16830-15-2]. *O*-6-Deoxy-α-L-mannopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl 2α,3β,23-trihydroxy-4α-urs-12-en-28-oate.

White or almost white powder, hygroscopic, soluble in methanol, slightly soluble in anhydrous ethanol, insoluble in acetonitrile.

mp: about 232 °C, with decomposition.

Water (*2.5.12*): 6.0 per cent.

Asiaticoside used in liquid chromatography complies with the following additional test.

Assay. Liquid chromatography (*2.2.29*) as prescribed in the monograph *Centella (1498)*.

Content: minimum 97.0 per cent, calculated by the normalisation procedure.

Storage: protected from humidity.

Asparagine. $C_4H_8N_2O_3$. (M_r 132.12). *1200000.* [70-47-3].

Aspartic acid. *1134100.* [56-84-8].

See *Aspartic acid (0797)*.

D-Aspartic acid. $C_4H_7NO_4$. (M_r 133.1). *1200100.* [1783-96-6].

L-Aspartyl-L-phenylalanine. $C_{13}H_{16}N_2O_5$. (M_r 280.3). *1008500.* [13433-09-5]. (*S*)-3-Amino-*N*-[(*S*)-1-carboxy-2-phenylethyl]-succinamic acid.

White or almost white powder.

mp: about 210 °C, with decomposition.

Astragaloside IV. $C_{41}H_{68}O_{14}$. (M_r 785). *1178200.* [84687-43-4]. (20*R*,24*S*)-20,24-Epoxy-16β,25-dihydroxy-3β-(β-D-xylopyranosyloxy)-9,19-cyclolanostan-6α-yl β-D-glucopyranoside.

Atropine sulfate. *1159000.* [5908-99-6].

See *Atropine sulfate (0068)*.

Aucubin. $C_{15}H_{22}O_9$. (M_r 346.3). *1145200.* [479-98-1]. [1*S*,4a*R*,5*S*,7a*S*)-5-Hydroxy-7-(hydroxymethyl)-1,4a,5,7a-tetrahydrocyclopenta[*c*]pyran-1-yl β-D-glucopyranoside.

Crystals, soluble in water, in ethanol (96 per cent) and in methanol, practically insoluble in light petroleum.

$[\alpha]_D^{20}$: about − 163.

mp: about 181 °C.

Azomethine H. $C_{17}H_{12}NNaO_8S_2$. (M_r 445.4). *1008700.* [5941-07-1]. Sodium hydrogeno-4-hydroxy-5-(2-hydroxybenzylideneamino)-2,7-naphthalenedisulfonate.

Azomethine H solution. *1008701.*

Dissolve 0.45 g of *azomethine H R* and 1 g of *ascorbic acid R* with gentle heating in *water R* and dilute to 100 mL with the same solvent.

Baicalin. $C_{21}H_{18}O_{11}$. (M_r 446.4). *1179200.* [21967-41-9].

5,6-Dihydroxy-4-oxo-2-phenyl-4*H*-1-benzopyran-7-yl-β-D-glucopyranosiduronic acid.

Barbaloin. $C_{21}H_{22}O_9,H_2O$. (M_r 436.4). *1008800.* [1415-73-2]. Aloin. 1,8-Dihydroxy-3-hydroxymethyl-10-β-D-glucopyranosyl-10*H*-anthracen-9-one.

Yellow to dark-yellow, crystalline powder, or yellow needles, darkening on exposure to air and light, sparingly soluble in water and in ethanol (96 per cent), soluble in acetone, in ammonia and in solutions of alkali hydroxides.

$A_{1\,cm}^{1\%}$: about 192 at 269 nm, about 226 at 296.5 nm, about 259 at 354 nm, determined on a solution in *methanol R* and calculated with reference to the anhydrous substance.

Chromatography. Thin-layer chromatography (*2.2.27*) as prescribed in the monograph *Frangula bark (0025)*; the chromatogram shows only one principal spot.

Barbital. *1008900.* [57-44-3].

See *Barbital (0170)*.

Barbital sodium. $C_8H_{11}N_2NaO_3$. (M_r 206.2). *1009000.* [144-02-5]. Sodium derivative of 5,5-diethyl-1*H*,3*H*,5*H*-pyrimidine-2,4,6-trione.

Content: minimum 98.0 per cent.

A white or almost white, crystalline powder or colourless crystals, freely soluble in water, slightly soluble in ethanol (96 per cent).

Barbituric acid. $C_4H_4N_2O_3$. (M_r 128.1). *1009100.* [67-52-7]. 1*H*,3*H*,5*H*-Pyrimidine-2,4,6-trione.

White or almost white powder, slightly soluble in water, freely soluble in boiling water and in dilute acids.

mp: about 253 °C.

Barium acetate. $C_4H_6BaO_4$. (M_r 255.4). *1162700.* [543-80-6]. Barium diacetate.

White or almost white powder, soluble in water.

d_{20}^{20}: 2.47.

Barium carbonate. $BaCO_3$. (M_r 197.3). *1009200.* [513-77-9].

White or almost white powder or friable masses, practically insoluble in water.

Barium chloride. $BaCl_2,2H_2O$. (M_r 244.3). *1009300.* [10326-27-9]. Barium dichloride.

Colourless crystals, freely soluble in water, slightly soluble in ethanol (96 per cent).

Barium chloride solution R1. *1009301.*

A 61 g/L solution of *barium chloride R*.

Barium chloride solution R2. *1009302.*

A 36.5 g/L solution of *barium chloride R*.

Barium hydroxide. $Ba(OH)_2,8H_2O$. (M_r 315.5). *1009400.* [12230-71-6]. Barium dihydroxide.

Colourless crystals, soluble in water.

Barium hydroxide solution. *1009401.*

A 47.3 g/L solution of *barium hydroxide R*.

Barium nitrate. $Ba(NO_3)_2$. (M_r 261.3). *1163800.* [10022-31-8].

Crystals or crystalline powder, freely soluble in water, very slightly soluble in ethanol (96 per cent) and in acetone.

mp: about 590 °C.

Barium sulfate. *1009500.* [7727-43-7].

See *Barium sulfate (0010)*.

Benzalacetone. $C_{10}H_{10}O$. (M_r 146.2). *1168500.* [122-57-6]. (3*E*)-4-phenylbut-3-en-2-one.

White or pale yellow mass.

Content: minimum 98.0 per cent.

bp: about 261 °C.

mp: about 39 °C.

Benzaldehyde. C_7H_6O. (M_r 106.1). *1009600.* [100-52-7].

Colourless or slightly yellow liquid, slightly soluble in water, miscible with ethanol (96 per cent).

d_{20}^{20}: about 1.05.

n_D^{20}: about 1.545.

Distillation range (*2.2.11*). Not less than 95 per cent distils between 177 °C and 180 °C.

Storage: protected from light.

Benzene. C_6H_6. (M_r 78.1). *1009800.* [71-43-2].

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

bp: about 80 °C.

Where benzene is used to prepare a reference solution, for safety reasons, the pure reagent may be replaced by a commercially available reference material containing a certified amount of benzene.

Benzene-1,2,4-triol. $C_6H_6O_3$. (M_r 126.1). *1177500.* [533-73-3]. Hydroxyhydroquinone. Hydroxyquinol.

Freely soluble in water, in ethanol (96 per cent) and in ethyl acetate.

mp: about 140 °C.

Benzethonium chloride. $C_{27}H_{42}ClNO_2,H_2O$. (M_r 466.1). *1009900.* [121-54-0]. Benzyldimethyl[2-[2-[4-(1,1,3,3-tetramethylbutyl)phenoxy]ethoxy]ethyl]ammonium chloride monohydrate.

Fine, white or almost white powder or colourless crystals, soluble in water and in ethanol (96 per cent).

mp: about 163 °C.

Storage: protected from light.

Benzidine. $C_{12}H_{12}N_2$. (M_r 184.2). *1145300.* [92-87-5]. Biphenyl-4,4′-diamine.

Content: minimum 95 per cent.

White or slightly yellowish or reddish powder, darkening on exposure to air and light.

mp: about 120 °C.

Storage: protected from light.

Benzil. $C_{14}H_{10}O_2$. (M_r 210.2). *1117800.* [134-81-6]. Diphenylethanedione.

Yellow, crystalline powder, practically insoluble in water, soluble in ethanol (96 per cent), ethyl acetate and toluene.

mp: 95 °C.

Benzocaine. $C_9H_{11}NO_2$. (M_r 165.2). *1123600.* [94-09-7].

See *Benzocaine (0011)*.

Benzohydrazide. $C_7H_8N_2O$. (M_r 136.2). *1194400.* [613-94-5]. Benzoyldiazane.

Benzoic acid. *1010100.* [65-85-0].

See *Benzoic acid (0066)*.

Benzoin. $C_{14}H_{12}O_2$. (M_r 212.3). *1010200.* [579-44-2]. 2-Hydroxy-1,2-diphenylethanone.

Slightly yellowish crystals, very slightly soluble in water, freely soluble in acetone, soluble in hot ethanol (96 per cent).

mp: about 137 °C.

Benzophenone. $C_{13}H_{10}O$. (M_r 182.2). *1010300.* [119-61-9]. Diphenylmethanone.

Prismatic crystals, practically insoluble in water, freely soluble in ethanol (96 per cent).

mp: about 48 °C.

1,4-Benzoquinone. $C_6H_4O_2$. (M_r 108.1). *1118500.* [106-51-4]. Cyclohexa-2,5-diene-1,4-dione.

Content: minimum 98.0 per cent.

Benzoylarginine ethyl ester hydrochloride. $C_{15}H_{23}ClN_4O_3$. (M_r 342.8). *1010500.* [2645-08-1]. *N*-Benzoyl-L-arginine ethyl ester hydrochloride. Ethyl (*S*)-2-benzamido-5-guanidinovalerate hydrochloride.

White or almost white, crystalline powder, very soluble in water and in anhydrous ethanol.

$[\alpha]_D^{20}$: – 15 to – 18, determined on a 10 g/L solution.

mp: about 129 °C.

$A_{1\,cm}^{1\%}$: 310 to 340, determined at 227 nm using a 0.01 g/L solution.

Benzoyl chloride. C_7H_5ClO. (M_r 140.6). *1010400.* [98-88-4].

Colourless, lachrymatory liquid, decomposed by water and by ethanol (96 per cent).

d_{20}^{20}: about 1.21.

bp: about 197 °C.

***N*-Benzoyl-L-prolyl-L-phenylalanyl-L-arginine 4-nitroanilide acetate.** $C_{35}H_{42}N_8O_8$. (M_r 703). *1010600.*

3-Benzoylpropionic acid. $C_{10}H_{10}O_3$. (M_r 178.2). *1171000.* [2051-95-8]. 4-Oxo-4-phenylbutanoic acid.

mp: about 118 °C.

2-Benzoylpyridine. $C_{12}H_9NO$. (M_r 183.2). *1134300.* [91-02-1]. Phenyl(pyridin-2-yl)methanone.

Colourless crystals, soluble in ethanol (96 per cent).

mp: about 43 °C.

Benzyl alcohol. *1010700.* [100-51-6].

See *Benzyl alcohol (0256)*.

Benzyl benzoate. *1010800.* [120-51-4].

See *Benzyl benzoate (0705)*.

Chromatography. Thin-layer chromatography (*2.2.27*) as prescribed in the monograph *Peru balsam (0754)*: apply 20 µL of a 0.3 per cent *V/V* solution in *ethyl acetate R*; after spraying and heating, the chromatogram shows a principal band with an R_F of about 0.8.

Benzyl cinnamate. $C_{16}H_{14}O_2$. (M_r 238.3). *1010900.* [103-41-3]. Benzyl 3-phenylprop-2-enoate.

Colourless or yellowish crystals, practically insoluble in water, soluble in ethanol (96 per cent).

mp: about 39 °C.

Chromatography. Thin-layer chromatography (*2.2.27*) as prescribed in the monograph *Peru balsam (0754)*: apply 20 µL of a 3 g/L solution in *ethyl acetate R*; after spraying and heating, the chromatogram shows a principal band with an R_F of about 0.6.

Benzyl cyanide. C_8H_7N. (M_r 117.2). *1171100.* [140-29-4]. Phenylacetonitrile.

Content: minimum 95.0 per cent.

Clear, colourless or light yellow liquid.

n_D^{20}: about 1.523.

bp: about 233 °C.

Benzyl ether. $C_{14}H_{14}O$. (M_r 198.3). *1140900.* [103-50-4]. Dibenzyl ether.

Clear, colourless liquid, practically insoluble in water, miscible with acetone and with anhydrous ethanol.

d_{20}^{20}: about 1.043.

n_D^{20}: about 1.562.

bp: about 296 °C, with decomposition.

Benzylpenicillin sodium. *1011000.* [69-57-8].

See *Benzylpenicillin sodium (0114)*.

2-Benzylpyridine. $C_{12}H_{11}N$. (M_r 169.2). *1112900.* [101-82-6].

Content: minimum 98.0 per cent.

Yellow liquid.

mp: 13 °C to 16 °C.

4-Benzylpyridine. $C_{12}H_{11}N$. (M_r 169.2). *1181200.* [2116-65-6].

Content: minimum 98.0 per cent.

Yellow liquid.

mp: 72 °C to 78 °C.

Benzyltrimethylammonium chloride. $C_{10}H_{16}ClN$. (M_r 185.7). *1155700.* [56-93-9]. *N*,*N*,*N*-Trimethylphenylmethanaminium chloride. *N*,*N*,*N*-Trimethylbenzenemethanaminium chloride.

White or almost white powder, soluble in water.

mp: about 230 °C, with decomposition.

Berberine chloride. $C_{20}H_{18}ClNO_4,2H_2O$. (M_r 407.8). *1153400.* [5956-60-5]. 9,10-Dimethoxy-5,6-dihydrobenzo[*g*]-1,3-benzodioxolo[5,6-*a*]quinolizinium chloride.

Yellow crystals, slightly soluble in water, practically insoluble in ethanol (96 per cent).

mp: 204 °C to 206 °C.

Berberine chloride used in liquid chromatography complies with the following additional test.

Assay. Liquid chromatography (*2.2.29*) as prescribed in the monograph *Goldenseal rhizome (1831)*.

Content: minimum 95 per cent, calculated by the normalisation procedure.

Bergapten. $C_{12}H_8O_4$. (M_r 216.2). *1103700.* [484-20-8]. 5-Methoxypsoralen.

Colourless crystals, practically insoluble in water, sparingly soluble in ethanol (96 per cent) and slightly soluble in glacial acetic acid.

mp: about 188 °C.

Betulin. $C_{30}H_{50}O_2$. (M_r 442.7). *1011100.* [473-98-3]. Lup-20(39)-ene-3β,28-diol.

White or almost white, crystalline powder.

mp: 248 °C to 251 °C.

Bibenzyl. $C_{14}H_{14}$. (M_r 182.3). *1011200.* [103-29-7]. 1,2-Diphenylethane.

White or almost white, crystalline powder, practically insoluble in water, very soluble in methylene chloride, freely soluble in acetone, soluble in ethanol (96 per cent).

mp: 50 °C to 53 °C.

Biphenyl. $C_{12}H_{10}$. (M_r 154.2). *1168600.* [92-52-4].

mp: 68 °C to 70 °C.

(−)-α-Bisabolol. $C_{15}H_{26}O$. (M_r 222.4). *1128800.* [23089-26-1]. (2*S*)-6-Methyl-2-[(1*S*)-4-methylcyclohex-3-enyl]hept-5-en-2-ol. Levomenol.

Colourless, viscous liquid with a slight, characteristic odour, practically insoluble in water, freely soluble in ethanol (96 per cent), in methanol, in toluene, in fatty oils and in essential oils.

d_{20}^{20}: 0.925 to 0.935.

n_D^{20}: 1.492 to 1.500.

$[\alpha]_D^{20}$: − 54.5 to − 58.0, determined on a 50 g/L solution in *ethanol (96 per cent) R*.

(−)-α-Bisabolol used for gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Matricaria oil (1836)*.

Test solution. A 4 g/L solution in *cyclohexane R*.

Content: minimum 95.0 per cent, calculated by the normalisation procedure.

Bisbenzimide. $C_{25}H_{27}Cl_3N_6O,5H_2O$. ($M_r$ 624). *1103800.* [23491-44-3]. 4-[5-[5-(4-Methylpiperazin-1-yl)benzimidazol-2-yl]benzimidazol-2-yl]phenol trihydrochloride pentahydrate.

Bisbenzimide stock solution. *1103801.*

Dissolve 5 mg of *bisbenzimide R* in *water R* and dilute to 100 mL with the same solvent.

Storage: in the dark.

Bisbenzimide working solution. *1103802.*

Immediately before use, dilute 100 µL of *bisbenzimide stock solution R* to 100 mL with *phosphate buffered saline pH 7.4 R*.

Bis(diphenylmethyl) ether. $C_{26}H_{22}O$. (M_r 350.5). *1203100.* [574-42-5].

[Oxybis(methanetriyl)]tetrakisbenzene. 1,1′,1″,1‴-(Oxymethylidyne)tetrakisbenzene.

Bismuth nitrate pentahydrate. $Bi(NO_3)_3,5H_2O$. (M_r 485.1). *1165600.* [10035-06-0].

mp: about 30 °C.

Bismuth subnitrate. $4BiNO_3(OH)_2,BiO(OH)$. (M_r 1462). *1011500.* [1304-85-4].

White or almost white powder, practically insoluble in water.

Bismuth subnitrate R1. *1011501.*

Content: 71.5 per cent to 74.0 per cent of bismuth (Bi), and 14.5 per cent to 16.5 per cent of nitrate, calculated as nitrogen pentoxide (N_2O_5).

Bismuth subnitrate solution. *1011502.*

Dissolve 5 g of *bismuth subnitrate R1* in a mixture of 8.4 mL of *nitric acid R* and 50 mL of *water R* and dilute to 250 mL with *water R*. Filter if necessary.

Acidity. To 10 mL add 0.05 mL of *methyl orange solution R*. 5.0 mL to 6.25 mL of *1 M sodium hydroxide* is required to change the colour of the indicator.

Bis-tris propane. $C_{11}H_{26}N_2O_6$. (M_r 282.3). *1185500.* [64431-96-5]. 2,2′-(Propane-1,3-diyldiimino)bis[2-(hydroxymethyl)-1,3-propanediol.

Content: minimum 99.0 per cent.

Biuret. $C_2H_5N_3O_2$. (M_r 103.1). *1011600.* [108-19-0].

White or almost white crystals, hygroscopic, soluble in water, sparingly soluble in ethanol (96 per cent).

mp: 188 °C to 190 °C, with decomposition.

Storage: in an airtight container.

Biuret reagent. *1011601.*

Dissolve 1.5 g of *copper sulfate pentahydrate R* and 6.0 g of *sodium potassium tartrate R* in 500 mL of *water R*. Add 300 mL of a carbonate-free 100 g/L solution of *sodium hydroxide R*, dilute to 1000 mL with the same solution and mix.

Blocking solution. *1122400.*

A 10 per cent *V/V* solution of *acetic acid R*.

Blue dextran 2000. *1011700.* [9049-32-5].

Prepared from dextran having an average relative molecular mass of 2×10^6 by introduction of a polycyclic chromophore that colours the substance blue. The degree of substitution is 0.017.

It is freeze-dried and dissolves rapidly and completely in water and aqueous saline solutions.

Absorbance (*2.2.25*). A 1 g/L solution in a *phosphate buffer solution pH 7.0 R* shows an absorption maximum at 280 nm.

Boldine. $C_{19}H_{21}NO_4$. (M_r 327.3). *1118800.* [476-70-0]. 1,10-Dimethoxy-6aα-aporphine-2,9-diol.

White or almost white crystalline powder, very slightly soluble in water, soluble in ethanol (96 per cent) and in dilute solutions of acids.

$[\alpha]_D^{25}$: about + 127, determined on a 1 g/L solution in *anhydrous ethanol R*.

mp: about 163 °C.

Boric acid. *1011800.* [10043-35-3].

See *Boric acid (0001)*.

Boric acid solution, saturated, cold. *1011801.*

To 3 g of *boric acid R* add 50 mL of *water R* and shake for 10 min. Place the solution for 2 h in the refrigerator.

Borneol. $C_{10}H_{18}O$. (M_r 154.3). *1011900.* [507-70-0]. *endo*-1,7,7-Trimethylbicyclo[2.2.1]heptan-2-ol.

Colourless crystals, readily sublimes, practically insoluble in water, freely soluble in ethanol (96 per cent) and in light petroleum.

mp: about 208 °C.

Chromatography. Thin-layer chromatography (*2.2.27*), using *silica gel G R* as the coating substance. Apply to the plate 10 µL of a 1 g/L solution in *toluene R*. Develop over a path of 10 cm using *chloroform R*. Allow the plate to dry in air, spray with *anisaldehyde solution R*, using 10 mL for a plate 200 mm square, and heat at 100-105 °C for 10 min. The chromatogram obtained shows only one principal spot.

Bornyl acetate. $C_{12}H_{20}O_2$. (M_r 196.3). *1012000.* [5655-61-8]. *endo*-1,7,7-Trimethylbicyclo[2.2.1]hept-2-yl acetate.

Colourless crystals or a colourless liquid, very slightly soluble in water, soluble in ethanol (96 per cent).

mp: about 28 °C.

Chromatography. Thin-layer chromatography (*2.2.27*), using *silica gel G R* as the coating substance. Apply to the plate 10 µL of a 2 g/L solution in *toluene R*. Develop over a path of 10 cm using *chloroform R*. Allow the plate to dry in air, spray with *anisaldehyde solution R*, using 10 mL for a plate 200 mm square, and heat at 100-105 °C for 10 min. The chromatogram obtained shows only one principal spot.

Boron trichloride. BCl_3. (M_r 117.2). *1112000.* [10294-34-5].

Colourless gas. Reacts violently with water. Available as solutions in suitable solvents (2-chloroethanol, methylene chloride, hexane, heptane, methanol).

n_D^{20}: about 1.420.

bp: about 12.6 °C.

Caution: toxic and corrosive.

Boron trichloride-methanol solution. *1112001.*

A 12 per cent *m/m* solution of *boron trichloride R* in *methanol R*.

Storage: protected from light at – 20 °C, preferably in sealed tubes.

Boron trifluoride. BF_3. (M_r 67.8). *1012100.* [7637-07-2].

Colourless gas.

Boron trifluoride-methanol solution. *1012101.*

A 140 g/L solution of *boron trifluoride R* in *methanol R*.

Brilliant blue. *1012200.* [6104-59-2].

See *acid blue 83 R*.

Bromelains. *1012300.* [37189-34-7].

Concentrate of proteolytic enzymes derived from *Ananas comosus* Merr.

Dull-yellow powder.

Activity. 1 g liberates about 1.2 g of amino-nitrogen from a solution of *gelatin R* in 20 min at 45 °C and pH 4.5.

Bromelains solution. *1012301.*

A 10 g/L solution of *bromelains R* in a mixture of 1 volume of *phosphate buffer solution pH 5.5 R* and 9 volumes of a 9 g/L solution of *sodium chloride R*.

Bromine. Br_2. (M_r 159.8). *1012400.* [7726-95-6].

Brownish-red fuming liquid, slightly soluble in water, soluble in ethanol (96 per cent).

d_{20}^{20}: about 3.1.

Bromine solution. *1012401.*

Dissolve 30 g of *bromine R* and 30 g of *potassium bromide R* in *water R* and dilute to 100 mL with the same solvent.

Bromine water. *1012402.*

Shake 3 mL of *bromine R* with 100 mL of *water R* to saturation.

Storage: over an excess of *bromine R*, protected from light.

Bromine water R1. *1012403.*

Shake 0.5 mL of *bromine R* with 100 mL of *water R*.

Storage: protected from light; use within 1 week.

Bromocresol green. $C_{21}H_{14}Br_4O_5S$. (M_r 698). *1012600.* [76-60-8]. 3′,3″,5′,5″-Tetrabromo-*m*-cresol-sulfonphthalein. 4,4′-(3*H*-2,1-Benzoxathiol-3-ylidene)bis(2,6-dibromo-3-methylphenol)-*S*,*S*-dioxide.

Brownish-white powder, slightly soluble in water, soluble in ethanol (96 per cent) and in dilute solutions of alkali hydroxides.

Bromocresol green-methyl red solution. *1012602.*

Dissolve 0.15 g of *bromocresol green R* and 0.1 g of *methyl red R* in 180 mL of *anhydrous ethanol R* and dilute to 200 mL with *water R*.

Bromocresol green solution. *1012601.*

Dissolve 50 mg of *bromocresol green R* in 0.72 mL of *0.1 M sodium hydroxide* and 20 mL of *ethanol (96 per cent) R* and dilute to 100 mL with *water R*.

Test for sensitivity. To 0.2 mL of the bromocresol green solution add 100 mL of *carbon dioxide-free water R*. The solution is blue. Not more than 0.2 mL of *0.02 M hydrochloric acid* is required to change the colour to green.

Colour change: pH 3.6 (yellow) to pH 5.2 (blue).

Bromocresol purple. $C_{21}H_{16}Br_2O_5S$. (M_r 540.2). *1012700.* [115-40-2]. 3′,3″-Dibromo-*o*-cresolsulfonphthalein. 4,4′-(3*H*-2,1-Benzoxathiol-3-ylidene)bis(2-bromo-6-methylphenol)-*S*,*S*-dioxide.

Pinkish powder, practically insoluble in water, soluble in ethanol (96 per cent) and in dilute solutions of alkali hydroxides.

Bromocresol purple solution. *1012701.*

Dissolve 50 mg of *bromocresol purple R* in 0.92 mL of *0.1 M sodium hydroxide* and 20 mL of *ethanol (96 per cent) R* and dilute to 100 mL with *water R*.

Test for sensitivity. To 0.2 mL of the bromocresol purple solution add 100 mL of *carbon dioxide-free water R* and 0.05 mL of *0.02 M sodium hydroxide*. The solution is bluish-violet. Not more than 0.2 mL of *0.02 M hydrochloric acid* is required to change the colour to yellow.

Colour change: pH 5.2 (yellow) to pH 6.8 (bluish-violet).

5-Bromo-2′-deoxyuridine. $C_9H_{11}BrN_2O_5$. (M_r 307.1). *1012500.* [59-14-3]. 5-Bromo-1-(2-deoxy-β-d-*erythro*-pentofuranosyl)-1*H*,3*H*-pyrimidine-2,4-dione.

mp: about 194 °C.

Chromatography. Thin-layer chromatography (*2.2.27*) as prescribed in the monograph *Idoxuridine (0669)*: apply 5 µL of a 0.25 g/L solution; the chromatogram shows only one principal spot.

Bromomethoxynaphthalene. $C_{11}H_9BrO$. (M_r 237.1). *1159100.* [5111-65-9]. 2-Bromo-6-methoxynaphthalene.

mp: about 109 °C.

Bromophenol blue. $C_{19}H_{10}Br_4O_5S$. (M_r 670). *1012800.* [115-39-9]. 3′,3″,5′,5″-Tetrabromophenolsulfonphthalein. 4,4′-(3*H*-2,1-Benzoxathiol-3-ylidene)bis(2,6-dibromophenol) *S*,*S*-dioxide.

Light orange-yellow powder, very slightly soluble in water, slightly soluble in ethanol (96 per cent), freely soluble in solutions of alkali hydroxides.

Bromophenol blue solution. *1012801.*

Dissolve 0.1 g of *bromophenol blue R* in 1.5 mL of *0.1 M sodium hydroxide* and 20 mL of *ethanol (96 per cent) R* and dilute to 100 mL with *water R*.

Test for sensitivity. To 0.05 mL of the bromophenol blue solution add 20 mL of *carbon dioxide-free water R* and 0.05 mL of *0.1 M hydrochloric acid*. The solution is yellow. Not more than 0.1 mL of *0.1 M sodium hydroxide* is required to change the colour to bluish-violet.

Colour change: pH 2.8 (yellow) to pH 4.4 (bluish-violet).

Bromophenol blue solution R1. *1012802.*

Dissolve 50 mg of *bromophenol blue R* with gentle heating in 3.73 mL of *0.02 M sodium hydroxide* and dilute to 100 mL with *water R*.

Bromophenol blue solution R2. *1012803.*

Dissolve with heating 0.2 g of *bromophenol blue R* in 3 mL of *0.1 M sodium hydroxide* and 10 mL of *ethanol (96 per cent) R*. After solution is effected, allow to cool and dilute to 100 mL with *ethanol (96 per cent) R*.

Bromophos. $C_8H_8BrCl_2O_3PS$. (M_r 366.0). *1123700.* [2104-96-3].

A suitable certified reference solution (10 ng/µL in iso-octane) may be used.

Bromophos-ethyl. $C_{10}H_{12}BrCl_2O_3PS$. (M_r 394.0). *1123800.* [4824-78-6].

A suitable certified reference solution (10 ng/µL in iso-octane) may be used.

Bromothymol blue. $C_{27}H_{28}Br_2O_5S$. (M_r 624). *1012900.* [76-59-5]. 3′,3″-Dibromothymolsulfonphthalein. 4,4′-(3*H*-2,1-Benzoxathiol-3-ylidene)bis(2-bromo-6-isopropyl-3-methylphenol) *S,S*-dioxide.

Reddish-pink or brownish powder, practically insoluble in water, soluble in ethanol (96 per cent) and in dilute solutions of alkali hydroxides.

Bromothymol blue solution R1. *1012901.*

Dissolve 50 mg of *bromothymol blue R* in a mixture of 4 mL of *0.02 M sodium hydroxide* and 20 mL of *ethanol (96 per cent) R* and dilute to 100 mL with *water R*.

Test for sensitivity. To 0.3 mL of *bromothymol blue solution R1* add 100 mL of *carbon dioxide-free water R*. The solution is yellow. Not more than 0.1 mL of *0.02 M sodium hydroxide* is required to change the colour to blue.

Colour change: pH 5.8 (yellow) to pH 7.4 (blue).

Bromothymol blue solution R2. *1012902.*

A 10 g/L solution of *bromothymol blue R* in *dimethylformamide R*.

Bromothymol blue solution R3. *1012903.*

Warm 0.1 g of *bromothymol blue R* with 3.2 mL of *0.05 M sodium hydroxide* and 5 mL of *ethanol (90 per cent V/V) R*. After solution is effected, dilute to 250 mL with *ethanol (90 per cent V/V) R*.

Bromothymol blue solution R4. *1012904.*

Dissolve 100 mg of *bromothymol blue R* in a mixture of equal volumes of *ethanol (96 per cent) R* and *water R* and dilute to 100 mL with the same mixture of solvents. Filter if necessary.

BRP indicator solution. *1013000.*

Dissolve 0.1 g of *bromothymol blue R*, 20 mg of *methyl red R* and 0.2 g of *phenolphthalein R* in *ethanol (96 per cent) R* and dilute to 100 mL with the same solvent. Filter.

Brucine. $C_{23}H_{26}N_2O_4$. (M_r 394.5). *1013100.* [357-57-3]. 2,3-Dimethoxystrychnidin-10-one. 2,3-Dimethoxystrychnine.

Colourless crystals, slightly soluble in water, freely soluble in ethanol (96 per cent).

mp: about 178 °C.

Butanal. C_4H_8O. (M_r 72.1). *1134400.* [123-72-8]. Butyraldehyde.

d_{20}^{20}: 0.806.

n_D^{20}: 1.380.

bp: 75 °C.

***i*-Butane.** C_4H_{10}. (M_r 58.12). *1189000.* [75-28-5]. Isobutane. 2-Methylpropane.

Content: minimum 99.0 per cent *V/V*.

***n*-Butane.** C_4H_{10}. (M_r 58.12). *1189100.* [106-97-8]. Butane.

Content: minimum 99.0 per cent *V/V*.

Butane-1,4-diol. $HO(CH_2)_4OH$. (M_r 90.12). *1174800.* [110-63-4].

Butanol. $C_4H_{10}O$. (M_r 74.1). *1013200.* [71-36-3]. Butan-1-ol.

Clear, colourless liquid, miscible with ethanol (96 per cent).

d_{20}^{20}: about 0.81.

bp: 116 °C to 119 °C.

2-Butanol R1. $C_4H_{10}O$. (M_r 74.1). *1013301.* [78-92-2]. Butan-2-ol. *sec*-Butyl alcohol.

Content: minimum 99.0 per cent.

Clear, colourless liquid, soluble in water, miscible with ethanol (96 per cent).

d_{20}^{20}: about 0.81.

Distillation range (*2.2.11*). Not less than 95 per cent distils between 99 °C and 100 °C.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Isopropyl alcohol (0970)*.

Butyl acetate. $C_6H_{12}O_2$. (M_r 116.2). *1013400.* [123-86-4].

Clear, colourless liquid, flammable, slightly soluble in water, miscible with ethanol (96 per cent).

d_{20}^{20}: about 0.88.

n_D^{20}: about 1.395.

Distillation range (*2.2.11*). Not less than 95 per cent distils between 123 °C and 126 °C.

Butyl acetate R1. *1013401.*

Content: minimum 99.5 per cent, determined by gas chromatography.

Clear, colourless liquid, flammable, slightly soluble in water, miscible with ethanol (96 per cent).

d_{20}^{20}: about 0.883.

n_D^{20}: about 1.395.

Butanol: maximum 0.2 per cent, determined by gas chromatography.

n-Butyl formate: maximum 0.1 per cent, determined by gas chromatography.

n-Butyl propionate: maximum 0.1 per cent, determined by gas chromatography.

Water: maximum 0.1 per cent.

Butylamine. $C_4H_{11}N$. (M_r 73.1). *1013600.* [109-73-9]. Butan-1-amine.

Distil and use within one month.

Colourless liquid, miscible with water, with ethanol (96 per cent).

n_D^{20}: about 1.401.

bp: about 78 °C.

***tert*-Butylamine.** *1100900.* [75-64-9].

See *1,1-dimethylethylamine R*.

4-(Butylamino)benzoic acid. $C_{11}H_{15}NO_2$. (M_r 193.2). *1206700.* [4740-24-3].

White or almost white powder.

Content: 96.5 per cent to 103.5 per cent.

Butylated hydroxytoluene. *1013800.* [128-37-0].

See *Butylhydroxytoluene R*.

Butylboronic acid. $C_4H_{11}BO_2$. (M_r 101.9). *1013700.* [4426-47-5].

Content: minimum 98 per cent.

mp: 90 °C to 92 °C.

***tert*-Butylhydroperoxide.** $C_4H_{10}O_2$. (M_r 90.1). *1118000.* [75-91-2]. 1,1-Dimethylethylhydroperoxide.

Flammable liquid, soluble in organic solvents.

d_{20}^{20}: 0.898.

n_D^{20}: 1.401.

bp: 35 °C.

Butyl 4-hydroxybenzoate. *1103900.* [94-26-8].

See *Butyl parahydroxybenzoate R*.

Butylhydroxytoluene. *1013800.* [128-37-0].

See *Butylhydroxytoluene (0581)*.

Butyl methacrylate. $C_8H_{14}O_2$. (M_r 142.2). *1145400.* [97-88-1]. Butyl 2-methylpropenoate.

Clear, colourless solution.

d_4^{20}: about 0.894.

n_D^{20}: about 1.424.

bp: about 163 °C.

***tert*-Butyl methyl ether.** *1013900.* [1634-04-4].

See *1,1-dimethylethyl methyl ether R*.

2-Butyloctanol. $C_{12}H_{26}O$. (M_r 186.3). *1206100.* [3913-02-8]. (2Ξ)-2-Butyloctan-1-ol.

Butyl parahydroxybenzoate. *1103900.* [94-26-8].

See *Butyl parahydroxybenzoate (0881)*.

Butyric acid. $C_4H_8O_2$. (M_r 88.1). *1014000.* [107-92-6]. Butanoic acid.

Content: minimum 99.0 per cent.

Oily liquid, miscible with water and with ethanol (96 per cent).

d_{20}^{20}: about 0.96.

n_D^{20}: about 1.398.

bp: about 163 °C.

Butyrolactone. $C_4H_6O_2$. (M_r 86.1). *1104000.* [96-48-0]. Dihydro-2(3*H*)-furanone. γ-Butyrolactone.

Oily liquid, miscible with water, soluble in methanol.

n_D^{25}: about 1.435.

bp: about 204 °C.

Cadmium. Cd. (A_r 112.4). *1014100.* [7440-43-9].

Silvery-white, lustrous metal, practically insoluble in water, freely soluble in nitric acid and in hot hydrochloric acid.

Cadmium nitrate tetrahydrate. $Cd(NO_3)_2,4H_2O$. (M_r 308.5). *1174900.* [10022-68-1].

Hygroscopic orthorhombic crystals, very soluble in water, soluble in acetone and in ethanol (96 per cent).

mp: about 59.5 °C.

Caesium chloride. CsCl. (M_r 168.4). *1014200.* [7647-17-8].

White or almost white powder, very soluble in water, freely soluble in methanol, practically insoluble in acetone.

Caffeic acid. $C_9H_8O_4$. (M_r 180.2). *1014300.* [331-39-5]. (*E*)-3-(3,4-Dihydroxyphenyl)propenoic acid.

White or almost white crystals or plates, freely soluble in hot water and in ethanol (96 per cent), sparingly soluble in cold water.

Absorbance (*2.2.25*). A freshly prepared solution at pH 7.6 shows 2 absorption maxima at about 288 nm and about 313 nm.

Caffeine. *1014400.* [58-08-2].

See *Caffeine (0267)*.

Calcium acetate. $C_4H_6CaO_4$. (M_r 158.2). *1191600.* [62-54-4]. Calcium diacetate. See *Calcium acetate (2128)*.

Calcium carbonate. *1014500.* [471-34-1].

See *Calcium carbonate (0014)*.

Calcium carbonate R1. *1014501.*

Complies with the requirements prescribed for *calcium carbonate R* with the following additional requirement.

Chlorides (*2.4.4*): maximum 50 ppm.

Calcium chloride. *1014600.* [10035-04-8].

See *Calcium chloride (0015)*.

Calcium chloride solution. *1014601.*

A 73.5 g/L solution of *calcium chloride R*.

Calcium chloride solution, 0.01 M. *1014602.*

Dissolve 0.147 g of *calcium chloride R* in *water R* and dilute to 100.0 mL with the same solvent.

Calcium chloride solution, 0.02 M. *1014603.*

Dissolve 2.94 g of *calcium chloride R* in 900 mL of *water R*, adjust to pH 6.0 to 6.2 and dilute to 1000.0 mL with *water R*.

Storage: at 2 °C to 8 °C.

Calcium chloride solution, 0.025 M. *1014604.*

Dissolve 0.368 g of *calcium chloride R* in *water R* and dilute to 100.0 mL with the same solvent.

Calcium chloride R1. $CaCl_2,4H_2O$. (M_r 183.1). *1014700.* Calcium chloride tetrahydrate.

Iron: maximum 0.05 ppm.

Calcium chloride, anhydrous. $CaCl_2$. (M_r 111.0). *1014800.* [10043-52-4].

Content: minimum 98.0 per cent (dried substance).

White or almost white granules, deliquescent, very soluble in water, freely soluble in ethanol (96 per cent) and in methanol.

Loss on drying (*2.2.32*): maximum 5.0 per cent, determined by drying in an oven at 200 °C.

Storage: in an airtight container, protected from moisture.

Calcium hydroxide. $Ca(OH)_2$. (M_r 74.1). *1015000.* [1305-62-0]. Calcium dihydroxide.

White or almost white powder, almost completely soluble in 600 parts of water.

Calcium hydroxide solution. *1015001.*

A freshly prepared saturated solution.

Calcium lactate pentahydrate. *1015100.* [41372-22-9].

See *Calcium lactate pentahydrate (0468)*.

Calcium phosphate monobasic monohydrate. $CaH_4O_8P_2,H_2O$. (M_r 252.1). *1157200.* [10031-30-8]. Calcium tetrahydrogen bisphosphate monohydrate. Phosphoric acid calcium salt (2:1) monohydrate.

White or almost white, crystalline powder, soluble in water.

Calcium sulfate. $CaSO_4,{}^1/_2H_2O$. (M_r 145.1). *1015200.* [10034-76-1]. Calcium sulfate hemihydrate.

White or almost white powder, soluble in about 1500 parts of water, practically insoluble in ethanol (96 per cent). When mixed with half its mass of water it rapidly solidifies to a hard and porous mass.

Calcium sulfate solution. *1015201.*

Shake 5 g of *calcium sulfate R* with 100 mL of *water R* for 1 h and filter.

Calconecarboxylic acid. $C_{21}H_{14}N_2O_7S$. (M_r 438.4). *1015300.* [3737-95-9]. 2-Hydroxy-1-(2-hydroxy-4-sulfo-1-naphthylazo)naphthalene-3-carboxylic acid.

Brownish-black powder, slightly soluble in water, very slightly soluble in acetone and in ethanol (96 per cent), sparingly soluble in dilute solutions of sodium hydroxide.

Calconecarboxylic acid triturate. *1015301.*

Mix 1 part of *calconecarboxylic acid R* with 99 parts of *sodium chloride R*.

Test for sensitivity. Dissolve 50 mg of calconecarboxylic acid triturate in a mixture of 2 mL of *strong sodium hydroxide solution R* and 100 mL of *water R*. The solution is blue but becomes violet on addition of 1 mL of a 10 g/L solution of *magnesium sulfate R* and 0.1 mL of a 1.5 g/L solution of *calcium chloride R* and turns pure blue on addition of 0.15 mL of *0.01 M sodium edetate*.

Camphene. $C_{10}H_{16}$. (M_r 136.2). *1139200.* [79-92-5]. 2,2-Dimethyl-3-methylenebicyclo[2.2.1]heptane.

Camphene used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Rosemary Oil (1846)*.

Content: minimum 90 per cent, calculated by the normalisation procedure.

Camphor. *1113000.* [76-22-2].

See *Camphor, racemic (0655).*

Camphor used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Lavender oil (1338).*

Test solution. A 10 g/L solution of the substance to be examined in *hexane R*.

Content: minimum 95.0 per cent, calculated by the normalisation procedure.

(1*S*)-(+)-10-Camphorsulfonic acid. $C_{10}H_{16}O_4S$. (M_r 232.3). *1104100.* [3144-16-9]. (1*S*,4*R*)-(+)-2-Oxo-10-bornenesulfonic acid. [(1*S*)-7,7-Dimethyl-2-oxobicyclo[2.2.1]heptan-1-yl]methanesulfonic acid. Reychler's acid.

Prismatic crystals, hygroscopic, soluble in water.

Content: minimum 99.0 per cent of (1*S*)-(+)-10-camphorsulfonic acid.

$[\alpha]_D^{20}$: + 20 ± 1, determined on a 43 g/L solution.

mp: about 194 °C, with decomposition.

ΔA (*2.2.41*): 10.2×10^3 determined at 290.5 nm on a 1.0 g/L solution.

Capric acid. $C_{10}H_{20}O_2$. (M_r 172.3). *1142000.* [334-48-5]. Decanoic acid.

Crystalline solid, very slightly soluble in water, soluble in anhydrous ethanol.

bp: about 270 °C.

mp: about 31.4 °C.

Capric acid used in the assay of total fatty acids in Saw palmetto fruit (1848) complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Saw palmetto fruit (1848).*

Content: minimum 98 per cent, calculated by the normalisation procedure.

Capric alcohol. *1024700.*

See *Decanol R*.

Caproic acid. $C_6H_{12}O_2$. (M_r 116.2). *1142100.* [142-62-1]. Hexanoic acid.

Oily liquid, sparingly soluble in water.

d_4^{20}: about 0.926.

n_D^{20}: about 1.417.

bp: about 205 °C.

Caproic acid used in the assay of total fatty acids in Saw palmetto fruit (1848) complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Saw palmetto fruit (1848).*

Content: minimum 98 per cent, calculated by the normalisation procedure.

ε-Caprolactam. $C_6H_{11}NO$. (M_r 113.2). *1104200.* [105-60-2]. Hexane-6-lactam.

Hygroscopic flakes, freely soluble in water, in anhydrous ethanol and in methanol.

mp: about 70 °C.

Caprylic acid. $C_8H_{16}O_2$. (M_r 144.2). *1142200.* [124-07-2]. Octanoic acid.

Slightly yellow, oily liquid.

d_4^{20}: about 0.910.

n_D^{20}: about 1.428.

bp: about 239.7 °C.

mp: about 16.7 °C.

Caprylic acid used in the assay of total fatty acids in Saw palmetto fruit (1848) complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Saw palmetto fruit (1848).*

Content: minimum 98 per cent, calculated by the normalisation procedure.

Capsaicin. $C_{18}H_{27}NO_3$. (M_r 305.4). *1147900.* [404-86-4]. (*E*)-*N*-[(4-Hydroxy-3-methoxyphenyl)methyl]-8-methylnon-6-enamide.

White or almost white, crystalline powder, practically insoluble in water, freely soluble in anhydrous ethanol.

mp: about 65 °C.

Capsaicin used in the assay in Capsicum (1859) complies with the following additional test.

Assay. Liquid chromatography (*2.2.29*) as prescribed in the monograph *Capsicum (1859).*

Content: minimum 95.0 per cent, calculated by the normalisation procedure.

Carbazole. $C_{12}H_9N$. (M_r 167.2). *1015400.* [86-74-8]. Dibenzopyrrole.

Crystals, practically insoluble in water, freely soluble in acetone, slightly soluble in anhydrous ethanol.

mp: about 245 °C.

Carbomer. *1015500.* [9007-20-9].

A cross-linked polymer of acrylic acid; it contains a large proportion (56 per cent to 68 per cent) of carboxylic acid (CO_2H) groups after drying at 80 °C for 1 h. Average relative molecular mass about 3×10^6.

pH (*2.2.3*): about 3 for a 10 g/L suspension.

Carbon dioxide. *1015600.* [124-38-9].

See *Carbon dioxide (0375).*

Carbon dioxide R1. CO_2. (M_r 44.01). *1015700.* [124-38-9].

Content: minimum 99.995 per cent *V/V*.

Carbon monoxide: less than 5 ppm.

Oxygen: less than 25 ppm.

Nitric oxide: less than 1 ppm.

Carbon dioxide R2. CO_2. (M_r 44.01). *1134500.* [124-38-9].

Content: minimum 99 per cent *V/V*.

Carbon disulfide. CS_2. (M_r 76.1). *1015800.* [75-15-0].

Colourless or yellowish, flammable liquid, practically insoluble in water, miscible with anhydrous ethanol.

d_{20}^{20}: about 1.26.

bp: 46 °C to 47 °C.

Carbon for chromatography, graphitised. *1015900.*

Carbon chains having a length greater than C_9.

Particle size: 400 µm to 850 µm.

Relative density: 0.72.

Surface area: 10 m^2/g.

Do not use at a temperature higher than 400 °C.

Carbon for chromatography, graphitised R1. *1153500.*

Porous spherical carbon particles comprised of flat sheets of hexagonally arranged carbon atoms.

Particle size: 5 µm to 7 µm.

Pore volume: 0.7 cm^3/g.

Carbon monoxide. CO. (M_r 28.01). *1016000.* [630-08-0].

Content: minimum 99.97 per cent *V/V*.

Carbon monoxide R1. CO. (M_r 28.01). *1134600.* [630-08-0].

Content: minimum 99 per cent *V/V*.

Carbon tetrachloride. CCl_4. (M_r 153.8). *1016100.* [56-23-5]. Tetrachloromethane.

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

d_{20}^{20}: 1.595 to 1.598.

bp: 76 °C to 77 °C.

Carbophenothion. $C_{11}H_{16}ClO_2PS_3$. (M_r 342.9). *1016200.* [786-19-6]. *O,O*-Diethyl *S*-[[(4-chlorophenyl)thio]methyl]-phosphorodithioate.

Yellowish liquid, practically insoluble in water, miscible with organic solvents.

d_4^{25}: about 1.27.

For the monograph *Wool Fat (0134)*, a suitable certified reference solution (10 ng/µL in iso-octane) may be used.

Car-3-ene. $C_{10}H_{16}$. (M_r 136.2). *1124000.* [498-15-7]. 3,7,7-Trimethylbicyclo[4.1.0]hept-3-ene. 4,7,7-Trimethyl-3-norcarene.

Liquid with a pungent odour, slightly soluble in water, soluble in organic solvents.

d_{20}^{20}: about 0.864.

n_D^{20}: 1.473 to 1.474.

$[\alpha]_D^{20}$: + 15 to + 17.

bp: 170 °C to 172 °C.

Car-3-ene used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Nutmeg oil (1552)*.

Content: minimum 95.0 per cent, calculated by the normalisation procedure.

Carminic acid. $C_{22}H_{20}O_{13}$. (M_r 492.4). *1156700.* [1260-17-9]. 7-α-D-Glucopyranosyl-3,5,6,8-tetrahydroxy-1-methyl-9,10-dioxo-9,10-dihydroanthracene-2-carboxylic acid.

Dark red powder, very slightly soluble in water, soluble in dimethyl sulfoxide, very slightly soluble in ethanol (96 per cent).

Carob bean gum. *1104500.*

The ground endosperm of the fruit kernels of *Ceratonia siliqua* L. Taub.

White or almost white powder containing 70 per cent to 80 per cent of a water-soluble gum consisting mainly of galactomannoglycone.

Carvacrol. $C_{10}H_{14}O$. (M_r 150.2). *1016400.* [499-75-2]. 5-Isopropyl-2-methylphenol.

Brownish liquid, practically insoluble in water, very soluble in ethanol (96 per cent).

d_{20}^{20}: about 0.975.

n_D^{20}: about 1.523.

bp: about 237 °C.

Carvacrol used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Peppermint oil (0405)*.

Test solution. Dissolve 0.1 g in about 10 mL of *acetone R*.

Content: minimum 95.0 per cent, calculated by the normalisation procedure.

Carveol. $C_{10}H_{16}O$. (M_r 152.2). *1160400.* [99-48-9]. *p*-Mentha-1(6),8-dien-2-ol. 2-Methyl-5-(1-methylethenyl)cyclohex-2-enol.

The substance contains a variable content of *trans*- and *cis*-carveol.

Carveol used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the test for chromatographic profile in the monograph *Caraway oil (1817)*.

Content: minimum 97 per cent, calculated by the normalisation procedure.

Carvone. $C_{10}H_{14}O$. (M_r 150.2). *1016500.* [2244-16-8]. (+)-*p*-Mentha-6,8-dien-2-one. (5*S*)-2-Methyl-5-(1-methylethenyl)-cyclohex-2-enone.

Liquid, practically insoluble in water, miscible with ethanol (96 per cent).

d_{20}^{20}: about 0.965

n_D^{20}: about 1.500.

$[\alpha]_D^{20}$: about + 61.

bp: about 230 °C.

Carvone used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Peppermint oil (0405)* using the substance to be examined as the test solution.

Content: minimum 98.0 per cent, calculated by the normalisation procedure.

Carvone R1. *1016501.*

Complies with the requirements prescribed for *carvone R* with the following additional requirement.

Assay. Gas chromatography (*2.2.28*) as prescribed in the test for chiral purity in the monograph *Caraway oil (1817)*.

Content: minimum 98 per cent.

(−)-Carvone. $C_{10}H_{14}O$. (M_r 150.2). *1160500.* [6485-40-1]. (−)-*p*-Mentha-1(6),8-dien-2-one. (5*R*)-2-Methyl-5-(1-methylethenyl)cyclohex-2-enone.

Liquid.

d_{20}^{20}: about 0.965.

n_D^{20}: about 1.4988.

$[\alpha]_D^{20}$: about − 62.

bp: about 230 °C.

Assay. Gas chromatography (*2.2.28*) as prescribed in the test for chiral purity in the monograph *Caraway oil (1817)*.

Content: minimum 99 per cent.

β-Caryophyllene. $C_{15}H_{24}$. (M_r 204.4). *1101000.* [87-44-5]. (*E*)-(1*R*,9*S*)-4,11,11-Trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene.

Oily liquid, practically insoluble in water, miscible with ethanol (96 per cent).

β-Caryophyllene used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Clove oil (1091)*.

Test solution. The substance to be examined.

Content: minimum 90.0 per cent, calculated by the normalisation procedure.

Caryophyllene oxide. $C_{15}H_{24}O$. (M_r 220.4). *1149000.* [1139-30-6]. (-)-β-Caryophyllene epoxide. (1*R*,4*R*,6*R*,10*S*)-4,12,12-Trimethyl-9-methylene-5-oxatricyclo[8.2.0.0^{4,6}]dodecane.

Colourless, fine crystals with lumps.

mp: 62 °C to 63 °C.

Caryophyllene oxide used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Turpentine oil, Pinus pinaster type (1627)*.

Content: minimum 99.0 per cent, calculated by the normalisation procedure.

Casein. *1016600.* [9000-71-9].

Mixture of related phosphoproteins obtained from milk.

White or almost white, amorphous powder or granules, very slightly soluble in water and in non-polar organic solvents. It dissolves in concentrated hydrochloric acid giving a pale-violet solution. It forms salts with acids and bases. Its isoelectric point is at about pH 4.7. Alkaline solutions are laevorotatory.

Casticin. $C_{19}H_{18}O_8$. (M_r 374.3). *1162200.* [479-91-4]. 5-Hydroxy-2-(3-hydroxy-4-methoxyphenyl)-3,6,7-trimethoxy-4*H*-1-benzopyran-4-one.

Yellow crystals.

Catalpol. $C_{15}H_{22}O_{10}$. (M_r 362.3). *1142300.* [2415-24-9]. (1a*S*,1b*S*,2*S*,5a*R*,6*S*,6a*S*)-6-Hydroxy-1a-(hydroxymethyl)-1a,1b,2,5a,6,6a-hexahydrooxireno[4,5]cyclopenta[1,2-*c*]pyran-2-yl β-D-glucopyranoside.

mp: 203 °C to 205 °C.

Catechin. $C_{15}H_{14}O_6,xH_2O$. (M_r 290.3 for the anhydrous substance). *1119000.* [154-23-4]. (+)-(2*R*,3*S*)-2-(3,4-Dihydroxyphenyl)-3,4-dihydro-2*H*-chromene-3,5,7-triol. Catechol. Cianidanol. Cyanidol.

Cathine hydrochloride. $C_9H_{14}ClNO$. (M_r 187.7). *1206800.* [2153-98-2]. (1*S*,2*S*)-2-Amino-1-phenylpropan-1-ol hydrochloride. Norpseudoephedrine hydrochloride.

White or almost white solid.

Content: minimum 95.0 per cent.

Cation-exchange resin. *1016700.*

A resin in protonated form with sulfonic acid groups attached to a polymer lattice consisting of polystyrene cross-linked with 8 per cent of divinylbenzene. It is available as spherical beads.

Cation-exchange resin R1. *1121900.*

A resin in protonated form with sulfonic acid groups attached to a polymer lattice consisting of polystyrene cross-linked with 4 per cent of divinylbenzene. It is available as spherical beads.

Cation-exchange resin R2. *1195400.*

Resin containing strongly acidic propylenesulfonic acid groups.

Cation-exchange resin (calcium form), strong. *1104600.*

Resin in calcium form with sulfonic acid groups attached to a polymer lattice consisting of polystyrene cross-linked with 8 per cent of divinylbenzene.

Cation-exchange resin (sodium form), strong. *1176100.*

Resin in sodium form with sulfonic acid groups attached to a polymer lattice consisting of polystyrene cross-linked with divinylbenzene.

Cation-exchange resin, strong. *1156800.*

Strong cation-exchange resin in protonated form with sulfonic acid groups attached to a polymer lattice consisting of polystyrene cross-linked with divinylbenzene.

Cation-exchange resin, weak. *1203200.*

Weak cation-exchange resin in protonated form with carboxylate functional groups attached to a polymer lattice consisting of polystyrene cross-linked with divinylbenzene.

Cellulose for chromatography. *1016800.* [9004-34-6].

Fine, white or almost white, homogeneous powder with an average particle size less than 30 µm.

Preparation of a thin layer. Suspend 15 g in 100 mL of *water R* and homogenise in an electric mixer for 60 s. Coat carefully cleaned plates with a layer 0.1 mm thick using a spreading device. Allow to dry in air.

Cellulose for chromatography R1. *1016900.*

Microcrystalline cellulose.

Preparation of a thin layer. Suspend 25 g in 90 mL of *water R* and homogenise in an electric mixer for 60 s. Coat carefully cleaned plates with a layer 0.1 mm thick using a spreading device. Allow to dry in air.

Cellulose for chromatography F_{254}. *1017000.*

Microcrystalline cellulose F_{254}. A fine, white or almost white, homogeneous powder with an average particle size less than 30 µm, containing a fluorescent indicator having an optimal intensity at 254 nm.

Preparation of a thin layer. Suspend 25 g in 100 mL of *water R* and homogenise using an electric mixer for 60 s. Coat carefully cleaned plates with a layer 0.1 mm thick using a spreading device. Allow to dry in air.

Cerium sulfate. $Ce(SO_4)_2,4H_2O$. (M_r 404.3). *1017300.* [10294-42-5]. Cerium(IV) sulfate tetrahydrate. Ceric sulfate.

Yellow or orange-yellow, crystalline powder or crystals, very slightly soluble in water, slowly soluble in dilute acids.

Cerous nitrate. $Ce(NO_3)_3,6H_2O$. (M_r 434.3). *1017400.* [10294-41-4]. Cerium trinitrate hexahydrate.

Colourless or pale yellow, crystalline powder, freely soluble in water and in ethanol (96 per cent).

Cetostearyl alcohol. *1017500.* [67762-27-0].

See *Cetostearyl alcohol (0702)*.

Cetrimide. *1017600.* [8044-71-1].

See *Cetrimide (0378)*.

Cetyl alcohol. $C_{16}H_{34}O$. (M_r 242.4). *1160600.* [36653-82-4]. Hexadecan-1-ol.

Content: minimum 95.0 per cent.

mp: about 48 °C.

Cetylpyridinium chloride monohydrate. $C_{21}H_{38}ClN,H_2O$. (M_r 358.0). *1162800.* [6004-24-6]. 1-Hexadecylpyridinium chloride monohydrate.

White or almost white powder, freely soluble in water and in ethanol (96 per cent).

mp: 80 °C to 83 °C.

Cetyltrimethylammonium bromide. $C_{19}H_{42}BrN$. (M_r 364.5). *1017700.* [57-09-0]. Cetrimonium bromide. *N*-Hexadecyl-*N*,*N*,*N*-trimethylammonium bromide.

White or almost white, crystalline powder, soluble in water, freely soluble in ethanol (96 per cent).

mp: about 240 °C.

Chamazulene. $C_{14}H_{16}$. (M_r 184.3). *1148000.* [529-05-5]. 7-Ethyl-1,4-dimethylazulene.

Blue liquid, very slightly soluble in water, soluble in ethanol (96 per cent), miscible with fatty oils, with essential oils and with liquid paraffin, soluble with discolouration in phosphoric acid (85 per cent *m/m*) and sulfuric acid (50 per cent *V/V*).

Appearance of solution. 50 mg is soluble in 2.5 mL of *hexane R*. The blue solution is clear in a thin-layer obtained by tilting the test-tube.

Chamazulene used for gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Matricaria oil (1836)*.

Test solution: a 4 g/L solution in *cyclohexane R*.

Content: minimum 95.0 per cent, calculated by the normalisation procedure.

Charcoal, activated. *1017800.* [64365-11-3].

See *Activated charcoal (0313)*.

Chloral hydrate. *1017900.* [302-17-0].

See *Choral hydrate (0265)*.

Chloral hydrate solution. *1017901.*

A solution of 80 g in 20 mL of *water R*.

Chloramine. *1018000.* [7080-50-4].

See *Tosylchloramide sodium (0381)*.

Chloramine solution. *1018001.*

A 20 g/L solution of *chloramine R*. Prepare immediately before use.

Chloramine solution R1. *1018002.*

A 0.1 g/L solution of *chloramine R*. Prepare immediately before use.

Chloramine solution R2. *1018003.*

A 0.2 g/L solution of *chloramine R*. Prepare immediately before use.

Chlordane. $C_{10}H_6Cl_8$. (M_r 409.8). *1124100.* [12789-03-6].

bp: about 175 °C.

mp: about 106 °C.

A suitable certified reference solution of technical grade (10 ng/µL in iso-octane) may be used.

Chlordiazepoxide. *1113200.* [58-25-3].

See *Chlordiazepoxide (0656)*.

Chlorfenvinphos. $C_{12}H_{14}Cl_3O_4P$. (M_r 359.6). *1124200.* [470-90-6].

A suitable certified reference solution (10 ng/µL in cyclohexane) may be used.

Chloroacetanilide. C_8H_8ClNO. (M_r 169.6). *1018100.* [539-03-7]. 4′-Chloroacetanilide.

Content: minimum 95 per cent.

Crystalline powder, practically insoluble in water, soluble in ethanol (96 per cent).

mp: about 178 °C.

Chloroacetic acid. $C_2H_3ClO_2$. (M_r 94.5). *1018200.* [79-11-8].

Colourless or white or almost white crystals, deliquescent, very soluble in water, soluble in ethanol (96 per cent).

Storage: in an airtight container.

Chloroaniline. C_6H_6ClN. (M_r 127.6). *1018300.* [106-47-8]. 4-Chloroaniline.

Crystals, soluble in hot water, freely soluble in ethanol (96 per cent).

mp: about 71 °C.

4-Chlorobenzenesulfonamide. $C_6H_6ClNO_2S$. (M_r 191.6). *1097400.* [98-64-6].

White or almost white powder.

mp: about 145 °C.

2-Chlorobenzoic acid. $C_7H_5ClO_2$. (M_r 156.6). *1139300.* [118-91-2].

Soluble in water, slightly soluble in anhydrous ethanol.

bp: about 285 °C.

mp: about 140 °C.

Chlorobutanol. *1018400.* [57-15-8].

See *Chlorobutanol (0382)*.

2-Chloro-2-deoxy-D-glucose. $C_6H_{11}ClO_5$. (M_r 198.6). *1134700.* [14685-79-1].

White or almost white crystalline, very hygroscopic powder, soluble in water and in dimethyl sulfoxide, practically insoluble in ethanol (96 per cent).

2-Chloroethanol. C_2H_5ClO. (M_r 80.5). *1097500.* [107-07-3].

Colourless liquid, soluble in ethanol (96 per cent).

d_{20}^{20}: about 1.197.

n_D^{20}: about 1.442.

bp: about 130 °C.

mp: about – 89 °C.

2-Chloroethanol solution. *1097501.*

Dissolve 125 mg of *2-chloroethanol R* in *2-propanol R* and dilute to 50 mL with the same solvent. Dilute 5 mL of the solution to 50 mL with *2-propanol R*.

Chloroethylamine hydrochloride. $C_2H_7Cl_2N$. (M_r 116.0). *1124300.* [870-24-6]. 2-Chloroethanamine hydrochloride.

mp: about 145 °C.

(2-Chloroethyl)diethylamine hydrochloride. $C_6H_{15}Cl_2N$. (M_r 172.1). *1018500.* [869-24-9].

White or almost white, crystalline powder, very soluble in water and in methanol, freely soluble in methylene chloride, practically insoluble in hexane.

mp: about 211 °C.

Chloroform. $CHCl_3$. (M_r 119.4). *1018600.* [67-66-3]. Trichloromethane.

Clear, colourless liquid, slightly soluble in water, miscible with ethanol (96 per cent).

d_{20}^{20}: 1.475 to 1.481.

bp: about 60 °C.

Ethanol: 0.4 per cent *m/m* to 1.0 per cent *m/m*.

Chloroform, acidified. *1018601.*

To 100 mL of *chloroform R* add 10 mL of *hydrochloric acid R*. Shake, allow to stand and separate the 2 layers.

Chloroform, ethanol-free. *1018602.*

Shake 200 mL of *chloroform R* with four quantities, each of 100 mL, of *water R*. Dry over 20 g of *anhydrous sodium sulfate R* for 24 h. Distil the filtrate over 10 g of *anhydrous sodium sulfate R*. Discard the first 20 mL of distillate. Prepare immediately before use.

Chlorogenic acid. $C_{16}H_{18}O_9$. (M_r 354.3). *1104700.* [327-97-9]. (1*S*,3*R*,4*R*,5*R*)-3-[(3,4-Dihydroxycinnamoyl)oxy]-1,4,5-trihydroxycyclohexanecarboxylic acid.

White or almost white, crystalline powder or needles, freely soluble in boiling water, in acetone and in ethanol (96 per cent).

$[\alpha]_D^{26}$: about – 35.2.

mp: about 208 °C.

Chromatography. Thin-layer chromatography (*2.2.27*) as prescribed on Identification A in the monograph *Belladonna leaf dry extract, standardised (1294)*; the chromatogram shows only one principal zone.

Chlorogenic acid used in liquid chromatography complies with the following additional test.

Assay. Liquid chromatography (*2.2.29*) as prescribed in the monograph *Artichoke Leaf (1866)*.

Content: minimum 97.0 per cent.

3-Chloro-2-methylaniline. C_7H_8ClN. (M_r 141.6). *1139400.* [87-60-5]. 6-Chloro-2-toluidine.

Not miscible with water, slightly soluble in anhydrous ethanol.

d_{20}^{20}: about 1.171.

n_D^{20}: about 1.587.

bp: about 115 °C.

mp: about 2 °C.

2-Chloro-*N*-(2,6-dimethylphenyl)acetamide. $C_{10}H_{12}ClNO$. (M_r 197.7). *1168700.* [1131-01-7].

2-Chloronicotinic acid. $C_6H_4ClNO_2$. (M_r 157.6). *1157300.* [2942-59-8]. 2-Chloropyridine-3-carboxylic acid.

White or almost white powder.

mp: about 177 °C.

Content: minimum 95 per cent.

2-Chloro-4-nitroaniline. $C_6H_5ClN_2O_2$. (M_r 172.6). *1018800.* [121-87-9].

Yellow, crystalline powder, freely soluble in methanol.

mp: about 107 °C.

Storage: protected from light.

2-Chloro-5-nitrobenzoic acid. $C_7H_4ClNO_4$. (M_r 201.6). *1183800.* [2516-96-3].

mp: 165 °C to 168 °C.

Chlorophenol. C_6H_5ClO. (M_r 128.6). *1018900.* [106-48-9]. 4-Chlorophenol.

Colourless or almost colourless crystals, slightly soluble in water, very soluble in ethanol (96 per cent) and in solutions of alkali hydroxides.

mp: about 42 °C.

2-[2-(4-Chlorophenyl)acetyl]benzoic acid. $C_{15}H_{11}ClO_3$. (M_r 274.7). *1194500.* [53242-76-5].

Chloroplatinic acid. $H_2Cl_6Pt,6H_2O$. (M_r 517.9). *1019000.* [18497-13-7]. Hydrogen hexachloroplatinate(IV) hexahydrate.

Content: minimum 37.0 per cent *m/m* of platinum (A_r 195.1).

Brownish-red crystals or a crystalline mass, very soluble in water, soluble in ethanol (96 per cent).

Assay. Ignite 0.200 g to constant mass at 900 ± 50 °C and weigh the residue (platinum).

Storage: protected from light.

3-Chloropropane-1,2-diol. $C_3H_7ClO_2$. (M_r 110.5). *1097600.* [96-24-2].

Colourless liquid, soluble in water and ethanol (96 per cent).

d_{20}^{20}: about 1.322.

n_D^{20}: about 1.480.

bp: about 213 °C.

5-Chloroquinolin-8-ol. C_9H_6ClNO. (M_r 179.6). *1156900.* [130-16-5]. 5-Chlorooxine.

Sparingly soluble in cold dilute hydrochloric acid.

mp: about 123 °C.

Content: minimum 95.0 per cent.

4-Chlororesorcinol. $C_6H_5ClO_2$. (M_r 144.6). *1177700.* [95-88-5]. 4-Chlorobenzene-1,3-diol. 1,3-Dihydroxy-4-chlorobenzene.

mp: 106 °C to 108 °C.

5-Chlorosalicylic acid. $C_7H_5ClO_3$. (M_r 172.6). *1019100.* [321-14-2].

White or almost white, crystalline powder, soluble in methanol.

mp: about 173 °C.

Chlorothiazide. $C_7H_6ClN_3O_4S_2$. (M_r 295.7). *1112100.* [58-94-6]. 6-Chloro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide.

Content: minimum 98.0 per cent.

White or almost white, crystalline powder, very slightly soluble in water, sparingly soluble in acetone, slightly soluble in ethanol (96 per cent). It dissolves in dilute solutions of alkali hydroxides.

Chlorotrimethylsilane. C_3H_9ClSi. (M_r 108.6). *1019300.* [75-77-4].

Clear, colourless liquid, fuming in air.

d_{20}^{20}: about 0.86.

n_D^{20}: about 1.388.

bp: about 57 °C.

Chlorpyriphos. $C_9H_{11}Cl_3NO_3PS$. (M_r 350.6). *1124400.* [2921-88-2].

bp: about 200 °C.

mp: 42 °C to 44 °C.

A suitable certified reference solution (10 ng/µL in cyclohexane) may be used.

Chlorpyriphos-methyl. $C_7H_7Cl_3NO_3PS$. (M_r 322.5). *1124500.* [5598-13-0].

mp: 45 °C to 47 °C.

A suitable certified reference solution (10 ng/µL in cyclohexane) may be used.

Chlortetracycline hydrochloride. *1145500.*

See *Chlortetracycline hydrochloride (0173)*.

(5α)-Cholestane. $C_{27}H_{48}$. (M_r 372.7). *1167900.* [481-21-0].

Slightly soluble in anhydrous ethanol.

mp: about 81 °C.

Cholesterol. *1019400.* [57-88-5].

See *Cholesterol (0993)*.

Choline chloride. $C_5H_{14}ClNO$. (M_r 139.6). *1019500.* [67-48-1]. (2-Hydroxyethyl)trimethylammonium chloride.

Deliquescent crystals, very soluble in water and in ethanol (96 per cent).

Chromatography. Thin-layer chromatography (*2.2.27*) as prescribed in the monograph *Suxamethonium chloride (0248)*: apply 5 µL of a 0.2 g/L solution in *methanol R*; the chromatogram shows one principal spot.

Storage: in an airtight container.

Chondroitinase ABC. *1162900.*

Pectin lyase-like enzyme secreted by *Flavobacterium heparinum*. Available in vials containing 5-10 units. It cleaves both glucuronate-containing disaccharides, e.g. chondroitin sulfate, and iduronate-containing disaccharides, e.g. dermatan sulfate.

Chondroitinase AC. *1163000.*

Pectin lyase-like enzyme secreted by *Flavobacterium heparinum*. Available in vials containing 5-10 units. It cleaves only glucuronate-containing disaccharides, e.g. chondroitin sulfate.

Chromazurol S. $C_{23}H_{13}Cl_2Na_3O_9S$. (M_r 605). *1019600.* [1667-99-8].

Schultz No. 841.

Colour Index No. 43825.

Trisodium 5-[(3-carboxylato-5-methyl-4-oxocyclohexa-2,5-dien-1-ylidene)(2,6-dichloro-3-sulfonatophenyl)methyl]-2-hydroxy-3-methylbenzoate.

Brownish-black powder, soluble in water, slightly soluble in ethanol (96 per cent).

Chromic potassium sulfate. $CrK(SO_4)_2,12H_2O$. (M_r 499.4). *1019800.* [7788-99-0]. Chrome alum.

Large, violet-red or black crystals, freely soluble in water, practically insoluble in ethanol (96 per cent).

Chromium(III) acetylacetonate. $C_{15}H_{21}CrO_6$. (M_r 349.3). *1172900.* [21679-31-2]. (*OC*-6-11)-Tris(2,4-pentanedionato-κ*O*,κ*O*′)chromium.

Chromium(III) trichloride hexahydrate. $[Cr(H_2O)_4Cl_2]Cl,2H_2O$. (M_r 266.5). *1104800.* [10060-12-5].

Dark green crystalline powder, hygroscopic.

Storage: protected from humidity and oxidising agents.

Chromium trioxide. CrO_3. (M_r 100.0). *1019900.* [1333-82-0].

Dark brownish-red needles or granules, deliquescent, very soluble in water.

Storage: in an airtight glass container.

Chromogenic substrate R1. *1020000.*

Dissolve *N*-α-benzyloxycarbonyl-D-arginyl-L-glycyl-L-arginine-4-nitroanilide dihydrochloride in *water R* to give a 0.003 M solution. Dilute in *tris(hydroxymethyl)aminomethane-EDTA buffer solution pH 8.4 R* to 0.0005 M before use.

Chromogenic substrate R2. *1020100.*

Dissolve D-phenylalanyl-L-pipecolyl-L-arginine-4-nitroanilide dihydrochloride in *water R* to give a 0.003 M solution. Dilute before use in titrating in *tris(hydroxymethyl)aminomethane-EDTA buffer solution pH 8.4 R* to give a 0.0005 M solution.

Chromogenic substrate R3. *1149100.*

Dissolve D-valyl-leucyl-lysyl-4-nitroanilide dihydrochloride in *water R* to give a 0.003 M solution.

Chromogenic substrate R4. *1163100.*

Dissolve D-phenylalanyl-L-pipecolyl-L-arginine-4-nitroanilide dihydrochloride in *water R* to give a 0.008 M solution. Dilute to 0.0025 M with *phosphate buffer solution pH 8.5 R* before use.

Chromogenic substrate R5. *1163200.*

Dissolve *N*-benzoyl-L-isoleucyl-L-glutamyl-glycyl-L-arginine-4-nitroanilide hydrochloride in *water R* to give a 0.003 M solution.

Chromotrope II B. $C_{16}H_9N_3Na_2O_{10}S_2$. ($M_r$ 513.4). *1020200.* [548-80-1].

Schultz No. 67.

Colour Index No. 16575.
Disodium 4,5-dihydroxy-3-(4-nitrophenylazo)naphthalene-2,7-disulfonate.

Reddish-brown powder, soluble in water giving a yellowish-red colour, practically insoluble in ethanol (96 per cent).

Chromotrope II B solution. *1020201.*

A 0.05 g/L solution of *chromotrope II B R* in *sulfuric acid R*.

Chromotropic acid, sodium salt. $C_{10}H_6Na_2O_8S_2,2H_2O$. ($M_r$ 400.3). *1020300.* [5808-22-0].

Schultz No. 1136.
Disodium 4,5-dihydroxynaphthalene-2,7-disulfonate dihydrate. Disodium 1,8-dihydroxynaphthalene-3,6-disulfonate dihydrate.

A yellowish-white powder, soluble in water, practically insoluble in ethanol (96 per cent).

Chromotropic acid, sodium salt solution. *1020301.*

Dissolve 0.60 g of *chromotropic acid, sodium salt R* in about 80 mL of *water R* and dilute to 100 mL with the same solvent. Use this solution within 24 h.

Chromotropic acid-sulfuric acid solution. *1020302.*

Dissolve 5 mg of *chromotropic acid, sodium salt R* in 10 mL of a mixture of 9 mL of *sulfuric acid R* and 4 mL of *water R*.

Chrysanthemin. $C_{21}H_{21}ClO_{11}$. (M_r 485.8). *1134800.* [7084-24-4]. Cyanidin 3-*O*-glucoside chloride. Kuromanin chloride. 2-(3,4-Dihydroxyphenyl)-3-(β-D-glucopyranosyl)oxy-5,7-dihydroxy-1-benzopyrylium chloride.

Reddish-brown crystalline powder, soluble in water and in ethanol (96 per cent).

Absorbance (*2.2.25*). A 0.01 g/L solution in a mixture of 1 volume of *hydrochloric acid R* and 999 volumes of *methanol R* shows an absorption maximum at 528 nm.

α-Chymotrypsin for peptide mapping. *1142400.*

α-Chymotrypsin of high purity, treated to eliminate tryptic activity.

Cimifugin. $C_{16}H_{18}O_6$. (M_r 306.3). *1181700.* [37921-38-3]. (2*S*)-7-(Hydroxymethyl)-2-(1-hydroxy-1-methylethyl)-4-methoxy-2,3-dihydro-5*H*-furo[3,2-*g*][1]benzopyran-5-one.

Cinchonidine. $C_{19}H_{22}N_2O$. (M_r 294.4). *1020400.* [485-71-2]. (*R*)-(Quinol-4-yl)[(2*S*,4*S*,5*R*)-5-vinylquinuclidin-2-yl]methanol.

White or almost white, crystalline powder, very slightly soluble in water and in light petroleum, soluble in ethanol (96 per cent).

$[\alpha]_D^{20}$: – 105 to – 110, determined on a 50 g/L solution in *ethanol (96 per cent) R*.

mp: about 208 °C, with decomposition.

Storage: protected from light.

Cinchonine. $C_{19}H_{22}N_2O$. (M_r 294.4). *1020500.* [118-10-5]. (*S*)-(Quinol-4-yl)[(2*R*,4*S*,5*R*)-5-vinylquinuclidin-2-yl]methanol.

White or almost white, crystalline powder, very slightly soluble in water, sparingly soluble in ethanol (96 per cent) and in methanol.

$[\alpha]_D^{20}$: + 225 to + 230, determined on a 50 g/L solution in *ethanol (96 per cent) R*.

mp: about 263 °C.

Storage: protected from light.

Cineole. $C_{10}H_{18}O$. (M_r 154.3). *1020600.* [470-82-6]. 1,8-Cineole. Eucalyptol. 1,8-Epoxy-*p*-menthane.

Colourless liquid, practically insoluble in water, miscible with anhydrous ethanol.

d_{20}^{20}: 0.922 to 0.927.

n_D^{20}: 1.456 to 1.459.

Freezing point (*2.2.18*): 0 °C to 1 °C.

Distillation range (*2.2.11*): 174 °C to 177 °C.

Phenol. Shake 1 g with 20 mL of *water R*. Allow to separate and add to 10 mL of the aqueous layer 0.1 mL of *ferric chloride solution R1*. No violet colour develops.

Turpentine oil. Dissolve 1 g in 5 mL of *ethanol (90 per cent V/V) R*. Add dropwise freshly prepared *bromine water R*. Not more than 0.5 mL is required to give a yellow colour lasting for 30 min.

Residue on evaporation: maximum 0.05 per cent.

To 10.0 mL add 25 mL of *water R*, evaporate on a water-bath and dry the residue to constant mass at 100-105 °C.

Cineole used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Peppermint oil (0405)*.

Test solution. The substance to be examined.

Content: minimum 98.0 per cent, calculated by the normalisation procedure.

1,4-Cineole. $C_{10}H_{18}O$. (M_r 154.3). *1142500.* [470-67-7]. 1-Methyl-4-(1-methylethyl)-7-oxabicyclo[2.2.1]heptane. 1-Isopropyl-4-methyl-7-oxabicyclo[2.2.1]heptane.

Colourless liquid.

d_4^{20}: about 0.900.

n_D^{20}: about 1.445.

bp: about 173 °C.

Cinnamamide. C_9H_9NO. (M_r 147.2). *1154800.* [621-79-4]. (*E*)-3-Phenylprop-2-enamide.

White or almost white powder.

mp: about 149 °C.

***trans*-Cinnamic acid.** $C_9H_8O_2$. (M_r 148.2). *1159200.* [140-10-3]. *trans*-3-Phenylacrylic acid. (2*E*)-3-Phenylprop-2-enoic acid.

Colourless crystals, very slightly soluble in water, freely soluble in ethanol (96 per cent).

mp: 133 °C.

Cinnamic aldehyde. C_9H_8O. (M_r 132.2). *1020700.* [104-55-2]. 3-Phenylpropenal.

Yellowish or greenish-yellow, oily liquid, slightly soluble in water, very soluble in ethanol (96 per cent).

n_D^{20}: about 1.620.

Storage: protected from light.

***trans*-Cinnamic aldehyde.** C_9H_8O. (M_r 132.2). *1124600.* [14371-10-9]. *(E)*-3-Phenylprop-2-enal.

trans-Cinnamic aldehyde used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Cassia oil (1496)*.

Content: minimum 99.0 per cent, calculated by the normalisation procedure.

Cinnamyl acetate. $C_{11}H_{12}O_2$. (M_r 176.2). *1124700.* [103-54-8]. 3-Phenylprop-2-en-1-yl acetate.

n_D^{20}: about 1.542.

bp: about 262 °C.

Cinnamyl acetate used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Cassia oil (1496)*.

Content: minimum 99.0 per cent, calculated by the normalisation procedure.

Citral. $C_{10}H_{16}O$. (M_r 152.2). *1020800.* [5392-40-5]. Mixture of (2*E*)- and (2*Z*)-3,7-Dimethylocta-2,6-dienal.

Light yellow liquid, practically insoluble in water, miscible with ethanol (96 per cent) and with propylene glycol.

Chromatography. Thin-layer chromatography (*2.2.27*), using *silica gel* GF_{254} *R* as the coating substance: apply to the plate 10 µL of a 1 g/L solution in *toluene R*. Develop over a path of 15 cm using a mixture of 15 volumes of *ethyl acetate R* and 85 volumes of *toluene R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. The chromatogram shows only one principal spot.

Citral used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Citronella oil (1609)*.

Content of citral (neral + geranial): minimum 95.0 per cent, calculated by the normalisation procedure.

Citrated rabbit plasma. *1020900.*

Collect blood by intracardiac puncture from a rabbit kept fasting for 12 h, using a plastic syringe with a No. 1 needle containing a suitable volume of 38 g/L solution of *sodium citrate R* so that the final volume ratio of citrate solution to blood is 1: 9. Separate the plasma by centrifugation at 1500 *g* to 1800 *g* at 15 °C to 20 °C for 30 min.

Storage: at 0 °C to 6 °C; use within 4 h of collection.

Citric acid, anhydrous. *1021200.* [77-92-9].

See *Citric acid (0455)*.

Citric acid monohydrate. *1021000.* [5949-29-1].

See *Citric acid monohydrate (0456)*.

When used in the test for iron, it complies with the following additional requirement.

Dissolve 0.5 g in 10 mL of *water R*, add 0.1 mL of *thioglycollic acid R*, mix and make alkaline with *ammonia R*. Dilute to 20 mL with *water R*. No pink colour appears in the solution.

Citronellal. $C_{10}H_{18}O$. (M_r 154.3). *1113300.* [106-23-0]. 3,7-Dimethyl-6-octenal.

Very slightly soluble in water, soluble in ethanol (96 per cent).

d_{20}^{20}: 0.848 to 0.856.

n_D^{20}: about 1.446.

Citronellal used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Citronella oil (1609)*.

Content: minimum 95.0 per cent, calculated by the normalisation procedure.

Citronellol. $C_{10}H_{20}O$. (M_r 156.3). *1134900.* [106-22-9]. 3,7-Dimethyloct-6-en-1-ol.

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

d_{20}^{20}: 0.857.

n_D^{20}: 1.456.

bp: 220 °C to 222 °C.

Citronellol used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Citronella oil (1609)*.

Content: minimum 95.0 per cent, calculated by the normalisation procedure.

Storage: in an airtight container, protected from light.

Citronellyl acetate. $C_{12}H_{22}O_2$. (M_r 198.3). *1135000.* [150-84-5]. 3,7-Dimethyl-6-octen-1-yl acetate.

d_{20}^{20}: 0.890.

n_D^{20}: 1.443.

bp: 229 °C.

Citronellyl acetate used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Citronella oil (1609)*.

Content: minimum 95.0 per cent, calculated by the normalisation procedure.

Storage: in an airtight container, protected from light.

Citropten. $C_{11}H_{10}O_4$. (M_r 206.2). *1021300.* [487-06-9]. Limettin. 5,7-Dimethoxy-2*H*-1-benzopyran-2-one.

Needle-shaped crystals, practically insoluble in water and in light petroleum, freely soluble in acetone and in ethanol (96 per cent).

mp: about 145 °C.

Chromatography. Thin-layer chromatography (*2.2.27*), using *silica gel* GF_{254} *R* as the coating substance: apply to the plate 10 µL of a 1 g/L solution in *toluene R*. Develop over a path of 15 cm using a mixture of 15 volumes of *ethyl acetate R* and

85 volumes of *toluene R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. The chromatogram obtained shows only one principal spot.

Clobetasol propionate. $C_{25}H_{32}ClFO_5$. (M_r 467.0). *1097700.* [25122-46-7]. 21-Chloro-9-fluoro-11β,17-dihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17-propionate.

White or almost white crystalline powder, insoluble in water, soluble in ethanol (96 per cent) and in acetone.

$[\alpha]_D^{20}$: about + 104 (in dioxan).

mp: about 196 °C.

Coagulation factor V solution. *1021400.*

Coagulation factor V solution may be prepared by the following method or by any other method which excludes factor VIII.

Prepare the factor V reagent from fresh oxalated bovine plasma, by fractionation at 4 °C with a saturated solution of *ammonium sulfate R* prepared at 4 °C. Separate the fraction which precipitates between 38 per cent and 50 per cent of saturation, which contains factor V without significant contamination with factor VIII. Remove the ammonium sulfate by dialysis and dilute the solution with a 9 g/L solution of *sodium chloride R* to give a solution containing between 10 per cent and 20 per cent of the quantity of factor V present in fresh human normal plasma.

Assay of factor V. Prepare two dilutions of the preparation of factor V in *imidazole buffer solution pH 7.3 R* containing 1 volume of the preparation in 10 volumes and in 20 volumes of the buffer solution respectively. Test each dilution as follows: mix 0.1 mL of *plasma substrate deficient in factor V R*, 0.1 mL of the solution to be examined, 0.1 mL of *thromboplastin R* and 0.1 mL of a 3.5 g/L solution of *calcium chloride R* and measure the coagulation times, i.e. the interval between the moment at which the calcium chloride solution is added and the first indication of the formation of fibrin, which may be observed visually or by means of a suitable apparatus.

In the same manner, determine the coagulation time (in duplicate) of four dilutions of human normal plasma in *imidazole buffer solution pH 7.3 R*, containing respectively, 1 volume in 10 (equivalent to 100 per cent of factor V), 1 volume in 50 (20 per cent), 1 volume in 100 (10 per cent), and 1 volume in 1000 (1 per cent). Using two-way logarithmic paper plot the average coagulation times for each dilution of human plasma against the equivalent percentage of factor V and read the percentage of factor V for the two dilutions of the factor V solution by interpolation. The mean of the two results gives the percentage of factor V in the solution to be examined.

Storage: in the frozen state at a temperature not higher than − 20 °C.

Cobalt chloride. $CoCl_2,6H_2O$. (M_r 237.9). *1021600.* [7791-13-1].

Red, crystalline powder or deep-red crystals, very soluble in water, soluble in ethanol (96 per cent).

Cobalt nitrate. $Co(NO_3)_2,6H_2O$. (M_r 291.0). *1021700.* [10026-22-9].

Small garnet-red crystals, very soluble in water.

Codeine. *1021800.* [6059-47-8].

See *Codeine monohydrate (0076)*.

Codeine phosphate. *1021900.* [52-28-8].

See *Codeine phosphate hemihydrate (0074)*.

Congo red. $C_{32}H_{22}N_6Na_2O_6S_2$. ($M_r$ 697). *1022000.* [573-58-0].

Schultz No. 360.

Colour Index No. 22120.

Disodium (biphenyl-4,4′-diyl-bis-2,2′-azo)bis(1-amino-naphthalene-4-sulfonate).

Brownish-red powder, soluble in water.

Congo red paper. *1022002.*

Immerse strips of filter paper for a few minutes in *congo red solution R*. Allow to dry.

Congo red solution. *1022001.*

Dissolve 0.1 g of *congo red R* in a mixture of 20 mL of *ethanol (96 per cent) R* and *water R* and dilute to 100 mL with *water R*.

Test for sensitivity. To 0.2 mL of the congo red solution add 100 mL of *carbon dioxide-free water R* and 0.3 mL of *0.1 M hydrochloric acid*. The solution is blue. Not more than 0.3 mL of *0.1 M sodium hydroxide* is required to change the colour to pink.

Colour change: pH 3.0 (blue) to pH 5.0 (pink).

Coomassie blue. *1001400.* [3861-73-2].

See *acid blue 92 R*.

Coomassie blue solution. *1001401.*

See *acid blue 92 solution R*.

Coomassie staining solution. *1012201.*

A 1.25 g/L solution of *acid blue 83 R* in a mixture consisting of 1 volume of *glacial acetic acid R*, 4 volumes of *methanol R* and 5 volumes of *water R*. Filter.

Coomassie staining solution R1. *1173000.*

Dissolve 0.275 g of *acid blue 83 R* in 200 mL of *methanol R*. Stir until complete dissolution of the crystals (for about 2 h). Add 750 mL of *water R* and 50 mL of *glacial acetic acid R*. Stir overnight (for at least 16 h); filter.

Copper. Cu. (A_r 63.55). *1022100.* [7440-50-8].

Cleaned foil, turnings, wire or powder of the pure metal of electrolytic grade.

Copper acetate. $C_4H_6CuO_4,H_2O$. (M_r 199.7). *1022200.* [6046-93-1].

Blue-green crystals or powder, freely soluble in boiling water, soluble in water and in ethanol (96 per cent), slightly soluble in glycerol (85 per cent).

Copper edetate solution. *1022300.*

To 2 mL of a 20 g/L solution of *copper acetate R* add 2 mL of *0.1 M sodium edetate* and dilute to 50 mL with *water R*.

Copper nitrate. $Cu(NO_3)_2,3H_2O$. (M_r 241.6). *1022400.* [10031-43-3]. Copper dinitrate trihydrate.

Dark blue crystals, hygroscopic, very soluble in water giving a strongly acid reaction, freely soluble in ethanol (96 per cent) and in dilute nitric acid.

Storage: in an airtight container.

Copper sulfate, anhydrous. $CuSO_4$. (M_r 159.6). *1199000.* [7758-98-7].

Greenish-grey powder, hygroscopic, freely soluble in water, slightly soluble in methanol and practically insoluble in ethanol (96 per cent).

Copper sulfate solution R1. *1199001.*

To 600 mL of *water R* slowly add 80 mL of *phosphoric acid R*. Dissolve with stirring 100 g of *anhydrous copper sulfate R* and dilute to 1 L with *water R*.

Copper sulfate pentahydrate. $CuSO_4,5H_2O$. (M_r 249.7). *1022500.* [7758-99-8].

Blue powder or deep-blue crystals, slowly efflorescent, very soluble in water, slightly soluble in ethanol (96 per cent).

Copper sulfate solution. *1022501.*

A 125 g/L solution of *copper sulfate pentahydrate R*.

Copper tetrammine, ammoniacal solution of. *1022600.*

Dissolve 34.5 g of *copper sulfate pentahydrate R* in 100 mL of *water R* and, whilst stirring, add dropwise *concentrated ammonia R* until the precipitate which forms dissolves completely. Keeping the temperature below 20 °C, add dropwise with continuous shaking 30 mL of *strong sodium hydroxide solution R*. Filter through a sintered-glass filter (40) (*2.1.2*), wash with *water R* until the filtrate is clear and take up the precipitate with 200 mL of *concentrated ammonia R*. Filter through a sintered-glass filter (*2.1.2*) and repeat the filtration to reduce the residue to a minimum.

Cortisone. $C_{21}H_{28}O_5$. (M_r 360.4). *1175000.* [53-06-5].

Content: minimum 95.0 per cent.

mp: 223-228 °C.

Cortisone acetate. *1097800.* [50-04-4].

See *Cortisone acetate (0321)*.

Corydaline. $C_{22}H_{27}NO_4$. (M_r 369.4). *1204400.* [518-69-4]. (13*S*,13a*R*)-5,8,13,13a-Tetrahydro-2,3,9,10-tetramethoxy-13-methyl-6*H*-dibenzo[*a*,*g*]quinolizine.

Costunolide. $C_{15}H_{20}O_2$. (M_r 232.3). *1194600.* [553-21-9]. (3a*S*,6*E*,10*E*,11a*R*)-6,10-Dimethyl-3-methylene-3a,4,5,8,9,11a-hexahydrocyclodeca[*b*]furan-2(3*H*)-one.

Coumaphos. $C_{14}H_{16}ClO_5PS$. (M_r 362.8). *1124800.* [56-72-4].

mp: 91 °C to 92 °C.

A suitable certified reference solution (10 ng/µL in iso-octane) may be used.

***o*-Coumaric acid.** $C_9H_8O_3$. (M_r 164.2). *1157400.* [614-60-8]. (*E*)-2-Hydroxycinnamic acid. (2*E*)-3-(2-Hydroxyphenyl)prop-2-enoic acid.

White or almost white powder.

mp: about 217 °C.

Coumarin. $C_9H_6O_2$. (M_r 146.1). *1124900.* [91-64-5]. 2*H*-Chromen-2-one. 2*H*-1-Benzopyran-2-one.

Colourless, crystalline powder or orthorhombic or rectangular crystals, very soluble in boiling water, soluble in ethanol (96 per cent). It dissolves in solutions of alkali hydroxides.

mp: 68 °C to 70 °C.

Coumarin used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Cassia oil (1496)*.

Content: minimum 98.0 per cent, calculated by the normalisation procedure.

Cresol. C_7H_8O. (M_r 108.1). *1022700.* [95-48-7]. *o*-Cresol. 2-Methylphenol.

Crystals or a super-cooled liquid becoming dark on exposure to light and air, miscible with anhydrous ethanol, soluble in about 50 parts of water and soluble in solutions of alkali hydroxides.

d_{20}^{20}: about 1.05.

n_D^{20}: 1.540 to 1.550.

bp: about 190 °C.

Freezing point (*2.2.18*): minimum 30.5 °C.

Residue on evaporation: maximum 0.1 per cent *m/m*, determined by evaporating on a water-bath and drying in an oven at 100-105 °C.

Storage: protected from light, moisture and oxygen.

Distil before use.

***m*-Cresol.** *1177100.* [108-39-4].

See *metacresol (2077)*.

***p*-Cresol.** C_7H_8O. (M_r 108.1). *1153100.* [106-44-5]. 4-Methylphenol.

Colourless or white or almost white crystals or crystalline mass.

d_{20}^{20}: about 1.02.

bp: about 202 °C.

***m*-Cresol purple.** $C_{21}H_{18}O_5S$. (M_r 382.44). *1121700.* [2303-01-7]. *m*-Cresolsulfonphthalein.

Olive-green, crystalline powder, slightly soluble in water, soluble in ethanol (96 per cent), in glacial acetic acid and in methanol.

***m*-Cresol purple solution.** *1121701.*

Dissolve 0.1 g of *m-cresol purple R* in 13 mL of *0.01 M sodium hydroxide*, dilute to 100 mL with *water R* and mix.

Colour change: pH 1.2 (red) to pH 2.8 (yellow); pH 7.4 (yellow) to pH 9.0 (purple).

Cresol red. $C_{21}H_{18}O_5S$. (M_r 382.4). *1022800.* [1733-12-6]. Cresolsulfonphthalein. 4,4′-(3*H*-2,1-Benzoxathiol-3-ylidene)bis-(2-methylphenol) *S*,*S*-dioxide.

A reddish-brown crystalline powder, slightly soluble in water, soluble in ethanol (96 per cent) and in dilute solutions of alkali hydroxides.

Cresol red solution. *1022801.*

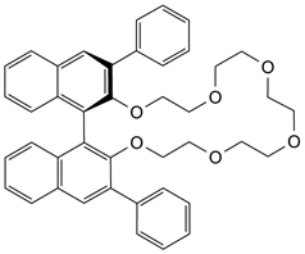
Dissolve 0.1 g of *cresol red R* in a mixture of 2.65 mL of *0.1 M sodium hydroxide* and 20 mL of *ethanol (96 per cent) R* and dilute to 100 mL with *water R*.

Test for sensitivity. A mixture of 0.1 mL of the cresol red solution and 100 mL of *carbon dioxide-free water R* to which 0.15 mL of *0.02 M sodium hydroxide* has been added is purple-red. Not more than 0.15 mL of *0.02 M hydrochloric acid* is required to change the colour to yellow.

Colour change: pH 7.0 (yellow) to pH 8.6 (red).

Crown-ether silica gel for chiral separation. *1192400.*

A very finely divided silica gel for chromatography coated with the following chiral crown ether:



(R_a)-6,23-Diphenyl-8,9,11,12,14,15,17,18,20,21-decahydrodinaphtho[2,1-*q*:1′,2′-*s*][1,4,7,10,13,16]-hexaoxacycloicosine.

Crystal violet. $C_{25}H_{30}ClN_3$. (M_r 408.0). *1022900.* [548-62-9].

Schultz No. 78.

Colour Index No. 42555.
Hexamethyl-pararosanilinium chloride.

Dark-green powder or crystals, soluble in water and in ethanol (96 per cent).

Crystal violet solution. *1022901.*

Dissolve 0.5 g of *crystal violet R* in *anhydrous acetic acid R* and dilute to 100 mL with the same solvent.

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 *0.1 M perchloric acid* the bluish-purple solution turns bluish-green.

Cupric chloride. $CuCl_2,2H_2O$. (M_r 170.5). *1023000.* [10125-13-0]. Cupric chloride dihydrate.

Greenish-blue powder or crystals, deliquescent in moist air, efflorescent in dry air, freely soluble in water, in ethanol (96 per cent) and in methanol, sparingly soluble in acetone.

Storage: in an airtight container.

Cupri-citric solution. *1023100.*

Dissolve 25 g of *copper sulfate pentahydrate R*, 50 g of *citric acid monohydrate R* and 144 g of *anhydrous sodium carbonate R* in *water R* and dilute to 1000 mL with the same solvent.

Cupri-citric solution R1. *1023200.*

Dissolve 25 g of *copper sulfate pentahydrate R*, 50 g of *citric acid monohydrate R* and 144 g of *anhydrous sodium carbonate R* in *water R* and dilute to 1000 mL with the same solvent.

Adjust the solution so that it complies with the following requirements.

a) To 25.0 mL add 3 g of *potassium iodide R*. Add 25 mL of a 25 per cent *m/m* solution of *sulfuric acid R* with precaution and in small quantities. Titrate with *0.1 M sodium thiosulfate* using 0.5 mL of *starch solution R*, added towards the end of the titration, as indicator.

24.5 mL to 25.5 mL of *0.1 M sodium thiosulfate* is used in the titration.

b) Dilute 10.0 mL to 100.0 mL with *water R* and mix. To 10.0 mL of the solution, add 25.0 mL of *0.1 M hydrochloric acid* and heat for 1 h on a water-bath. Cool, adjust with *water R* to the initial volume and titrate with *0.1 M sodium hydroxide*, using 0.1 mL of *phenolphthalein solution R1* as indicator.

5.7 mL to 6.3 mL of *0.1 M sodium hydroxide* is used in the titration.

c) Dilute 10.0 mL to 100.0 mL with *water R* and mix. Titrate 10.0 mL of the solution with *0.1 M hydrochloric acid*, using 0.1 mL of *phenolphthalein solution R1* as indicator.

6.0 mL to 7.5 mL of *0.1 M hydrochloric acid* is used in the titration.

Cupriethylenediamine hydroxide solution. *3008700.* [14552-35-3].

The molar ratio of ethylenediamine to copper is 2.00 ± 0.04.

This solution is commercially available.

Cupri-tartaric solution. *1023300.*

Solution A. Dissolve 34.6 g of *copper sulfate pentahydrate R* in *water R* and dilute to 500 mL with the same solvent.

Solution B. Dissolve 173 g of *sodium potassium tartrate R* and 50 g of *sodium hydroxide R* in 400 mL of *water R*. Heat to boiling, allow to cool and dilute to 500 mL with *carbon dioxide-free water R*.

Mix equal volumes of the 2 solutions immediately before use.

Cupri-tartaric solution R2. *1023302.*

Add 1 mL of a solution containing 5 g/L of *copper sulfate pentahydrate R* and 10 g/L of *potassium tartrate R* to 50 mL of *sodium carbonate solution R1*. Prepare immediately before use.

Cupri-tartaric solution R3. *1023303.*

Prepare a solution containing 10 g/L of *copper sulfate pentahydrate R* and 20 g/L of *sodium tartrate R*. To 1.0 mL of the solution add 50 mL of *sodium carbonate solution R2*. Prepare immediately before use.

Cupri-tartaric solution R4. *1023304.*

Solution A. 150 g/L *copper sulfate pentahydrate R*.

Solution B. Dissolve 2.5 g of *anhydrous sodium carbonate R*, 2.5 g of *sodium potassium tartrate R*, 2.0 g of *sodium hydrogen carbonate R*, and 20.0 g of *anhydrous sodium sulfate R* in *water R* and dilute to 100 mL with the same solvent.

Mix 1 part of solution A with 25 parts of solution B immediately before use.

Curcumin. $C_{21}H_{20}O_6$. (M_r 368.4). *1023500.* [458-37-7]. 1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione.

Orange-brown, crystalline powder, practically insoluble in water, soluble in glacial acetic acid.

mp: about 183 °C.

Curcuminoids. *1183900.*

A mixture of curcumin ($C_{21}H_{20}O_6$; M_r 368.4), demethoxycurcumin ($C_{20}H_{18}O_5$; M_r 338.4) and bis-demethoxycurcumin ($C_{19}H_{16}O_4$; M_r 308.3).

Cyanoacetic acid. $C_3H_3NO_2$. (M_r 85.1). *1097900.* [372-09-8].

White or yellowish-white, hygroscopic crystals, very soluble in water.

Storage: in an airtight container.

Cyanocobalamin. *1023600.* [68-19-9].

See *Cyanocobalamin (0547)*.

Cyanogen bromide solution. *1023700.* [506-68-3].

Add dropwise, with cooling *0.1 M ammonium thiocyanate* to *bromine water R* until the yellow colour disappears. Prepare immediately before use.

Cyanoguanidine. $C_2H_4N_4$. (M_r 84.1). *1023800.* [461-58-5]. Dicyandiamide. 1-Cyanoguanidine.

White or almost white, crystalline powder, sparingly soluble in water and in ethanol (96 per cent), practically insoluble in methylene chloride.

mp: about 210 °C.

Cyanopropyl(3)phenyl(3)methyl(94)polysiloxane. *1114800.*

Polysiloxane substituted with 3 per cent of cyanopropyl groups, 3 per cent of phenyl groups and 94 per cent of methyl groups.

Cyanopropyl(7)phenyl(7)methyl(86)polysiloxane. *1109200.*

Polysiloxane substituted with 7 per cent of cyanopropyl groups, 7 per cent of phenyl groups and 86 per cent of methyl groups.

Cyanopropyl(25)phenyl(25)methyl(50)polysiloxane. *1066500.*

Polysiloxane substituted with 25 per cent of cyanopropyl groups, 25 per cent of phenyl groups and 50 per cent of methyl groups.

Cyanopropylpolysiloxane. *1066700.*

Polysiloxane substituted with 100 per cent of cyanopropyl groups.

Cyasterone. $C_{29}H_{44}O_8$. (M_r 520.7). *1204500.* [17086-76-9]. (2β,3β,5β,22*R*,24*S*,24^1*R*,25*S*)-24^1,26-Epoxy-2,3,14,20,22-pentahydroxystigmast-7-ene-6,26-dione.

α-Cyclodextrin. $C_{36}H_{60}O_{30}$. (M_r 972). *1176200.* [10016-20-3]. Cyclohexakis-(1→4)-(α-D-glucopyranosyl). Cyclomaltohexaose. Alfadex.

β-Cyclodextrin. *1184000.* [7585-39-9].

See *Betadex (1070)*.

β-Cyclodextrin for chiral chromatography, modified. *1154600.*

30 per cent of 2,3-di-*O*-ethyl-6-*O*-*tert*-butyldimethylsilyl-β-cyclodextrin dissolved in polysiloxane substituted with 15 per cent of phenyl groups and 85 per cent of methyl groups.

β-Cyclodextrin for chiral chromatography, modified R1. *1160700.*

30 per cent of 2,3-di-*O*-acetyl-6-*O*-*tert*-butylsilyl-β-cyclodextrin dissolved in polysiloxane substituted with 15 per cent of phenyl groups and 85 per cent of methyl groups.

Cyclohexane. C_6H_{12}. (M_r 84.2). *1023900.* [110-82-7].

Clear, colourless, flammable liquid, practically insoluble in water, miscible with organic solvents.

d_{20}^{20}: about 0.78.

bp: about 80.5 °C.

Cyclohexane used in spectrophotometry complies with the following additional test.

Absorbance (*2.2.25*): maximum 0.35 at 220 nm, 0.16 at 235 nm, 0.05 at 240 nm, 0.01 at 250 nm, determined using *water R* as compensation liquid.

Cyclohexane R1. *1023901.*

Complies with the requirements prescribed for *cyclohexane R* with the following additional requirement.

The fluorescence, measured at 460 nm, under illumination with an excitant light beam at 365 nm, is not more intense than that of a solution containing 0.002 ppm of *quinine R* in *dilute sulfuric acid R1*.

Cyclohexylamine. $C_6H_{13}N$. (M_r 99.2). *1024000.* [108-91-8]. Cyclohexanamine.

Colourless liquid, soluble in water, miscible with usual organic solvents.

n_D^{20}: about 1.460.

bp: 134 °C to 135 °C.

Cyclohexylenedinitrilotetra-acetic acid. $C_{14}H_{22}N_2O_8,H_2O$. (M_r 364.4). *1024100. trans*-Cyclohexylene-1,2-dinitrilo-*N,N,N',N'*-tetra-acetic acid.

White or almost white, crystalline powder.

mp: about 204 °C.

Cyclohexylmethanol. $C_7H_{14}O$. (M_r 114.2). *1135200.* [100-49-2]. Cyclohexylcarbinol.

Liquid with a slight odour of camphor, soluble in ethanol (96 per cent).

n_D^{25}: about 1.464.

bp: about 185 °C.

3-Cyclohexylpropionic acid. $C_9H_{16}O_2$. (M_r 156.2). *1119200.* [701-97-3].

Clear liquid.

d_{20}^{20}: about 0.998.

n_D^{20}: about 1.4648.

bp: about 130 °C.

Cyhalothrin. $C_{23}H_{19}ClF_3NO_3$. (M_r 449.9). *1125000.* [91465-08-6].

bp: 187 °C to 190 °C.

mp: about 49 °C.

A suitable certified reference solution (10 ng/µL in cyclohexane) may be used.

***p*-Cymene.** $C_{10}H_{14}$. (M_r 134.2). *1113400.* [99-87-6]. 1-Isopropyl-4-methylbenzene.

Colourless liquid, practically insoluble in water, soluble in ethanol (96 per cent).

d_{20}^{20}: about 0.858.

n_D^{20}: about 1.4895.

bp: 175 °C to 178 °C.

p-Cymene used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Peppermint oil (0405)*.

Test solution. The substance to be examined.

Content: minimum 96.0 per cent, calculated by the normalisation procedure.

Cynarin. $C_{25}H_{24}O_{12}$. (M_r 516.4). *1159300.* [30964-13-7]. (1α,3α,4α,5β)-1,3-Bis[[3-(3,4-Dihydroxyphenyl)-1-oxo-2-propenyl]oxy]-4,5-dihydroxycyclohexanecarboxylic acid.

White or almost white amorphous mass, odourless.

Cypermethrin. $C_{22}H_{19}Cl_2NO_3$. (M_r 416.3). *1125100.* [52315-07-8].

bp: 170 °C to 195 °C.

mp: 60 °C to 80 °C.

A suitable certified reference solution (10 ng/µL in cyclohexane) may be used.

L-Cysteine. $C_3H_7NO_2S$. (M_r 121.1). *1024200.* [52-90-4].

Powder, freely soluble in water, in ethanol (96 per cent) and in acetic acid, practically insoluble in acetone.

Cysteine hydrochloride. *1024300.* [7048-04-6].

See *Cysteine hydrochloride monohydrate (0895)*.

L-Cystine. $C_6H_{12}N_2O_4S_2$. (M_r 240.3). *1024400.* [56-89-3].

White or almost white, crystalline powder, practically insoluble in water and in ethanol (96 per cent). It dissolves in dilute solutions of alkali hydroxides.

$[\alpha]_D^{20}$: − 218 to − 224, determined in *1 M hydrochloric acid*.

mp: 250 °C, with decomposition.

Cytosine. $C_4H_5N_3O$. (M_r 111.1). *1160800.* [71-30-7].

Content: minimum 95.0 per cent.

Daidzein. $C_{15}H_{10}O_4$. (M_r 254.2). *1178400.* [486-66-8]. 7-Hydroxy-3-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one.

Daidzin. $C_{21}H_{20}O_9$. (M_r 416.4). *1178300.* [552-66-9]. Daidzein-7-*O*-glucoside. 7-(β-D-Glucopyranosyloxy)-3-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one.

Dantron. $C_{14}H_8O_4$. (M_r 240.2). *1024500.* [117-10-2]. 1,8-Dihydroxyanthraquinone. 1,8-Dihydroxyanthracene-9,10-dione.

Crystalline orange powder, practically insoluble in water, slightly soluble in ethanol (96 per cent), soluble in solutions of alkali hydroxides.

mp: about 195 °C.

***o,p'*-DDD.** $C_{14}H_{10}Cl_4$. (M_r 320.0). *1125200.* [53-19-0]. 1-(2-Chlorophenyl)-1-(4-chlorophenyl)-2,2-dichloroethane.

A suitable certified reference solution (10 ng/µL in cyclohexane) may be used.

***p,p'*-DDD.** $C_{14}H_{10}Cl_4$. (M_r 320.0). *1125300.* [72-54-8]. 1,1-Bis(4-chlorophenyl)-2,2-dichloroethane.

bp: about 193 °C.

mp: about 109 °C.

A suitable certified reference solution (10 ng/µL in cyclohexane) may be used.

***o,p'*-DDE.** $C_{14}H_8Cl_4$. (M_r 318.0). *1125400.* [3424-82-6].

1-(2-Chlorophenyl)-1-(4-chlorophenyl)-2,2-dichloroethylene.

A suitable certified reference solution (10 ng/µL in cyclohexane) may be used.

***p,p'*-DDE.** $C_{14}H_8Cl_4$. (M_r 318.0). *1125500.* [72-55-9]. 1,1-Bis(4-chlorophenyl)-2,2-dichloroethylene.

bp: 316 °C to 317 °C.

mp: 88 °C to 89 °C.

A suitable certified reference solution (10 ng/µL in cyclohexane) may be used.

***o,p′*-DDT.** $C_{14}H_9Cl_5$. (M_r 354.5). *1125600.* [789-02-6]. 1-(2-Chlorophenyl)-1-(4-chlorophenyl)-2,2,2-trichloroethane.

A suitable certified reference solution (10 ng/µL in cyclohexane) may be used.

***p,p′*-DDT.** $C_{14}H_9Cl_5$. (M_r 354.5). *1125700.* [50-29-3]. 1,1-Bis(4-chlorophenyl)-2,2,2-trichloroethane.

bp: about 260 °C.

mp: 108 °C to 109 °C.

A suitable certified reference solution (10 ng/µL in cyclohexane) may be used.

Decanal. $C_{10}H_{20}O$. (M_r 156.3). *1149200.* [112-31-2]. Decyl aldehyde.

Oily, colourless liquid, practically insoluble in water.

Decanal used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Sweet orange oil (1811)*.

Content: minimum 97 per cent, calculated by the normalisation procedure.

Decane. $C_{10}H_{22}$. (M_r 142.3). *1024600.* [124-18-5].

Colourless liquid, practically insoluble in water.

n_D^{20}: about 1.411.

bp: about 174 °C.

Decanol. $C_{10}H_{22}O$. (M_r 158.3). *1024700.* [112-30-1]. Decan-1-ol.

Viscous liquid, solidifying at about 6 °C, practically insoluble in water, soluble in ethanol (96 per cent).

n_D^{20}: about 1.436.

bp: about 230 °C.

Dehydrocostus lactone. $C_{15}H_{18}O_2$. (M_r 230.3). *1194700.* [477-43-0]. (3a*S*,6a*R*,9a*R*,9b*S*)-3,6,9-Trismethylenedecahydroazuleno[4,5-*b*]furan-2(3*H*)-one.

Deltamethrin. $C_{22}H_{19}Br_2NO_3$. (M_r 505.2). *1125800.* [52918-63-5].

bp: about 300 °C.

mp: about 98 °C.

A suitable certified reference solution (10 ng/µL in cyclohexane) may be used.

Demeclocycline hydrochloride. *1145600.*

See *Demeclocycline hydrochloride (0176)*.

Demethylflumazenil. $C_{14}H_{12}FN_3O_3$. (M_r 289.3). *1149300.* [79089-72-8]. Ethyl 8-fluoro-6-oxo-5,6-dihydro-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate.

Colourless needles, soluble in dimethyl sulfoxide and in hot methanol.

mp: about 288 °C.

14-Deoxy-11,12-didehydroandrographolide. $C_{20}H_{28}O_4$. (M_r 332.4). *1198300.* [42895-58-9]. 3-[(1*E*)-2-[(1*R*,4a*S*,5*R*,6*R*,8a*R*)-6-Hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylenedecahydronaphthalen-1-yl]ethenyl]furan-2(5*H*)-one.

2-Deoxy-D-ribose. $C_5H_{10}O_4$. (M_r 134.1). *1163900.* [533-67-5]. Thyminose. 2-Deoxy-D-*erythro*-pentose.

2′-Deoxyuridine. $C_9H_{12}N_2O_5$. (M_r 228.2). *1024800.* [951-78-0]. 1-(2-Deoxy-β-d-*erythro*-pentofuranosyl)-1*H*,3*H*-pyrimidine-2,4-dione.

mp: about 165 °C.

Chromatography. Thin-layer chromatography (*2.2.27*) as prescribed in the monograph *Idoxuridine (0669)*: apply 5 µL of a 0.25 g/L solution; the chromatogram shows only one principal spot.

4-Deoxypyridoxine hydrochloride. $C_8H_{12}NO_2Cl$. (M_r 189.6). *1175500.* [148-51-6]. 5-(Hydroxymethyl)-2,4-dimethylpyridin-3-ol.

Desmethylmisonidazole. $C_6H_9N_3O_4$. (M_r 187.2). *1185600.* [13551-92-3]. (2*RS*)-3-(2-Nitro-1*H*-imidazol-1-yl)propane-1,2-diol.

Content: minimum 95 per cent.

Yellow powder.

Destaining solution. *1012202.*

A mixture consisting of 1 volume of *glacial acetic acid R*, 4 volumes of *methanol R* and 5 volumes of *water R*.

Deuterated acetic acid. $C_2{}^2H_4O_2$. (M_r 64.1). *1101100.* [1186-52-3]. Tetradeuteroacetic acid. Acetic-d_3 acid-*d*.

Degree of deuteration: minimum 99.7 per cent.

d_{20}^{20}: about 1.12.

n_D^{20}: about 1.368.

bp: about 115 °C.

mp: about 16 °C.

Deuterated acetone. $C_3{}^2H_6O$. (M_r 64.1). *1024900.* [666-52-4]. Acetone-d_6. ($^2H_6$)-Acetone.

Degree of deuteration: minimum 99.5 per cent.

Clear, colourless liquid, miscible with water, with dimethylformamide, with anhydrous ethanol and with methanol.

d_{20}^{20}: about 0.87.

n_D^{20}: about 1.357.

bp: about 55 °C.

Water and deuterium oxide. Not more than 0.1 per cent.

Deuterated acetonitrile. $C_2{}^2H_3N$. (M_r 44.1). *1173100.* [2206-26-0].

Degree of deuteration: minimum 99.8 per cent.

Clear, colourless liquid, miscible with water, with acetone and with methanol.

d_{20}^{20}: about 0.78.

n_D^{20}: about 1.344.

Deuterated chloroform. C^2HCl_3. (M_r 120.4). *1025000.* [865-49-6]. (^2H)-Chloroform. Chloroform-*d*.

Degree of deuteration: minimum 99.7 per cent.

Clear, colourless liquid, practically insoluble in water, miscible with acetone and with ethanol (96 per cent). It may be stabilised over silver foil.

d_{20}^{20}: about 1.51.

n_D^{20}: about 1.445.

bp: about 60 °C.

Water and deuterium oxide: maximum 0.05 per cent.

Deuterated dimethyl sulfoxide. $C_2{}^2H_6OS$. (M_r 84.2). *1025100.* [2206-27-1]. ($^2H_6$)-Dimethyl sulfoxide. Dimethyl sulfoxide-d_6.

Degree of deuteration: minimum 99.8 per cent.

Very hygroscopic liquid, practically colourless, viscous, soluble in water, in acetone and in anhydrous ethanol.

d_{20}^{20}: about 1.18.

mp: about 20 °C.

Water and deuterium oxide: maximum 0.1 per cent.

Storage: in an airtight container.

Deuterated methanol. C^2H_4O. (M_r 36.1). *1025200.* [811-98-3]. (^2H)-Methanol. Methanol-*d*.

Degree of deuteration: minimum 99.8 per cent.

Clear, colourless liquid miscible with water, with ethanol (96 per cent) and with methylene chloride.

d_{20}^{20}: about 0.888.

n_D^{20}: about 1.326.

bp: 65.4 °C.

Deuterated sodium trimethylsilylpropionate. $C_6H_9{}^2H_4NaO_2Si$. (M_r 172.3). *1179100.* [24493-21-8]. Sodium 3-(trimethylsilyl)(2,2,3,3-$^2H_4$)propionate. TSP-d_4.

Degree of deuteration: minimum 98 per cent.

White or almost white powder.

Deuterium chloride. ^2HCl. (M_r 37.47). *1178800.* [7698-05-7]. Deuterated hydrochloric acid.

Gas.

Degree of deuteration: minimum 99 per cent.

Caution: *toxic*.

Deuterium chloride solution. *1178801.*

Dilute 1 mL of *deuterium chloride R* (38 per cent *m*/*m*) with 5 mL of *deuterium oxide R*.

Deuterium oxide. ^2H_2O. (M_r 20.03). *1025300.* [7789-20-0]. Deuterated water.

Degree of deuteration: minimum 99.7 per cent.

d_{20}^{20}: about 1.11.

n_D^{20}: about 1.328.

bp: about 101 °C.

Deuterium oxide R1. ^2H_2O. (M_r 20.03). *1025301.* [7789-20-0]. Deuterated water.

Degree of deuteration: minimum 99.95 per cent.

Developer solution. *1122500.*

Dilute 2.5 mL of a 20 g/L solution of *citric acid monohydrate R* and 0.27 mL of *formaldehyde R* to 500.0 mL with *water R*.

Dextran for chromatography, cross-linked R2. *1025500.*

Bead-form dextran with a fraction range suitable for the separation of peptides and proteins with relative molecular masses of 15×10^2 to 30×10^3. When dry, the beads have a diameter of 20-80 µm.

Dextran for chromatography, cross-linked R3. *1025600.*

Bead-form dextran with a fraction range suitable for the separation of peptides and proteins with relative molecular masses of 4×10^3 to 15×10^4. When dry, the beads have a diameter of 40-120 µm.

Dextrose. *1025700.* [50-99-7].

See *glucose R*.

3,3′-Diaminobenzidine tetrahydrochloride. $C_{12}H_{18}Cl_4N_4$, $2H_2O$. (M_r 396.1). *1098000.* [7411-49-6]. 3,3′,4,4′-Biphenyl-tetramine.

Almost white or slightly pink powder, soluble in water.

mp: about 280 °C, with decomposition.

1,2-Diamino-4,5-methylenedioxybenzene dihydrochloride. $C_7H_{10}Cl_2N_2O_2$. (M_r 225.1). *1202100.* [81864-15-5]. 2*H*-1,3-Benzodioxole-5,6-diamine dihydrochloride.

Content: minimum 99 per cent (HPLC).

Diammonium 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate). $C_{18}H_{24}N_6O_6S_4$. (M_r 548.7). *1153000.* [30931-67-0]. ABTS. Diammonium 2,2′-(diazanediylidene)bis[3-ethyl-2,3-dihydrobenzothiazole-6-sulfonate].

Chromogenic substrate suitable for use in ELISA procedures.

Green tablets, freely soluble in water.

pH (*2.2.3*): 4.2 to 5.8 for a 0.1 g/L solution.

Diatomaceous earth. *1025900.* [91053-39-3].

White or almost white, fine granular powder, made up of siliceous frustules of fossil diatoms or of debris of fossil diatoms, practically insoluble in water and in ethanol (96 per cent).

The substance may be identified by microscopic examination with a magnification of × 500.

Diatomaceous earth for gas chromatography. *1026000.*

White or almost white, fine granular powder, practically insoluble in water and in ethanol (96 per cent), made up of siliceous frustules of fossil diatoms or of debris of fossil diatoms. The substance may be identified by microscopic examination with a magnification of × 500. The substance is acid-washed, then water-washed until neutral.

Diatomaceous earth for gas chromatography, silanised. *1026300.*

Diatomaceous earth for gas chromatography R silanised with dimethyldichlorosilane or other suitable silanising agents.

Diazinon. $C_{12}H_{21}N_2O_3PS$. (M_r 304.3). *1125900.* [333-41-5].

bp: about 306 °C.

A suitable certified reference solution (10 ng/µL in iso-octane) may be used.

Diazobenzenesulfonic acid solution R1. *1026500.*

Dissolve 0.9 g of *sulfanilic acid R* in a mixture of 30 mL of *dilute hydrochloric acid R* and 70 mL of *water R*. To 3 mL of the solution add 3 mL of a 50 g/L solution of *sodium nitrite R*. Cool in an ice-bath for 5 min, add 12 mL of the sodium nitrite solution and cool again. Dilute to 100 mL with *water R* and keep the reagent in an ice-bath. Prepare extemporaneously but allow to stand for 15 min before use.

Dibromomethane. CH_2Br_2. (M_r 173.8). *1195500.* [74-95-3].

Colourless liquid, slightly soluble in water.

bp: about 96 °C.

Dibutylamine. $C_8H_{19}N$. (M_r 129.3). *1126000.* [111-92-2]. *N*-Butylbutan-1-amine.

Colourless liquid.

n_D^{20}: about 1.417.

bp: about 159 °C.

Dibutylammonium phosphate for ion-pairing. *1168800.*

A colourless solution of 10 per cent to 15 per cent *V/V* of di-*n*-butylamine and 12 per cent to 17 per cent *V/V* of phosphoric acid in water, suitable for ion-pairing in liquid chromatography.

Dibutyl ether. $C_8H_{18}O$. (M_r 130.2). *1026700.* [142-96-1].

Colourless, flammable liquid, practically insoluble in water, miscible with anhydrous ethanol.

d_{20}^{20}: about 0.77.

n_D^{20}: about 1.399.

Do not distil if the dibutyl ether does not comply with the test for peroxides.

Peroxides. Place 8 mL of *potassium iodide and starch solution R* in a 12 mL ground-glass-stoppered cylinder about 1.5 cm in diameter. Fill completely with the substance to be examined, shake vigorously and allow to stand protected from light for 30 min. No colour is produced.

The name and concentration of any added stabiliser are stated on the label.

Dibutyl phthalate. $C_{16}H_{22}O_4$. (M_r 278.3). *1026800.* [84-74-2]. Dibutyl benzene-1,2-dicarboxylate.

Clear, colourless or faintly coloured, oily liquid, very slightly soluble in water, miscible with acetone and with ethanol (96 per cent).

d_{20}^{20}: 1.043 to 1.048.

n_D^{20}: 1.490 to 1.495.

Dicarboxidine hydrochloride. $C_{20}H_{26}Cl_2N_2O_6$. (M_r 461.3). *1026900.* [56455-90-4]. 4,4′-[(4,4′-Diaminobiphenyl-3,3′-diyl)dioxy]dibutanoic acid dihydrochloride.

Dichlofenthion. $C_{10}H_{13}Cl_2O_3PS$. (M_r 315.2). *1126100.* [97-17-6].

A suitable certified reference solution (10 ng/µL in cyclohexane) may be used.

Dichloroacetic acid. $C_2H_2Cl_2O_2$. (M_r 128.9). *1027000.* [79-43-6].

Colourless liquid, miscible with water and ethanol (96 per cent).

d_{20}^{20}: about 1.566.

n_D^{20}: about 1.466.

bp: about 193 °C.

Dichloroacetic acid solution. *1027001.*

Dilute 67 mL of *dichloroacetic acid R* to 300 mL with *water R* and neutralise to *blue litmus paper R* using *ammonia R*. Cool, add 33 mL of *dichloroacetic acid R* and dilute to 600 mL with *water R*.

3,5-Dichloroaniline. $C_6H_5Cl_2N$. (M_r 162.0). *1177800.* [626-43-7]. 3,5-dichlorophenylamine.

mp: 46 °C to 52 °C.

Dichlorobenzene. $C_6H_4Cl_2$. (M_r 147.0). *1027100.* [95-50-1]. 1,2-Dichlorobenzene.

Colourless, oily liquid, practically insoluble in water, soluble in anhydrous ethanol.

d_{20}^{20}: about 1.31.

bp: about 180 °C.

2,4-Dichlorobenzoic acid. $C_7H_4Cl_2O_2$. (M_r 191.0). *1185700.* [50-84-0].

Faintly beige powder.

mp: about 160 °C.

2,3-Dichloro-5,6-dicyanobenzoquinone. $C_8Cl_2N_2O_2$. (M_r 227.0). *1153600.* [84-58-2]. 4,5-Dichloro-3,6-dioxocyclohexa-1,4-diene-1,2-dicarbonitrile.

Yellow or orange crystals, soluble in dioxan and in acetic acid, slightly soluble in methylene chloride. It decomposes in water.

mp: about 214 °C.

Storage: at a temperature of 2 °C to 8 °C.

(*S*)-3,5-Dichloro-2,6-dihydroxy-*N*-[(1-ethylpyrrolidin-2-yl)methyl]benzamide hydrobromide. $C_{14}H_{19}BrCl_2N_2O_3$. (M_r 414.1). *1142600.* [113310-88-6].

White or almost white, crystalline powder.

$[\alpha]_D^{22}$: + 11.4, determined on a 15.0 g/L solution in *anhydrous ethanol R*.

mp: about 212 °C.

Dichlorofluorescein. $C_{20}H_{10}Cl_2O_5$. (M_r 401.2). *1027200.* [76-54-0]. 2,7-Dichlorofluorescein. 2-(2,7-Dichloro-6-hydroxy-3-oxo-3*H*-xanthen-9-yl)benzoic acid.

Yellowish-brown or yellow-orange powder, slightly soluble in water, freely soluble in ethanol (96 per cent) and in dilute solutions of alkali hydroxides giving a solution showing a yellowish-green fluorescence.

2,6-Dichlorophenol. $C_6H_4Cl_2O$. (M_r 163.0). *1177600.* [87-65-0].

mp: 64 °C to 66 °C.

Dichlorophenolindophenol, sodium salt. $C_{12}H_6Cl_2NNaO_2,2H_2O$. ($M_r$ 326.1). *1027300.* [620-45-1]. The sodium derivative of 2,6-dichloro-*N*-(4-hydroxyphenyl)-1,4-benzoquinone monoimine dihydrate.

Dark-green powder, freely soluble in water and in anhydrous ethanol. The aqueous solution is dark blue; when acidified it becomes pink.

Dichlorophenolindophenol standard solution. *1027301.*

Dissolve 50.0 mg of *dichlorophenolindophenol, sodium salt R* in 100.0 mL of *water R* and filter.

Assay. Dissolve 20.0 mg of *ascorbic acid R* in 10 mL of a freshly prepared 200 g/L solution of *metaphosphoric acid R* and dilute to 250.0 mL with *water R*. Titrate 5.0 mL rapidly with the dichloro-phenolindophenol standard solution, added from a microburette graduated in 0.01 mL, until the pink colour persists for 10 s, the titration occupying not more than 2 min. Dilute the dichlorophenolindophenol solution with *water R* to make 1 mL of the solution equivalent to 0.1 mg of ascorbic acid ($C_6H_8O_6$).

Storage: use within 3 days.

Standardise immediately before use.

5,7-Dichloroquinolin-8-ol. $C_9H_5Cl_2NO$. (M_r 214.1). *1157000.* [773-76-2]. 5,7-Dichlorooxine.

Yellow, crystalline powder, soluble in acetone, slightly soluble in ethanol (96 per cent).

mp: about 179 °C.

Content: minimum 95.0 per cent.

Dichloroquinonechlorimide. $C_6H_2Cl_3NO$. (M_r 210.4). *1027400.* [101-38-2]. 2,6-Dichloro-*N*-chloro-1,4-benzoquinone mono-imine.

Pale yellow or greenish-yellow crystalline powder, practically insoluble in water, soluble in ethanol (96 per cent) and in dilute alkaline solutions.

mp: about 66 °C.

Dichlorvos. $C_4H_7Cl_2O_4P$. (M_r 221). *1101200.* [62-73-7]. 2,2-Dichlorovinyl dimethyl phosphate.

Colourless or brownish-yellow liquid, soluble in water, miscible with most organic solvents.

n_D^{25}: about 1.452.

Dicyclohexyl. $C_{12}H_{22}$. (M_r 166.3). *1135300.* [92-51-3]. Bicyclohexyl.

d_{20}^{20}: about 0.864.

bp: about 227 °C.

mp: about 4 °C.

Dicyclohexylamine. $C_{12}H_{23}N$. (M_r 181.3). *1027500.* [101-83-7]. *N,N*-Dicyclohexylamine.

Colourless liquid, sparingly soluble in water, miscible with the usual organic solvents.

n_D^{20}: about 1.484.

bp: about 256 °C.

Freezing point (*2.2.18*): 0 °C to 1 °C.

Dicyclohexylurea. $C_{13}H_{24}N_2O$. (M_r 224.4). *1027600.* [2387-23-7]. 1,3-Dicyclohexylurea.

White or almost white, crystalline powder.

mp: about 232 °C.

Didocosahexaenoin. $C_{47}H_{68}O_5$. (M_r 713.0). *1142700.* [88315-12-2]. Diglyceride of docosahexaenoic acid (C22:6). Glycerol didocosahexaenoate. (*all-Z*)-Docosahexaenoic acid, diester with propane-1,2,3-triol.

Didodecyl 3,3′-thiodipropionate. $C_{30}H_{58}O_4S$. (M_r 514.8). *1027700.* [123-28-4].

White or almost white, crystalline powder, practically insoluble in water, freely soluble in acetone and in light petroleum, slightly soluble in ethanol (96 per cent).

mp: about 39 °C.

Dieldrin. $C_{12}H_8Cl_6O$. (M_r 380.9). *1126200.* [60-57-1].

bp: about 385 °C.

mp: about 176 °C.

A suitable certified reference solution (10 ng/µL in cyclohexane) may be used.

Diethanolamine. $C_4H_{11}NO_2$. (M_r 105.1). *1027800.* [111-42-2]. 2,2′-Iminobisethanol.

Viscous, clear, slightly yellow liquid or deliquescent crystals melting at about 28 °C, very soluble in water, in acetone and in methanol.

d_{20}^{20}: about 1.09.

pH (*2.2.3*): 10.0 to 11.5 for a 50 g/L solution.

Diethanolamine used in the test for alkaline phosphatase complies with the following additional test.

Ethanolamine. Gas chromatography (*2.2.28*).

Internal standard solution. Dissolve 1.00 g of *3-aminopropanol R* in *acetone R* and dilute to 10.0 mL with the same solvent.

Test solution (a). Dissolve 5.00 g of the substance to be examined in *acetone R* and dilute to 10.0 mL with the same solvent.

Test solution (b). Dissolve 5.00 g of the substance to be examined in *acetone R*, add 1.0 mL of the internal standard solution and dilute to 10.0 mL with the same solvent.

Reference solutions. Dissolve 0.50 g of *ethanolamine R* in *acetone R* and dilute to 10.0 mL with the same solvent. To 0.5 mL, 1.0 mL and 2.0 mL of this solution, add 1.0 mL of the internal standard solution and dilute to 10.0 mL with *acetone R*.

Column:

– *size*: *l* = 1 m, Ø = 4 mm;

– *stationary phase*: *diphenylphenylene oxide polymer R* (180-250 µm).

Carrier gas: *nitrogen for chromatography R*.

Flow rate: 40 mL/min.

Temperature:

	Time (min)	Temperature (°C)
Column	0 → 3	125
	3 → 17.6	125 → 300
Injection port		250
Detector		280

Detection: flame-ionisation.

Injection: 1.0 µL.

Limit:

– *ethanolamine*: maximum 1.0 per cent.

Diethoxytetrahydrofuran. $C_8H_{16}O_3$. (M_r 160.2). *1027900.* [3320-90-9]. 2,5-Diethoxytetrahydrofuran. A mixture of the *cis* and *trans* isomers.

Clear, colourless or slightly yellowish liquid, practically insoluble in water, soluble in ethanol (96 per cent) and in most other organic solvents.

d_{20}^{20}: about 0.98.

n_D^{20}: about 1.418.

Diethylamine. $C_4H_{11}N$. (M_r 73.1). *1028000.* [109-89-7].

Clear, colourless, flammable liquid, strongly alkaline, miscible with water and with ethanol (96 per cent).

d_{20}^{20}: about 0.71.

bp: about 55 °C.

Diethylamine R1. $C_4H_{11}N$. (M_r 73.1). *1028001.* [109-89-7]. *N*-Ethylethanamine.

Content: minimum 99.5 per cent.

Clear, colourless, flammable liquid, strongly alkaline, miscible with water and with ethanol (96 per cent).

d_{20}^{20}: about 0.71.

bp: about 55 °C.

Diethylaminoethyldextran. *1028200.*

Anion-exchange resin presented as the hydrochloride.

Powder forming gels with water.

***N,N*-Diethylaniline.** $C_{10}H_{15}N$. (M_r 149.2). *1028400.* [91-66-7].

d_{20}^{20}: about 0.938.

bp: about 217 °C.

mp: about − 38 °C.

Diethylene glycol. $C_4H_{10}O_3$. (M_r 106.1). *1028300.* [111-46-6]. 2,2′-Oxydiethanol.

Content: minimum 99.5 per cent *m/m*.

Clear, colourless liquid, hygroscopic, miscible with water, with acetone and with ethanol (96 per cent).

d_{20}^{20}: about 1.118.

n_D^{20}: about 1.447.

bp: 244 °C to 246 °C.

Storage: in an airtight container.

***N,N*-Diethylethane-1,2-diamine.** $C_6H_{16}N_2$. (M_r 116.2). *1028500.* [100-36-7]. *N,N*-Diethylethylenediamine.

Content: minimum 98.0 per cent.

Slightly oily liquid, colourless or slightly yellow, strong odour of ammonia, irritant to the skin, eyes and mucous membranes.

d_{20}^{20}: 0.827.

bp: 145 °C to 147 °C.

Water (*2.5.12*): maximum 1.0 per cent, determined on 0.500 g.

Di(2-ethylhexyl) phthalate. $C_{24}H_{38}O_4$. (M_r 390.5). *1028100.* Di(2-ethylhexyl) benzene-1,2-dicarboxylate.

Colourless, oily liquid, practically insoluble in water, soluble in organic solvents.

d_{20}^{20}: about 0.98.

n_D^{20}: about 1.486.

Viscosity (*2.2.9*): about 80 mPa·s.

Diethylphenylenediamine sulfate. $C_{10}H_{18}N_2O_4S$. (M_r 262.3). *1028600.* [6283-63-2]. *N,N'*-Diethyl-*p*-phenylenediamine sulfate. *N,N'*-Diethylbenzene-1,4-diamine sulfate.

White or slightly yellow powder, soluble in water.

mp: about 185 °C, with decomposition.

Storage: protected from light.

Diethylphenylenediamine sulfate solution. *1028601.*

To 250 mL of *water R* add 2 mL of *sulfuric acid R* and 25 mL of *0.02 M sodium edetate*. Dissolve in this solution 1.1 g of *diethylphenylenediamine sulfate R* and dilute to 1000 mL with *water R*.

Do not use if the solution is not colourless.

Storage: protected from light and heat for 1 month.

Diethyl sulfone. $C_4H_{10}O_2S$. (M_r 122.2). *1203300.* [597-35-3]. 1-(Ethylsulfonyl)ethane. 1-(Ethanesulfonyl)ethane.

Content: minimum 97 per cent.

Crystalline powder.

mp: about 73 °C.

Diflubenzuron. $C_{14}H_9ClF_2N_2O_2$. (M_r 310.7). *1180000.* [35367-38-5]. 1-(4-Chlorophenyl)-3-(2,6-difluorobenzoyl)urea.

Colourless or white or almost white crystals, practically insoluble in water, freely soluble in dimethyl sulfoxide, slightly soluble in acetone.

mp: 230 to 232 °C.

Digitonin. $C_{56}H_{92}O_{29}$. (M_r 1229). *1028700.* [11024-24-1]. 3β-[*O*-β-D-Glucopyranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-*O*-β-D-galactopyranosyl-(1→4)-*O*-β-D-galactopyranosyloxy]-(25*R*)-5α-spirostan-2α,15β-diol.

Crystals, practically insoluble in water, sparingly soluble in anhydrous ethanol, slightly soluble in ethanol (96 per cent).

Digitoxin. *1028800.* [71-63-6].

See *Digitoxin (0078)*.

Diglycine. $C_4H_8N_2O_3$. (M_r 132.1). *1191700.* [556-50-3]. 2-[(2-Aminoacetyl)amino]acetic acid. Glycylglycine.

Digoxin. *1203400.*

See *Digoxin (0079)*.

Dihydrocapsaicin. $C_{18}H_{29}NO_3$. (M_r 307.4). *1148100.* [19408-84-5]. *N*-[(4-Hydroxy-3-methoxyphenyl)methyl]-8-methylnonanamide.

White or almost white, crystalline powder, practically insoluble in cold water, freely soluble in anhydrous ethanol.

10,11-Dihydrocarbamazepine. $C_{15}H_{14}N_2O$. (M_r 238.3). *1028900.* [3564-73-6]. 10,11-Dihydro-5*H*-dibenzo[*b*,*f*]azepine-5-carboxamide.

mp: 205 °C to 210 °C.

Dihydrocarvone. $C_{10}H_{16}O$. (M_r 152.2). *1160900.* [7764-50-3]. *p*-Menth-8-en-2-one. 2-Methyl-5-(1-methylethenyl)cyclohexanone.

Dihydrocarvone used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the test for chromatographic profile in the monograph *Caraway oil (1817)*.

Content calculated by the normalisation procedure:

- *major component (trans-dihydrocarvone)*: minimum 70 per cent;
- *sum of cis- and trans-dihydrocarvone*: minimum 98 per cent.

2,5-Dihydroxybenzoic acid. $C_7H_6O_4$. (M_r 154.1). *1148200.* [490-79-9]. Gentisic acid.

Light yellow crystals.

mp: about 200 °C.

5,7-Dihydroxy-4-methylcoumarin. $C_{10}H_8O_4$. (M_r 192.2). *1149400.* [2107-76-8]. 5,7-Dihydroxy-4-methyl-2*H*-1-benzopyran-2-one.

Light yellowish powder, practically insoluble in water, sparingly soluble in ethanol (96 per cent).

mp: 295 °C to 303 °C.

Dihydroxynaphthalene. *1029000.* [132-86-5].

See *1,3-dihydroxynaphthalene R*.

1,3-Dihydroxynaphthalene. $C_{10}H_8O_2$. (M_r 160.2). *1029000.* [132-86-5]. Naphthalene-1,3-diol.

Crystalline, generally brownish-violet powder, freely soluble in water and in ethanol (96 per cent).

mp: about 125 °C.

2,7-Dihydroxynaphthalene. $C_{10}H_8O_2$. (M_r 160.2). *1029100.* [582-17-2]. Naphthalene-2,7-diol.

Needles, soluble in water and in ethanol (96 per cent).

mp: about 190 °C.

2,7-Dihydroxynaphthalene solution. *1029101.*

Dissolve 10 mg of *2,7-dihydroxynaphthalene R* in 100 mL of *sulfuric acid R* and allow to stand until decolorised.

Storage: use within 2 days.

5,7-Diiodoquinolin-8-ol. $C_9H_5I_2NO$. (M_r 397.0). *1157100.* [83-73-8]. 5,7-Diiodooxine.

Yellowish-brown powder, sparingly soluble in acetone and in ethanol (96 per cent).

Content: minimum 95.0 per cent.

Di-isobutyl ketone. $C_9H_{18}O$. (M_r 142.2). *1029200.* [108-83-8].

Clear, colourless liquid, slightly soluble in water, miscible with most organic solvents.

n_D^{20}: about 1.414

bp: about 168 °C.

Di-isopropyl ether. $C_6H_{14}O$. (M_r 102.2). *1029300.* [108-20-3].

Clear, colourless liquid, very slightly soluble in water, miscible with ethanol (96 per cent).

d_{20}^{20}: 0.723 to 0.728.

bp: 67 °C to 69 °C.

Do not distil if the di-isopropyl ether does not comply with the test for peroxides.

Peroxides. Place 8 mL of *potassium iodide and starch solution R* in a 12 mL ground-glass-stoppered cylinder about 1.5 cm in diameter. Fill completely with the substance to be examined, shake vigorously and allow to stand protected from light for 30 min. No colour is produced.

The name and concentration of any added stabiliser are stated on the label.

Storage: protected from light.

***N,N*-Diisopropylethylamine.** $C_8H_{19}N$. (M_r 129.2). *1204600.* [7087-68-5]. *N*-Ethyl-*N*-(propan-2-yl)propan-2-amine. *N*-Ethyldiisopropylamine.

Clear, colourless or light yellow liquid.

bp: 127 °C.

***N,N′*-Diisopropylethylenediamine.** $C_8H_{20}N_2$. (M_r 144.3). *1140600.* [4013-94-9]. *N,N′*-Bis(1-methylethyl)-1,2-ethanediamine.

Colourless or yellowish, corrosive, flammable, hygroscopic liquid.

d_{20}^{20}: about 0.798.

n_D^{20}: about 1.429.

bp: about 170 °C.

4,4′-Dimethoxybenzophenone. $C_{15}H_{14}O_3$. (M_r 242.3). *1126300.* [90-96-0]. Bis(4-methoxyphenyl)methanone.

White or almost white powder, practically insoluble in water and slightly soluble in ethanol (96 per cent).

mp: about 142 °C.

3,4-Dimethoxy-L-phenylalanine. $C_{11}H_{15}NO_4$. (M_r 225.2). *1191800.* [32161-30-1]. (2*S*)-2-Amino-3-(3,4-dimethoxyphenyl)propanoic acid.

Content: minimum 95 per cent.

White or almost white powder.

Dimethoxypropane. $C_5H_{12}O_2$. (M_r 104.1). *1105200.* [77-76-9]. 2,2-Dimethoxypropane.

Colourless liquid, decomposing on exposure to moist air or water.

d_{20}^{20}: about 0.847.

n_D^{20}: about 1.378.

bp: about 83 °C.

Dimethylacetamide. C_4H_9NO. (M_r 87.1). *1029700.* [127-19-5]. *N*,*N*-Dimethylacetamide.

Content: minimum 99.5 per cent.

Colourless liquid, miscible with water and with many organic solvents.

d_{20}^{20}: about 0.94.

n_D^{20}: about 1.437.

bp: about 165 °C.

Dimethylamine. C_2H_7N. (M_r 45.08). *1168900.* [124-40-3]. *N*-Methylmethanamine.

Colourless, flammable gas.

bp: about 7 °C.

mp: about − 92.2 °C.

Dimethylamine solution. *1168901.*

A 400 g/L solution of *dimethylamine R*.

Clear, colourless solution.

Density: about 0.89.

bp: about 54 °C.

mp: about − 37 °C.

Dimethylaminobenzaldehyde. $C_9H_{11}NO$. (M_r 149.2). *1029800.* [100-10-7]. 4-Dimethylaminobenzaldehyde.

White or yellowish-white crystals, soluble in ethanol (96 per cent) and in dilute acids.

mp: about 74 °C.

Dimethylaminobenzaldehyde solution R1. *1029801.*

Dissolve 0.2 g of *dimethylaminobenzaldehyde R* in 20 mL of *ethanol (96 per cent) R* and add 0.5 mL of *hydrochloric acid R*. Shake the solution with *activated charcoal R* and filter. The colour of the reagent is less intense than that of *iodine solution R3*. Prepare immediately before use.

Dimethylaminobenzaldehyde solution R2. *1029802.*

Dissolve 0.2 g of *dimethylaminobenzaldehyde R*, without heating, in a mixture of 4.5 mL of *water R* and 5.5 mL of *hydrochloric acid R*. Prepare immediately before use.

Dimethylaminobenzaldehyde solution R6. *1029803.*

Dissolve 0.125 g of *dimethylaminobenzaldehyde R* in a cooled mixture of 35 mL of *water R* and 65 mL of *sulfuric acid R*. Add 0.1 mL of a 50 g/L solution of *ferric chloride R*. Before use allow to stand for 24 h, protected from light.

Storage: when stored at room temperature, use within 1 week; when stored in a refrigerator use within several months.

Dimethylaminobenzaldehyde solution R7. *1029804.*

Dissolve 1.0 g of *dimethylaminobenzaldehyde R* in 50 mL of *hydrochloric acid R* and add 50 mL of *ethanol (96 per cent) R*.

Storage: protected from light; use within 4 weeks.

Dimethylaminobenzaldehyde solution R8. *1029805.*

Dissolve 0.25 g of *dimethylaminobenzaldehyde R* in a mixture of 5 g of *phosphoric acid R*, 45 g of *water R* and 50 g of *anhydrous acetic acid R*. Prepare immediately before use.

Dimethylaminobenzaldehyde solution R9. *1029806.*

Dissolve 1.0 g of *dimethylaminobenzaldehyde R* in 3.5 mL of perchloric acid (600 g/L $HClO_4$) and slowly add 6.5 mL of *2-propanol R*. Prepare immediately before use.

4-Dimethylaminocinnamaldehyde. $C_{11}H_{13}NO$. (M_r 175.2). *1029900.* [6203-18-5]. 3-(4-Dimethylaminophenyl)prop-2-enal.

Orange or orange-brown crystals or powder. Sensitive to light.

mp: about 138 °C.

4-Dimethylaminocinnamaldehyde solution. *1029901.*

Dissolve 2 g of *4-dimethylaminocinnamaldehyde R* in a mixture of 100 mL of *hydrochloric acid R1* and 100 mL of *anhydrous ethanol R*. Dilute the solution to four times its volume with *anhydrous ethanol R* immediately before use.

Dimethylaminoethanol. $C_4H_{11}NO$. (M_r 89.1). *1195600.* [108-01-0]. 2-(Dimethylamino)ethan-1-ol.

Colourless or slightly yellow liquid, miscible with water.

bp: about 135 °C.

2-(Dimethylamino)ethyl methacrylate. $C_8H_{15}NO_2$. (M_r 157.2). *1147200.* [2867-47-2]. 2-(Dimethylamino)ethyl 2-methylpropenoate.

d_4^{20}: about 0.930.

bp: about 187 °C.

Dimethylaminonaphthalenesulfonyl chloride. $C_{12}H_{12}ClNO_2S$. (M_r 269.8). *1030000.* [605-65-2]. 5-Dimethyl-amino-1-naphthalenesulfonyl chloride.

Yellow, crystalline powder, slightly soluble in water, soluble in methanol.

mp: about 70 °C.

3-Dimethylaminophenol. $C_8H_{11}NO$. (M_r 137.2). *1156500.* [99-07-0]. 3-(Dimethylamino)phenol.

Grey powder, slightly soluble in water.

mp: about 80 °C.

2-(Dimethylamino)thioacetamide hydrochloride. $C_4H_{11}ClN_2S$. (M_r 154.7). *1181800.* [27366-72-9].

Dimethylaniline. *1030100.* [121-69-7].

See *N,N-Dimethylaniline R*.

***N*,*N*-Dimethylaniline.** $C_8H_{11}N$. (M_r 121.2). *1030100.* [121-69-7].

Clear, oily liquid, almost colourless when freshly distilled, darkening on storage to reddish-brown, practically insoluble in water, freely soluble in ethanol (96 per cent).

n_D^{20}: about 1.558.

Distillation range (*2.2.11*). Not less than 95 per cent distils between 192 °C and 194 °C.

2,3-Dimethylaniline. $C_8H_{11}N$. (M_r 121.2). *1105300.* [87-59-2]. 2,3-Xylidine.

Yellowish liquid, sparingly soluble in water, soluble in ethanol (96 per cent).

d_{20}^{20}: 0.993 to 0.995.

n_D^{20}: about 1.569.

bp: about 224 °C.

2,6-Dimethylaniline. $C_8H_{11}N$. (M_r 121.2). *1030200.* [87-62-7]. 2,6-Xylidine.

Colourless liquid, sparingly soluble in water, soluble in ethanol (96 per cent).

d_{20}^{20}: about 0.98.

2,6-Dimethylaniline hydrochloride. $C_8H_{12}ClN$. (M_r 157.6). *1169000.* [21436-98-6]. 2,6-Dimethylbenzenamide hydrochloride. 2,6-Xylidine hydrochloride.

Content: minimum 98.0 per cent.

2,4-Dimethyl-6-*tert*-butylphenol. $C_{12}H_{18}O$. (M_r 178.3). *1126500.* [1879-09-0].

Dimethyl carbonate. $C_3H_6O_3$. (M_r 90.1). *1119300.* [616-38-6]. Carbonic acid dimethyl ester.

Liquid, insoluble in water, miscible with ethanol (96 per cent).

d_4^{17}: 1.065.

n_D^{20}: 1.368.

bp: about 90 °C.

Dimethyl-β-cyclodextrin. $C_{56}H_{98}O_{35}$. (M_r 1331). *1169100.* [51166-71-3]. Heptakis(2,6-di-*O*-methyl)cyclomaltoheptaose. Cycloheptakis-(1→4)-(2,6-di-*O*-methyl-α-D-glucopyranosyl). $2^A,2^B,2^C,2^D,2^E,2^F,2^G,6^A,6^B,6^C,6^D,6^E,6^F,6^G$-Tetradeca-*O*-methyl-β-cyclodextrin.

White or almost white powder.

Dimethyldecylamine. $C_{12}H_{27}N$. (M_r 185.4). *1113500.* [1120-24-7]. *N*,*N*-dimethyldecylamine.

Content: minimum 98.0 per cent *m/m*.

bp: about 234 °C.

1,1-Dimethylethylamine. $C_4H_{11}N$. (M_r 73.1). *1100900.* [75-64-9]. 2-Amino-2-methylpropane. *tert*-Butylamine.

Liquid, miscible with ethanol (96 per cent).

d_{20}^{20}: about 0.694.

n_D^{20}: about 1.378.

bp: about 46 °C.

1,1-Dimethylethyl methyl ether. $C_5H_{12}O$. (M_r 88.1). *1013900.* [1634-04-4]. 2-Methoxy-2-methylpropane. *tert*-Butyl methyl ether.

Colourless, clear, flammable liquid.

n_D^{20}: about 1.376.

Absorbance (*2.2.25*): maximum 0.30 at 240 nm, 0.10 at 255 nm, 0.01 at 280 nm, determined using *water R* as compensation liquid.

1,1-Dimethylethyl methyl ether R1. $C_5H_{12}O$. (M_r 88.1). *1126400.* [1634-04-4]. 2-Methoxy-2-methylpropane. *tert*-Butyl methyl ether.

Content: minimum 99.5 per cent.

d_{20}^{20}: about 0.741.

n_D^{20}: about 1.369.

bp: about 55 °C.

Dimethylformamide. C_3H_7NO. (M_r 73.1). *1030300.* [68-12-2].

Clear, colourless neutral liquid, miscible with water and with ethanol (96 per cent).

d_{20}^{20}: 0.949 to 0.952.

bp: about 153 °C.

Water (*2.5.12*): maximum 0.1 per cent.

Dimethylformamide diethylacetal. $C_7H_{17}NO_2$. (M_r 147.2). *1113600.* [1188-33-6]. *N*,*N*-Dimethylformamide diethylacetal.

n_D^{20}: about 1.40.

bp: 128 °C to 130 °C.

***N*,*N*-Dimethylformamide dimethylacetal.** $C_5H_{13}NO_2$. (M_r 119.2). *1140700.* [4637-24-5]. 1,1-Dimethoxytrimethylamine.

Clear, colourless liquid.

d_{20}^{20}: about 0.896.

n_D^{20}: about 1.396.

bp: about 103 °C.

Dimethylglyoxime. $C_4H_8N_2O_2$. (M_r 116.1). *1030400.* [95-45-4]. 2,3-Butanedione dioxime.

White or almost white, crystalline powder or colourless crystals, practically insoluble in cold water, very slightly soluble in boiling water, soluble in ethanol (96 per cent).

mp: about 240 °C, with decomposition.

Sulfated ash (*2.4.14*): maximum 0.05 per cent.

1,3-Dimethyl-2-imidazolidinone. $C_5H_{10}N_2O$. (M_r 114.2). *1135400.* [80-73-9]. *N*,*N*′-Dimethylethylene urea. 1,3-Dimethyl-2-imidazolidone.

n_D^{20}: 1.4720.

bp: about 224 °C.

***N*,*N*-Dimethyloctylamine.** $C_{10}H_{23}N$. (M_r 157.3). *1030500.* [7378-99-6]. Octyldimethylamine.

Colourless liquid.

d_{20}^{20}: about 0.765.

n_D^{20}: about 1.424.

bp: about 195 °C.

2,5-Dimethylphenol. $C_8H_{10}O$. (M_r 122.2). *1162300.* [95-87-4]. *p*-Xylenol.

White or almost white crystals.

2,6-Dimethylphenol. $C_8H_{10}O$. (M_r 122.2). *1030600.* [576-26-1].

Colourless needles, slightly soluble in water, very soluble in ethanol (96 per cent).

bp: about 203 °C.

mp: 46 °C to 48 °C.

3,4-Dimethylphenol. $C_8H_{10}O$. (M_r 122.2). *1098100.* [95-65-8].

White or almost white crystals, slightly soluble in water, freely soluble in ethanol (96 per cent).

bp: about 226 °C.

mp: 25 °C to 27 °C.

***N*,*N*-Dimethyl-L-phenylalanine.** $C_{11}H_{15}NO_2$. (M_r 193.2). *1164000.* [17469-89-5]. (2*S*)-2-(Dimethylamino)-3-phenylpropanoic acid.

mp: about 226 °C.

Dimethylpiperazine. $C_6H_{14}N_2$. (M_r 114.2). *1030700.* [106-58-1]. 1,4-Dimethylpiperazine.

A colourless liquid, miscible with water and with ethanol (96 per cent).

d_{20}^{20}: about 0.85.

n_D^{20}: about 1.446.

bp: about 131 °C.

Dimethylstearamide. $C_{20}H_{41}NO$. (M_r 311.6). *1030800.* *N*,*N*-Dimethylstearamide.

White or almost white solid mass, soluble in many organic solvents, including acetone.

mp: about 51 °C.

Dimethylstearylamide. *1030800.*

See *dimethylstearamide R*.

Dimethyl sulfone. $C_2H_6O_2S$. (M_r 94.1). *1030900.* [67-71-0].

White or almost white, crystalline powder, freely soluble in water, soluble in acetone and ethanol (96 per cent).

mp: 108 °C to 110 °C.

Dimethyl sulfoxide. *1029500.* [67-68-5].

See *Dimethyl sulfoxide (0763)*.

Dimethyl sulfoxide used in spectrophotometry complies with the following additional test.

Absorbance (*2.2.25*): maximum 1.00 at 262 nm, 0.46 at 270 nm, 0.16 at 290 nm, 0.01 at 340 nm and higher wavelengths, determined using *water R* as compensation liquid.

Dimethyl sulfoxide R1. *1029501.*

Content: minimum 99.7 per cent, determined by gas chromatography.

Dimethyl sulfoxide R2. *1029502.*

Content: minimum 99.9 per cent, determined by gas chromatography.

Residue on evaporation: maximum 0.0005 per cent.

Water (2.5.32): maximum 0.005 per cent.

Dimeticone. *1105400.* [9006-65-9].

See *Dimeticone (0138)*.

Dimidium bromide. $C_{20}H_{18}BrN_3$. (M_r 380.3). *1031100.* [518-67-2]. 3,8-Diamino-5-methyl-6-phenylphenanthridinium bromide.

Dark-red crystals, slightly soluble in water at 20 °C, sparingly soluble in water at 60 °C and in ethanol (96 per cent).

Dimidium bromide-sulfan blue mixed solution. *1031101.*

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: protected from light.

Dinitrobenzene. $C_6H_4N_2O_4$. (M_r 168.1). *1031200.* [99-65-0]. 1,3-Dinitrobenzene.

Yellowish crystalline powder or crystals, practically insoluble in water, slightly soluble in ethanol (96 per cent).

mp: about 90 °C.

Dinitrobenzene solution. *1031201.*

A 10 g/L solution of *dinitrobenzene R* in *ethanol (96 per cent) R*.

Dinitrobenzoic acid. $C_7H_4N_2O_6$. (M_r 212.1). *1031300.* [99-34-3]. 3,5-Dinitrobenzoic acid.

Almost colourless crystals, slightly soluble in water, very soluble in ethanol (96 per cent).

mp: about 206 °C.

Dinitrobenzoic acid solution. *1031301.*

A 20 g/L solution of *dinitrobenzoic acid R* in *ethanol (96 per cent) R*.

Dinitrobenzoyl chloride. $C_7H_3ClN_2O_5$. (M_r 230.6). *1031400.* [99-33-2]. 3,5-Dinitrobenzoyl chloride.

Translucent, yellow or greenish-yellow powder or yellowish crystals, soluble in acetone and in toluene.

mp: about 68 °C.

Suitability test. To 1 mL of *anhydrous ethanol R* and 0.1 g of *dinitrobenzoyl chloride R* add 0.05 mL of *dilute sulfuric acid R* and boil under a reflux condenser for 30 min. After evaporation on a water-bath add 5 mL of *heptane R* to the residue and heat to boiling. Filter the hot solution. Wash the crystals formed on cooling to room temperature with a small quantity of *heptane R* and dry in a desiccator. The crystals melt (*2.2.14*) at 94 °C to 95 °C.

Dinitrophenylhydrazine. $C_6H_6N_4O_4$. (M_r 198.1). *1031500.* [119-26-6]. 2,4-Dinitrophenylhydrazine.

Reddish-orange crystals, very slightly soluble in water, slightly soluble in ethanol (96 per cent).

mp: about 203 °C (instantaneous method).

Dinitrophenylhydrazine-aceto-hydrochloric solution. *1031501.*

Dissolve 0.2 g of *dinitrophenylhydrazine R* in 20 mL of *methanol R* and add 80 mL of a mixture of equal volumes of *acetic acid R* and *hydrochloric acid R1*. Prepare immediately before use.

Dinitrophenylhydrazine-hydrochloric solution. *1031502.*

Dissolve by heating 0.50 g of *dinitrophenylhydrazine R* in *dilute hydrochloric acid R* and dilute to 100 mL with the same solvent. Allow to cool and filter. Prepare immediately before use.

Dinitrophenylhydrazine-sulfuric acid solution. *1031503.*

Dissolve 1.5 g of *dinitrophenylhydrazine R* in 50 mL of a 20 per cent *V/V* solution of *sulfuric acid R*. Prepare immediately before use.

Dinonyl phthalate. $C_{26}H_{42}O_4$. (M_r 418.6). *1031600.* [28553-12-0].

Colourless to pale yellow, viscous liquid.

d_{20}^{20}: 0.97 to 0.98.

n_D^{20}: 1.482 to 1.489.

Acidity. Shake 5.0 g with 25 mL of *water R* for 1 min. Allow to stand, filter the separated aqueous layer and add 0.1 mL of *phenolphthalein solution R*. Not more than 0.3 mL of *0.1 M sodium hydroxide* is required to change the colour of the solution (0.05 per cent, calculated as phthalic acid).

Water (*2.5.12*): maximum 0.1 per cent.

Dioctadecyl disulfide. $C_{36}H_{74}S_2$. (M_r 571.1). *1031700.* [2500-88-1].

White or almost white powder, practically insoluble in water.

mp: 53 °C to 58 °C.

2,2′-Di(octadecyloxy)-5,5′-spirobi(1,3,2-dioxaphosphorinane). $C_{41}H_{82}O_6P_2$. (M_r 733). *1031800.*

White or almost white, waxy solid, practically insoluble in water, soluble in hydrocarbons.

mp: 40 °C to 70 °C.

Dioctadecyl 3,3′-thiodipropionate. $C_{42}H_{82}O_4S$. (M_r 683). *1031900.* [693-36-7].

White or almost white, crystalline powder, practically insoluble in water, freely soluble in methylene chloride, sparingly soluble in acetone, in ethanol (96 per cent) and in light petroleum.

mp: 58 °C to 67 °C.

Di-*n*-octyl phthalate. $C_{24}H_{38}O_4$. (M_r 390.6). *1203500.* [117-84-0]. Dioctyl benzene-1,2-dicarboxylate.

Colourless viscous liquid, insoluble in water.

Density: about 0.98 g/mL (20 °C).

Dioxan. $C_4H_8O_2$. (M_r 88.1). *1032000.* [123-91-1]. 1,4-Dioxane.

Clear, colourless liquid, miscible with water and with most organic solvents.

d_{20}^{20}: about 1.03.

Freezing point (*2.2.18*): minimum 11.0 °C.

Water (*2.5.12*): maximum 0.5 per cent.

Do not distil if the dioxan does not comply with the test for peroxides.

Peroxides. Place 8 mL of *potassium iodide and starch solution R* in a 12 mL ground-glass-stoppered cylinder about 1.5 cm in diameter. Fill completely with the substance to be examined, shake vigorously and allow to stand in the dark for 30 min. No colour is produced.

Dioxan used for liquid scintillation is of a suitable analytical grade.

Dioxan solution. *1032002.*

Dilute 50.0 mL of *dioxan stock solution R* to 100.0 mL with *water R*. (0.5 mg/mL of dioxan).

Dioxan solution R1. *1032003.*

Dilute 10.0 mL of *dioxan solution R* to 50.0 mL with *water R*. (0.1 mg/mL of dioxan).

Dioxan solution R2. *1032004.*

Dilute 2.0 mL of *dioxan solution R* to 50.0 mL with *water R* (0.02 mg/mL of dioxan).

Dioxan stock solution. *1032001.*

Dissolve 1.00 g of *dioxan R* in *water R* and dilute to 100.0 mL with the same solvent. Dilute 5.0 mL of this solution to 50.0 mL with *water R* (1.0 mg/mL).

Diphenylamine. $C_{12}H_{11}N$. (M_r 169.2). *1032100.* [122-39-4].

White or almost white crystals, slightly soluble in water, soluble in ethanol (96 per cent).

mp: about 55 °C.

Storage: protected from light.

Diphenylamine solution. *1032101.*

A 1 g/L solution of *diphenylamine R* in *sulfuric acid R*.

Storage: protected from light.

Diphenylamine solution R1. *1032102.*

A 10 g/L solution of *diphenylamine R* in *sulfuric acid R*. The solution is colourless.

Diphenylamine solution R2. *1032103.*

Dissolve 1 g of *diphenylamine R* in 100 mL of *glacial acetic acid R* and add 2.75 mL of *sulfuric acid R*. Use immediately.

Diphenylanthracene. $C_{26}H_{18}$. (M_r 330.4). *1032200.* [1499-10-1]. 9,10-Diphenylanthracene.

Yellowish or yellow, crystalline powder, practically insoluble in water.

mp: about 248 °C.

Diphenylbenzidine. $C_{24}H_{20}N_2$. (M_r 336.4). *1032300.* [531-91-9]. *N,N*'-Diphenylbenzidine. *N,N*'-Diphenylbiphenyl-4,4′-diamine.

White or faintly grey, crystalline powder, practically insoluble in water, slightly soluble in acetone and in ethanol (96 per cent).

mp: about 248 °C.

Nitrates. Dissolve 8 mg in a cooled mixture of 5 mL of *water R* and 45 mL of *nitrogen-free sulfuric acid R*. The solution is colourless or very pale blue.

Sulfated ash (*2.4.14*): maximum 0.1 per cent.

Storage: protected from light.

Diphenylboric acid aminoethyl ester. $C_{14}H_{16}BNO$. (M_r 225.1). *1032400.* [524-95-8].

White or slightly yellow, crystalline powder, practically insoluble in water, soluble in ethanol (96 per cent).

mp: about 193 °C.

Diphenylcarbazide. $C_{13}H_{14}N_4O$. (M_r 242.3). *1032500.* [140-22-7]. 1,5-Diphenylcarbonodihydrazide.

White or almost white, crystalline powder which gradually becomes pink on exposure to air, very slightly soluble in water, soluble in acetone, in ethanol (96 per cent) and in glacial acetic acid.

mp: about 170 °C.

Sulfated ash (*2.4.14*): maximum 0.1 per cent.

Storage: protected from light.

Diphenylcarbazide solution. *1032501.*

Dissolve 0.2 g of *diphenylcarbazide R* in 10 mL of *glacial acetic acid R* and dilute to 100 mL with *anhydrous ethanol R*. Prepare immediately before use.

Diphenylcarbazone. $C_{13}H_{12}N_4O$. (M_r 240.3). *1032600.* [538-62-5]. 1,5-Diphenylcarbazone.

Orange-yellow, crystalline powder, practically insoluble in water, freely soluble in ethanol (96 per cent).

mp: about 157 °C, with decomposition.

Diphenylcarbazone mercuric reagent. *1032601.*

Solution A. Dissolve 0.1 g of *diphenylcarbazone R* in *anhydrous ethanol R* and dilute to 50 mL with the same solvent.

Solution B. Dissolve 1 g of *mercuric chloride R* in *anhydrous ethanol R* and dilute to 50 mL with the same solvent.

Mix equal volumes of the two solutions.

2,2-Diphenylglycine. $C_{14}H_{13}NO_2$. (M_r 227.26). *1174300.* [3060-50-2]. Amino(diphenyl)acetic acid.

1,2-Diphenylhydrazine. $C_{12}H_{12}N_2$. (M_r 184.3). *1140800.* [122-66-7]. Hydrazobenzene. 1,2-Diphenyldiazane.

Orange powder.

mp: about 125 °C.

Diphenylmethanol. $C_{13}H_{12}O$. (M_r 184.2). *1145700.* [91-01-0]. Benzhydrol.

White or almost white, crystalline powder.

mp: about 66 °C.

Diphenyloxazole. $C_{15}H_{11}NO$. (M_r 221.3). *1032700.* [92-71-7]. 2,5-Diphenyloxazole.

White or almost white powder, practically insoluble in water, soluble in methanol, sparingly soluble in dioxan and in glacial acetic acid.

mp: about 70 °C.

$A_{1\text{ cm}}^{1\%}$: about 1260 determined at 305 nm in *methanol R*.

Diphenyloxazole used for liquid scintillation is of a suitable analytical grade.

Diphenylphenylene oxide polymer. *1032800.* 2,6-Diphenyl-*p*-phenylene oxide polymer.

White or almost white, porous beads. The size range of the beads is specified after the name of the reagent in the tests where it is used.

Diphosphorus pentoxide. P_2O_5. (M_r 141.9). *1032900.* [1314-56-3]. Phosphorus pentoxide. Phosphoric anhydride.

White or almost white powder, amorphous, deliquescent. It is hydrated by water with the evolution of heat.

Storage: in an airtight container.

Dipotassium hydrogen phosphate. K_2HPO_4. (M_r 174.2). *1033000.* [7758-11-4].

White or almost white, crystalline powder, hygroscopic, very soluble in water, slightly soluble in ethanol (96 per cent).

Storage: in an airtight container.

Dipotassium hydrogen phosphate trihydrate. $K_2HPO_4,3H_2O$. (M_r 228.2). *1157600.* [16788-57-1].

Colourless or white or almost white powder or crystals, freely soluble in water.

Dipotassium sulfate. K_2SO_4. (M_r 174.3). *1033100.* [7778-80-5].

Colourless crystals, soluble in water.

2,2′-Dipyridylamine. $C_{10}H_9N_3$. (M_r 171.2). *1157700.* [1202-34-2]. *N*-(Pyridin-2-yl)pyridin-2-amine.

mp: about 95 °C.

Disodium arsenate. $Na_2HAsO_4,7H_2O$. (M_r 312.0). *1102500.* [10048-95-0]. Disodium hydrogen arsenate heptahydrate. Dibasic sodium arsenate.

Crystals, efflorescent in warm air, freely soluble in water, soluble in glycerol, slightly soluble in ethanol (96 per cent). The aqueous solution is alcaline to litmus.

d_{20}^{20}: about 1.87.

mp: about 57 °C when rapidly heated.

Disodium bicinchoninate. $C_{20}H_{10}N_2Na_2O_4$. (M_r 388.3). *1126600.* [979-88-4]. Disodium 2,2′-biquinoline-4-4′-dicarboxylate.

Disodium hydrogen citrate. $C_6H_6Na_2O_7,1^1/_2H_2O$. (M_r 263.1). *1033200.* [144-33-2]. Sodium acid citrate. Disodium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate sesquihydrate.

White or almost white powder, soluble in less than 2 parts of water, practically insoluble in ethanol (96 per cent).

Disodium hydrogen phosphate, anhydrous. Na_2HPO_4. (M_r 142.0). *1033400.* [7558-79-4].

Disodium hydrogen phosphate dihydrate. *1033500.* [10028-24-7].

See *Disodium phosphate dihydrate (0602)*.

Disodium hydrogen phosphate dodecahydrate. *1033300.* [10039-32-4].

See *Disodium phosphate dodecahydrate (0118)*.

Disodium hydrogen phosphate solution. *1033301.*

A 90 g/L solution of *disodium hydrogen phosphate dodecahydrate R*.

Disodium hydrogen phosphate heptahydrate. $Na_2HPO_4,7H_2O$. (M_r 268.1). *1206900.* [7782-85-6].

Disodium tetraborate. *1033600.* [1303-96-4].

See *Borax (0013)*.

Borate solution. *1033601.*

Dissolve 9.55 g of *disodium tetraborate R* in *sulfuric acid R*, heating on a water-bath, and dilute to 1 L with the same acid.

Ditalimphos. $C_{12}H_{14}NO_4PS$. (M_r 299.3). *1126700.* [5131-24-8]. *O*,*O*-Diethyl (1,3-dihydro-1,3-dioxo-2*H*-isoindol-2-yl)phosphonothioate.

Very slightly soluble in water, in ethyl acetate and in anhydrous ethanol.

A suitable certified reference solution may be used.

5,5′-Dithiobis(2-nitrobenzoic acid). $C_{14}H_8N_2O_8S_2$. (M_r 396.4). *1097300.* [69-78-3]. 3-Carboxy-4-nitrophenyldisulfide. Ellman's reagent. DTNB.

Yellow powder sparingly soluble in ethanol (96 per cent).

mp: about 242 °C.

Dithioerythritol. $C_4H_{10}O_2S_2$. (M_r 154.3). *1187500.* [6892-68-8].

(2*R*,3*S*)-1,4-Disulfanylbutane-2,3-diol. DTE.

mp: about 83 °C.

Dithiol. $C_7H_8S_2$. (M_r 156.3). *1033800.* [496-74-2]. Toluene-3,4-dithiol. 4-Methylbenzene-1,2-dithiol.

White or almost white crystals, hygroscopic, soluble in methanol and in solutions of alkali hydroxides.

mp: about 30 °C.

Storage: in an airtight container.

Dithiol reagent. *1033801.*

To 1 g of *dithiol R* add 2 mL of *thioglycollic acid R* and dilute to 250 mL with a 20 g/L solution of *sodium hydroxide R*. Prepare immediately before use.

Dithiothreitol. $C_4H_{10}O_2S_2$. (M_r 154.2). *1098200.* [27565-41-9]. *threo*-1,4-Dimercaptobutane-2,3-diol.

Slightly hygroscopic needles, freely soluble in water, in acetone and in anhydrous ethanol.

Storage: in an airtight container.

Dithizone. $C_{13}H_{12}N_4S$. (M_r 256.3). *1033900.* [60-10-6]. 1,5-Diphenylthiocarbazone.

A bluish-black, brownish-black or black powder, practically insoluble in water, soluble in ethanol (96 per cent).

Storage: protected from light.

Dithizone solution. *1033901.*

A 0.5 g/L solution of *dithizone R* in *chloroform R*. Prepare immediately before use.

Dithizone solution R2. *1033903.*

Dissolve 40.0 mg of *dithizone R* in *chloroform R* and dilute to 1000.0 mL with the same solvent. Dilute 30.0 mL of the solution to 100.0 mL with *chloroform R*.

Assay. Dissolve a quantity of *mercuric chloride R* equivalent to 0.1354 g of $HgCl_2$ in a mixture of equal volumes of *dilute sulfuric acid R* and *water R* and dilute to 100.0 mL with the same mixture of solvents. Dilute 2.0 mL of this solution to 100.0 mL with a mixture of equal volumes of *dilute sulfuric acid R* and *water R*. (This solution contains 20 ppm of Hg). Transfer 1.0 mL of the solution to a separating funnel and add 50 mL of *dilute sulfuric acid R*, 140 mL of *water R* and 10 mL of a 200 g/L solution of *hydroxylamine hydrochloride R*. Titrate with the dithizone solution; after each addition, shake the mixture twenty times and towards the end of the titration allow to separate and discard the chloroform layer. Titrate until a bluish-green colour is obtained. Calculate the equivalent in micrograms of mercury per millilitre of the dithizone solution from the expression 20/*V*, where *V* is the volume in millilitres of the dithizone solution used in the titration.

Dithizone R1. $C_{13}H_{12}N_4S$. (M_r 256.3). *1105500.* [60-10-6]. 1,5-Diphenylthiocarbazone.

Content: minimum 98.0 per cent.

Bluish-black, brownish-black or black powder, practically insoluble in water, soluble in ethanol (96 per cent).

Storage: protected from light.

Divanadium pentoxide. V_2O_5. (M_r 181.9). *1034000.* [1314-62-1]. Vanadic anhydride.

Content: minimum 98.5 per cent.

Yellow-brown or rust-brown powder, slightly soluble in water, soluble in strong mineral acids and in solutions of alkali hydroxides with formation of salts.

Appearance of solution. Heat 1 g for 30 min with 10 mL of *sulfuric acid R*. Allow to cool and dilute to 10 mL with the same acid. The solution is clear (*2.2.1*).

Sensitivity to hydrogen peroxide. Dilute 1.0 mL of the solution prepared for the test for appearance of solution cautiously to 50.0 mL with *water R*. To 0.5 mL of the solution add 0.1 mL of a solution of hydrogen peroxide (0.1 g/L of H_2O_2) prepared from *dilute hydrogen peroxide solution R*. The solution has a distinct orange colour compared with a blank prepared from 0.5 mL of the solution to be examined and 0.1 mL of *water R*. After the addition of 0.4 mL of a solution of hydrogen peroxide (0.1 g/L of H_2O_2) prepared from *dilute hydrogen peroxide solution R*, the orange solution becomes orange-yellow.

Loss on ignition: maximum 1.0 per cent, determined on 1.00 g at 700 ± 50 °C.

Assay. Dissolve 0.200 g with heating in 20 mL of a 70 per cent *m/m* solution of *sulfuric acid R*. Add 100 mL of *water R* and *0.02 M potassium permanganate* until a reddish colour is obtained. Decolorise the excess of potassium permanganate by the addition of a 30 g/L solution of *sodium nitrite R*. Add 5 g of *urea R* and 80 mL of a 70 per cent *m/m* solution of

sulfuric acid R. Cool. Using 0.1 mL of *ferroin R* as indicator, titrate the solution immediately with *0.1 M ferrous sulfate* until a greenish-red colour is obtained.

1 mL of *0.1 M ferrous sulfate* is equivalent to 9.095 mg of V_2O_5.

Divanadium pentoxide solution in sulfuric acid. *1034001.*

Dissolve 0.2 g of *divanadium pentoxide R* in 4 mL of *sulfuric acid R* and dilute to 100 mL with *water R*.

Docosahexaenoic acid methyl ester. $C_{23}H_{34}O_2$. (M_r 342.5). *1142800.* [301-01-9]. DHA methyl ester. Cervonic acid methyl ester. (all-*Z*)-Docosa-4,7,10,13,16,19-hexaenoic acid methyl ester.

Content: minimum 90.0 per cent, determined by gas chromatography.

Docusate sodium. *1034100.* [577-11-7].

See *Docusate sodium (1418)*.

Dodecyltrimethylammonium bromide. $C_{15}H_{34}BrN$. (M_r 308.4). *1135500.* [1119-94-4]. *N,N,N*-Trimethyldodecan-1-aminium bromide.

White or almost white crystals.

mp: about 246 °C.

D-Dopa. $C_9H_{11}NO_4$. (M_r 197.2). *1164100.* [5796-17-8]. (2*R*)-2-Amino-3-(3,4-dihydroxyphenyl)propanoic acid. 3-Hydroxy-D-tyrosine. 3,4-Dihydroxy-D-phenylalanine.

$[\alpha]_D^{20}$: + 9.5 to + 11.5, determined on a 10 g/L solution in *1 M hydrochloric acid*.

mp: about 277 °C.

Dotriacontane. $C_{32}H_{66}$. (M_r 450.9). *1034200.* [544-85-4]. *n*-Dotriacontane.

White or almost white plates, practically insoluble in water, sparingly soluble in hexane.

mp: about 69 °C.

Impurities. Not more than 0.1 per cent of impurities with the same t_R value as α-tocopherol acetate, determined by the gas chromatographic method prescribed in the monograph *α-Tocopherol acetate (0439)*.

Doxycycline. *1145800.*

See *Doxycycline monohydrate (0820)*.

β-Ecdysterone. $C_{27}H_{44}O_7$. (M_r 480.6). *1204700.* [5289-74-7]. (2β,3β,5β,22*R*)-2,3,14,20,22,25-Hexahydroxycholest-7-en-6-one.

Echinacoside. $C_{35}H_{46}O_{20}$. (M_r 786.5). *1159400.* [82854-37-3]. β-(3′,4′-Dihydroxyphenyl)-ethyl-*O*-α-L-rhamnopyranosyl (1→3)-*O*-β-D-[β-D-glucopyranosyl(1→6)]-(4-*O*-caffeoyl)-glucopyranoside.

Pale yellow powder, odourless.

Edotreotide. $C_{65}H_{92}N_{14}O_{18}S_2$. ($M_r$ 1422). *1182400.* [204318-14-9]. *N*-[[4,7,10-Tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]acetyl]-D-phenylalanyl-L-cysteinyl-L-tyrosyl-D-tryptophyl-L-lysyl-L-threonyl-*N*-[(1*R*,2*R*)-2-hydroxy-1-(hydroxymethyl)propyl]-L-cysteinamide cyclic (2→7)-disulfide. DOTATOC. DOTA-[Tyr^3]-octreotide.

White or almost white powder.

Content: minimum 95.0 per cent.

Electrolyte reagent for the micro determination of water. *1113700.*

Commercially available anhydrous reagent or a combination of anhydrous reagents for the coulometric titration of water, containing suitable organic bases, sulfur dioxide and iodide dissolved in a suitable solvent.

Elementary standard solution for atomic spectrometry (1.000 g/L). *5004000.*

This solution is prepared, generally in acid conditions, from the element or a salt of the element whose minimum content is not less than 99.0 per cent. The quantity per litre of solution is greater than 0.995 g throughout the guaranteed period, as long as the vial has not been opened. The starting material (element or salt) and the characteristics of the final solvent (nature and acidity, etc.) are mentioned on the label.

Emodin. $C_{15}H_{10}O_5$. (M_r 270.2). *1034400.* [518-82-1]. 1,3,8-Trihydroxy-6-methylanthraquinone.

Orange-red needles, practically insoluble in water, soluble in ethanol (96 per cent) and in solutions of alkali hydroxides.

Chromatography. Thin-layer chromatography (*2.2.27*) as prescribed in the monograph *Rhubarb (0291)*; the chromatogram shows only one principal spot.

Endoprotease LysC. *1173200.*

Microbial extracellular proteolytic enzyme secreted by *Achromobacter lyticus*. A lyophilised powder, free of salts.

α-Endosulfan. $C_9H_6Cl_6O_3S$. (M_r 406.9). *1126800.* [959-98-8].

bp: about 200 °C.

mp: about 108 °C.

A suitable certified reference solution (10 ng/µL in cyclohexane) may be used.

β-Endosulfan. $C_9H_6Cl_6O_3S$. (M_r 406.9). *1126900.* [33213-65-9].

bp: about 390 °C.

mp: about 207 °C.

A suitable certified reference solution (10 ng/µL in cyclohexane) may be used.

Endrin. $C_{12}H_8Cl_6O$. (M_r 380.9). *1127000.* [72-20-8].

A suitable certified reference solution (10 ng/µL in cyclohexane) may be used.

(−)-Epicatechin. $C_{15}H_{14}O_6$. (M_r 290.3). *1201300.* [490-46-0]. (2*R*,3*R*)-2-(3,4-Dihydroxyphenyl)-3,4-dihydro-2*H*-1-benzopyran-3,5,7-triol.

(−)-Epigallocatechin-3-*O*-gallate. $C_{22}H_{18}O_{11}$. (M_r 458.4). *1201400.* [989-51-5]. (2*R*,3*R*)-5,7-Dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2*H*-1-benzopyran-3-yl 3,4,5-trihydroxybenzoate.

Epilactose. $C_{12}H_{22}O_{11}$. (M_r 342.3). *1189200.* [20869-27-6]. 4-*O*-β-D-Galactopyranosyl-D-mannopyranose.

Content: minimum 98 per cent.

Erucamide. $C_{22}H_{43}NO$. (M_r 337.6). *1034500.* [112-84-5]. (*Z*)-Docos-13-enoamide.

Yellowish or white powder or granules, practically insoluble in water, very soluble in methylene chloride, soluble in anhydrous ethanol.

mp: about 70 °C.

Erythritol. *1113800.* [149-32-6].

See *Erythritol (1803)*.

Esculetin. $C_9H_6O_4$. (M_r 178.1). *1185800.* [305-01-1]. 6,7-Dihydroxy-2*H*-1-benzopyran-2-one. Aesculetin.

Esculin. $C_{15}H_{16}O_9$,$1^1/_2H_2O$. (M_r 367.3). *1119400.* [531-75-9]. 6-(β-D-Glucopyranosyloxy)-7-hydroxy-2*H*-chromen-2-one.

White or almost white powder or colourless crystals, sparingly soluble in water and in ethanol (96 per cent), freely soluble in hot water and in hot ethanol (96 per cent).

Chromatography (*2.2.27*). Thin-layer chromatography (*2.2.27*) as prescribed in the monograph *Eleutherococcus (1419)*; the chromatogram shows only one principal spot.

Estradiol. $C_{18}H_{24}O_2$. (M_r 272.4). *1135600.* [50-28-2]. Estra-1,3,5(10)-triene-3,17β-diol. β-Estradiol.

Prisms stable in air, practically insoluble in water, freely soluble in ethanol (96 per cent), soluble in acetone and in dioxan, sparingly soluble in vegetable oils.

mp: 173 °C to 179 °C.

17α-Estradiol. $C_{18}H_{24}O_2$. (M_r 272.4). *1034600.* [57-91-0].

White or almost white, crystalline powder or colourless crystals.

mp: 220 °C to 223 °C.

Estragole. $C_{10}H_{12}O$. (M_r 148.2). *1034700.* [140-67-0]. 1-Methoxy-4-prop-2-enylbenzene.

Liquid, miscible with ethanol (96 per cent).

n_D^{20}: about 1.52.

bp: about 216 °C.

Estragole used in gas chromatography complies with the following test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Anise oil (0804)*.

Test solution. The substance to be examined.

Content: minimum 98.0 per cent, calculated by the normalisation procedure.

Ethane. C_2H_6. (M_r 30.07). *1189300.* [74-84-0].

Content: minimum 99.0 per cent *V/V*.

Ethanol. *1034800.* [64-17-5].

See *Ethanol, anhydrous R*.

Ethanol, anhydrous. *1034800.* [64-17-5].

See *Ethanol, anhydrous (1318)*.

Ethanol R1. *1034801.*

Complies with the requirements prescribed for the monograph *Ethanol, anhydrous (1318)* with the following additional requirement.

Methanol. Gas chromatography (*2.2.28*).

Test solution. The substance to be examined.

Reference solution. Dilute 0.50 mL of *anhydrous methanol R* to 100.0 mL with the substance to be examined. Dilute 1.0 mL of this solution to 100.0 mL with the substance to be examined.

Column:

– *material*: glass;

– *size*: *l* = 2 m, Ø = 2 mm;

– *stationary phase*: *ethylvinylbenzene-divinylbenzene copolymer R* (75-100 µm).

Carrier gas: *nitrogen for chromatography R*.

Flow rate: 30 mL/min.

Temperature:

– *column*: 130 °C;

– *injection port*: 150 °C;

– *detector*: 200 °C.

Detection: flame-ionisation.

Injection: 1 µL of the test solution and 1 µL of the reference solution, alternately, three times.

After each chromatography, heat the column to 230 °C for 8 min. Integrate the methanol peak. Calculate the percentage methanol content from the following expression:

$$\frac{a \times b}{c - b}$$

a = percentage *V/V* content of methanol in the reference solution,

b = area of the methanol peak in the chromatogram obtained with the test solution,

c = area of the methanol peak in the chromatogram obtained with the reference solution.

Limit:

– *methanol*: maximum 0.005 per cent *V/V*.

Ethanol (96 per cent). *1002500.* [64-17-5].

See *Ethanol (96 per cent) (1317)*.

Ethanol (*x* per cent *V/V*). *1002502.*

Mix appropriate volumes of *water R* and *ethanol (96 per cent) R*, allowing for the effects of warming and volume contraction inherent to the preparation of such a mixture, to obtain a solution whose final content of ethanol corresponds to the value of *x*.

Ethanolamine. C_2H_7NO. (M_r 61.1). *1034900.* [141-43-5]. 2-Aminoethanol.

Clear, colourless, viscous, hygroscopic liquid, miscible with water and with methanol.

d_{20}^{20}: about 1.014.

n_D^{20}: about 1.454.

mp: about 11 °C.

Storage: in an airtight container.

Ether. $C_4H_{10}O$. (M_r 74.1). *1035000.* [60-29-7].

Clear, colourless, volatile and very mobile liquid, very flammable, hygroscopic, soluble in water, miscible with ethanol (96 per cent).

d_{20}^{20}: 0.713 to 0.715.

bp: 34 °C to 35 °C.

Do not distil if the ether does not comply with the test for peroxides.

Peroxides. Place 8 mL of *potassium iodide and starch solution R* in a 12 mL ground-glass-stoppered cylinder about 1.5 cm in diameter. Fill completely with the substance to be examined, shake vigorously and allow to stand in the dark for 30 min. No colour is produced.

The name and concentration of any added stabilisers are stated on the label.

Storage: in an airtight container, protected from light, at a temperature not exceeding 15 °C.

Ether, peroxide-free. *1035100.*

See *Anaesthetic ether (0367)*.

Ethion. $C_9H_{22}O_4P_2S_4$. (M_r 384.5). *1127100.* [563-12-2].

mp: – 24 °C to – 25 °C.

A suitable certified reference solution (10 ng/µL in cyclohexane) may be used.

Ethoxychrysoidine hydrochloride. $C_{14}H_{17}ClN_4O$. (M_r 292.8). *1035200.* [2313-87-3]. 4-[(4-Ethoxyphenyl)diazenyl]phenylene-1,3-diamine hydrochloride.

Reddish powder, soluble in ethanol (96 per cent).

Ethoxychrysoidine solution. *1035201.*

A 1 g/L solution of *ethoxychrysoidine hydrochloride R* in *ethanol (96 per cent) R*.

Test for sensitivity. To a mixture of 5 mL of *dilute hydrochloric acid R* and 0.05 mL of the ethoxy-chrysoidine solution add 0.05 mL of *0.0167 M bromide-bromate*. The colour changes from red to light yellow within 2 min.

Ethyl acetate. $C_4H_8O_2$. (M_r 88.1). *1035300.* [141-78-6].

Clear, colourless liquid, soluble in water, miscible with ethanol (96 per cent).

d_{20}^{20}: 0.901 to 0.904.

bp: 76 °C to 78 °C.

Ethyl acetate, treated. *1035301.*

Disperse 200 g of *sulfamic acid R* in *ethyl acetate R* and make up to 1000 mL with the same solvent. Stir the suspension obtained for three days and filter through a filter paper.

Storage: use within 1 month.

Ethyl acrylate. $C_5H_8O_2$. (M_r 100.1). *1035400.* [140-88-5]. Ethyl prop-2-enoate.

Colourless liquid.

d_{20}^{20}: about 0.924.

n_D^{20}: about 1.406.

bp: about 99 °C.

mp: about − 71 °C.

4-[(Ethylamino)methyl]pyridine. $C_8H_{12}N_2$. (M_r 136.2). *1101300.* [33403-97-3].

Pale yellow liquid.

d_{20}^{20}: about 0.98.

n_D^{20}: about 1.516.

bp: about 98 °C.

Ethylbenzene. C_8H_{10}. (M_r 106.2). *1035800.* [100-41-4].

Content: minimum 99.5 per cent *m/m*, determined by gas chromatography.

Clear, colourless liquid, practically insoluble in water, soluble in acetone, and in ethanol (96 per cent).

d_{20}^{20}: about 0.87.

n_D^{20}: about 1.496.

bp: about 135 °C.

Ethyl benzenesulfonate. $C_8H_{10}O_3S$. (M_r 186.2). *1194800.* [515-46-8].

Content: minimum 97.0 per cent.

Colourless or slightly yellow liquid, slightly soluble in water, miscible with ethanol (96 per cent).

Density: about 1.22 g/mL (25 °C).

Ethyl benzoate. $C_9H_{10}O_2$. (M_r 150.2). *1135700.* [93-89-0].

A clear, colourless, refractive liquid, practically insoluble in water, miscible with ethanol (96 per cent) and with light petroleum.

d_4^{25}: about 1.050.

n_D^{20}: about 1.506.

bp: 211 °C to 213 °C.

Ethyl 5-bromovalerate. $C_7H_{13}BrO_2$. (M_r 209.1). *1142900.* [14660-52-7]. Ethyl 5-bromopentanoate.

Clear, colourless liquid.

d_{20}^{20}: about 1.321.

bp: 104 °C to 109 °C.

Ethyl clorazepate. $C_{18}H_{15}ClN_2O_3$. (M_r 342.8). *1204800.* [5606-55-3]. Ethyl (3*RS*)-7-chloro-2-oxo-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepine-3-carboxylate.

Ethyl cyanoacetate. $C_5H_7NO_2$. (M_r 113.1). *1035500.* [105-56-6].

Colourless or pale yellow liquid, slightly soluble in water, miscible with ethanol (96 per cent).

bp: 205 °C to 209 °C, with decomposition.

Ethylene chloride. $C_2H_4Cl_2$. (M_r 99.0). *1036000.* [107-06-2]. 1,2-Dichloroethane.

Clear, colourless liquid, soluble in about 120 parts of water and in 2 parts of ethanol (96 per cent).

d_{20}^{20}: about 1.25.

Distillation range (*2.2.11*). Not less than 95 per cent distils between 82 °C and 84 °C.

Ethylenediamine. $C_2H_8N_2$. (M_r 60.1). *1036500.* [107-15-3]. Ethane-1,2-diamine.

Clear, colourless, fuming liquid, strongly alkaline, miscible with water and with ethanol (96 per cent).

bp: about 116 °C.

Ethylene bis[3,3-di(3-*tert*-butyl-4-hydroxyphenyl)butyrate]. *1035900.* [32509-66-3].

See *ethylene bis[3,3-di(3-(1,1-dimethylethyl)-4-hydroxyphenyl)butyrate] R*.

Ethylene bis[3,3-di(3-(1,1-dimethylethyl)-4-hydroxyphenyl)butyrate]. $C_{50}H_{66}O_8$. (M_r 795). *1035900.* [32509-66-3]. Ethylene bis[3,3-di(3-*tert*-butyl-4-hydroxyphenyl)butyrate].

Crystalline powder, practically insoluble in water and in light petroleum, very soluble in acetone and in methanol.

mp: about 165 °C.

(Ethylenedinitrilo)tetra-acetic acid. $C_{10}H_{16}N_2O_8$. (M_r 292.2). *1105800.* [60-00-4]. *N,N'*-1,2-Ethanediylbis[*N*-(carboxymethyl)glycine]. Edetic acid.

White or almost white crystalline powder, very slightly soluble in water.

mp: about 250 °C, with decomposition.

Ethylene glycol. $C_2H_6O_2$. (M_r 62.1). *1036100.* [107-21-1]. Ethane-1,2-diol.

Content: minimum 99.0 per cent.

Colourless, slightly viscous liquid, hygroscopic, miscible with water and with ethanol (96 per cent).

d_{20}^{20}: 1.113 to 1.115.

n_D^{20}: about 1.432.

bp: about 198 °C.

mp: about − 12 °C.

Acidity. To 10 mL add 20 mL of *water R* and 1 mL of *phenolphthalein solution R*. Not more than 0.15 mL of *0.02 M sodium hydroxide* is required to change the colour of the indicator to pink.

Water (*2.5.12*): maximum 0.2 per cent

Ethylene glycol monododecyl ether. $C_{14}H_{30}O_2$. (M_r 230.4). *1191900.* [4536-30-5]. 2-(Dodecyloxy)ethan-1-ol.

Colourless or faintly green liquid.

Ethylene glycol monoethyl ether. $C_4H_{10}O_2$. (M_r 90.1). *1036200.* [110-80-5]. 2-Ethoxyethanol.

Content: minimum 99.0 per cent.

Clear, colourless liquid, miscible with water, with acetone and with ethanol (96 per cent).

d_{20}^{20}: about 0.93.

n_D^{25}: about 1.406.

bp: about 135 °C.

Ethylene glycol monomethyl ether. $C_3H_8O_2$. (M_r 76.1). *1036300.* [109-86-4]. 2-Methoxyethanol.

Content: minimum 99.0 per cent.

Clear, colourless liquid, miscible with water, with acetone and with ethanol (96 per cent).

d_{20}^{20}: about 0.97.

n_D^{20}: about 1.403.

bp: about 125 °C.

Ethylene oxide. C_2H_4O. (M_r 44.05). *1036400.* [75-21-8]. Oxirane.

Colourless, flammable gas, very soluble in water and in anhydrous ethanol.

Liquefaction point: about 12 °C.

Ethylene oxide solution. *1036402.*

Weigh a quantity of cool *ethylene oxide stock solution R* equivalent to 2.5 mg of ethylene oxide into a cool flask and dilute to 50.0 g with *macrogol 200 R1*. Mix well and dilute 2.5 g of this solution to 25.0 mL with *macrogol 200 R1* (5 µg of ethylene oxide per gram of solution). *Prepare immediately before use.*

The solution can be prepared using commercially available reagents instead of *ethylene oxide stock solution R*, making appropriate dilutions.

Ethylene oxide solution R1. *1036403.*

Dilute 1.0 mL of cooled *ethylene oxide stock solution R* (check the exact volume by weighing) to 50.0 mL with *macrogol 200 R1*. Mix well and dilute 2.5 g of this solution to 25.0 mL with *macrogol 200 R1*. Calculate the exact amount of ethylene oxide in parts per million from the volume determined by weighing and taking the relative density of *macrogol 200 R1* as 1.127. *Prepare immediately before use.*

The solution can be prepared using commercially available reagents instead of *ethylene oxide stock solution R*, making appropriate dilutions.

Ethylene oxide solution R2. *1036404.*

Weigh 1.00 g of cold *ethylene oxide stock solution R* (equivalent to 2.5 mg of ethylene oxide) into a cold flask containing 40.0 g of cold *macrogol 200 R1*. Mix and determine the exact mass and dilute to a calculated mass to obtain a solution containing 50 µg of ethylene oxide per gram of solution. Weigh 10.00 g into a flask containing about 30 mL of *water R*, mix and dilute to 50.0 mL with *water R* (10 µg/mL of ethylene oxide). *Prepare immediately before use.*

The solution can be prepared using commercially available reagents instead of *ethylene oxide stock solution R*, making appropriate dilutions.

Ethylene oxide solution R3. *1036405.*

Dilute 10.0 mL of *ethylene oxide solution R2* to 50.0 mL with *water R* (2 µg/mL of ethylene oxide). *Prepare immediately before use.*

Ethylene oxide solution R4. *1036407.*

Dilute 1.0 mL of *ethylene oxide stock solution R1* to 100.0 mL with *water R*. Dilute 1.0 mL of this solution to 25.0 mL with *water R*.

Ethylene oxide stock solution. *1036401.*

All operations carried out in the preparation of these solutions must be conducted in a fume cupboard. The operator must protect both hands and face by wearing polyethylene protective gloves and an appropriate face mask.

Store all solutions in an airtight container in a refrigerator at 4 °C to 8 °C. Carry out all determinations three times.

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 R1*. Determine the absorbed quantity of ethylene oxide by weighing before and after absorption (M_{eo}). Dilute to 100.0 mL with *macrogol 200 R1*. Mix well before use.

Assay. To 10 mL of a 500 g/L suspension of *magnesium chloride R* in *anhydrous ethanol R* add 20.0 mL of *0.1 M alcoholic hydrochloric acid R* in a flask. Stopper and shake to obtain a saturated solution and allow to stand overnight to equilibrate. Weigh 5.00 g of ethylene oxide stock solution (2.5 g/L) into the flask and allow to stand for 30 min. Titrate with *0.1 M alcoholic potassium hydroxide R* determining the end-point potentiometrically (*2.2.20*).

Carry out a blank titration, replacing the substance to be examined with the same quantity of *macrogol 200 R1*.

Ethylene oxide content in milligrams per gram is given by:

$$\frac{(V_0 - V_1) \times f \times 4.404}{m}$$

V_0, V_1 = volumes of *0.1 M alcoholic potassium hydroxide* used respectively for the blank titration and the assay,

f = factor of the alcoholic potassium hydroxide solution,

m = mass of the sample taken, in grams.

Ethylene oxide stock solution R1. *1036406.*

A 50 g/L solution of *ethylene oxide R* in *methanol R*.

Either use a commercially available reagent or prepare the solution corresponding to the aforementioned composition.

Ethylene oxide stock solution R2. *1036408.*

A 50 g/L solution of *ethylene oxide R* in *methylene chloride R*.

Either use a commercially available reagent or prepare the solution corresponding to the aforementioned composition.

Ethyl formate. $C_3H_6O_2$. (M_r 74.1). *1035600.* [109-94-4]. Ethyl methanoate.

Clear, colourless, flammable liquid, freely soluble in water, miscible with ethanol (96 per cent).

d_{20}^{20}: about 0.919.

n_D^{20}: about 1.36.

bp: about 54 °C.

2-Ethylhexane-1,3-diol. $C_8H_{18}O_2$. (M_r 146.2). *1105900.* [94-96-2].

Slightly oily liquid, soluble in anhydrous ethanol, 2-propanol, propylene glycol and castor oil.

d_{20}^{20}: about 0.942.

n_D^{20}: about 1.451.

bp: about 244 °C.

2-Ethylhexanoic acid. $C_8H_{16}O_2$. (M_r 144.2). *1036600.* [149-57-5].

Colourless liquid.

d_{20}^{20}: about 0.91.

n_D^{20}: about 1.425.

Related substances. Gas chromatography (*2.2.28*).

Injection: 1 µL of the test solution.

Test solution: suspend 0.2 g of the 2-ethylhexanoic acid in 5 mL of *water R*, add 3 mL of *dilute hydrochloric acid R* and 5 mL of *hexane R*, shake for 1 min, allow the layers to separate and use the upper layer. Carry out the chromatographic procedure as prescribed in the test for 2-ethylhexanoic acid in the monograph on *Amoxicillin sodium (0577)*.

Limit: the sum of the area of any peaks, apart from the principal peak and the peak due to the solvent, is not greater than 2.5 per cent of the area of the principal peak.

Ethyl 4-hydroxybenzoate. *1035700.* [120-47-8].

See *Ethyl parahydroxybenzoate R*.

***N*-Ethylmaleimide.** $C_6H_7NO_2$. (M_r 125.1). *1036700.* [128-53-0]. 1-Ethyl-1*H*-pyrrole-2,5-dione.

Colourless crystals, sparingly soluble in water, freely soluble in ethanol (96 per cent).

mp: 41 °C to 45 °C.

Storage: at a temperature of 2 °C to 8 °C.

Ethyl methanesulfonate. $C_3H_8O_3S$. (M_r 124.2). *1179300.* [62-50-0].

Clear, colourless liquid.

Content: minimum 99.0 per cent.

Density: about 1.206 g/cm^3 (20 °C).

n_D^{20}: about 1.418.

bp: about 213 °C.

Ethyl methyl ketone. *1054100.* [78-93-3].

See *methyl ethyl ketone R*.

2-Ethyl-2-methylsuccinic acid. $C_7H_{12}O_4$. (M_r 160.2). *1036800.* [631-31-2]. 2-Ethyl-2-methylbutanedioic acid.

mp: 104 °C to 107 °C.

Ethyl parahydroxybenzoate. *1035700.* [120-47-8].

See *Ethyl parahydroxybenzoate (0900)*.

2-Ethylpyridine. C_7H_9N. (M_r 107.2). *1133400.* [100-71-0].

Colourless or brownish liquid.

d_{20}^{20}: about 0.939.

n_D^{20}: about 1.496.

bp: about 149 °C.

Ethyl toluenesulfonate. $C_9H_{12}O_3S$. (M_r 200.3). *1191000.* [80-40-0]. Ethyl 4-methylbenzenesulfonate. Ethyl tosilate.

Content: minimum 97.0 per cent.

Density: about 1.17 g/mL (25 °C).

bp: about 160 °C.

mp: about 33 °C.

Ethylvinylbenzene-divinylbenzene copolymer. *1036900.*

Porous, rigid, cross-linked polymer beads. Several grades are available with different sizes of bead. The size range of the beads is specified after the name of the reagent in the tests where it is used.

Eugenol. $C_{10}H_{12}O_2$. (M_r 164.2). *1037000.* [97-53-0]. 4-Allyl-2-methoxyphenol.

Colourless or pale yellow, oily liquid, darkening on exposure to air and light and becoming more viscous, practically insoluble in water, miscible with ethanol (96 per cent) and with fatty and essential oils.

d_{20}^{20}: about 1.07.

bp: about 250 °C.

Eugenol used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Clove oil (1091)*.

Test solution. The substance to be examined.

Content: minimum 98.0 per cent, calculated by the normalisation procedure.

Storage: protected from light.

Euglobulins, bovine. *1037100.*

Use fresh bovine blood collected into an anticoagulant solution (for example, sodium citrate solution). Discard any haemolysed blood. Centrifuge at 1500-1800 *g* at 15-20 °C to obtain a supernatant plasma poor in platelets.

To 1 L of bovine plasma add 75 g of *barium sulfate R* and shake for 30 min. Centrifuge at not less than 1500-1800 *g* at 15-20 °C and draw off the clear supernatant. Add 10 mL of a 0.2 mg/mL solution of *aprotinin R* and shake to ensure mixing. In a container with a minimum capacity of 30 L in a chamber at 4 °C introduce 25 L of *distilled water R* at 4 °C and add about 500 g of solid carbon dioxide. Immediately add, while stirring, the supernatant obtained from the plasma. A white precipitate is formed. Allow to settle at 4 °C for 10-15 h. Remove the clear supernatant solution by siphoning. Collect the precipitate by centrifuging at 4 °C. Suspend the precipitate by dispersing mechanically in 500 mL of *distilled water R* at 4 °C, shake for 5 min and collect the precipitate by centrifuging at 4 °C. Disperse the precipitate mechanically in 60 mL of a solution containing 9 g/L of *sodium chloride R* and 0.9 g/L *sodium citrate R* and adjust to pH 7.2-7.4 by adding a 10 g/L solution of *sodium hydroxide R*. Filter through a sintered glass filter (*2.1.2*); to facilitate the dissolution of the precipitate crush the particles of the precipitate with a suitable instrument. Wash the filter and the instrument with 40 mL of the chloride-citrate solution described above and dilute to 100 mL with the same solution. Freeze-dry the solution. The yields are generally 6 g to 8 g of euglobulins per litre of bovine plasma.

Test for suitability. For this test, prepare the solutions using *phosphate buffer solution pH 7.4 R* containing 30 g/L of *bovine albumin R*.

Into a test-tube 8 mm in diameter placed in a water-bath at 37 °C introduce 0.2 mL of a solution of a reference preparation of urokinase containing 100 IU/mL and 0.1 mL of a solution of *human thrombin R* containing 20 IU/mL. Add rapidly 0.5 mL of a solution containing 10 mg of bovine euglobulins per millilitre. A firm clot forms in less than 10 s. Note the time that elapses between the addition of the solution of bovine euglobulins and the lysis of the clot. The lysis time does not exceed 15 min.

Storage: protected from moisture at 4 °C; use within 1 year.

Euglobulins, human. *1037200.*

For the preparation, use fresh human blood collected into an anticoagulant solution (for example sodium citrate solution) or human blood for transfusion that has been collected in plastic blood bags and which has just reached its expiry date. Discard any haemolysed blood. Centrifuge at 1500-1800 *g* at 15 °C to obtain a supernatant plasma poor in platelets. Iso-group plasmas may be mixed.

To 1 L of the plasma add 75 g of *barium sulfate R* and shake for 30 min. Centrifuge at not less than 15 000 *g* at 15 °C and draw off the clear supernatant. Add 10 mL of a solution of *aprotinin R* containing 0.2 mg/mL and shake to ensure mixing. In a container with a minimum capacity of 30 L in a chamber at 4 °C introduce 25 L of *distilled water R* at 4 °C and add about 500 g of solid carbon dioxide. Immediately add while stirring the supernatant obtained from the plasma. A white precipitate is formed. Allow to settle at 4 °C for 10-15 h. Remove the clear supernatant solution by siphoning. Collect the precipitate by centrifuging at 4 °C. Suspend the precipitate by dispersing mechanically in 500 mL of *distilled water R* at 4 °C, shake for 5 min and collect the precipitate by centrifuging at 4 °C. Disperse the precipitate mechanically in 60 mL of a solution containing 9 g/L of *sodium chloride R* and 0.9 g/L of *sodium citrate R*, and adjust the pH to 7.2-7.4 by adding a 10 g/L solution of *sodium hydroxide R*. Filter through a sintered-glass filter (*2.1.2*); to facilitate the dissolution of the precipitate crush the particles of the precipitate with a suitable instrument. Wash the filter and the instrument with 40 mL of the chloride-citrate solution described above and dilute to 100 mL with the same solution. Freeze-dry the solution. The yields are generally 6 g to 8 g of euglobulins per litre of human plasma.

Test for suitability. For this test, prepare the solutions using *phosphate buffer solution pH 7.2 R* containing 30 g/L of *bovine albumin R*. Into a test-tube 8 mm in diameter placed in a water-bath at 37 °C introduce 0.1 mL of a solution of a reference preparation of streptokinase containing 10 IU of streptokinase activity per millilitre and 0.1 mL of a solution of *human thrombin R* containing 20 IU/mL. Add rapidly 1 mL

of a solution containing 10 mg of human euglobulins per millilitre. A firm clot forms in less than 10 s. Note the time that elapses between the addition of the solution of human euglobulins and the lysis of the clot. The lysis time does not exceed 15 min.

Storage: in an airtight container at 4 °C; use within 1 year.

Evodiamine. $C_{19}H_{17}N_3O$. (M_r 303.4). *1199400.* [518-17-2]. (13b*S*)-14-Methyl-8,13,13b,14-tetrahydro-indolo[2′,3′:3,4]pyrido[2,1-*b*]quinazolin-5(7*H*)-one.

Extraction resin. *1204900.*

Solid phase extraction resin containing 2,2′-oxybis(*N*,*N*-dioctylacetamide) (*N*,*N*,*N*′,*N*′-tetra-*n*-octyldiglycolamide).

Factor VII-deficient plasma. *1185900.*

Plasma that is deficient in factor VII.

Factor Xa, bovine, coagulation. *1037300.* [9002-05-5].

An enzyme which converts prothrombin to thrombin. The semi-purified preparation is obtained from liquid bovine plasma and it may be prepared by activation of the zymogen factor X with a suitable activator such as Russell's viper venom.

Storage: freeze-dried preparation at − 20 °C and frozen solution at a temperature lower than − 20 °C.

Factor Xa solution, bovine. *1037301.*

Reconstitute as directed by the manufacturer and dilute with *tris(hydroxymethyl)aminomethane sodium chloride buffer solution pH 7.4 R*.

Any change in the absorbance of the solution, measured at 405 nm (*2.2.25*) against *tris(hydroxymethyl)aminomethane sodium chloride buffer solution pH 7.4 R* and from which the blank absorbance has been substracted, is not more than 0.20 per minute.

Factor Xa solution, bovine R1. *1037302.*

Reconstitute as directed by the manufacturer and dilute to 1.4 nkat/mL with *tris(hydroxymethyl)aminomethane-EDTA buffer solution pH 8.4 R*.

Factor Xa solution, bovine R2. *1037303.*

Reconstitute as directed by the manufacturer and dilute with *tris(hydroxymethyl)aminomethane-EDTA buffer solution pH 8.4 R1* to obtain a solution that gives an absorbance between 0.65 and 1.25 at 405 nm when determining the blank amidolytic activity according to general chapter *2.7.5* using the end-point method.

Fargesin. $C_{21}H_{22}O_6$. (M_r 370.4). *1200200.* [31008-19-2].

5-[(3*SR*,3a*RS*,6*RS*,6a*RS*)-6-(3,4-Dimethoxyphenyl)-1,3,3a,4,6,6a-hexahydrofuro[3,4-*c*]furan-3-yl]-1,3-benzodioxole.

***(E,E)*-Farnesol.** $C_{15}H_{26}O$. (M_r 222.4). *1161000.* [106-28-5]. *trans,trans*-Farnesol. (2*E*,6*E*)-3,7,11-Trimethyldodeca-2,6,10-trien-1-ol.

Fast blue B salt. $C_{14}H_{12}Cl_2N_4O_2$. (M_r 339.2). *1037400.* [84633-94-3].

Schultz No. 490.

Colour Index No. 37235.

3,3′-Dimethoxy(biphenyl)-4,4′-bisdiazonium dichloride.

Dark green powder, soluble in water. It is stabilised by addition of zinc chloride.

Storage: in an airtight container, at a temperature between 2 °C and 8 °C.

Fast blue B salt solution. *1037401.*

Dissolve 140 mg of *fast blue B salt R* in 10 mL of *water R* and mix with 50 mL of *methylene chloride R* and 140 mL of *methanol R*.

Storage: protected from light at a temperature of 4 °C; use within 1 week.

Fast red B salt. $C_{17}H_{13}N_3O_9S_2$. (M_r 467.4). *1037500.* [49735-71-9].

Schultz No. 155.

Colour Index No. 37125.

2-Methoxy-4-nitrobenzenediazonium hydrogen naphthalene-1,5-disulfonate.

Orange-yellow powder, soluble in water, slightly soluble in ethanol (96 per cent).

Storage: in an airtight container, protected from light, at 2 °C to 8 °C.

Fenchlorphos. $C_8H_8Cl_3O_3PS$. (M_r 321.5). *1127200.* [299-84-3].

mp: about 35 °C.

A suitable certified reference solution (10 ng/µL in cyclohexane) may be used.

Fenchone. $C_{10}H_{16}O$. (M_r 152.2). *1037600.* [7787-20-4]. (1*R*)-1,3,3-Trimethylbicyclo[2.2.1]heptan-2-one.

Oily liquid, miscible with ethanol (96 per cent), practically insoluble in water.

n_D^{20}: about 1.46.

bp_{15mm}: 192 °C to 194 °C.

Fenchone used in gas chromatography complies with the following test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Bitter fennel (0824)*.

Test solution. The substance to be examined.

Content: minimum 98.0 per cent, calculated by the normalisation procedure.

Fenvalerate. $C_{25}H_{22}ClNO_3$. (M_r 419.9). *1127300.* [51630-58-1].

bp: about 300 °C.

A suitable certified reference solution (10 ng/µL in cyclohexane) may be used.

Ferric ammonium sulfate. $FeNH_4(SO_4)_2,12H_2O$. (M_r 482.2). *1037700.* [7783-83-7]. Ammonium iron disulfate dodecahydrate.

Pale-violet crystals, efflorescent, very soluble in water, practically insoluble in ethanol (96 per cent).

Ferric ammonium sulfate solution R2. *1037702.*

A 100 g/L solution of *ferric ammonium sulfate R*. If necessary filter before use.

Ferric ammonium sulfate solution R5. *1037704.*

Shake 30.0 g of *ferric ammonium sulfate R* with 40 mL of *nitric acid R* and dilute to 100 mL with *water R*. If the solution is turbid, centrifuge or filter it.

Storage: protected from light.

Ferric ammonium sulfate solution R6. *1037705.*

Dissolve 20 g of *ferric ammonium sulfate R* in 75 mL of *water R*, add 10 mL of a 2.8 per cent *V/V* solution of *sulfuric acid R* and dilute to 100 mL with *water R*.

Ferric chloride. $FeCl_3,6H_2O$. (M_r 270.3). *1037800.* [10025-77-1]. Iron trichloride hexahydrate.

Yellowish-orange or brownish crystalline masses, deliquescent, very soluble in water, soluble in ethanol (96 per cent). On exposure to light, ferric chloride and its solutions are partly reduced.

Storage: in an airtight container.

Ferric chloride solution R1. *1037801.*

A 105 g/L solution of *ferric chloride R*.

Ferric chloride solution R2. *1037802.*

A 13 g/L solution of *ferric chloride R*.

Ferric chloride solution R3. *1037803.*

Dissolve 2.0 g of *ferric chloride R* in *anhydrous ethanol R* and dilute to 100.0 mL with the same solvent.

Ferric chloride-ferricyanide-arsenite reagent. *1037805.*

Immediately before use mix 10 mL of a 27 g/L solution of *ferric chloride R* in *dilute hydrochloric acid R*, 7 mL of *potassium ferricyanide solution R*, 3 mL of *water R* and 10 mL of *sodium arsenite solution R*.

Ferric chloride-sulfamic acid reagent. *1037804.*

A solution containing 10 g/L of *ferric chloride R* and 16 g/L of *sulfamic acid R*.

Ferric nitrate. $Fe(NO_3)_3,9H_2O$. (M_r 404). *1106100.* [7782-61-8].

Content: minimum 99.0 per cent *m/m* of $Fe(NO_3)_3,9H_2O$.

Light-purple crystals or crystalline mass, very soluble in water.

Free acid: not more than 0.3 per cent (as HNO_3).

Ferric sulfate. $Fe_2(SO_4)_3,xH_2O$. *1037900.* [15244-10-7]. Iron(III) trisulfate hydrated.

Yellowish-white powder, very hygroscopic, decomposes in air, slightly soluble in water and in ethanol (96 per cent).

Storage: in an airtight container, protected from light.

Ferric sulfate solution. *1037901.*

Dissolve 50 g of *ferric sulfate R* in an excess of *water R*, add 200 mL of *sulfuric acid R* and dilute to 1000 mL with *water R*.

Ferric sulfate pentahydrate. $Fe_2(SO_4)_3,5H_2O$. (M_r 489.9). *1153700.* [142906-29-4].

White or yellowish powder.

Ferrocyphene. $C_{26}H_{16}FeN_6$. (M_r 468.3). *1038000.* [14768-11-7]. Dicyanobis(1,10-phenanthroline)iron(II).

Violet-bronze, crystalline powder, practically insoluble in water and in ethanol (96 per cent).

Storage: protected from light and moisture.

Ferroin. *1038100.* [14634-91-4].

Dissolve 0.7 g of *ferrous sulfate R* and 1.76 g of *phenanthroline hydrochloride R* in 70 mL of *water R* and dilute to 100 mL with the same solvent.

Test for sensitivity. To 50 mL of *dilute sulfuric acid R* add 0.1 mL of *ferroin R*. After the addition of 0.1 mL of *0.1 M ammonium and cerium nitrate* the colour changes from red to light blue.

Ferrous ammonium sulfate. $Fe(NH_4)_2(SO_4)_2,6H_2O$. (M_r 392.2). *1038200.* [7783-85-9]. Diammonium iron disulfate hexahydrate.

Pale bluish-green crystals or granules, freely soluble in water, practically insoluble in ethanol (96 per cent).

Storage: protected from light.

Ferrous sulfate. *1038300.* [7782-63-0].

See *Ferrous sulfate heptahydrate (0083)*.

Ferrous sulfate solution R2. *1038301.*

Dissolve 0.45 g of *ferrous sulfate R* in 50 mL of *0.1 M hydrochloric acid* and dilute to 100 mL with *carbon dioxide-free water R*. Prepare immediately before use.

Ferulic acid. $C_{10}H_{10}O_4$. (M_r 194.2). *1149500.* [1135-24-6]. 4-Hydroxy-3-methoxycinnamic acid. 3-(4-Hydroxy-3-methoxyphenyl)propenoic acid.

Faint yellow powder, freely soluble in methanol.

mp: 172.9 °C to 173.9 °C.

Ferulic acid used in the assay of eleutherosides in Eleutherococcus (1419) complies with the following additional test.

Assay. Liquid chromatography (*2.2.29*) as prescribed in the monograph *Eleutherococcus (1419)*.

Content: minimum 99 per cent, calculated by the normalisation procedure.

Fibrin blue. *1101400.*

Mix 1.5 g of fibrin with 30 mL of a 5 g/L solution of *indigo carmine R* in 1 per cent *V/V dilute hydrochloric acid R*. Heat the mixture to 80 °C and maintain at this temperature whilst stirring for about 30 min. Allow to cool. Filter. Wash extensively by resuspension in 1 per cent *V/V dilute hydrochloric acid R* and mixing for about 30 min; filter. Repeat the washing operation three times. Dry at 50 °C. Grind.

Fibrin congo red. *1038400.*

Take 1.5 g of fibrin and leave overnight in 50 mL of a 20 g/L solution of *congo red R* in *ethanol (90 per cent V/V) R*. Filter, rinse the fibrin with *water R* and store under *ether R*.

Fibrinogen. *1038500.* [9001-32-5].

See *Human fibrinogen, freeze-dried (0024)*.

Fixing solution. *1122600.*

To 250 mL of *methanol R*, add 0.27 mL of *formaldehyde R* and dilute to 500.0 mL with *water R*.

Fixing solution for isoelectric focusing in polyacrylamide gel. *1138700.*

A solution containing 35 g of *sulfosalicylic acid R* and 100 g of *trichloroacetic acid R* per litre of *water R*.

Flufenamic acid. $C_{14}H_{10}F_3NO_2$. (M_r 281.2). *1106200.* [530-78-9]. 2-[[3-(Trifluoromethyl)phenyl]amino]benzoic acid.

Pale yellow, crystalline powder or needles, practically insoluble in water, freely soluble in ethanol (96 per cent).

mp: 132 °C to 135 °C.

Flumazenil. *1149600.* [78755-81-4].

See *Flumazenil (1326)*.

Flunitrazepam. *1153800.* [1622-62-4].

See *Flunitrazepam (0717)*.

Fluorene. $C_{13}H_{10}$. (M_r 166.2). *1127400.* [86-73-7]. Diphenylenemethane.

White or almost white crystals, freely soluble in anhydrous acetic acid, soluble in hot ethanol (96 per cent).

mp: 113 °C to 115 °C.

(9-Fluorenyl)methyl chloroformate. $C_{15}H_{11}ClO_2$. (M_r 258.7). *1180100.* [28920-43-6]. Fluoren-9-ylmethyl chloromethanoate.

mp: about 63 °C.

Fluorescamine. $C_{17}H_{10}O_4$. (M_r 278.3). *1135800.* [38183-12-9]. 4-Phenylspiro[furan-2(3*H*),1'(3'*H*)-isobenzofuran]-3,3'-dione.

mp: 154 °C to 155 °C.

Fluorescein. $C_{20}H_{12}O_5$. (M_r 332.3). *1106300.* [2321-07-5]. 3′,6′-Dihydroxyspiro[isobenzofurane-1(3*H*),9′-[9*H*]xanthen]-3-one.

Orange-red powder, practically insoluble in water, soluble in warm ethanol (96 per cent), soluble in alkaline solutions. In solution, fluorescein displays a green fluorescence.

mp: about 315 °C.

Fluorescein-conjugated rabies antiserum. *1038700.*

Immunoglobulin fraction with a high rabies antibody titre, prepared from the sera of suitable animals that have been immunised with inactivated rabies virus; the immunoglobulin is conjugated with fluorescein isothiocyanate.

Fluorocholine chloride. $C_5H_{13}ClFNO$. (M_r 157.6). *1195700.* [459424-38-5]. *N*-(Fluoromethyl)-2-hydroxy-*N*,*N*-dimethylethan-1-aminium chloride.

Colourless, hygroscopic crystals.

mp: about 184 °C.

2-Fluoro-2-deoxy-D-glucose. $C_6H_{11}FO_5$. (M_r 182.2). *1113900.* [86783-82-6].

White or almost white crystalline powder.

mp: 174 °C to 176 °C.

2-Fluoro-2-deoxy-D-mannose. $C_6H_{11}FO_5$. (M_r 182.1). *1172100.* [38440-79-8].

Colourless semi-solid.

Fluorodinitrobenzene. $C_6H_3FN_2O_4$. (M_r 186.1). *1038800.* [70-34-8]. 1-Fluoro-2,4-dinitrobenzene.

Pale yellow liquid or crystals, soluble in propylene glycol.

mp: about 29 °C.

Content: minimum 99.0 per cent, determined by gas chromatography.

1-Fluoro-2,4-dinitrophenyl-5-L-alaninamide. $C_9H_9FN_4O_5$. (M_r 272.2). *1194900.* [95713-52-3]. N^{α}-(5-Fluoro-2,4-dinitrophenyl)-L-alaninamide. Marfey's reagent. FDAA.

Yellow or orange powder.

mp: about 228 °C.

Enantiomeric purity: minimum 99.5 per cent.

DL-6-Fluorodopa hydrochloride. $C_9H_{11}ClFNO_4$. (M_r 251.6). *1169200.* (2*RS*)-2-Amino-3-(2-fluoro-4,5-dihydroxyphenyl)propanoic acid hydrochloride. 2-Fluoro-5-hydroxy-DL-tyrosine hydrochloride.

White or almost white powder.

Fluoroethyl(2-hydroxyethyl)dimethylammonium chloride. $C_6H_{15}ClFNO$. (M_r 171.6). *1195800.* [479407-08-4]. *N*-(2-Fluoroethyl)-2-hydroxy-*N*,*N*-dimethylethan-1-aminium chloride.

Slightly yellow powder.

Fluoroethyl-D-tyrosine hydrochloride. $C_{11}H_{15}FNO_3Cl$. (M_r 263.7). *1192000.* (2*R*)-2-Amino-3-[4-(2-fluoroethoxy)phenyl]propanoic acid hydrochloride.

Content: minimum 95 per cent.

Colourless or almost colourless crystals.

Fluoroethyl-L-tyrosine hydrochloride. $C_{11}H_{15}FNO_3Cl$. (M_r 263.7). *1192100.* (2*S*)-2-Amino-3-[4-(2-fluoroethoxy)phenyl]propanoic acid hydrochloride.

Content: minimum 95 per cent.

Colourless or almost colourless crystals.

6-Fluorolevodopa hydrochloride. $C_9H_{11}ClFNO_4$. (M_r 251.6). *1169300.* [144334-59-8]. (2*S*)-2-Amino-3-(2-fluoro-4,5-dihydroxyphenyl)propanoic acid hydrochloride. 2-Fluoro-5-hydroxy-L-tyrosine hydrochloride.

Colourless or almost colourless solid, soluble in water.

Fluoromisonidazole. $C_6H_8FN_3O_3$. (M_r 189.1). *1186000.* [13551-89-8]. (2*RS*)-1-Fluoro-3-(2-nitro-1*H*-imidazol-1-yl)propan-2-ol. FMISO.

Content: minimum 95 per cent.

Yellow crystals.

1-Fluoro-2-nitro-4-(trifluoromethyl)benzene. $C_7H_3F_4NO_2$. (M_r 209.1). *1038900.* [367-86-2].

mp: about 197 °C.

Folic acid. *1039000.* [75708-92-8].

See *Folic acid hydrate (0067)*.

Formaldehyde. *1039100.* [50-00-0].

See *Formaldehyde solution R*.

Formaldehyde solution. *1039101.*

See *Formaldehyde solution (35 per cent) (0826)*.

Formaldehyde solution R1. *1039102.*

Complies with the requirements prescribed in the monograph *Formaldehyde solution (35 per cent) (0826)* with the following modification.

Content: 36.5 per cent *m/m* to 38.0 per cent *m/m* of formaldehyde (CH_2O; M_r 30.03).

Formamide. CH_3NO. (M_r 45.0). *1039200.* [75-12-7].

Clear, colourless, oily liquid, hygroscopic, miscible with water and with ethanol (96 per cent). It is hydrolysed by water.

d_{20}^{20}: about 1.134.

bp: about 210 °C.

Content: minimum 99.5 per cent.

Storage: in an airtight container.

Formamide R1. *1039202.*

Complies with the requirements prescribed for *formamide R* with the following additional requirement.

Water (*2.5.12*): maximum 0.1 per cent determined with an equal volume of *anhydrous methanol R*.

Formamide, treated. *1039201.*

Disperse 1.0 g of *sulfamic acid R* in 20.0 mL of *formamide R* containing 5 per cent *V/V* of *water R*.

Formic acid, anhydrous. CH_2O_2. (M_r 46.03). *1039300.* [64-18-6].

Content: minimum 98.0 per cent *m/m*.

Colourless liquid, corrosive, miscible with water and with ethanol (96 per cent).

d_{20}^{20}: about 1.22.

Assay. Weigh accurately a conical flask containing 10 mL of *water R*, quickly add about 1 mL of the acid and weigh again. Add 50 mL of *water R* and titrate with *1 M sodium hydroxide*, using 0.5 mL of *phenolphthalein solution R* as indicator.

1 mL of *1 M sodium hydroxide* is equivalent to 46.03 mg of CH_2O_2.

Fructose. *1106400.* [57-48-7].

See *Fructose (0188)*.

Fuchsin, basic. *1039400.* [632-99-5].

A mixture of rosaniline hydrochloride ($C_{20}H_{20}ClN_3$; M_r 337.9; Colour Index No. 42510; Schultz No. 780) and *para*-rosaniline hydrochloride ($C_{19}H_{18}ClN_3$; M_r 323.8; Colour Index No. 42500; Schultz No. 779).

If necessary, purify in the following manner. Dissolve 1 g in 250 mL of *dilute hydrochloric acid R*. Allow to stand for 2 h at room temperature, filter and neutralise with *dilute sodium hydroxide solution R* and add 1 mL to 2 mL in excess. Filter the precipitate through a sintered-glass filter (40) (*2.1.2*) and wash with *water R*. Dissolve the precipitate in 70 mL of *methanol R*, previously heated to boiling, and add 300 mL of *water R* at 80 °C. Allow to cool to room temperature, filter and dry the crystals *in vacuo*.

Crystals with a greenish-bronze sheen, soluble in water and in ethanol (96 per cent).

Storage: protected from light.

Fuchsin solution, decolorised. *1039401.*

Dissolve 0.1 g of *basic fuchsin R* in 60 mL of *water R*. Add a solution containing 1 g of *anhydrous sodium sulfite R* or 2 g of *sodium sulfite heptahydrate R* in 10 mL of *water R*. Slowly and with continuous shaking add 2 mL of *hydrochloric acid R*. Dilute to 100 mL with *water R*. Allow to stand protected from light for at least 12 h, decolorise

with *activated charcoal R* and filter. If the solution becomes cloudy, filter before use. If on standing the solution becomes violet, decolorise again by adding *activated charcoal R*.

Test for sensitivity. To 1.0 mL add 1.0 mL of *water R* and 0.1 mL of *aldehyde-free alcohol R*. Add 0.2 mL of a solution containing 0.1 g/L of formaldehyde (CH_2O, M_r 30.03). A pale-pink colour develops within 5 min.

Storage: protected from light.

Fuchsin solution, decolorised R1. *1039402.*

To 1 g of *basic fuchsin R* add 100 mL of *water R*. Heat to 50 °C and allow to cool with occasional shaking. Allow to stand for 48 h, shake and filter. To 4 mL of the filtrate add 6 mL of *hydrochloric acid R*, mix and dilute to 100 mL with *water R*. Allow to stand for at least 1 h before use.

Fucose. $C_6H_{12}O_5$. (M_r 164.2). *1039500.* [6696-41-9]. 6-Deoxy-L-galactose.

White or almost white powder, soluble in water and in ethanol (96 per cent).

$[\alpha]_D^{20}$: about – 76, determined on a 90 g/L solution 24 h after dissolution.

mp: about 140 °C.

Fumaric acid. $C_4H_4O_4$. (M_r 116.1). *1153200.* [110-17-8]. (*E*)-Butenedioic acid.

White or almost white crystals, slightly soluble in water, soluble in ethanol (96 per cent), slightly soluble in acetone.

mp: about 300 °C.

Furfural. $C_5H_4O_2$. (M_r 96.1). *1039600.* [98-01-1]. 2-Furaldehyde. 2-Furanecarbaldehyde.

Clear, colourless to brownish-yellow, oily liquid, miscible in 11 parts of water, miscible with ethanol (96 per cent).

d_{20}^{20}: 1.155 to 1.161.

Distillation range (*2.2.11*). Not less than 95 per cent distils between 159 °C and 163 °C.

Storage: in a dark place.

Gadolinium chloride hexahydrate. $GdCl_3,6H_2O$. (M_r 371.7). *1198400.* [13450-84-5]. Gadolinium trichloride hexahydrate.

Content: minimum 99.9 per cent.

Gadolinium sulfate octahydrate. $Gd_2(SO_4)_3,8H_2O$. (M_r 747). *1195300.* [13450-87-8].

Colourless, crystalline powder.

Galactose. $C_6H_{12}O_6$. (M_r 180.2). *1039700.* [59-23-4]. D-(+)-Galactose.

White or almost white, crystalline powder, freely soluble in water.

$[\alpha]_D^{20}$: + 79 to + 81, determined on a 100 g/L solution in *water R* containing about 0.05 per cent of NH_3.

1,6-Galactosylgalactose. $C_{12}H_{22}O_{11}$. (M_r 342.3). *1195900.* [5077-31-6]. 6-*O*-β-D-Galactopyranosyl-D-galactopyranose.

White or almost white powder.

Galacturonic acid. $C_6H_{10}O_7$. (M_r 194.1). *1196000.* [685-73-4]. D-(+)-galacturonic acid. (2*S*,3*R*,4*S*,5*R*)-2,3,4,5-Tetrahydroxy-6-oxo-hexanoic acid.

$[\alpha]_D^{20}$: about + 53°, determined on a 100 g/L solution.

Gallic acid. $C_7H_6O_5,H_2O$. (M_r 188.1). *1039800.* [5995-86-8]. 3,4,5-Trihydroxybenzoic acid monohydrate.

Crystalline powder or long needles, colourless or slightly yellow, soluble in water, freely soluble in hot water, in ethanol (96 per cent) and in glycerol.

It loses its water of crystallisation at 120 °C.

mp: about 260 °C, with decomposition.

Chromatography. Thin-layer chromatography (*2.2.27*) as prescribed in the monograph *Bearberry leaf (1054)*; the chromatogram shows only one principal spot.

Gallium (^{68}Ga) chloride solution. $^{68}GaCl_3$. (M_r 174.3). *1182500.*

Solution containing gallium-68 in the form of gallium chloride in *dilute hydrochloric acid R*.

Content: 90 per cent to 110 per cent of the declared gallium-68 radioactivity at the date and time stated on the label.

Gastric juice, artificial. *1039900.*

Dissolve 2.0 g of *sodium chloride R* and 3.2 g of *pepsin powder R* in *water R*. Add 80 mL of *1 M hydrochloric acid* and dilute to 1000 mL with *water R*.

Gastrodin. $C_{13}H_{18}O_7$. (M_r 286.3). *1203600.* [62499-27-8]. 4-(Hydroxymethyl)phenyl α-D-glucopyranoside. (2*R*,3*S*,4*S*,5*R*,6*S*)-2-(Hydroxymethyl)-6-[4-(hydroxymethyl)-phenoxy]oxane-3,4,5-triol.

GC concentrical column. *1135100.*

A commercially available system consisting of 2 concentrically arranged tubes. The outer tube is packed with molecular sieves and the inner tube is packed with a porous polymer mixture. The main application is the separation of gases.

Gelatin. *1040000.* [9000-70-8].

See *Gelatin (0330)*.

Gelatin, hydrolysed. *1040100.*

Dissolve 50 g of *gelatin R* in 1000 mL of *water R*. Autoclave in saturated steam at 121 °C for 90 min and freeze dry.

Geniposide. $C_{17}H_{24}O_{10}$. (M_r 388.4). *1196800.* [24512-63-8]. Methyl (1*S*,4a*S*,7a*S*)-1-(β-D-glucopyranosyloxy)-7-(hydroxymethyl)-1,4a,5,7a-tetrahydrocyclopenta[*c*]pyran-4-carboxylate.

Geraniol. $C_{10}H_{18}O$. (M_r 154.2). *1135900.* [106-24-1]. (*E*)-3,7-Dimethylocta-2,6-dien-1-ol.

Oily liquid, slight odour of rose, practically insoluble in water, miscible with ethanol (96 per cent).

Geraniol used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Citronella oil (1609)*.

Content: minimum 98.5 per cent, calculated by the normalisation procedure.

Storage: in an airtight container, protected from light

Geranyl acetate. $C_{12}H_{20}O_2$. (M_r 196.3). *1106500.* [105-87-3]. (*E*)-3,7-Dimethylocta-2,6-dien-1-yl acetate.

Colourless or slightly yellow liquid, slight odour of rose and lavender.

Geranyl acetate used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Bitter-orange-flower oil (1175)*.

Test solution. The substance to be examined.

Content: minimum 98.0 per cent, calculated by the normalisation procedure.

Ginsenoside Rb1. $C_{54}H_{92}O_{23},3H_2O$. (M_r 1163). *1127500.* [41753-43-9]. (20*S*)-3β-Di-D-glucopyranosyl-20-di-D-glucopyranosylprotopanaxadiol. (20*S*)-3β-[(2-*O*-β-D-Glucopyranosyl-β-D-glucopyranosyl)oxy]-20-[(6-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]-5α-dammar-24-en-12β-ol. (20*S*)-3β-[(2-*O*-β-D-Glucopyranosyl-β-D-glucopyranosyl)oxy]-20-[(6-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]-4,4,8,14-tetramethyl-18-nor-5α-cholest-24-en-12β-ol.

A colourless solid, soluble in water, in anhydrous ethanol and in methanol.

$[\alpha]_D^{20}$: + 11.3 determined on a 10 g/L solution in *methanol R*.

mp: about 199 °C.

Water (2.5.12): maximum 6.8 per cent.

Assay. Liquid chromatography (*2.2.29*) as prescribed in the monograph *Ginseng (1523)*.

Test solution. Dissolve 3.0 mg, accurately weighed, of *ginsenoside Rb1* in 10 mL of *methanol R*.

Content: minimum 95.0 per cent, calculated by the normalisation procedure.

Ginsenoside Re. $C_{48}H_{82}O_{18}$. (M_r 947.2). *1157800.* [52286-59-6]. (3β,6α,12β)-20-(β-D-Glucopyranosyloxy)-3,12-dihydroxydammar-24-en-6-yl 2-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranoside.

Colourless solid, soluble in water, in ethanol (96 per cent) and in methanol.

Ginsenoside Rf. $C_{42}H_{72}O_{14},2H_2O$. (M_r 837). *1127700.* [52286-58-5]. (20*S*)-6-*O*-[β-D-Glucopyranosyl-(1→2)-β-D-glycopyranoside]-dammar-24-ene-3β,6α,12β,20-tetrol.

A colourless solid, soluble in water, in anhydrous ethanol and in methanol.

$[\alpha]_D^{20}$: + 12.8 determined on a 10 g/L solution in *methanol R*.

mp: about 198 °C.

Ginsenoside Rg1. $C_{42}H_{72}O_{14},2H_2O$. (M_r 837). *1127600.* [22427-39-0]. (20*S*)-6β-D-Glucopyranosyl-D-glucopyranosylprotopanaxatriol. (20*S*)-6α,20-Bis(β-D-glucopyranosyloxy)-5α-dammar-24-ene-3β,12β-diol. (20*S*)-6α,20-Bis(β-D-glucopyranosyloxy)-4,4,8,14-tetramethyl-18-nor-5α-cholest-24-ene-3β,12β-diol.

A colourless solid, soluble in water, in anhydrous ethanol and in methanol.

$[\alpha]_D^{20}$: + 31.2 determined on a 10 g/L solution in *methanol R*.

mp: 188 °C to 191 °C.

Water (2.5.12): maximum 4.8 per cent.

Assay. Liquid chromatography (*2.2.29*) as prescribed in the monograph *Ginseng (1523)*.

Test solution. Dissolve 3.0 mg, accurately weighed, of *ginsenoside Rg1* in 10 mL of *methanol R*.

Content: minimum 95.0 per cent, calculated by the normalisation procedure.

Ginsenoside Rg2. $C_{42}H_{72}O_{13}$. (M_r 785). *1182600.* [52286-74-5]. 3β,12β,20-Trihydroxydammar-24-en-6α-yl 2-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranoside.

Ginsenoside Ro. $C_{48}H_{76}O_{19}$. (M_r 957). *1205000.* [34367-04-9]. (3β)-28-(β-D-Glucopyranosyloxy)-28-oxoolean-12-en-3-yl 2-*O*-β-D-glucopyranosyl-β-D-glucopyranosiduronic acid.

Gitoxin. $C_{41}H_{64}O_{14}$. (M_r 781). *1040200.* [4562-36-1]. Glycoside of *Digitalis purpurea* L. 3β-(*O*-2,6-Dideoxy-β-d-*ribo*-hexopyranosyl-(1→4)-*O*-2,6-dideoxy-β-d-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-d-*ribo*-hexopyranosyloxy)-14,16β-dihydroxy-5β,14β-card-20(22)-enolide.

A white or almost white, crystalline powder, practically insoluble in water and in most common organic solvents, soluble in pyridine.

$[\alpha]_D^{20}$: + 20 to + 24, determined on a 5 g/L solution in a mixture of equal volumes of *chloroform R* and *methanol R*.

Chromatography. Thin-layer chromatography (*2.2.27*) as prescribed in the monograph *Digitalis leaf (0117)*; the chromatogram shows only one principal spot.

Glucosamine hydrochloride. $C_6H_{14}ClNO_5$. (M_r 215.6). *1040300.* [66-84-2]. D-Glucosamine hydrochloride.

Crystals, soluble in water.

$[\alpha]_D^{20}$: + 100, decreasing to + 47.5 after 30 min, determined on a 100 g/L solution.

Glucose. *1025700.* [50-99-7].

See *Glucose (0177)*.

D-Glucuronic acid. $C_6H_{10}O_7$. (M_r 194.1). *1119700.* [6556-12-3].

Content: minimum 96.0 per cent, calculated with reference to the substance dried *in vacuo* (*2.2.32*).

Soluble in water and in ethanol (96 per cent).

Shows mutarotation: $[\alpha]_D^{24}$: + 11.7 → + 36.3.

Assay. Dissolve 0.150 g in 50 mL of *anhydrous methanol R* while stirring under nitrogen. Titrate with *0.1 M tetrabutylammonium hydroxide*, protecting the solution from atmospheric carbon dioxide throughout solubilisation and titration. Determine the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M tetrabutylammonium hydroxide* is equivalent to 19.41 mg of $C_6H_{10}O_7$.

Glutamic acid. *1040400.* [56-86-0].

See *Glutamic acid (0750)*.

L-Glutamine. $C_5H_{10}N_2O_3$. (M_r 146.2). *1203700.* [56-85-9]. (*S*)-2,5-Diamino-5-oxopentanoic acid.

White crystalline powder.

mp: about 185 °C, with decomposition.

Glutamyl endopeptidase for peptide mapping. *1173300.* [137010-42-5]. Endoproteinase Glu-C of high purity from *Staphylococcus aureus* strain V8 (EC 3.4.21.19).

L-γ-Glutamyl-L-cysteine. $C_8H_{14}N_2O_5S$. (M_r 250.3). *1157900.* [636-58-8].

Glutaraldehyde. $C_5H_8O_2$. (M_r 100.1). *1098300.* [111-30-8].

Oily liquid, soluble in water.

n_D^{25}: about 1.434.

bp: about 188 °C.

Glutaric acid. $C_5H_8O_4$. (M_r 132.1). *1149700.* [110-94-1]. Pentanedioic acid.

White or almost white, crystalline powder.

L-Glutathione, oxidised. $C_{20}H_{32}N_6O_{12}S_2$. (M_r 612.6). *1158000.* [27025-41-8]. Bis(L-γ-glutamyl-L-cysteinylglycine) disulfide.

Glycerol. *1040500.* [56-81-5].

See *Glycerol (0496)*.

Glycerol R1. *1040501.*

Complies with the requirements prescribed for the monograph *Glycerol (0496)* and free from diethylene glycol when examined as prescribed in the test for impurity A and related substances in that monograph.

Glycerol (85 per cent). *1040600.*

See *Glycerol (85 per cent) (0497)*.

Glycerol (85 per cent) R1. *1040601.*

Complies with the requirements prescribed for the monograph *Glycerol 85 per cent (0497)* and free from diethylene glycol when examined as prescribed in the test for impurity A and related substances in that monograph.

Glycerol 1-decanoate. $C_{13}H_{26}O_4$. (M_r 246.3). *1169400.* [2277-23-8]. (2*RS*)-2,3-Dihydroxypropyl decanoate. α-Monocaprin. 1-Monodecanoyl-*rac*-glycerol.

Content: about 99 per cent.

Glycerol 1-octanoate. $C_{11}H_{22}O_4$. (M_r 218.3). *1169500.* [502-54-5]. (2*RS*)-2,3-Dihydroxypropyl octanoate. α-Monocaprylin. 1-Monooctanoyl-*rac*-glycerol.

Content: about 99 per cent.

Glycidol. $C_3H_6O_2$. (M_r 74.1). *1127800.* [556-52-5].

Slightly viscous liquid, miscible with water.

d_4^{20}: about 1.115.

n_D^{20}: about 1.432.

Glycine. *1040700.* [56-40-6].

See *Glycine (0614)*.

Glycine anhydride. $C_4H_6N_2O_2$. (M_r 114.1). *1192200.* [106-57-0]. Piperazine-2,5-dione (2,5-DKP).

Glycolic acid. $C_2H_4O_3$. (M_r 76.0). *1040800.* [79-14-1]. 2-Hydroxyacetic acid.

Crystals, soluble in water, in acetone, in ethanol (96 per cent) and in methanol.

mp: about 80 °C.

Glycyrrhetic acid. $C_{30}H_{46}O_4$. (M_r 470.7). *1040900.* [471-53-4]. Glycyrrhetinic acid. 12,13-Didehydro-3β-hydroxy-11-oxo-olean-30-oic acid.

A mixture of α- and β-glycyrrhetic acids in which the β-isomer is predominant.

White or yellowish-brown powder, practically insoluble in water, soluble in anhydrous ethanol and in glacial acetic acid.

$[\alpha]_D^{20}$: + 145 to + 155, determined on a 10.0 g/L solution in *anhydrous ethanol R*.

Chromatography. Thin-layer chromatography (*2.2.27*) using *silica gel* GF_{254} *R* as the coating substance; prepare the slurry using a 0.25 per cent *V/V* solution of *phosphoric acid R*. Apply to the plate 5 µL of a 5 g/L solution of the glycyrrhetic acid in a mixture of equal volumes of *chloroform R* and *methanol R*. Develop over a path of 10 cm using a mixture of 5 volumes of *methanol R* and 95 volumes of *chloroform R*. Examine the chromatogram in ultraviolet light at 254 nm. The chromatogram shows a dark spot (R_F about 0.3) corresponding to β-glycyrrhetic acid and a smaller spot (R_F about 0.5) corresponding to α-glycyrrhetic acid. Spray with *anisaldehyde solution R* and heat at 100-105 °C for 10 min. Both spots are coloured bluish-violet. Between them a smaller bluish-violet spot may be present.

18α-Glycyrrhetinic acid. $C_{30}H_{46}O_4$. (M_r 470.7). *1127900.* [1449-05-4]. (20β)-3β-Hydroxy-11-oxo-18α-olean-12-en-29-oic acid.

White or almost white powder, practically insoluble in water, soluble in anhydrous ethanol, sparingly soluble in methylene chloride.

Glyoxalhydroxyanil. $C_{14}H_{12}N_2O_2$. (M_r 240.3). *1041000.* [1149-16-2]. Glyoxal bis(2-hydroxyanil).

White or almost white crystals, soluble in hot ethanol (96 per cent).

mp: about 200 °C.

Glyoxal solution. *1098400.* [107-22-2].

Contains about 40 per cent (*m/m*) glyoxal.

Assay. In a ground-glass stoppered flask place 1.000 g of glyoxal solution, 20 mL of a 70 g/L solution of *hydroxylamine hydrochloride R* and 50 mL of *water R*. Allow to stand for 30 min and add 1 mL of *methyl red mixed solution R* and titrate with *1 M sodium hydroxide* until the colour changes from red to green. Carry out a blank titration.

1 mL of *1 M sodium hydroxide* is equivalent to 29.02 mg of glyoxal ($C_2H_2O_2$).

Gonadotrophin, chorionic. *1041100.* [9002-61-3].

See *Chorionic gonadotrophin (0498)*.

Gonadotrophin, serum. *1041200.*

See *Equine serum gonadotrophin for veterinary use (0719)*.

Gramine. $C_{11}H_{14}N_2$. (M_r 174.2). *1189400.* [87-52-5]. 1-(1*H*-Indol-3-yl)-*N*,*N*-dimethylmethanamine.

Flakes, practically insoluble in water, soluble in ethanol (96 per cent), slightly soluble in acetone.

mp: 132 °C to 134 °C.

Guaiacol. $C_7H_8O_2$. (M_r 124.1). *1148300.* [90-05-1]. 2-Methoxyphenol. 1-Hydroxy-2-methoxybenzene.

Crystalline mass or colourless or yellowish liquid, hygroscopic, slightly soluble in water, very soluble in methylene chloride, freely soluble in ethanol (96 per cent).

bp: about 205 °C.

mp: about 28 °C.

Guaiacum resin. *1041400.*

Resin obtained from the heartwood of *Guaiacum officinale* L. and *Guaiacum sanctum* L.

Reddish-brown or greenish-brown, hard, glassy fragments; fracture shiny.

Guaiazulene. $C_{15}H_{18}$. (M_r 198.3). *1041500.* [489-84-9]. 1,4-Dimethyl-7-isopropylazulene.

Dark-blue crystals or blue liquid, very slightly soluble in water, miscible with fatty and essential oils and with liquid paraffin, sparingly soluble in ethanol (96 per cent), soluble in 500 g/L sulfuric acid and 80 per cent *m/m* phosphoric acid, giving a colourless solution.

mp: about 30 °C.

Storage: protected from light and air.

Guanidine hydrochloride. CH_5N_3HCl. (M_r 95.5). *1098500.* [50-01-1].

Crystalline powder, freely soluble in water and in ethanol (96 per cent).

Guanine. $C_5H_5N_5O$. (M_r 151.1). *1041600.* [73-40-5]. 2-Amino-1,7-dihydro-6*H*-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.

Haemoglobin. *1041700.* [9008-02-0].

Nitrogen: 15 per cent to 16 per cent.

Iron: 0.2 per cent to 0.3 per cent.

Loss on drying (*2.2.32*): maximum 2 per cent.

Sulfated ash (*2.4.14*): maximum 1.5 per cent.

Haemoglobin solution. *1041701.*

Transfer 2 g of *haemoglobin R* to a 250 mL beaker and add 75 mL of *dilute hydrochloric acid R2*. Stir until solution is complete. Adjust the pH to 1.6 ± 0.1 using *1 M hydrochloric acid*. Transfer to a 100 mL flask with the aid of *dilute hydrochloric acid R2*. Add 25 mg of *thiomersal R*. Prepare daily, store at 5 ± 3 °C and readjust to pH 1.6 before use.

Storage: at 2 °C to 8 °C.

Hamamelitannin. $C_{20}H_{20}O_{14}$. (M_r 484.4). *1192700.* [469-32-9]. (2*R*,3*R*,4*R*)-2-Formyl-2,3,4-trihydroxypentane-1,5-diyl bis(3,4,5-trihydroxybenzoate). 2-*C*-[(Galloyloxy)methyl]-D-ribose 5-gallate.

Harpagoside. $C_{24}H_{30}O_{11}$. (M_r 494.5). *1098600.*

White or almost white, crystalline powder, very hygroscopic, soluble in water and in ethanol (96 per cent).

mp: 117 °C to 121 °C.

Storage: in an airtight container.

Hederacoside C. $C_{59}H_{96}O_{26}$. (M_r 1221). *1158100.* [14216-03-6]. *O*-6-Deoxy-α-L-mannopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl (4*R*)-3β-[[2-*O*(-6-deoxy-α-L-mannopyranosyl)-α-L-arabinopyranosyl]oxy]-23-hydroxyolean-12-en-28-oate.

Colourless crystals or white or almost white powder.

mp: about 220 °C.

Hederacoside C used in liquid chromatography complies with the following additional test.

Assay. Liquid chromatography (*2.2.29*) as prescribed in the monograph *Ivy leaf (2148)*.

Test solution. Dissolve 5.0 mg of hederacoside C in 5.0 mL of *methanol R*.

Content: minimum 95 per cent, calculated by the normalisation procedure.

Hederagenin. $C_{30}H_{48}O_4$. (M_r 472.7). *1184100*. [465-99-6]. Astrantiagenin E. Caulosapogenin. 3β,23-Dihydroxy-4α-olean-12-en-28-oic acid.

α-Hederin. $C_{41}H_{66}O_{12}$. (M_r 751.0). *1158200*. [27013-91-8]. (+)-(4*R*)-3β-[[2-*O*-(6-Deoxy-α-L-mannopyranosyl)-α-L-arabinopyranosyl]oxy]-23-hydroxyolean-12-en-28-oic acid.

White or almost white powder.

mp: about 256 °C.

Helium for chromatography. He. (A_r 4.003). *1041800*. [7440-59-7].

Content: minimum 99.995 per cent *V/V* of He.

Heparin. *1041900*. [9041-08-1].

See *Heparin sodium (0333)*.

Heparinase I. *1187600*. [9025-39-2]. Heparin lyase (EC *4.2.2.7*).

Enzyme from *Flavobacterium heparinum* that performs eliminative cleavage of polysaccharides containing (1→4)-linked D-glucuronate or L-iduronate residues and (1→4)-α-linked 2-sulfoamino-2-deoxy-6-sulfo-D-glucose residues to give oligosaccharides with terminal 4-deoxy-α-D-gluc-4-enuronosyl groups at their non-reducing ends.

Heparinase II. *1187700*. [149371-12-0].

Enzyme from *Flavobacterium heparinum* that depolymerises sulfated polysaccharide chains containing 1→4 linkages between hexosamines and uronic acid residues (both iduronic and glucuronic acid residues). The reaction yields oligosaccharide products (mainly disaccharides) containing unsaturated uronic acids.

Heparinase III. *1187800*. [37290-86-1]. Heparin-sulfate lyase (EC *4.2.2.8*).

Enzyme from *Flavobacterium heparinum* that depolymerises selectively sulfated polysaccharide chains containing 1→4 linkages between hexosamines and glucuronic acid residues to give oligosaccharide products (mainly disaccharides) containing unsaturated uronic acids.

HEPES. $C_8H_{18}N_2O_4S$. (M_r 238.3). *1106800*. [7365-45-9]. 2-[4-(2-Hydroxyethyl)piperazin-1-yl]ethanesulfonic acid.

White or almost white powder.

mp: about 236 °C, with decomposition

Heptachlor. $C_{10}H_5Cl_7$. (M_r 373.3). *1128000*. [76-44-8].

bp: about 135 °C.

mp: about 95 °C.

A suitable certified reference solution (10 ng/µL in cyclohexane) may be used.

Heptachlor epoxide. $C_{10}H_5Cl_7O$. (M_r 389.3). *1128100*. [1024-57-3].

bp: about 200 °C.

mp: about 160 °C.

A suitable certified reference solution (10 ng/µL in cyclohexane) may be used.

Heptafluorobutyric acid. $C_4HF_7O_2$. (M_r 214.0). *1162400*. [375-22-4]. HFBA.

Clear, colourless liquid. Corrosive.

d_{20}^{20}: about 1.645.

n_D^{20}: about 1.300.

bp: about 120 °C.

Content: minimum 99.5 per cent.

Heptafluoro-*N*-methyl-*N*-(trimethylsilyl)butanamide. $C_8H_{12}F_7NOSi$. (M_r 299.3). *1139500*. [53296-64-3]. 2,2,3,3,4,4,4-Heptafluoro-*N*-methyl-*N*-(trimethylsilyl)-butyramide.

Clear, colourless liquid, flammable.

n_D^{20}: about 1.351.

bp: about 148 °C.

Heptane. C_7H_{16}. (M_r 100.2). *1042000*. [142-82-5].

Colourless, flammable liquid, practically insoluble in water, miscible with anhydrous ethanol.

d_{20}^{20}: 0.683 to 0.686.

n_D^{20}: 1.387 to 1.388.

Distillation range (*2.2.11*). Not less than 95 per cent distils between 97 °C and 98 °C.

Hesperidin. $C_{28}H_{34}O_{15}$. (M_r 611). *1139000*. [520-26-3]. (*S*)-7-[[6-*O*-(6-Deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-2,3-dihydro-4*H*-1-benzopyran-4-one.

Hygroscopic powder, slightly soluble in water and in methanol.

mp: 258 °C to 262 °C.

Hexachlorobenzene. C_6Cl_6. (M_r 284.8). *1128200*. [118-74-1].

bp: about 332 °C.

mp: about 230 °C.

A suitable certified reference solution (10 ng/µL in cyclohexane) may be used.

α-Hexachlorocyclohexane. $C_6H_6Cl_6$. (M_r 290.8). *1128300*. [319-84-6].

bp: about 288 °C.

mp: about 158 °C.

A suitable certified reference solution (10 ng/µL in cyclohexane) may be used.

β-Hexachlorocyclohexane. $C_6H_6Cl_6$. (M_r 290.8). *1128400*. [319-85-7].

A suitable certified reference solution (10 ng/µL in cyclohexane) may be used.

δ-Hexachlorocyclohexane. $C_6H_6Cl_6$. (M_r 290.8). *1128500*. [319-86-8].

A suitable certified reference solution (10 ng/µL in cyclohexane) may be used.

Hexacosane. $C_{26}H_{54}$. (M_r 366.7). *1042200*. [630-01-3].

Colourless or white or almost white flakes.

mp: about 57 °C.

Hexadimethrine bromide. $(C_{13}H_{30}Br_2N_2)_n$. *1042300*. [28728-55-4]. 1,5-Dimethyl-1,5-diazaundecamethylene polymethobromide. Poly(1,1,5,5-tetramethyl-1,5-azonia-undecamethylene dibromide).

White or almost white, amorphous powder, hygroscopic, soluble in water.

Storage: in an airtight container.

2,2′,2″,6,6′,6″-Hexa(1,1-dimethylethyl)-4,4′,4″-[(2,4,6-trimethyl-1,3,5-benzenetriyl)trismethylene]triphenol. $C_{54}H_{78}O_3$. (M_r 775). *1042100*. 2,2′,2″,6,6′,6″-Hexa-*tert*-butyl-4,4′,4″-[(2,4,6-trimethyl-1,3,5-benzenetriyl)trismethylene]triphenol.

Crystalline powder, practically insoluble in water, soluble in acetone, slightly soluble in ethanol (96 per cent).

mp: about 244 °C.

1,1,1,3,3,3-Hexafluoropropan-2-ol. $C_3H_2F_6O$. (M_r 168.0). *1136000.* [920-66-1].

Content: minimum 99.0 per cent, determined by gas chromatography.

Clear, colourless liquid, miscible with water and with anhydrous ethanol.

d_{20}^{20}: about 1.596.

bp: about 59 °C.

Hexamethyldisilazane. $C_6H_{19}NSi_2$. (M_r 161.4). *1042400.* [999-97-3].

Clear, colourless liquid.

d_{20}^{20}: about 0.78.

n_D^{20}: about 1.408.

bp: about 125 °C.

Storage: in an airtight container.

Hexamethylenetetramine. $C_6H_{12}N_4$. (M_r 140.2). *1042500.* [100-97-0]. Hexamine. 1,3,5,7-Tetraazatricyclo[3.3.1.1^{3,7}]-decane.

Colourless, crystalline powder, very soluble in water.

Hexane. C_6H_{14}. (M_r 86.2). *1042600.* [110-54-3].

Colourless, flammable liquid, practically insoluble in water, miscible with anhydrous ethanol.

d_{20}^{20}: 0.659 to 0.663.

n_D^{20}: 1.375 to 1.376.

Distillation range (*2.2.11*). Not less than 95 per cent distils between 67 °C and 69 °C.

Hexane used in spectrophotometry complies with the following additional test.

Absorbance (*2.2.25*): maximum 0.01 from 260 nm to 420 nm, determined using *water R* as compensation liquid.

Hexylamine. $C_6H_{15}N$. (M_r 101.2). *1042700.* [111-26-2]. Hexan-1-amine.

Colourless liquid, slightly soluble in water, soluble in ethanol (96 per cent).

d_{20}^{20}: about 0.766.

n_D^{20}: about 1.418.

bp: 127 °C to 131 °C.

Hibifolin. $C_{21}H_{18}O_{14}$. (M_r 494.4). *1207000.* [55366-56-8]. 2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4-oxo-4*H*-1-benzopyran-8-yl β-D-glucopyranosiduronic acid. Gossypetin 8-*O*-glucuronide. Gossypetin 8-*O*-β-D-glucuropyranoside.

Storage: protected from light, at a temperature not exceeding 8 °C, in a dry place.

Histamine dihydrochloride. *1042800.* [56-92-8].

See *Histamine dihydrochloride (0143)*.

Histamine solution. *1042901.*

A 9 g/L solution of *sodium chloride R* containing 0.1 µg per millilitre of histamine base (as the phosphate or dihydrochloride).

Histidine. *1187900.* [71-00-1]. (2*S*)-2-Amino-3-(1*H*-imidazol-4-yl)propanoic acid.

Histidine monohydrochloride. $C_6H_{10}ClN_3O_2,H_2O$. (M_r 209.6). *1043000.* [123333-71-1]. (*RS*)-2-Amino-3-(imidazol-4-yl)propionic acid hydrochloride monohydrate.

Crystalline powder or colourless crystals, soluble in water.

mp: about 250 °C, with decomposition.

Chromatography. Thin-layer chromatography (*2.2.27*) as prescribed in the monograph *Histamine dihydrochloride (0143)*; the chromatogram shows only one principal spot.

Holmium oxide. Ho_2O_3. (M_r 377.9). *1043100.* [12055-62-8]. Diholmium trioxide.

Yellowish powder, practically insoluble in water.

Holmium perchlorate solution. *1043101.*

A 40 g/L solution of *holmium oxide R* in a solution of *perchloric acid R* containing 141 g/L of $HClO_4$.

DL-Homocysteine. $C_4H_9NO_2S$. (M_r 135.2). *1136100.* [454-29-5]. (2*RS*)-2-Amino-4-sulfanylbutanoic acid.

White or almost white, crystalline powder.

mp: about 232 °C.

L-Homocysteine thiolactone hydrochloride. C_4H_8ClNOS. (M_r 153.6). *1136200.* [31828-68-9]. (3*S*)-3-Aminodihydrothiophen-2(3*H*)-one hydrochloride.

White or almost white, crystalline powder.

mp: about 202 °C.

Homoorientin. $C_{21}H_{20}O_{11}$. (M_r 448.4). *1189500.* [4261-42-1]. 2-(3,4-Dihydroxyphenyl)-6-β-D-glucopyranosyl-5,7-dihydroxy-4*H*-1-benzopyran-4-one. Isoorientin. Luteolin-6-*C*-glucoside.

Honokiol. $C_{18}H_{18}O_2$. (M_r 266.3). *1182700.* [35354-74-6]. 3′,5-Di(prop-2-enyl)biphenyl-2,4′-diol. 3′,5-Diallyl-2,4′-dihydroxybiphenyl. 3′,5-Di-2-propenyl-[1,1′-biphenyl]-2,4′-diol.

Human tissue factor solution. *1186100.*

Solution containing human tissue factor, which may be produced by recombinant DNA technology, combined with phospholipids and calcium buffers. Suitable stabilisers may be added.

Hyaluronidase diluent. *1043300.*

Mix 100 mL of *phosphate buffer solution pH 6.4 R* with 100 mL of *water R*. Dissolve 0.140 g of *hydrolysed gelatin R* in the solution at 37 °C.

Storage: use within 2 h.

Hydrastine hydrochloride. $C_{21}H_{22}ClNO_6$. (M_r 419.9). *1154000.* [5936-28-7]. (3*S*)-6,7-Dimethoxy-3-[(5*R*)-6-methyl-5,6,7,8-tetrahydro-1,3-dioxolo[4,5-*g*]isoquinolin-5-yl]isobenzofuran-1(3*H*)-one hydrochloride.

White or almost white powder, hygroscopic, very soluble in water and in ethanol (96 per cent).

$[\alpha]_D^{17}$: about + 127.

mp: about 116 °C.

Hydrastine hydrochloride used in liquid chromatography complies with the following additional test.

Assay. Liquid chromatography (*2.2.29*) as prescribed in the monograph *Goldenseal rhizome (1831)*.

Content: minimum 98 per cent, calculated by the normalisation procedure.

Hydrazine. H_4N_2. (M_r 32.05). *1136300.* [302-01-2]. Diazane.

Slightly oily liquid, colourless, with a strong odour of ammonia, miscible with water. Dilute solutions in water are commercially available.

n_D^{20}: about 1.470.

bp: about 113 °C.

mp: about 1.5 °C.

Caution: toxic and corrosive.

Hydrazine sulfate. $H_6N_2O_4S$. (M_r 130.1). *1043400.* [10034-93-2].

Colourless crystals, sparingly soluble in cold water, soluble in hot water (50 °C) and freely soluble in boiling water, practically insoluble in ethanol (96 per cent).

Content: minimum 99 per cent.

Hydriodic acid. HI. (M_r 127.9). *1098900.* [10034-85-2].

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.

Place the acid in small, amber, glass-stoppered bottles previously flushed with *carbon dioxide R* or *nitrogen R*, seal with paraffin.

Storage: in a dark place.

Hydrobromic acid, 30 per cent. *1098700.* [10035-10-6].

A 30 per cent solution of hydrobromic acid in *glacial acetic acid R*.

Degas with caution the contents before opening.

Hydrobromic acid, dilute. *1098701.*

Place 5.0 mL of *30 per cent hydrobromic acid R* in amber vials equipped with polyethylene stoppers. Seal under *argon R* and store in the dark. Add 5.0 mL of *glacial acetic acid R* immediately before use. Shake.

Storage: in the dark.

Hydrobromic acid, 47 per cent. *1118900.*

A 47 per cent *m/m* solution of hydrobromic acid.

Hydrobromic acid, dilute R1. *1118901.*

Contains 7,9 g/L of HBr.

Dissolve 16.81 g of *47 per cent hydrobromic acid R* in *water R* and dilute to 1000 mL with the same solvent.

Hydrochloric acid. *1043500.* [7647-01-0].

See *Concentrated hydrochloric acid (0002)*.

0.1 M Hydrochloric acid, alcoholic. *3008800.*

Dilute 9.0 mL of *hydrochloric acid R* to 1000.0 mL with *aldehyde-free alcohol R*.

2 M Hydrochloric acid. *3001700.*

Dilute 206.0 g of *hydrochloric acid R* to 1000.0 mL with *water R*.

3 M Hydrochloric acid. *3001600.*

Dilute 309.0 g of *hydrochloric acid R* to 1000.0 mL with *water R*.

6 M Hydrochloric acid. *3001500.*

Dilute 618.0 g of *hydrochloric acid R* to 1000.0 mL with *water R*.

Hydrochloric acid R1. *1043501.*

Contains 250 g/L of HCl.

Dilute 70 g of *hydrochloric acid R* to 100 mL with *water R*.

Hydrochloric acid, brominated. *1043507.*

To 1 mL of *bromine solution R* add 100 mL of *hydrochloric acid R*.

Hydrochloric acid, dilute. *1043503.*

Contains 73 g/L of HCl.

Dilute 20 g of *hydrochloric acid R* to 100 mL with *water R*.

Hydrochloric acid, dilute, heavy metal-free. *1043509.*

Complies with the requirements prescribed for *dilute hydrochloric acid R* with the following maximum contents of heavy metals.

As: 0.005 ppm.

Cd: 0.003 ppm.

Cu: 0.003 ppm.

Fe: 0.05 ppm.

Hg: 0.005 ppm.

Ni: 0.004 ppm.

Pb: 0.001 ppm.

Zn: 0.005 ppm.

Hydrochloric acid, dilute R1. *1043504.*

Contains 0.37 g/L of HCl.

Dilute 1.0 mL of *dilute hydrochloric acid R* to 200.0 mL with *water R*.

Hydrochloric acid, dilute R2. *1043505.*

Dilute 30 mL of *1 M hydrochloric acid* to 1000 mL with *water R*; adjust to pH 1.6 ± 0.1.

Hydrochloric acid, dilute R3. *1203800.*

Contains 3.7 g/L of HCl.

Dilute 10.0 mL of *dilute hydrochloric acid R* to 200.0 mL with *water R*.

Hydrochloric acid, ethanolic. *1043506.*

Dilute 5.0 mL of *1 M hydrochloric acid* to 500.0 mL with *ethanol (96 per cent) R*.

Hydrochloric acid, heavy metal-free. *1043510.*

Complies with the requirements prescribed for *hydrochloric acid R* with the following maximum contents of heavy metals.

As: 0.005 ppm.

Cd: 0.003 ppm.

Cu: 0.003 ppm.

Fe: 0.05 ppm.

Hg: 0.005 ppm.

Ni: 0.004 ppm.

Pb: 0.001 ppm.

Zn: 0.005 ppm.

Hydrochloric acid, lead-free. *1043508.*

Complies with the requirements prescribed for *hydrochloric acid R* with the following additional requirement.

Lead: maximum 20 ppb.

Atomic emission spectrometry (*2.2.22, Method I*).

Test solution. In a quartz crucible evaporate 200 g of the acid to be examined almost to dryness. Take up the residue in 5 mL of nitric acid prepared by sub-boiling distillation of *nitric acid R* and evaporate to dryness. Take up the residue in 5 mL of nitric acid prepared by sub-boiling distillation of *nitric acid R*.

Reference solutions. Prepare the reference solutions using *lead standard solution (0.1 ppm Pb) R* diluted with nitric acid prepared by sub-boiling distillation of *nitric acid R*.

Wavelength: 220.35 nm.

Hydrochloric acid, methanolic. *1043511.*

Dilute 4.0 mL of *hydrochloric acid R* to 1000.0 mL with *methanol R2*.

Hydrocortisone acetate. *1098800.* [50-03-3].

See *Hydrocortisone acetate (0334)*.

Hydrofluoric acid. HF. (M_r 20.01). *1043600.* [7664-39-3].

Content: minimum 40.0 per cent *m/m*.

Clear, colourless liquid.

Loss on ignition: not more than 0.05 per cent *m/m*; evaporate the hydrofluoric acid in a platinum crucible and gently ignite the residue to constant mass.

Assay. Weigh accurately a glass-stoppered flask containing 50.0 mL of *1 M sodium hydroxide*. Introduce 2 g of the hydrofluoric acid and weigh again. Titrate the solution with *0.5 M sulfuric acid*, using 0.5 mL of *phenolphthalein solution R* as indicator.

1 mL of *1 M sodium hydroxide* is equivalent to 20.01 mg of HF.

Storage: in a polyethylene container.

Hydrogen for chromatography. H_2. (M_r 2.016). *1043700.* [1333-74-0].
Content: minimum 99.95 per cent *V/V*.

Hydrogen peroxide solution, dilute. *1043800.* [7722-84-1].
See *Hydrogen peroxide solution (3 per cent) (0395)*.

Hydrogen peroxide solution, strong. *1043900.* [7722-84-1].
See *Hydrogen peroxide solution (30 per cent) (0396)*.

Hydrogen sulfide. H_2S. (M_r 34.08). *1044000.* [7783-06-4].
Gas, slightly soluble in water.

Hydrogen sulfide solution. *1136400.*
A recently prepared solution of *hydrogen sulfide R* in *water R*. The saturated solution contains about 0.4 per cent to 0.5 per cent of H_2S at 20 °C.

Hydrogen sulfide R1. H_2S. (M_r 34.08). *1106600.* [7783-06-4].
Content: minimum 99.7 per cent *V/V*.

Hydroquinone. $C_6H_6O_2$. (M_r 110.1). *1044100.* [123-31-9].
Benzene-1,4-diol.
Fine, colourless or white or almost white needles, darkening on exposure to air and light, soluble in water and in ethanol (96 per cent).
mp: about 173 °C.
Storage: protected from light and air.

Hydroquinone solution. *1044101.*
Dissolve 0.5 g of *hydroquinone R* in *water R*, add 20 µL of *sulfuric acid R* and dilute to 50 mL with *water R*.

4′-Hydroxyacetophenone. $C_8H_8O_2$. (M_r 136.2). *1196900.* [99-93-4]. 1-(4-Hydroxyphenyl)ethan-1-one.

2-Hydroxybenzimidazole. $C_7H_6N_2O$. (M_r 134.1). *1169600.* [615-16-7]. 1*H*-benzimidazol-2-ol.

4-Hydroxybenzohydrazide. $C_7H_8N_2O_2$. (M_r 152.2). *1145900.* [5351-23-5]. *p*-Hydroxybenzohydrazide.

4-Hydroxybenzoic acid. $C_7H_6O_3$. (M_r 138.1). *1106700.* [99-96-7].
Crystals, slightly soluble in water, very soluble in ethanol (96 per cent), soluble in acetone.
mp: 214 °C to 215 °C.

4-Hydroxycoumarin. $C_9H_6O_3$. (M_r 162.2). *1169700.* [1076-38-6]. 4-Hydroxy-2*H*-1-benzopyran-2-one.
White or almost white powder, freely soluble in methanol.
Content: minimum 98.0 per cent.

6-Hydroxydopa. $C_9H_{11}NO_5$. (M_r 213.2). *1169800.* [21373-30-8]. (2*RS*)-2-Amino-3-(2,4,5-trihydroxyphenyl)-propanoic acid. 2,5-Dihydroxy-DL-tyrosine.
mp: about 257 °C.

4-Hydroxyisophthalic acid. $C_8H_6O_5$. (M_r 182.1). *1106900.* [636-46-4]. 4-Hydroxybenzene-1,3-dicarboxylic acid.
Needles or platelets, very slightly soluble in water, freely soluble in ethanol (96 per cent).
mp: about 314 °C, with decomposition.

Hydroxylamine hydrochloride. NH_4ClO. (M_r 69.5). *1044300.* [5470-11-1].
White or almost white, crystalline powder, very soluble in water, soluble in ethanol (96 per cent).

Hydroxylamine hydrochloride solution R2. *1044304.*
Dissolve 2.5 g of *hydroxylamine hydrochloride R* in 4.5 mL of hot *water R* and add 40 mL of *ethanol (96 per cent) R* and 0.4 mL of *bromophenol blue solution R2*. Add *0.5 M alcoholic potassium hydroxide* until a greenish-yellow colour is obtained. Dilute to 50.0 mL with *ethanol (96 per cent) R*.

Hydroxylamine solution, alcoholic. *1044301.*
Dissolve 3.5 g of *hydroxylamine hydrochloride R* in 95 mL of *ethanol (60 per cent V/V) R*, add 0.5 mL of a 2 g/L solution of *methyl orange R* in *ethanol (60 per cent V/V) R* and sufficient *0.5 M potassium hydroxide in alcohol (60 per cent V/V)* to give a pure yellow colour. Dilute to 100 mL with *ethanol (60 per cent V/V) R*.

Hydroxylamine solution, alkaline. *1044302.*
Immediately before use, mix equal volumes of a 139 g/L solution of *hydroxylamine hydrochloride R* and a 150 g/L solution of *sodium hydroxide R*.

Hydroxylamine solution, alkaline R1. *1044303.*
Solution A. Dissolve 12.5 g of *hydroxylamine hydrochloride R* in *methanol R* and dilute to 100 mL with the same solvent.
Solution B. Dissolve 12.5 g of *sodium hydroxide R* in *methanol R* and dilute to 100 mL with the same solvent.
Mix equal volumes of solution A and solution B immediately before use.

Hydroxymethylfurfural. $C_6H_6O_3$. (M_r 126.1). *1044400.* [67-47-0]. 5-Hydroxymethylfurfural.
Acicular crystals, freely soluble in water, in acetone and in ethanol (96 per cent).
mp: about 32 °C.

Hydroxynaphthol blue, sodium salt. $C_{20}H_{11}N_2Na_3O_{11}S_3$. ($M_r$ 620). *1044500.* [63451-35-4]. Trisodium 2,2′-dihydroxy-1,1′-azonaphthalene-3′,4,6′-trisulfonate.

2-Hydroxypropylbetadex for chromatography. *1146000.*
Betacyclodextrin modified by the bonding of (*R*) or (*RS*) propylene oxide groups on the hydroxyl groups.

Hydroxypropyl-β-cyclodextrin. *1128600.* [94035-02-6].
See *Hydroxypropylbetadex (1804)*.
pH (*2.2.3*): 5.0 to 7.5 for a 20 g/L solution.

Hydroxyquinoline. C_9H_7NO. (M_r 145.2). *1044600.* [148-24-3]. 8-Hydroxyquinoline. Quinolin-8-ol.
White or slightly yellowish, crystalline powder, slightly soluble in water, freely soluble in acetone, in ethanol (96 per cent) and in dilute mineral acids.
mp: about 75 °C.
Sulfated ash (*2.4.14*): maximum 0.05 per cent.

12-Hydroxystearic acid. $C_{18}H_{36}O_3$. (M_r 300.5). *1099000.* [106-14-9]. 12-Hydroxyoctadecanoic acid.
White or almost white powder.
mp: 71 °C to 74 °C.

5-Hydroxyuracil. $C_4H_4N_2O_3$. (M_r 128.1). *1044700.* [496-76-4]. Isobarbituric acid. Pyrimidine-2,4,5-triol.
White or almost white, crystalline powder.
mp: about 310 °C, with decomposition.
Chromatography. Thin-layer chromatography (*2.2.27*) as prescribed in the monograph *Fluorouracil (0611)*; the chromatogram shows a principal spot with an R_F of about 0.3.
Storage: in an airtight container.

Hyoscine hydrobromide. *1044800.* [6533-68-2].
See *Hyoscine hydrobromide (0106)*.

Hyoscyamine sulfate. *1044900.* [620-61-1].
See *Hyoscyamine sulfate (0501)*.

Hypericin. $C_{30}H_{16}O_8$. (M_r 504.4). *1149800.* [548-04-9]. 1,3,4,6,8,13-Hexahydroxy-10,11-dimethylphenanthro[1,10,9,8-*opqra*]perylene-7,14-dione.
Content: minimum 85 per cent.

Hyperoside. $C_{21}H_{20}O_{12}$. (M_r 464.4). *1045000.* 2-(3,4-Dihydroxyphenyl)-3-β-D-galactopyranosyloxy-5,7-dihydroxychromen-4-one.

Faint yellow needles, soluble in methanol.

Absorbance (*2.2.25*). A solution in *methanol R* shows 2 absorption maxima at about 257 nm and at about 359 nm.

Hypophosphorous reagent. *1045200.*

Dissolve with the aid of gentle heat, 10 g of *sodium hypophosphite R* in 20 mL of *water R* and dilute to 100 mL with *hydrochloric acid R*. Allow to settle and decant or filter through glass wool.

Hypoxanthine. $C_5H_4N_4O$. (M_r 136.1). *1045300.* [68-94-0]. 1*H*-Purin-6-one.

White or almost white, crystalline powder, very slightly soluble in water, sparingly soluble in boiling water, soluble in dilute acids and in dilute alkali hydroxide solutions, decomposes without melting at about 150 °C.

Chromatography. Thin-layer chromatography (*2.2.27*) as prescribed in the monograph *Mercaptopurine (0096)*; the chromatogram shows only one principal spot.

Ibuprofen. *1197000.* [15687-27-1].

See *Ibuprofen (0721)*.

Imidazole. $C_3H_4N_2$. (M_r 68.1). *1045400.* [288-32-4].

White or almost white, crystalline powder, soluble in water and in ethanol (96 per cent).

mp: about 90 °C.

Iminodiacetic acid. $C_4H_7NO_4$. (M_r 133.1). *1192300.* [142-73-4]. 2,2′-Iminodiacetic acid.

Iminodibenzyl. $C_{14}H_{13}N$. (M_r 195.3). *1045500.* [494-19-9]. 10,11-Dihydrodibenz[*b,f*]azepine.

Pale yellow, crystalline powder, practically insoluble in water, freely soluble in acetone.

mp: about 106 °C.

Imipramine hydrochloride. *1207100.* [113-52-0].

See *Imipramine hydrochloride (0029)*.

Imperatorin. $C_{16}H_{14}O_4$. (M_r 270.3). *1180200.* [482-44-0]. 9-[(3-Methylbut-2-enyl)oxy]-7*H*-furo[3,2-*g*][1]benzopyran-7-one.

2-Indanamine hydrochloride. $C_9H_{12}ClN$. (M_r 169.7). *1175800.* [2338-18-3]. 2-Aminoindane hydrochloride.

2,3-Dihydro-1*H*-inden-2-amine hydrochloride.

Indigo. $C_{16}H_{10}N_2O_2$. (M_r 262.3). *1192800.* [482-89-3]. Indigotin. 1,1′,3,3′-Tetrahydro-2-2′-bi(indolylidene)-3,3′-dione.

Indigo carmine. $C_{16}H_8N_2Na_2O_8S_2$. (M_r 466.3). *1045600.* [860-22-0].

Schultz No. 1309.

Colour Index No. 73015.
3,3′-Dioxo-2,2′-bisindolylidene-5,5′-disulfonate disodium. E 132.

It usually contains sodium chloride.

Blue or violet-blue powder or blue granules with a coppery lustre, sparingly soluble in water, practically insoluble in ethanol (96 per cent). It is precipitated from an aqueous solution by sodium chloride.

Indigo carmine solution. *1045601.*

To a mixture of 10 mL of *hydrochloric acid R* and 990 mL of 200 g/L *nitrogen-free sulfuric acid R* add 0.2 g of *indigo carmine R*.

The solution complies with the following test: add 10 mL to a solution of 1.0 mg of *potassium nitrate R* in 10 mL of *water R*, rapidly add 20 mL of *nitrogen-free sulfuric acid R* and heat to boiling. The blue colour is discharged within 1 min.

Indigo carmine solution R1. *1045602.*

Dissolve 4 g of *indigo carmine R* in about 900 mL of *water R* added in several portions. Add 2 mL of *sulfuric acid R* and dilute to 1000 mL with *water R*.

Assay. Place in a 100 mL conical flask with a wide neck 10.0 mL of *nitrate standard solution (100 ppm NO_3) R*, 10 mL of *water R*, 0.05 mL of the *indigo carmine solution R1*, and then in a single addition, but with caution, 30 mL of *sulfuric acid R*. Titrate the solution immediately, using the *indigo carmine solution R1*, until a stable blue colour is obtained.

The number of millilitres used, *n*, is equivalent to 1 mg of NO_3.

Indirubin. $C_{16}H_{10}N_2O_2$. (M_r 262.3). *1192900.* [479-41-4].

1,1′,2′,3-Tetrahydro-2,3′-bi(indolylidene)-2′,3-dione.

Indometacin. *1101500.* [53-86-1].

See *Indometacin (0092)*.

Inosine. $C_{10}H_{12}N_4O_5$. (M_r 268.2). *1169900.* [58-63-9]. 9-β-D-Ribofuranosylhypoxanthine. 9-β-D-Ribofuranosyl-1,9-dihydro-6*H*-purin-6-one.

mp: 222 °C to 226 °C.

***myo*-Inositol.** *1161100.*

See *myo-Inositol (1805)*.

Iodine. *1045800.* [7553-56-2].

See *Iodine (0031)*.

Iodine solution R1. *1045801.*

To 10.0 mL of *0.05 M iodine* add 0.6 g of *potassium iodide R* and dilute to 100.0 mL with *water R*. Prepare immediately before use.

Iodine solution R2. *1045802.*

To 10.0 mL of *0.05 M iodine* add 0.6 g of *potassium iodide R* and dilute to 1000.0 mL with *water R*. Prepare immediately before use.

Iodine solution R3. *1045803.*

Dilute 2.0 mL of *iodine solution R1* to 100.0 mL with *water R*. Prepare immediately before use.

Iodine solution R4. *1045806.*

Dissolve 14 g of *iodine R* in 100 mL of a 400 g/L solution of *potassium iodide R*, add 1 mL of *dilute hydrochloric acid R* and dilute to 1000 mL with *water R*.

Storage: protected from light.

Iodine solution R5. *1045807.*

Dissolve 12.7 g of *iodine R* and 20 g of *potassium iodide R* in *water R* and dilute to 1000.0 mL with the same solvent (0.05 M solution).

Iodine solution, alcoholic. *1045804.*

A 10 g/L solution of *iodine R* in *ethanol (96 per cent) R*.

Storage: protected from light.

Iodine solution, chloroformic. *1045805.*

A 5 g/L solution of *iodine R* in *chloroform R*.

Storage: protected from light.

Iodine-123 and ruthenium-106 spiking solution. *1166700.*

Prepare immediately before use. Mix 3.5 mL of an 18.5 kBq/mL solution of ruthenium-106 in the form of ruthenium trichloride in a mixture of equal volumes of *glacial acetic acid R* and *water R* with 200 µL of a 75 kBq/mL solution of iodine-123 in the form of sodium iodide in *water R*.

Iodine bromide. IBr. (M_r 206.8). *1045900.* [7789-33-5].

Bluish-black or brownish-black crystals, freely soluble in water, in ethanol (96 per cent) and in glacial acetic acid.

bp: about 116 °C.

mp: about 40 °C.

Storage: protected from light.

Iodine bromide solution. *1045901.*

Dissolve 20 g of *iodine bromide R* in *glacial acetic acid R* and dilute to 1000 mL with the same solvent.

Storage: protected from light.

Iodine chloride. ICl. (M_r 162.4). *1143000.* [7790-99-0].

Black crystals, soluble in water, in acetic acid and in ethanol (96 per cent).

bp: about 97.4 °C.

Iodine chloride solution. *1143001.*

Dissolve 1.4 g of *iodine chloride R* in *glacial acetic acid R* and dilute to 100 mL with the same acid.

Storage: protected from light.

Iodine pentoxide, recrystallised. I_2O_5. (M_r 333.8). *1046000.* [12029-98-0]. Di-iodine pentoxide. Iodic anhydride.

Content: minimum 99.5 per cent.

White or almost white, crystalline powder, or white or greyish-white granules, hygroscopic, very soluble in water forming HIO_3.

Stability on heating. Dissolve 2 g, previously heated for 1 h at 200 °C, in 50 mL of *water R*. A colourless solution is obtained.

Assay. Dissolve 0.100 g in 50 mL of *water R*, add 3 g of *potassium iodide R* and 10 mL of *dilute hydrochloric acid R*. Titrate the liberated iodine with *0.1 M sodium thiosulfate*, using 1 mL of *starch solution R* as indicator.

1 mL of *0.1 M sodium thiosulfate* is equivalent to 2.782 mg of I_2O_5.

Storage: in an airtight container, protected from light.

Iodoacetamide. C_2H_4INO. (M_r 185.0). *1186200.* [144-48-9]. 2-Iodoacetamide.

Slightly yellow, crystalline powder, soluble in water.

mp: about 92 °C.

Iodoacetic acid. $C_2H_3IO_2$. (M_r 185.9). *1107000.* [64-69-7].

Colourless or white or almost white crystals, soluble in water and in ethanol (96 per cent).

mp: 82 °C to 83 °C.

2-Iodobenzoic acid. $C_7H_5IO_2$. (M_r 248.0). *1046100.* [88-67-5].

White or slightly yellow, crystalline powder, slightly soluble in water, soluble in ethanol (96 per cent).

mp: about 160 °C.

Chromatography. Thin-layer chromatography (*2.2.27*), using cellulose for chromatography f_{254} R as the coating substance: apply to the plate 20 µL of a solution of the 2-iodobenzoic acid, prepared by dissolving 40 mg in 4 mL of *0.1 M sodium hydroxide* and diluting to 10 mL with *water R*. Develop over a path of about 12 cm using as the mobile phase the upper layer obtained by shaking together 20 volumes of *water R*, 40 volumes of *glacial acetic acid R* and 40 volumes of *toluene R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. The chromatogram shows only one principal spot.

3-Iodobenzylammonium chloride. C_7H_9ClIN. (M_r 269.5). *1168000.* [3718-88-5]. 1-(3-Iodophenyl)methanamine hydrochloride. 1-(3-Iodophenyl)methanaminium chloride. *m*-Iodobenzylamine hydrochloride.

White or almost white crystals.

mp: 188 °C to 190 °C.

Iodoethane. C_2H_5I. (M_r 156.0). *1099100.* [75-03-6].

Content: minimum 99 per cent.

Colourless or slightly yellowish liquid, darkening on exposure to air and light, miscible with ethanol (96 per cent) and most organic solvents.

d_{20}^{20}: about 1.95.

n_D^{20}: about 1.513.

bp: about 72 °C.

Storage: in an airtight container, protected from light.

2-Iodohippuric acid. $C_9H_8INO_3,2H_2O$. (M_r 341.1). *1046200.* [147-58-0]. 2-(2-Iodobenzamido)acetic acid.

White or almost white, crystalline powder, sparingly soluble in water.

mp: about 170 °C.

Water (*2.5.12*): 9 per cent to 13 per cent, determined on 1.000 g.

Chromatography. Thin-layer chromatography (*2.2.27*), using *cellulose for chromatography F_{254} R* as the coating substance: apply to the plate 20 µL of a solution of the 2-iodohippuric acid, prepared by dissolving 40 mg in 4 mL of *0.1 M sodium hydroxide* and diluting to 10 mL with *water R*. Develop over a path of about 12 cm using as the mobile phase the upper layer obtained by shaking together 20 volumes of *water R*, 40 volumes of *glacial acetic acid R* and 40 volumes of *toluene R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. The chromatogram shows only one principal spot.

Iodoplatinate reagent. *1046300.*

To 3 mL of a 100 g/L solution of *chloroplatinic acid R* add 97 mL of *water R* and 100 mL of a 60 g/L solution of *potassium iodide R*.

Storage: protected from light.

Iodoplatinate reagent R1. *1172200.*

Mix 2.5 mL of a 50 g/L solution of *chloroplatinic acid R*, 22.5 mL of a 100 g/L solution of *potassium iodide R* and 50 mL of *water R*.

Storage: protected from light, at a temperature of 2-8 °C.

Iodosulfurous reagent. *1046400.*

The apparatus, which must be kept closed and dry during the preparation, consists of a 3000 mL to 4000 mL round-bottomed flask with three inlets for a stirrer and a thermometer and fitted with a drying tube. To 700 mL of *anhydrous pyridine R* and 700 mL of *ethylene glycol monomethyl ether R* add, with constant stirring, 220 g of finely powdered *iodine R*, previously dried over *diphosphorus pentoxide R*. Continue stirring until the iodine has completely dissolved (about 30 min). Cool to − 10 °C, and add quickly, still stirring, 190 g of *sulfur dioxide R*. Do not allow the temperature to exceed 30 °C. Cool.

Assay. Add about 20 mL of *anhydrous methanol R* to a titration vessel and titrate to the end-point with the iodosulfurous reagent (*2.5.12*). Introduce in an appropriate form a suitable amount of *water R*, accurately weighed, and repeat the determination of water. Calculate the water equivalent in milligrams per millilitre of iodosulfurous reagent.

The minimum water equivalent is 3.5 mg of water per millilitre of reagent.

Work protected from humidity. Standardise immediately before use.

Storage: in a dry container.

5-Iodouracil. $C_4H_3IN_2O_2$. (M_r 238.0). *1046500.* [696-07-1]. 5-Iodo-1*H*,3*H*-pyrimidine-2,4-dione.

mp: about 276 °C, with decomposition.

Chromatography. Thin-layer chromatography (*2.2.27*) as prescribed in the monograph *Idoxuridine (0669)*: apply 5 µL of a 0.25 g/L solution; the chromatogram obtained shows only one principal spot.

Ion-exclusion resin for chromatography. *1131000.*

A resin with sulfonic acid groups attached to a polymer lattice consisting of polystyrene cross-linked with divinylbenzene.

Ion-exchange resin, strongly acidic. *1085400.*

Resin in protonated form with sulfonic acid groups attached to a lattice consisting of polystyrene cross-linked with 8 per cent of divinylbenzene. It is available as spherical beads; unless otherwise prescribed, the particle size is 0.3 mm to 1.2 mm.

Capacity. 4.5 mmol to 5 mmol per gram, with a water content of 50 per cent to 60 per cent.

Preparation of a column. Unless otherwise prescribed, use a tube with a fused-in sintered glass disc having a length of 400 mm, an internal diameter of 20 mm and a filling height of about 200 mm. Introduce the resin, mixing it with *water R* and pouring the slurry into the tube, ensuring that no air bubbles are trapped between the particles. When in use, the liquid must not be allowed to fall below the surface of the resin. If the resin is in its protonated form, wash with *water R* until 50 mL requires not more than 0.05 mL of *0.1 M sodium hydroxide* for neutralisation, using 0.1 mL of *methyl orange solution R* as indicator.

If the resin is in its sodium form or if it requires regeneration, pass about 100 mL of a mixture of equal volumes of *hydrochloric acid R1* and *water R* slowly through the column and then wash with *water R* as described above.

Irisflorentin. $C_{20}H_{18}O_8$. (M_r 386.4). *1186300.* [41743-73-1]. 9-Methoxy-7-(3,4,5-trimethoxyphenyl)-8*H*-1,3-dioxolo-[4,5-*g*][1]benzopyran-8-one.

Iron. Fe. (A_r 55.85). *1046600.* [7439-89-6].

Grey powder or wire, soluble in dilute mineral acids.

Iron salicylate solution. *1046700.*

Dissolve 0.1 g of *ferric ammonium sulfate R* in a mixture of 2 mL of *dilute sulfuric acid R* and 48 mL of *water R* and dilute to 100 mL with *water R*. Add 50 mL of a 11.5 g/L solution of *sodium salicylate R*, 10 mL of *dilute acetic acid R*, 80 mL of a 136 g/L solution of *sodium acetate R* and dilute to 500 mL with *water R*. The solution should be recently prepared.

Storage: in an airtight container, protected from light.

Isatin. $C_8H_5NO_2$. (M_r 147.1). *1046800.* [91-56-5]. Indoline-2,3-dione.

Small, yellowish-red crystals, slightly soluble in water, soluble in hot water and in ethanol (96 per cent), soluble in solutions of alkali hydroxides giving a violet colour becoming yellow on standing.

mp: about 200 °C, with partial sublimation.

Sulfated ash (*2.4.14*): maximum 0.2 per cent.

Isatin reagent. *1046801.*

Dissolve 6 mg of *ferric sulfate R* in 8 mL of *water R* and add cautiously 50 mL of *sulfuric acid R*. Add 6 mg of *isatin R* and stir until dissolved.

The reagent should be pale yellow, but not orange or red.

Isoamyl alcohol. $C_5H_{12}O$. (M_r 88.1). *1046900.* [123-51-3]. 3-Methylbutan-1-ol.

Colourless liquid, slightly soluble in water, miscible with ethanol (96 per cent).

bp: about 130 °C.

Isoamyl benzoate. $C_{12}H_{16}O_2$. (M_r 192.3). *1164200.* [94-46-2]. Isopentyl benzoate. 3-Methylbutyl benzoate.

n_D^{20}: about 1.494.

bp: about 261 °C.

Colourless or pale yellow liquid.

Isoandrosterone. $C_{19}H_{30}O_2$. (M_r 290.4). *1107100.* [481-29-8]. Epiandrosterone. 3β-Hydroxy-5α-androstan-17-one.

White or almost white powder, practically insoluble in water, soluble in organic solvents.

$[\alpha]_D^{20}$: + 88, determined on 20 g/L solution in *methanol R*.

mp: 172 °C to 174 °C.

ΔA (*2.2.41*): 14.24×10^3, determined at 304 nm on a 1.25 g/L solution.

***N*-Isobutyldodecatetraenamide.** $C_{16}H_{25}NO$. (M_r 247.4). *1159500.* [866602-52-0]. (2*E*,4*E*,8*Z*,10*EZ*)-*N*-2-(Methylpropyl)dodeca-2,4,8,10-tetraenamide.

White or almost white or non-coloured crystals.

mp: about 70 °C.

***N*-Isobutyldodecatetraenamide solution.** *1159501.*

A solution of *N-isobutyldodecatetraenamide R*, exactly weighed, in *methanol R* at a concentration of about 10 mg/mL.

Isodrin. $C_{12}H_8Cl_6$. (M_r 364.9). *1128700.* [465-73-6]. 1,2,3,4,10,10-Hexachloro-1,4,4a,5,8,8a-hexahydro-*endo*,*endo*-1,4:5,8-dimethanonaphthalene.

Practically insoluble in water, soluble in common organic solvents such as acetone.

A suitable certified reference solution may be used.

Isoeugenol. $C_{10}H_{12}O_2$. (M_r 164.2). *1206200.* [97-54-1]. 2-Methoxy-4-[(1Ξ)-prop-1-en-1-yl]phenol.

Isoleucine. *1185000.* [73-32-5].

See *Isoleucine (0770)*.

Isomalt. $C_{12}H_{24}O_{11}$. (M_r 344.3). *1164300.* [64519-82-0].

Mixture of 6-*O*-α-D-glucopyranosyl-D-glucitol and of 1-*O*-α-D-glucopyranosyl-D-mannitol.

White or almost white powder or granules, freely soluble in water.

Isomaltitol. $C_{12}H_{24}O_{11}$. (M_r 344.3). *1161200.* [534-73-6]. 6-*O*-α-D-Glucopyranosyl-D-glucitol.

White or almost white powder, freely soluble in water.

Isomenthol. $C_{10}H_{20}O$. (M_r 156.3). *1047000.* [23283-97-8]. (+)-*Isomenthol:* (1*S*,2*R*,5*R*)-2-isopropyl-5-methylcyclohexanol. (±)-*Isomenthol:* a mixture of equal parts of (1*S*,2*R*,5*R*)- and (1*R*,2*S*,5*S*)-2-isopropyl-5-methylcyclohexanol.

Colourless crystals, practically insoluble in water, very soluble in ethanol (96 per cent).

$[\alpha]_D^{20}$: (+)-*Isomenthol*: about + 24, determined on a 100 g/L solution in *ethanol (96 per cent) R*.

bp: (+)-*Isomenthol*: about 218 °C. (±)-*Isomenthol*: about 218 °C.

mp: (+)-*Isomenthol*: about 80 °C. (±)-*Isomenthol*: about 53 °C.

(+)-Isomenthone. $C_{10}H_{18}O$. (M_r 154.2). *1047100.* (1*R*)-*cis*-*p*-Menthan-3-one. (1*R*)-*cis*-2-Isopropyl-5-methylcyclohexanone.

Contains variable amounts of menthone. A colourless liquid, very slightly soluble in water, soluble in ethanol (96 per cent).

d_{20}^{20}: about 0.904.

n_D^{20}: about 1.453.

$[\alpha]_D^{20}$: about + 93.2.

Isomenthone used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Peppermint oil (0405)*.

Test solution. The substance to be examined.

Content: minimum 80.0 per cent, calculated by the normalisation procedure.

Isomethyleugenol. $C_{11}H_{14}O_2$. (M_r 178.2). *1181900.* [93-16-3]. 1,2-Dimethoxy-4-prop-1-enylbenzene.

Isomethyleugenol used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Niaouli oil, cineole type (2468)*.

Content: minimum 97.0 per cent, calculated by the normalisation procedure.

Isonicotinamide. $C_6H_6N_2O$. (M_r 122.1). *1193000.* [1453-82-3]. 4-Pyridinecarboxamide. Pyridine-4-carboxamide.

White or almost white, crystalline powder, soluble in water.

Isonicotinic acid. $C_6H_5NO_2$. (M_r 123.1). *1202200.* [55-22-1]. Pyridine-4-carboxylic acid.

Creamish-white powder, sparingly soluble in water.

mp: about 311 °C.

Isopropylamine. C_3H_9N. (M_r 59.1). *1119800.* [75-31-0]. Propan-2-amine.

Colourless, highly volatile, flammable liquid.

n_D^{20}: about 1.374.

bp: 32 °C to 34 °C.

Isopropyl iodide. C_3H_7I. (M_r 170.0). *1166600.* [75-30-9]. 2-Iodopropane.

Content: minimum 99 per cent.

Isopropyl methanesulfonate. $C_4H_{10}O_3S$. (M_r 138.2). *1179400.* [926-06-7]. 1-methylethyl methanesulfonate.

Clear, colourless liquid.

Content: minimum 99.0 per cent.

Density: about 1.129 g/cm^3 (20 °C).

n_D^{20}: 1.418-1.421.

bp: about 82 °C at 6 mm Hg.

Isopropyl myristate. *1047200.* [110-27-0].

See *Isopropyl myristate (0725)*.

4-Isopropylphenol. $C_9H_{12}O$. (M_r 136.2). *1047300.* [99-89-8].

Content: minimum 98 per cent.

bp: about 212 °C.

mp: 59 °C to 61 °C.

Isopropyl toluenesulfonate. $C_{10}H_{14}O_3S$. (M_r 214.3). *1191100.* [2307-69-9]. 1-Methylethyl 4-methylbenzenesulfonate. Propan-2-yl 4-methylbenzenesulfonate. Isopropyl tosilate.

Content: minimum 97.0 per cent.

Clear liquid.

mp: about 20 °C.

Isopulegol. $C_{10}H_{18}O$. (M_r 154.2). *1139600.* [89-79-2]. (−)-Isopulegol. (1*R*,2*S*,5*R*)-2-Isopropenyl-5-methylcyclohexanol.

d_4^{20}: about 0.911.

n_D^{20}: about 1.472.

bp: about 91 °C.

Isopulegol used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Mint oil, partly dementholised (1838)*.

Content: minimum 99 per cent, calculated by the normalisation procedure.

Isoquercitrin. $C_{21}H_{20}O_{12}$. (M_r 464.4). *1201600.* [482-35-9]. 2-(3,4-Dihydroxyphenyl)-3-(β-D-glucopyranosyloxy)-5,7-dihydroxy-4*H*-1-benzopyran-4-one.

Isoquercitroside. $C_{21}H_{20}O_{12}$. (M_r 464.4). *1136500.* [21637-25-2]. 2-(3,4-Dihydroxyphenyl)-3-(β-D-glucofuranosyloxy)-5,7-dihydroxy-4*H*-1-benzopyran-4-one.

Isorhamnetin-3-*O*-neohesperidoside. $C_{28}H_{32}O_{16}$. (M_r 625). *1205100.* [55033-90-4]. 3-[6-Deoxy-α-L-mannopyranosyl-(1→2)-β-D-glucopyranosyloxy]-5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-4*H*-1-benzopyran-4-one.

Isorhynchophylline. $C_{22}H_{28}N_2O_4$. (M_r 384.5). *1197100.* [6859-01-4]. Methyl (16*E*)-17-methoxy-2-oxo-16,17-didehydro-20α-corynoxan-16-carboxylate. Methyl (16*E*)-16-(methoxymethylidene)-2-oxo-20α-corynoxan-17-oate.

Isosilibinin. $C_{25}H_{22}O_{10}$. (M_r 482.4). *1149900.* [72581-71-6]. 3,5,7-Trihydroxy-2-[2-(4-hydroxy-3-methoxyphenyl)-3-hydroxymethyl-2,3-dihydro-1,4-benzodioxin-6-yl]chroman-4-one.

White to yellowish powder, practically insoluble in water, soluble in acetone and in methanol.

Kaempferol. $C_{15}H_{10}O_6$. (M_r 286.2). *1197200.* [520-18-3]. 3,5,7-Trihydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one.

Kaolin, light. *1047400.* [1332-58-7].

A purified native hydrated aluminium silicate. It contains a suitable dispersing agent.

Light, white or almost white powder free from gritty particles, unctuous to the touch, practically insoluble in water and in mineral acids.

Coarse particles: maximum 0.5 per cent.

Place 5.0 g in a ground-glass-stoppered cylinder about 160 mm long and 35 mm in diameter and add 60 mL of a 10 g/L solution of *sodium pyrophosphate R*. Shake vigorously and allow to stand for 5 min. Using a pipette, remove 50 mL of the liquid from a point about 5 cm below the surface. To the remaining liquid add 50 mL of *water R*, shake, allow to stand for 5 min and remove 50 mL as before. Repeat the operations until a total of 400 mL has been removed. Transfer the remaining suspension to an evaporating dish. Evaporate to dryness on a water-bath and dry the residue to constant mass at 100-105 °C. The residue weighs not more than 25 mg.

Fine particles. Disperse 5.0 g in 250 mL of *water R* by shaking vigorously for 2 min. Immediately pour into a glass cylinder 50 mm in diameter and, using a pipette, transfer 20 mL to a glass dish, evaporate to dryness on a water-bath and dry to constant mass at 100-105 °C. Allow the remainder of the suspension to stand at 20 °C for 4 h and, using a pipette with its tip exactly 5 cm below the surface, withdraw a further 20 mL without disturbing the sediment, place in a glass dish, evaporate to dryness on a water-bath and dry to constant mass at 100-105 °C. The mass of the second residue is not less than 70 per cent of that of the first residue.

11-Keto-β-boswellic acid. $C_{30}H_{46}O_4$. (M_r 470.7). *1167600.* [17019-92-0]. 3α-Hydroxy-11-oxours-12-en-24-oic acid. (4β)-3α-Hydroxy-11-oxours-12-en-23-oic acid.

White or almost white powder, insoluble in water, soluble in acetone, in anhydrous ethanol and in methanol.

mp: 195 °C to 197 °C.

11-Keto-β-boswellic acid used in liquid chromatography complies with the following additional test.

Assay. Liquid chromatography (*2.2.29*) as prescribed in the monograph *Indian frankincense (2310)*.

Content: minimum 90 per cent, calculated by the normalisation procedure.

Kieselguhr for chromatography. *1047500.*

White or yellowish-white, light powder, practically insoluble in water, in dilute acids and in organic solvents.

Filtration rate. Use a chromatography column 0.25 m long and 10 mm in internal diameter with a sintered-glass (100) plate and two marks at 0.10 m and 0.20 m above the plate. Place sufficient of the substance to be examined in the column to reach the first mark and fill to the second mark with *water R*. When the first drops begin to flow from the column, fill to the second mark again with *water R* and measure the time required for the first 5 mL to flow from the column. The flow rate is not less than 1 mL/min.

Appearance of the eluate. The eluate obtained in the test for filtration rate is colourless (*2.2.2, Method I*).

Acidity or alkalinity. To 1.00 g add 10 mL of *water R*, shake vigorously and allow to stand for 5 min. Filter the suspension on a filter previously washed with hot *water R* until the washings are neutral. To 2.0 mL of the filtrate add 0.05 mL of *methyl red solution R*; the solution is yellow. To 2.0 mL of the filtrate add 0.05 mL of *phenolphthalein solution R1*; the solution is at most slightly pink.

Water-soluble substances. Place 10.0 g in a chromatography column 0.25 m long and 10 mm in internal diameter and elute with *water R*. Collect the first 20 mL of eluate, evaporate to dryness and dry the residue at 100 °C to 105 °C. The residue weighs not more than 10 mg.

Iron (*2.4.9*): maximum 200 ppm.

To 0.50 g add 10 mL of a mixture of equal volumes of *hydrochloric acid R1* and *water R*, shake vigorously, allow to stand for 5 min and filter. 1.0 mL of the filtrate complies with the test for iron.

Loss on ignition: maximum 0.5 per cent. During heating to red heat (600 ± 50 °C) the substance does not become brown or black.

Kieselguhr G. *1047600.*

Consists of kieselguhr treated with hydrochloric acid and calcined, to which is added about 15 per cent of calcium sulfate hemihydrate.

A fine greyish-white powder; the grey colour becomes more pronounced on triturating with water. The average particle size is 10-40 µm.

Calcium sulfate content. Determine by the method prescribed for *silica gel G R*.

pH (*2.2.3*). Shake 1 g with 10 mL of *carbon dioxide-free water R* for 5 min. The pH of the suspension is 7 to 8.

Chromatographic separation. Thin-layer chromatography (*2.2.27*). Prepare plates using a slurry of the kieselguhr G with a 2.7 g/L solution of *sodium acetate R*. Apply 5 µL of a solution containing 0.1 g/L of lactose, sucrose, glucose and fructose in *pyridine R*. Develop over a path of 14 cm using a mixture of 12 volumes of *water R*, 23 volumes of *2-propanol R* and 65 volumes of *ethyl acetate R*. The migration time of the solvent is about 40 min. Dry, spray onto the plate about 10 mL of *anisaldehyde solution R* and heat for 5-10 min at 100-105 °C. The chromatogram shows four well-defined spots without tailing and well separated from each other.

Lactic acid. *1047800.* [50-21-5].

See *Lactic acid (0458)*.

Lactic reagent. *1047801.*

Solution A. To 60 mL of *lactic acid R* add 45 mL of previously filtered *lactic acid R* saturated without heating with *Sudan red G R*; as lactic acid saturates slowly without heating, an excess of colorant is always necessary.

Solution B. Prepare 10 mL of a saturated solution of *aniline R*. Filter.

Solution C. Dissolve 75 mg of *potassium iodide R* in water and dilute to 70 mL with the same solvent. Add 10 mL of *ethanol (96 per cent) R* and 0.1 g of *iodine R*. Shake.

Mix solutions A and B. Add solution C.

Lactobionic acid. $C_{12}H_{22}O_{12}$. (M_r 358.3). *1101600.* [96-82-2].

White or almost white, crystalline powder, freely soluble in water, practically insoluble in ethanol (96 per cent).

mp: about 115 °C.

β-Lactose. $C_{12}H_{22}O_{11}$. (M_r 342.3). *1150100.* [5965-66-2]. β-D-Lactose.

White or slightly yellowish powder.

Content: minimum 99 per cent.

α-D-Lactose: not greater than 35 per cent.

Assay. Gas chromatography (*2.2.28*): use the normalisation procedure.

Column:

- *size*: *l* = 30 m, Ø = 0.25 mm;
- *stationary phase*: *cyanopropyl(3)phenyl(3)methyl(94)polysiloxane R* (film thickness 1 µm).

Carrier gas: *helium for chromatography R*.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 32.5	20 → 280
Injection port		250
Detector		250

Detection: flame ionisation.

Injection: an appropriate derivatised sample.

Lactose monohydrate. *1047900.* [5989-81-1].

See *Lactose monohydrate (0187)*.

α-Lactose monohydrate. $C_{12}H_{22}O_{11},H_2O$. (M_r 360.3). *1150000.* [5989-81-1]. α-D-Lactose monohydrate.

White or almost white powder.

Content: minimum 97 per cent.

β-D-Lactose: less than 3 per cent.

Assay. Gas chromatography (*2.2.28*): use the normalisation procedure.

Column:

- *size*: *l* = 30 m, Ø = 0.25 mm;
- *stationary phase*: *methylpolysiloxane R* (film thickness 1 µm).

Carrier gas: *helium for chromatography R*.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 12.5	230 → 280
Injection port		250
Detector		280

Detection: flame ionisation.

Injection: an appropriate derivatised sample.

Lactulose. *1189600.* [4618-18-2].

See *Lactulose (1230)*.

Lanatoside C. $C_{49}H_{76}O_{20}$. (M_r 985). *1163300.* [17575-22-3]. 3β-[(β-D-Glucopyranosyl-(1→4)-3-*O*-acetyl-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl)oxy]-12β,14-dihydroxy-5β-card-20(22)-enolide.

Long, flat prisms obtained after recrystallisation in ethanol (96 per cent), freely soluble in pyridine and in dioxan.

Lanthanum chloride heptahydrate. $LaCl_3,7H_2O$. (M_r 371.4). *1167200.*

White or almost white powder or colourless crystals, freely soluble in water.

Lanthanum chloride solution. *1114001.*

To 58.65 g of *lanthanum trioxide R* slowly add 100 mL of *hydrochloric acid R*. Heat to boiling. Allow to cool and dilute to 1000.0 mL with *water R*.

Lanthanum nitrate. $La(NO_3)_3,6H_2O$. (M_r 433.0). *1048000.* [10277-43-7]. Lanthanum trinitrate hexahydrate.

Colourless crystals, deliquescent, freely soluble in water.

Storage: in an airtight container.

Lanthanum nitrate solution. *1048001.*

A 50 g/L solution of *lanthanum nitrate R*.

Lanthanum trioxide. La_2O_3. (M_r 325.8). *1114000.* [1312-81-8].

An almost white, amorphous powder, practically insoluble in *water R*. It dissolves in dilute solutions of mineral acids and absorbs atmospheric carbon dioxide.

Calcium: maximum 5 ppm.

Lauric acid. $C_{12}H_{24}O_2$. (M_r 200.3). *1143100.* [143-07-7]. Dodecanoic acid.

White or almost white, crystalline powder, practically insoluble in water, freely soluble in ethanol (96 per cent).

mp: about 44 °C.

Lauric acid used in the assay of total fatty acids in Saw palmetto fruit (1848) complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Saw palmetto fruit (1848)*.

Content: minimum 98 per cent, calculated by the normalisation procedure.

Lauryl alcohol. $C_{12}H_{26}O$. (M_r 186.3). *1119900.* [112-53-8]. Dodecan-1-ol.

d_{20}^{20}: about 0.820.

mp: 24 °C to 27 °C.

Content: minimum 98.0 per cent, determined by gas chromatography.

Lavandulol. $C_{10}H_{18}O$. (M_r 154.2). *1114100.* [498-16-8]. (*R*)-5-Methyl-2-(1-methylethenyl)-4-hexen-1-ol.

Oily liquid with a characteristic odour.

Lavandulol used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Lavender oil (1338)*.

Test solution. The substance to be examined.

Content: minimum 90.0 per cent, calculated by the normalisation procedure.

Lavandulyl acetate. $C_{12}H_{20}O_2$. (M_r 196.3). *1114200.* [25905-14-0]. 2-Isopropenyl-5-methylhex-4-en-1-yl acetate.

Colourless liquid with a characteristic odour.

Lavandulyl acetate used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Lavender oil (1338)*.

Test solution. The substance to be examined.

Content: minimum 93.0 per cent, calculated by the normalisation procedure.

Lead acetate. $C_4H_6O_4Pb,3H_2O$. (M_r 379.3). *1048100.* [6080-56-4]. Lead di-acetate.

Colourless crystals, efflorescent, freely soluble in water, soluble in ethanol (96 per cent).

Lead acetate cotton. *1048101.*

Immerse absorbent cotton in a mixture of 1 volume of *dilute acetic acid R* and 10 volumes of *lead acetate solution R*. Drain off the excess of liquid, without squeezing the cotton, by placing it on several layers of filter paper. Allow to dry in air.

Storage: in an airtight container.

Lead acetate paper. *1048102.*

Immerse filter paper weighing about 80 g/m^2 in a mixture of 1 volume of *dilute acetic acid R* and 10 volumes of *lead acetate solution R*. After drying, cut the paper into strips 15 mm by 40 mm.

Lead acetate solution. *1048103.*

A 95 g/L solution of *lead acetate R* in *carbon dioxide-free water R*.

Lead dioxide. PbO_2. (M_r 239.2). *1048200.* [1309-60-0].

Dark brown powder, evolving oxygen when heated, practically insoluble in water, soluble in hydrochloric acid with evolution of chlorine, soluble in dilute nitric acid in the presence of hydrogen peroxide, oxalic acid or other reducing agents, soluble in hot, concentrated alkali hydroxide solutions.

Lead nitrate. $Pb(NO_3)_2$. (M_r 331.2). *1048300.* [10099-74-8]. Lead dinitrate.

White or almost white, crystalline powder or colourless crystals, freely soluble in water.

Lead nitrate solution. *1048301.*

A 33 g/L solution of *lead nitrate R*.

Lead subacetate solution. *1048400.* [1335-32-6]. Basic lead acetate solution.

Content: 16.7 per cent *m/m* to 17.4 per cent *m/m* of Pb (A_r 207.2) in a form corresponding approximately to the formula $C_8H_{14}O_{10}Pb_3$.

Dissolve 40.0 g of *lead acetate R* in 90 mL of *carbon dioxide-free water R*. Adjust the pH to 7.5 with *strong sodium hydroxide solution R*. Centrifuge and use the clear colourless supernatant solution.

The solution remains clear when stored in a well-closed container.

Leiocarposide. $C_{27}H_{34}O_{16}$. (M_r 614.5). *1150200.* [71953-77-0]. 2-(β-D-Glucopyranosyloxy)benzyl 3-(β-D-glucopyranosyloxy)-6-hydroxy-2-methoxybenzoate. 2-[[[3-(β-D-Glucopyranosyloxy)-6-hydroxy-2-methoxybenzoyl]oxy]methyl]phenyl-β-D-glucopyranoside.

White or almost white powder, soluble in water, freely soluble in methanol, slightly soluble in ethanol (96 per cent).

mp: 190 °C to 193 °C.

Lemon oil. *1101700.*

See *Lemon oil (0620)*.

Leucine. *1048500.* [61-90-5].

See *Leucine (0771)*.

Levodopa. *1170000.* [59-92-7].

See *Levodopa (0038)*.

(*Z*)-Ligustilide. $C_{12}H_{14}O_2$. (M_r 190.2). *1180300.* [81944-09-4]. (3*Z*)-3-Butylidene-1,3,4,5-tetrahydroisobenzofuran-1-one.

Limonene. $C_{10}H_{16}$. (M_r 136.2). *1048600.* [5989-27-5]. D-Limonene. (+)-*p*-Mentha-1,8-diene. (*R*)-4-Isopropenyl-1-methylcyclohex-1-ene.

Colourless liquid, practically insoluble in water, soluble in ethanol (96 per cent).

d_{20}^{20}: about 0.84.

n_D^{20}: 1.471 to 1.474.

$[\alpha]_D^{20}$: about + 124.

bp: 175 °C to 177 °C.

Limonene used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Peppermint oil (0405)*.

Test solution. The substance to be examined.

Content: minimum 99.0 per cent, calculated by the normalisation procedure.

Linalol. $C_{10}H_{18}O$. (M_r 154.2). *1048700.* [78-70-6]. (*RS*)-3,7-Dimethylocta-1,6-dien-3-ol.

Mixture of two stereoisomers (licareol and coriandrol).

Liquid, practically insoluble in water.

d_{20}^{20}: about 0.860.

n_D^{20}: about 1.462.

bp: about 200 °C.

Linalol used in gas chromatography complies with the following test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Anise oil (0804)*.

Test solution. The substance to be examined.

Content: minimum 98.0 per cent, calculated by the normalisation procedure.

Linalyl acetate. $C_{12}H_{20}O_2$. (M_r 196.3). *1107200.* [115-95-7]. (*RS*)-1,5-Dimethyl-1-vinylhex-4-enyl acetate.

Colourless or slightly yellow liquid with a strong odour of bergamot and lavender.

d_{25}^{25}: 0.895 to 0.912.

n_D^{20}: 1.448 to 1.451.

bp: about 215 °C.

Linalyl acetate used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Bitter-orange-flower oil (1175)*.

Test solution. The substance to be examined.

Content: minimum 95.0 per cent, calculated by the normalisation procedure.

Lindane. $C_6H_6Cl_6$. (M_r 290.8). *1128900.* [58-89-9]. γ-Hexachlorocyclohexane.

For the monograph *Wool fat (0134)*, a suitable certified reference solution (10 ng/µL in cyclohexane) may be used.

Linoleic acid. $C_{18}H_{32}O_2$. (M_r 280.5). *1143200.* [60-33-3]. (9*Z*,12*Z*)-Octadeca-9,12-dienoic acid.

Colourless, oily liquid.

d_4^{20}: about 0.903.

n_D^{20}: about 1.470.

Linoleic acid used in the assay of total fatty acids in Saw palmetto fruit (1848) complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Saw palmetto fruit (1848)*.

Content: minimum 98 per cent, calculated by the normalisation procedure.

Linolenic acid. $C_{18}H_{30}O_2$. (M_r 278.4). *1143300.* [463-40-1]. (9*Z*,12*Z*,15*Z*)-Octadeca-9,12,15-trienoic acid. α-Linolenic acid.

Colourless liquid, practically insoluble in water, soluble in organic solvents.

d_4^{20}: about 0.915.

n_D^{20}: about 1.480.

Linolenic acid used in the assay of total fatty acids in Saw palmetto fruit (1848) complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Saw palmetto fruit (1848)*.

Content: minimum 98 per cent, calculated by the normalisation procedure.

Linolenyl alcohol. $C_{18}H_{32}O$. (M_r 264.4). *1156200.* [24149-05-1]. (9*Z*,12*Z*,15*Z*)-Octadeca-9,12,15-trien-1-ol. α-Linolenyl alcohol.

Content: minimum 96 per cent.

Linoleyl alcohol. $C_{18}H_{34}O$. (M_r 266.5). *1155900.* [506-43-4]. (9*Z*,12*Z*)-Octadeca-9,12-dien-1-ol.

Relative density: 0.830.

Content: minimum 85 per cent.

Linsidomine hydrochloride. $C_6H_{11}ClN_4O_2$. (M_r 206.6). *1171200.* [16142-27-1]. 3-(Morpholin-4-yl)sydnonimine hydrochloride. 3-(Morpholin-4-yl)-1,2,3-oxadiazol-3-ium-5-aminide hydrochloride.

White or almost white powder.

Liquid scintillation cocktail. *1167300.*

Commercially available solution for the determination of radioactivity by liquid scintillation counting. It contains one or more fluorescent agents and mostly one or more emulsifying agents in a suitable organic solvent or mixture of organic solvents.

Liquid scintillation cocktail R1. *1176800.*

To 1000 mL of *dioxan R*, add 0.3 g of *methylphenyloxazolylbenzene R*, 7 g of *diphenyloxazole R* and 100 g of *naphthalene R*.

Lithium. Li. (A_r 6.94). *1048800.* [7439-93-2].

A soft metal whose freshly cut surface is silvery-grey. It rapidly tarnishes in contact with air. It reacts violently with water, yielding hydrogen and giving a solution of lithium hydroxide; soluble in methanol, yielding hydrogen and a solution of lithium methoxide; practically insoluble in light petroleum.

Storage: under light petroleum or liquid paraffin.

Lithium carbonate. Li_2CO_3. (M_r 73.9). *1048900.* [554-13-2]. Dilithium carbonate.

White or almost white, light powder, sparingly soluble in water, very slightly soluble in ethanol (96 per cent). A saturated solution at 20 °C contains about 13 g/L of Li_2CO_3.

Lithium chloride. LiCl. (M_r 42.39). *1049000.* [7447-41-8].

Crystalline powder or granules or cubic crystals, deliquescent, freely soluble in water, soluble in acetone and in ethanol (96 per cent). Aqueous solutions are neutral or slightly alkaline.

Storage: in an airtight container.

Lithium hydroxide. $LiOH,H_2O$. (M_r 41.96). *1049100.* [1310-66-3]. Lithium hydroxide monohydrate.

White or almost white, granular powder, strongly alkaline, it rapidly absorbs water and carbon dioxide, soluble in water, sparingly soluble in ethanol (96 per cent).

Storage: in an airtight container.

Lithium metaborate, anhydrous. $LiBO_2$. (M_r 49.75). *1120000.* [13453-69-5].

Lithium sulfate. Li_2SO_4,H_2O. (M_r 128.0). *1049200.* [10102-25-7]. Dilithium sulfate monohydrate.

Colourless crystals, freely soluble in water, practically insoluble in ethanol (96 per cent).

Lithium trifluoromethanesulfonate. CF_3LiO_3S. (M_r 156.0). *1173400.* [33454-82-9].

Litmus. *1049300.* [1393-92-6].

Schultz No. 1386.

Indigo-blue fragments prepared from various species of Rocella, Lecanora or other lichens, soluble in water, practically insoluble in ethanol (96 per cent).

Colour change: pH 5 (red) to pH 8 (blue).

Litmus paper, blue. *1049301.*

Boil 10 parts of coarsely powdered *litmus R* for 1 h with 100 parts of *ethanol (96 per cent) R*. Decant the alcohol and add to the residue a mixture of 45 parts of *ethanol (96 per cent) R* and 55 parts of *water R*. After 2 days decant the clear liquid. Impregnate strips of filter paper with the solution and allow to dry.

Test for sensitivity. Immerse a strip measuring 10 mm by 60 mm in a mixture of 10 mL of *0.02 M hydrochloric acid* and 90 mL of *water R*. On shaking the paper turns red within 45 s.

Litmus paper, red. *1049302.*

To the blue litmus extract, add *dilute hydrochloric acid R* dropwise until the blue colour becomes red. Impregnate strips of filter paper with the solution and allow to dry.

Test for sensitivity. Immerse a strip measuring 10 mm by 60 mm in a mixture of 10 mL of *0.02 M sodium hydroxide* and 90 mL of *water R*. On shaking the paper turns blue within 45 s.

Loganin. $C_{17}H_{26}O_{10}$. (M_r 390.4). *1136700.* [18524-94-2]. Methyl (1*S*,4a*S*,6*S*,7*R*,7a*S*)-1-(β-D-glucopyranosyloxy)-6-hydroxy-7-methyl-1,4a,5,6,7,7a-hexahydrocyclopenta[*c*]pyran-4-carboxylate.

mp: 220 °C to 221 °C.

Longifolene. $C_{15}H_{24}$. (M_r 204.4). *1150300.* [475-20-7]. (1*S*,3a*R*,4*S*,8a*S*)-4,8,8-Trimethyl-9-methylenedecahydro-1,4-methanoazulene.

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

d_4^{18}: 0.9319.

n_D^{20}: 1.5050.

$[\alpha]_D^{20}$: + 42.7.

bp: 254 °C to 256 °C.

Longifolene used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Turpentine oil, Pinus pinaster type (1627).*

Content: minimum 98.0 per cent, calculated by the normalisation procedure.

Low-vapour-pressure hydrocarbons (type L). *1049400.*

Unctuous mass, soluble in benzene and in toluene.

Lumiflavine. $C_{13}H_{12}N_4O_2$. (M_r 256.3). *1141000.* [1088-56-8]. 7,8,10-Trimethylbenzo[*g*]pteridine-2,4(3*H*,10*H*)-dione.

Yellow powder or orange crystals, very slightly soluble in water, freely soluble in methylene chloride.

Luteolin. $C_{15}H_{10}O_6$. (M_r 286.2). *1198500.* [491-70-3]. 2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4*H*-1-benzopyran-4-one.

Luteolin-7-glucoside. $C_{21}H_{20}O_{11}$. (M_r 448.4). *1163400.* [5373-11-5]. 2-(3,4-Dihydroxyphenyl)-7-(β-D-glucopyranosyloxy)-5-hydroxy-4*H*-1-benzopyran-4-one.

Yellow powder.

Absorbance (*2.2.25*). A solution in *methanol R* shows absorption maxima at 255 nm, 267 nm and 350 nm.

mp: about 247 °C.

Lutetium chloride hexahydrate. $LuCl_3,6H_2O$. (M_r 389.4). *1199600.* [15230-79-2].

White to yellow, crystalline powder, freely soluble in water.

Lysyl endopeptidase. *1188000.* [78642-25-8].

Achromobacter endoproteinase I. Lysyl bond specific proteinase (EC 3.4.21.50).

It belongs to the serine endopeptidase family. Initially isolated from *Achromobacter lyticus*. Enzymes with similar specificity are produced by *Lysobacter enzymogenes* (endoproteinase Lys-C) and *Pseudomonas aeruginosa* (Ps-1). It cleaves peptide bonds at the carboxy-terminal of both lysine residues and *S*-aminoethylcysteine residues with a high degree of specificity. 1 amidase unit (U) is the amount of enzyme that will produce 1 micromole of *p*-nitroaniline from *N*-benzoyl-DL-lysine-*p*-nitroaniline per minute at 30 °C at pH 9.5.

Macrogol 23 lauryl ether. *1129000.*

See *Macrogol lauryl ether (1124)*, the number of moles of ethylene oxide reacted per mole of lauryl alcohol being 23 (nominal value).

Macrogol 200. *1099200.* [25322-68-3]. Polyethyleneglycol 200.

Clear, colourless or almost colourless viscous liquid, very soluble in acetone and in anhydrous ethanol, practically insoluble in fatty oils.

d_{20}^{20}: about 1.127.

n_D^{20}: about 1.450.

Macrogol 200 R1. *1099201.*

Introduce 500 mL of *macrogol 200 R* into a 1000 mL round bottom flask. Using a rotation evaporator remove any volatile components applying for 6 h a temperature of 60 °C and a vacuum with a pressure of 1.5-2.5 kPa.

Macrogol 300. *1067100.* [25322-68-3]. Polyethyleneglycol 300.

See *Macrogols (1444)*.

Macrogol 400. *1067200.* [25322-68-3]. Polyethyleneglycol 400.

See *Macrogols (1444)*.

Macrogol 600. *1189700.* [25322-68-3]. Polyethyleneglycol 600.

See *Macrogols (1444)*.

Macrogol 1000. *1067300.* [25322-68-3]. Polyethyleneglycol 1000.

See *Macrogols (1444)*.

Macrogol 1500. *1067400.* [25322-68-3]. Polyethyleneglycol 1500.

See *Macrogols (1444)*.

Macrogol 4000. *1198000.* [25322-68-3]. Polyethyleneglycol 4000.

See *Macrogols (1444)*.

Macrogol 6000. *1189800.* [25322-68-3]. Polyethyleneglycol 6000.

White or almost white solid with a waxy or paraffin-like appearance, very soluble in water and in methylene chloride, practically insoluble in ethanol (96 per cent), in fatty oils and in mineral oils.

Macrogol 20 000. *1067600.* Polyethyleneglycol 20 000.

See *Macrogols (1444)*.

Macrogol 20 000 2-nitroterephthalate. *1067601.*

Polyethyleneglycol 20 000 with embedded 2-nitroterephthalate groups.

Macrogol, base-deactivated. *1170300.*
Base-deactivated polyethyleneglycol.

Macrogol cetostearyl ether. *1196100.*
See *Macrogol cetostearyl ether (1123)*.

Macrogol, polar-deactivated. *1179000.*
Polar-deactivated polyethyleneglycol.

Magnesium. Mg. (A_r 24.30). *1049500.* [7439-95-4].
Silver-white ribbon, turnings or wire, or a grey powder.

Magnesium acetate. $C_4H_6MgO_4,4H_2O$. (M_r 214.5). *1049600.* [16674-78-5]. Magnesium diacetate tetrahydrate.
Colourless crystals, deliquescent, freely soluble in water and in ethanol (96 per cent).
Storage: in an airtight container.

Magnesium chloride. *1049700.* [7791-18-6].
See *Magnesium chloride hexahydrate (0402)*.

Magnesium nitrate. $Mg(NO_3)_2,6H_2O$. (M_r 256.4). *1049800.* [13446-18-9]. Magnesium nitrate hexahydrate.
Colourless, clear crystals, deliquescent, very soluble in water, freely soluble in ethanol (96 per cent).
Storage: in an airtight container.

Magnesium nitrate solution. *1049801.*
Dissolve 17.3 g of *magnesium nitrate R* in 5 mL of *water R* warming gently and add 80 mL of *ethanol (96 per cent) R*. Cool and dilute to 100.0 mL with the same solvent.

Magnesium oxide. *1049900.* [1309-48-4].
See *Light magnesium oxide (0040)*.

Magnesium oxide R1. *1049901.*
Complies with the requirements prescribed for *magnesium oxide R* with the following modifications.
Arsenic (*2.4.2, Method A*): maximum 2 ppm.
Dissolve 0.5 g in a mixture of 5 mL of *water R* and 5 mL of *hydrochloric acid R1*.
Heavy metals (*2.4.8*): maximum 10 ppm.
Dissolve 1.0 g in a mixture of 3 mL of *water R* and 7 mL of *hydrochloric acid R1*. Add 0.05 mL of *phenolphthalein solution R* and *concentrated ammonia R* until a pink colour is obtained. Neutralise the excess of ammonia by the addition of *glacial acetic acid R*. Add 0.5 mL in excess and dilute to 20 mL with *water R*. Filter, if necessary. 12 mL of the solution complies with test A. Prepare the reference solution using a mixture of 5 mL of *lead standard solution (1 ppm Pb) R* and 5 mL of *water R*.
Iron (*2.4.9*): maximum 50 ppm.
Dissolve 0.2 g in 6 mL of *dilute hydrochloric acid R* and dilute to 10 mL with *water R*.

Magnesium oxide, heavy. *1050000.* [1309-48-4].
See *Heavy magnesium oxide (0041)*.

Magnesium silicate for pesticide residue analysis. *1129100.* [1343-88-0].
Magnesium silicate for chromatography (60-100 mesh).

Magnesium sulfate. *1050200.* [10034-99-8].
See *Magnesium sulfate heptahydrate (0044)*.

Magnolin. $C_{23}H_{28}O_7$. (M_r 416.5). *1200300.* [31008-18-1].
(3*S*,3a*R*,6*S*,6a*R*)-3-(3,4-Dimethoxyphenyl)-6-(3,4,5-trimethoxyphenyl)-1,3,3a,4,6,6a-hexahydrofuro[3,4-*c*]furan.

Magnolol. $C_{18}H_{18}O_2$. (M_r 266.3). *1182800.* [528-43-8]. 5,5′-Di(prop-2-enyl)biphenyl-2,2′-diol. 5,5′-Diallyl-2,2′-dihydroxybiphenyl. 5,5′-Di-2-propenyl-[1,1′-biphenyl]-2,2′-diol.

Maize oil. *1050400.*
See *Maize oil, refined (1342)*.

Makisterone A. $C_{28}H_{46}O_7$. (M_r 494.7). *1207200.* [20137-14-8]. (22*R*)-2β,3β,14,20,22,25-Hexahydroxy-5β-ergost-7-en-6-one.

Malachite green. $C_{23}H_{25}ClN_2$. (M_r 364.9). *1050500.* [123333-61-9].
Schultz No. 754.
Colour Index No. 42000.
[4-[[4-(Dimethylamino)phenyl]phenylmethylene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium chloride.
Green crystals with a metallic lustre, very soluble in water giving a bluish-green solution, soluble in ethanol (96 per cent) and in methanol.
Absorbance (*2.2.25*). A 0.01 g/L solution in *ethanol (96 per cent) R* shows an absorption maximum at 617 nm.

Malachite green solution. *1050501.*
A 5 g/L solution of *malachite green R* in *anhydrous acetic acid R*.

Malathion. $C_{10}H_{19}O_6PS_2$. (M_r 330.3). *1129200.* [121-75-5].
bp: about 156 °C.
A suitable certified reference solution (10 ng/µL in iso-octane) may be used.

Maleic acid. *1050600.* [110-16-7].
See *Maleic acid (0365)*.

Maleic anhydride. $C_4H_2O_3$. (M_r 98.1). *1050700.* [108-31-6]. Butenedioic anhydride. 2,5-Furandione.
White or almost white crystals, soluble in water forming maleic acid, very soluble in acetone and in ethyl acetate, freely soluble in toluene, soluble in ethanol (96 per cent) with ester formation, very slightly soluble in light petroleum.
mp: about 52 °C.
Any residue insoluble in toluene does not exceed 5 per cent (maleic acid).

Maleic anhydride solution. *1050701.*
Dissolve 5 g of *maleic anhydride R* in *toluene R* and dilute to 100 mL with the same solvent. Use within one month. If the solution becomes turbid, filter.

Malic acid. *1200400.* [6915-15-7].
See *Malic acid (2080)*.

Maltitol. *1136800.* [585-88-6].
See *Maltitol (1235)*.

Maltol. $C_6H_6O_3$. (M_r 126.1). *1202300.* [118-71-8]. 3-Hydroxy-2-methyl-4*H*-pyran-4-one.
White or almost white crystalline powder, soluble in hot water.
mp: 161 °C to 162 °C.

Maltose monohydrate. $C_{12}H_{22}O_{11},H_2O$. (M_r 360.3). *1193100.* [6363-53-7]. 4-*O*-α-D-glucopyranosyl-D-glucopyranose monohydrate.

Maltotriose. $C_{18}H_{32}O_{16}$. (M_r 504.4). *1176300.* [1109-28-0]. α-D-Glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-D-glucose.
White or almost white, crystalline powder, very soluble in water.
mp: about 134 °C.

Mandelic acid. $C_8H_8O_3$. (M_r 152.1). *1171300.* [90-64-2]. 2-Hydroxy-2-phenylacetic acid.
White crystalline flakes, soluble in water.
mp: 118 to 121 °C.

Manganese sulfate. $MnSO_4,H_2O$. (M_r 169.0). *1050900.* [10034-96-5]. Manganese sulfate monohydrate.

Pale-pink, crystalline powder or crystals, freely soluble in water, practically insoluble in ethanol (96 per cent).

Loss on ignition: 10.0 per cent to 12.0 per cent, determined on 1.000 g at 500 ± 50 °C.

Mannitol. *1051000.* [69-65-8].

See *Mannitol (0559)*.

Mannose. $C_6H_{12}O_6$. (M_r 180.2). *1051100.* [3458-28-4]. D-(+)-Mannose.

white or almost white, crystalline powder or small crystals, very soluble in water, slightly soluble in anhydrous ethanol.

$[\alpha]_D^{20}$: + 13.7 + 14.7, determined on a 200 g/L solution in *water R* containing about 0.05 per cent of NH_3.

mp: about 132 °C, with decomposition.

Marrubiin. $C_{20}H_{28}O_4$. (M_r 332.4). *1158300.* [465-92-9]. (2a*S*,5a*S*,6*R*,7*R*,8a*R*,8b*R*)-6-[2-(Furan-3-yl)ethyl]-6-hydroxy-2a,5a,7-trimethyldecahydro-2*H*-naphtho[1,8-*bc*]furan-2-one.

Colourless, microcrystalline powder.

Marrubiin used in liquid chromatography complies with the following additional test.

Assay. Liquid chromatography (*2.2.29*) as prescribed in the monograph *White horehound (1835)*.

Content: minimum 95.0 per cent, calculated by the normalisation procedure.

Meclozine dihydrochloride. *1051200.* [1104-22-9].

See *Meclozine dihydrochloride (0622)*.

Medronic acid. *1193200.* [1984-15-2].

See *Medronic acid for radiopharmaceutical preparations (2350)*.

Melamine. $C_3H_6N_6$. (M_r 126.1). *1051300.* [108-78-1]. 1,3,5-Triazine-2,4,6-triamine.

A white or almost white, amorphous powder, very slightly soluble in water and in ethanol (96 per cent).

Menadione. *1051400.* [58-27-5].

See *Menadione (0507)*.

Menthofuran. $C_{10}H_{14}O$. (M_r 150.2). *1051500.* [17957-94-7]. 3,9-Epoxy-*p*-mentha-3,8-diene. 3,6-Dimethyl-4,5,6,7-tetrahydro-benzofuran.

Slightly bluish liquid, very slightly soluble in water, soluble in ethanol (96 per cent).

d_{15}^{20}: about 0.965.

n_D^{20}: about 1.480.

$[\alpha]_D^{20}$: about + 93.

bp: 196 °C.

Menthofuran used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Peppermint oil (0405)*.

Test solution. The substance to be examined.

Content: minimum 97.0 per cent, calculated by the normalisation procedure.

Menthol. *1051600.* [2216-51-5].

See *Levomenthol (0619)* and *Racemic menthol (0623)*.

Menthol used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the related substances test included in the monograph *Racemic menthol (0623)*.

Content: minimum 98.0 per cent, calculated by the normalisation procedure.

Menthone. $C_{10}H_{18}O$. (M_r 154.2). *1051700.* [14073-97-3]. (2*S*,5*R*)-2-Isopropyl-5-methylcyclohexanone. (–)-*trans*-*p*-Menthan-3-one.

Contains variable amounts of isomenthone.

Colourless liquid, very slightly soluble in water, very soluble in ethanol (96 per cent).

d_{20}^{20}: about 0.897.

n_D^{20}: about 1.450.

Menthone used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Peppermint oil (0405)*.

Test solution. The substance to be examined.

Content: minimum 90.0 per cent, calculated by the normalisation procedure.

Menthyl acetate. $C_{12}H_{22}O_2$. (M_r 198.3). *1051800.* [2623-23-6]. (1*R*,2*S*,5*R*)-5-Methyl-2-(propan-2-yl)cyclohexyl acetate.

Colourless liquid, slightly soluble in water, miscible with ethanol (96 per cent).

d_{20}^{20}: about 0.92.

n_D^{20}: about 1.447.

bp: about 228 °C.

Menthyl acetate used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Peppermint oil (0405)*.

Test solution. The substance to be examined.

Content: minimum 97.0 per cent, calculated by the normalisation procedure.

2-Mercaptobenzimidazole. $C_7H_6N_2S$. (M_r 150.2). *1170100.* [583-39-1]. 1*H*-benzimidazole-2-thiol.

mp: about 302 °C.

2-Mercaptoethanol. C_2H_6OS. (M_r 78.1). *1099300.* [60-24-2].

Liquid, miscible with water.

d_{20}^{20}: about 1.116.

bp: about 157 °C.

Mercaptopurine. *1051900.* [6112-76-1].

See *Mercaptopurine (0096)*.

Mercuric acetate. $C_4H_6HgO_4$. (M_r 318.7). *1052000.* [1600-27-7]. Mercury diacetate.

White or almost white crystals, freely soluble in water, soluble in ethanol (96 per cent).

Mercuric acetate solution. *1052001.*

Dissolve 3.19 g of *mercuric acetate R* in *anhydrous acetic acid R* and dilute to 100 mL with the same acid. If necessary, neutralise the solution with *0.1 M perchloric acid* using 0.05 mL of *crystal violet solution R* as indicator.

Mercuric chloride. *1052200.* [7487-94-7].

See *Mercuric chloride (0120)*.

Mercuric chloride solution. *1052201.*

A 54 g/L solution of *mercuric chloride R*.

Mercuric iodide. HgI_2. (M_r 454.4). *1052300.* [7774-29-0]. Mercury di-iodide.

Dense, scarlet, crystalline powder, slightly soluble in water, sparingly soluble in acetone and in ethanol (96 per cent), soluble in an excess of *potassium iodide solution R*.

Storage: protected from light.

Mercuric nitrate. $Hg(NO_3)_2,H_2O$. (M_r 342.6). *1052400.* [7783-34-8]. Mercury dinitrate monohydrate.

Colourless or slightly coloured crystals, hygroscopic, soluble in water in the presence of a small quantity of nitric acid.

Storage: in an airtight container, protected from light.

Mercuric oxide. HgO. (M_r 216.6). *1052500.* [21908-53-2]. Yellow mercuric oxide. Mercury oxide.

A yellow to orange-yellow powder, practically insoluble in water and in ethanol (96 per cent).

Storage: protected from light.

Mercuric sulfate solution. *1052600.* [7783-35-9].

Dissolve 1 g of *mercuric oxide R* in a mixture of 20 mL of *water R* and 4 mL of *sulfuric acid R*.

Mercuric thiocyanate. $Hg(SCN)_2$. (M_r 316.7). *1052700.* [592-85-8]. Mercury di(thiocyanate).

White or almost white, crystalline powder, very slightly soluble in water, slightly soluble in ethanol (96 per cent), soluble in solutions of sodium chloride.

Mercuric thiocyanate solution. *1052701.*

Dissolve 0.3 g of *mercuric thiocyanate R* in *anhydrous ethanol R* and dilute to 100 mL with the same solvent.

Storage: use within 1 week.

Mesityl oxide. $C_6H_{10}O$. (M_r 98.1). *1120100.* [141-79-7]. 4-Methylpent-3-en-2-one.

Colourless, oily liquid, soluble in 30 parts of water, miscible with most organic solvents.

d_{20}^{20}: about 0.858.

bp: 129 °C to 130 °C.

Metanil yellow. $C_{18}H_{14}N_3NaO_3S$. (M_r 375.4). *1052900.* [587-98-4].

Schultz No. 169.

Colour Index No. 13065.

Sodium 3-[4-(phenylamino)phenylazo]benzenesulfonate.

A brownish-yellow powder, soluble in water and in ethanol (96 per cent).

Metanil yellow solution. *1052901.*

A 1 g/L solution of *metanil yellow R* in *methanol R*.

Test for sensitivity. To 50 mL of *anhydrous acetic acid R* add 0.1 mL of the metanil yellow solution. Add 0.05 mL of *0.1 M perchloric acid*; the colour changes from pinkish-red to violet.

Colour change: pH 1.2 (red) to pH 2.3 (orange-yellow).

Metaphosphoric acid. $(HPO_3)_x$. *1053000.* [37267-86-0].

Glassy lumps or sticks containing a proportion of sodium metaphosphate, hygroscopic, very soluble in water.

Nitrates. Boil 1.0 g with 10 mL of *water R*, cool, add 1 mL of *indigo carmine solution R*, 10 mL of *nitrogen-free sulfuric acid R* and heat to boiling. The blue colour is not entirely discharged.

Reducing substances: maximum 0.01 per cent, calculated as H_3PO_3.

Dissolve 35.0 g in 50 mL of *water R*. Add 5 mL of a 200 g/L solution of *sulfuric acid R*, 50 mg of *potassium bromide R* and 5.0 mL of *0.02 M potassium bromate* and heat on a water-bath for 30 min. Allow to cool and add 0.5 g of *potassium iodide R*. Titrate the liberated iodine with *0.1 M sodium thiosulfate*, using 1 mL of *starch solution R* as indicator. Carry out a blank test.

1 mL of *0.02 M potassium bromate* is equivalent to 4.10 mg of H_3PO_3.

Storage: in an airtight container.

Methacrylic acid. $C_4H_6O_2$. (M_r 86.1). *1101800.* [79-41-4]. 2-Methylprop-2-enoic acid.

Colourless liquid.

n_D^{20}: about 1.431.

bp: about 160 °C.

mp: about 16 °C.

Methane. CH_4. (M_r 16). *1166300.* [74-82-8].

Content: minimum 99.0 per cent *V/V*.

Methane R1. CH_4. (M_r 16). *1176400.* [74-82-8].

Content: minimum 99.995 per cent *V/V*.

Methanesulfonic acid. CH_4O_3S. (M_r 96.1). *1053100.* [75-75-2].

Clear, colourless liquid, solidifying at about 20 °C, miscible with water, slightly soluble in toluene, practically insoluble in hexane.

d_{20}^{20}: about 1.48.

n_D^{20}: about 1.430.

Methanesulfonyl chloride. CH_3ClO_2S. (M_r 114.6). *1181300.* [124-63-0].

Clear, colourless or slightly yellow liquid.

Content: minimum 99.0 per cent.

Density: 1.48 g/cm^3.

n_D^{20}: about 1.452.

bp: about 161 °C.

Methanol. CH_4O. (M_r 32.04). *1053200.* [67-56-1].

Clear, colourless, flammable liquid, miscible with water and with ethanol (96 per cent).

d_{20}^{20}: 0.791 to 0.793.

bp: 64 °C to 65 °C.

Methanol R1. *1053201.*

Complies with the requirements prescribed for *methanol R* with the following additional requirement.

Absorbance (*2.2.25*): maximum 0.70 at 210 nm, 0.30 at 220 nm, 0.13 at 230 nm, 0.02 at 250 nm, 0.01 at 260 nm and higher wavelengths, determined using *water R* as compensation liquid.

Methanol R2. *1053202.*

Complies with the requirements prescribed for *methanol R* and the following additional requirements.

Content: minimum 99.8 per cent.

Absorbance (2.2.25): maximum 0.17, determined at 225 nm using *water R* as the compensation liquid.

Methanol, hydrochloric. *1053203.*

Dilute 1.0 mL of *hydrochloric acid R1* to 100.0 mL with *methanol R*.

Methanol, aldehyde-free. *1053300.*

Dissolve 25 g of *iodine R* in 1 L of *methanol R* and pour the solution, with constant stirring, into 400 mL of *1 M sodium hydroxide*. Add 150 mL of *water R* and allow to stand for 16 h. Filter. Boil under a reflux condenser until the odour of iodoform disappears. Distil the solution by fractional distillation.

Aldehydes and ketones: maximum 0.001 per cent.

Methanol, anhydrous. *1053400.* [67-56-1].

Treat 1000 mL of *methanol R* with 5 g of *magnesium R*. If necessary initiate the reaction by adding 0.1 mL of *mercuric chloride solution R*. When the evolution of gas has ceased, distil the liquid and collect the distillate in a dry container protected from moisture.

Water (*2.5.12*): maximum 0.3 g/L.

DL-Methionine. *1129400.* [59-51-8].

See *DL-Methionine (0624)*.

L-Methionine. *1053500.* [63-68-3].

See *Methionine (1027)*.

L-Methionine sulfoxide. $C_5H_{11}NO_3S$. (M_r 165.2). *1193300.* [3226-65-1]. (2*S*)-2-Amino-4-[(*RS*)-methylsulfinyl]butanoic acid.

(*RS*)-Methotrexate. $C_{20}H_{22}N_8O_5$. *1120200.* [60388-53-6]. (*RS*)-2-[4-[[(2,4-diaminopteridin-6-yl)methyl]-methylamino]benzoylamino]pentanedioic acid.

Content: minimum 96.0 per cent.

mp: about 195 °C.

Methoxychlor. $C_{16}H_{15}Cl_3O_2$. (M_r 345.7). *1129300.* [72-43-5]. 1,1-(2,2,2-Trichloroethylidene)-bis(4-methoxybenzene).

Practically insoluble in water, freely soluble in most organic solvents.

bp: about 346 °C.

mp: 78 °C to 86 °C.

A suitable certified reference solution (10 ng/µL in iso-octane) may be used.

***trans*-2-Methoxycinnamaldehyde.** $C_{10}H_{10}O_2$. (M_r 162.2). *1129500.* [60125-24-8].

mp: 44 °C to 46 °C.

trans-2-Methoxycinnamaldehyde used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Cassia oil (1496)*.

Content: minimum 96.0 per cent, calculated by the normalisation procedure.

(1*RS*)-1-(6-Methoxynaphthalen-2-yl)ethanol. $C_{13}H_{14}O_2$. (M_r 202.3). *1159600.* [77301-42-9]. 6-Methoxy-α-methyl-2-naphthalenemethanol.

White or almost white powder.

mp: about 113 °C.

1-(6-Methoxynaphthalen-2-yl)ethanone. $C_{13}H_{12}O_2$. (M_r 200.2). *1159700.* [3900-45-6]. 6′-Methoxy-2′-acetonaphthone.

White or almost white powder.

mp: about 108 °C.

6-Methoxy-2-naphthoic acid. $C_{12}H_{10}O_3$. (M_r 202.2). *1184200.* [2471-70-7]. 6-Methoxynaphthalene-2-carboxylic acid.

White or almost white, crystalline powder.

mp: 201 °C to 206 °C.

Methoxyphenylacetic acid. $C_9H_{10}O_3$. (M_r 166.2). *1053600.* [7021-09-2]. (*RS*)-2-Methoxy-2-phenylacetic acid.

White, crystalline powder or white or almost white crystals, sparingly soluble in water, freely soluble in ethanol (96 per cent).

mp: about 70 °C.

Methoxyphenylacetic reagent. *1053601.*

Dissolve 2.7 g of *methoxyphenylacetic acid R* in 6 mL of *tetramethylammonium hydroxide solution R* and add 20 mL of *anhydrous ethanol R*.

Storage: in a polyethylene container.

3-Methoxy-L-tyrosine. $C_{10}H_{13}NO_4H_2O$. (M_r 229.2). *1164400.* [200630-46-2].

Off-white or yellow powder.

Methyl acetate. $C_3H_6O_2$. (M_r 74.1). *1053700.* [79-20-9].

Clear, colourless liquid, soluble in water, miscible with ethanol (96 per cent).

d_{20}^{20}: about 0.933.

n_D^{20}: about 1.361.

bp: 56 °C to 58 °C.

Methyl 4-acetylbenzoate. $C_{10}H_{10}O_3$. (M_r 178.2). *1154100.* [3609-53-8].

mp: about 94 °C.

Methyl 4-acetylbenzoate reagent. *1154101.*

Dissolve 0.25 g of *methyl 4-acetylbenzoate R* in a mixture of 5 mL of *sulfuric acid R* and 85 mL of cooled *methanol R*.

Methyl acrylate. $C_4H_6O_2$. (M_r 86.1). *1199200.* [96-33-3]. Methyl prop-2-enoate.

Clear, colourless liquid.

bp: about 80 °C.

Methylal. $C_3H_8O_2$. (M_r 76.1). *1173500.* [109-87-5]. Dimethoxymethane. Dioxapentane. Formaldehyde dimethyl acetal. Methylene dimethyl ether.

Clear, colourless, volatile, flammable liquid, soluble in water and miscible with ethanol (96 per cent).

d_{20}^{20}: about 0.860.

n_D^{20}: about 1.354.

bp: about 41 °C.

Methylal used in gas chromatography complies with the following additional test.

Content: minimum 99.5 per cent, determined by gas chromatography.

Methylamine hydrochloride. CH_6ClN. (M_r 67.5). *1198600.* [593-51-1]. Methanamine hydrochloride.

White or almost white powder.

Content: minimum 98.0 per cent.

Methyl 4-aminobenzoate. $C_8H_9NO_2$. (M_r 151.2). *1175600.* [619-45-4].

mp: 110 °C to 113 °C.

4-Methylaminophenol sulfate. $C_{14}H_{20}N_2O_6S$. (M_r 344.4). *1053800.* [55-55-0].

Colourless crystals, very soluble in water, slightly soluble in ethanol (96 per cent).

mp: about 260 °C.

3-(Methylamino)-1-phenylpropan-1-ol. $C_{10}H_{15}NO$. (M_r 165.2). *1186400.* [42142-52-9].

White or almost white powder.

mp: 59 °C to 64 °C.

Methyl anthranilate. $C_8H_9NO_2$. (M_r 151.2). *1107300.* [134-20-3]. Methyl 2-aminobenzoate.

Colourless crystals or a colourless or yellowish liquid, soluble in water, freely soluble in ethanol (96 per cent).

mp: 24 °C to 25 °C.

Methyl anthranilate used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Bitter-orange-flower oil (1175)*.

Test solution. The substance to be examined.

Content: minimum 95.0 per cent, calculated by the normalisation procedure.

Methyl arachidate. $C_{21}H_{42}O_2$. (M_r 326.6). *1053900.* [1120-28-1]. Methyl eicosanoate.

Content: minimum 98.0 per cent, determined by gas chromatography (*2.4.22*).

White or yellow, crystalline mass, soluble in ethanol (96 per cent) and in light petroleum.

mp: about 46 °C.

Methyl behenate. $C_{23}H_{46}O_2$. (M_r 354.6). *1107500.* [929-77-1]. Methyl docosanoate.

mp: 54 °C to 55 °C.

Methyl benzenesulfonate. $C_7H_8O_3S$. (M_r 172.2). *1159800.* [80-18-2].

Content: minimum 98.0 per cent.

Clear, colourless liquid.

bp: about 148 °C.

Methyl benzoate. $C_8H_8O_2$. (M_r 136.2). *1164500.* [93-58-3]. Benzoic acid, methyl ester.

Colourless liquid.

d_4^{20}: 1.088.

bp: about 200 °C.

Methylbenzothiazolone hydrazone hydrochloride. $C_8H_{10}ClN_3S,H_2O$. (M_r 233.7). *1055300.* [38894-11-0]. 3-Methylbenzothiazol-2(3*H*)-one hydrazone hydrochloride monohydrate.

Almost white or yellowish, crystalline powder.

mp: about 270 °C.

Suitability for determination of aldehydes. To 2 mL of *aldehyde-free methanol R* add 60 µL of a 1 g/L solution of *propionaldehyde R* in *aldehyde-free methanol R* and 5 mL of a 4 g/L solution of methylbenzothiazolone hydrazone hydrochloride. Mix. Allow to stand for 30 min. Prepare a blank omitting the propionaldehyde solution. Add 25.0 mL of a 2 g/L solution of *ferric chloride R* to the test solution and to the blank, dilute to 100.0 mL with *acetone R* and mix. The absorbance (*2.2.25*) of the test solution, measured at 660 nm using the blank as compensation liquid, is not less than 0.62.

(*R*)-(+)-α-Methylbenzyl isocyanate. C_9H_9NO. (M_r 147.2). *1171400.* [33375-06-3]. (+)-(*R*)-α-Methylbenzyl isocyanate. (+)-[(1*R*)-1-Isocyanatoethyl]benzene. (+)-(1*R*)-1-Phenylethyl isocyanate.

Content: minimum 99.0 per cent.

Colourless liquid.

d_{20}^{20}: about 1.045.

n_D^{20}: about 1.513.

bp: 55 °C to 56 °C at 2.5 mm Hg.

Enantiomeric purity: minimum 99.5.

Storage: at a temperature of 2 °C to 8 °C.

(*S*)-(−)-α-Methylbenzyl isocyanate. C_9H_9NO. (M_r 147.2). *1170200.* [14649-03-7]. (−)-(*S*)-α-Methylbenzyl isocyanate. (−)-[(1*S*)-1-Isocyanatoethyl]benzene. (−)-(1*S*)-1-Phenylethyl isocyanate.

Content: minimum 99.0 per cent.

Colourless liquid.

d_{20}^{20}: about 1.045.

n_D^{20}: about 1.514.

bp: 55 °C to 56 °C at 2.5 mm Hg.

Enantiomeric purity: minimum 99.5 per cent.

Storage: at a temperature of 2 °C to 8 °C.

NOTE: do not use the reagent if it is coloured.

2-Methylbutane. C_5H_{12}. (M_r 72.2). *1099500.* [78-78-4]. Isopentane.

Content: minimum 99.5 per cent of C_5H_{12}.

Very flammable colourless liquid.

d_{20}^{20}: about 0.621.

n_D^{20}: about 1.354.

bp: about 29 °C.

Water (2.5.12): maximum 0.02 per cent.

Residue on evaporation: maximum 0.0003 per cent.

Absorbance (*2.2.25*): maximum 0.30 at 210 nm, 0.07 at 220 nm, 0.01 at 240 nm and higher wavelengths, determined using *water R* as compensation liquid.

2-Methylbut-2-ene. C_5H_{10}. (M_r 70.1). *1055400.* [513-35-9].

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

bp: 37.5 °C to 38.5 °C.

Methyl 4-(butylamino)benzoate. $C_{12}H_{17}NO_2$. (M_r 207.3). *1207300.* [71839-12-8].

White or almost white solid.

Content: minimum 99.9 per cent.

Methyl caprate. *1054000.*

See *Methyl decanoate R*.

Methyl caproate. $C_7H_{14}O_2$. (M_r 130.2). *1120300.* [106-70-7]. Methyl hexanoate.

d_{20}^{20}: about 0.885.

n_D^{20}: about 1.405.

bp: 150 °C to 151 °C.

Methyl caprylate. $C_9H_{18}O_2$. (M_r 158.2). *1120400.* [111-11-5]. Methyl octanoate.

d_{20}^{20}: about 0.876.

n_D^{20}: about 1.417.

bp: 193 °C to 194 °C.

Methylcellulose 450. *1055500.* [9004-67-5].

See *Methylcellulose (0345)*.

Nominal viscosity: 450 mPa·s.

Methyl cinnamate. $C_{10}H_{10}O_2$. (M_r 162.2). *1099400.* [103-26-4].

Colourless crystals practically insoluble in water, soluble in ethanol (96 per cent).

n_D^{20}: about 1.56.

bp: about 260 °C.

mp: 34 °C to 36 °C.

Methylcyclohexane. C_7H_{14}. (M_r 98.2). *1189900.* [108-87-2].

Methyl decanoate. $C_{11}H_{22}O_2$. (M_r 186.3). *1054000.* [110-42-9].

Content: minimum 99.0 per cent.

Clear, colourless or yellow liquid, soluble in light petroleum.

d_{20}^{20}: 0.871 to 0.876.

n_D^{20}: 1.425 to 1.426.

Foreign substances. Gas chromatography (*2.2.28*), injecting equal volumes of each of the following:

A 0.02 g/L solution of the substance to be examined in *carbon disulfide R* (solution A), a 2 g/L solution of the substance to be examined in *carbon disulfide R* (solution B), and *carbon disulfide R* (solution C). Carry out the chromatographic procedure under the conditions of the test for butylated hydroxytoluene prescribed in the monograph *Wool fat (0134)*. The total area of any peaks, apart from the solvent peak and the principal peak, in the chromatogram obtained with solution B is less than the area of the principal peak in the chromatogram obtained with solution A.

Methyldopa, racemic. $C_{10}H_{13}NO_4,1½H_2O$. (M_r 238.2). *1175100.*

Mixture of equal volumes of (2*S*)- and (2*R*)-2-amino-3-(3,4-dihydroxyphenyl)-2-methylpropanoic acids.

3-*O*-Methyldopamine hydrochloride. $C_9H_{14}ClNO_2$. (M_r 203.7). *1055600.* [1477-68-5]. 4-(2-Aminoethyl)-2-methoxyphenol hydrochloride.

mp: 213 °C to 215 °C.

4-*O*-Methyldopamine hydrochloride. $C_9H_{14}ClNO_2$. (M_r 203.7). *1055700.* [645-33-0]. 5-(2-Aminoethyl)-2-methoxyphenol hydrochloride.

mp: 207 °C to 208 °C.

Methylenebisacrylamide. $C_7H_{10}N_2O_2$. (M_r 154.2). *1056000.* [110-26-9]. *N,N′*-Methylenebispropenamide.

Fine, white or almost white powder, slightly soluble in water, soluble in ethanol (96 per cent).

mp: 300 °C, with decomposition.

Methylene blue. $C_{16}H_{18}ClN_3S,xH_2O$. (M_r 319.9 for the anhydrous substance). *1055800.* [122965-43-9].

Schultz No. 1038.

Colour Index No. 52015.
3,7-Dimethylaminophenothiazin-5-ium chloride.
It occurs in different hydrated forms and may contain up to 22 per cent of water.

Dark-green or bronze, crystalline powder, freely soluble in water, soluble in ethanol (96 per cent).

Methylene blue solution. *1055801.*

Dissolve 3 mg of *methylene blue R*, 1.2 g of *sulfuric acid R* and 5.0 g of *anhydrous sodium sulfate R* in 100 mL of *water R*.

Methylene chloride. CH_2Cl_2. (M_r 84.9). *1055900.* [75-09-2]. Dichloromethane.

Colourless liquid, sparingly soluble in water, miscible with ethanol (96 per cent).

bp: 39 °C to 42 °C.

Methylene chloride used in fluorimetry complies with the following additional test.

Fluorescence. Under irradiation at 365 nm, the fluorescence (*2.2.21*) measured at 460 nm in a 1 cm cell is not more intense than that of a solution containing 0.002 ppm of *quinine R* in *0.5 M sulfuric acid* measured in the same conditions.

Methylene chloride, acidified. *1055901.*

To 100 mL of *methylene chloride R* add 10 mL of *hydrochloric acid R*, shake, allow to stand and separate the two layers. Use the lower layer.

Methyl eicosenoate. $C_{21}H_{40}O_2$. (M_r 324.5). *1120500.* [2390-09-2]. Methyl (11*Z*)-eicos-11-enoate.

Methyl erucate. $C_{23}H_{44}O_2$. (M_r 352.6). *1146100.* [1120-34-9]. Methyl (13*Z*)-docos-13-enoate.

d_{20}^{20}: about 0.871.

n_D^{20}: about 1.456.

3-*O*-Methylestrone. $C_{19}H_{24}O_2$. (M_r 284.4). *1137000.* [1624-62-0]. 3-Methoxy-1,3,5(10)-estratrien-17-one.

White to yellowish-white powder.

$[\alpha]_D^{20}$: about + 157.

mp: about 173 °C.

Methyl ethyl ketone. C_4H_8O. (M_r 72.1). *1054100.* [78-93-3]. Ethyl methyl ketone. 2-Butanone.

Clear, colourless, flammable liquid, very soluble in water, miscible with ethanol (96 per cent).

d_{20}^{20}: about 0.81.

bp: 79 °C to 80 °C.

Methyleugenol. $C_{11}H_{14}O_2$. (M_r 178.2). *1182000.* [93-15-2]. 1,2-Dimethoxy-4-prop-2-enylbenzene.

Methyleugenol used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Niaouli oil, cineole type (2468)*.

Content: minimum 97.0 per cent, calculated by the normalisation procedure.

Methyl 4-hydroxybenzoate. *1055000.* [99-76-3].

See *Methyl parahydroxybenzoate R*.

1-Methylimidazole. $C_4H_6N_2$. (M_r 82.1). *1139700.* [616-47-7]. 1-Methyl-1*H*-imidazole.

Colourless or slightly yellowish liquid.

n_D^{20}: about 1.495.

bp: 195 °C to 197 °C.

Storage: in an airtight container, protected from light.

1-Methylimidazole R1. *1139701.*

Complies with the requirements prescribed for *1-methylimidazole R* with the following additional requirement.

Content: minimum 95.0 per cent.

2-Methylimidazole. $C_4H_6N_2$. (M_r 82.1). *1143400.* [693-98-1].

White or almost white, crystalline powder.

mp: about 145 °C.

Methyl iodide. CH_3I. (M_r 141.9). *1166400.* [74-88-4]. Iodomethane.

Content: minimum 99.0 per cent.

Methyl isobutyl ketone. $C_6H_{12}O$. (M_r 100.2). *1054300.* [108-10-1]. 4-Methyl-2-pentanone.

Clear, colourless liquid, slightly soluble in water, miscible with most organic solvents.

d_{20}^{20}: about 0.80.

bp: about 115 °C.

Distillation range (*2.2.11*). Distil 100 mL. The range of temperature of distillation from 1 mL to 95 mL of distillate does not exceed 4.0 °C.

Residue on evaporation: maximum 0.01 per cent, determined by evaporating on a water-bath and drying at 100-105 °C.

Methyl isobutyl ketone R1. *1054301.*

Shake 50 mL of freshly distilled *methyl isobutyl ketone R* with 0.5 mL of *hydrochloric acid R1* for 1 min. Allow the phases to separate and discard the lower phase. Prepare immediately before use.

Methyl isobutyl ketone R3. *1054302.*

Complies with the requirements for *methyl isobutyl ketone R* and with the following limits.

Cr: maximum 0.02 ppm.

Cu: maximum 0.02 ppm.

Pb: maximum 0.1 ppm.

Ni: maximum 0.02 ppm.

Sn: maximum 0.1 ppm.

Methyl isobutyl ketone, water-saturated. *1054303.*

Shake *methyl isobutyl ketone R* with *water R* prior to use.

Methyl laurate. $C_{13}H_{26}O_2$. (M_r 214.4). *1054400.* [111-82-0]. Methyl dodecanoate.

Content: minimum 98.0 per cent, determined by gas chromatography (*2.4.22*).

Colourless or yellow liquid, soluble in ethanol (96 per cent) and in light petroleum.

d_{20}^{20}: about 0.87.

n_D^{20}: about 1.431.

mp: about 5 °C.

Methyl lignocerate. $C_{25}H_{50}O_2$. (M_r 382.7). *1120600.* [2442-49-1]. Methyl tetracosanoate.

Flakes.

mp: about 58 °C.

Methyl linoleate. $C_{19}H_{34}O_2$. (M_r 294.5). *1120700.* [112-63-0]. Methyl (9*Z*,12*Z*)-octadeca-9,12-dienoate.

d_{20}^{20}: about 0.888.

n_D^{20}: about 1.466.

bp: 207 °C to 208 °C.

Methyl linolenate. $C_{19}H_{32}O_2$. (M_r 292.5). *1120800.* [301-00-8]. Methyl (9*Z*,12*Z*,15*Z*)-octadeca-9,12,15-trienoate. Methyl α-linolenate.

d_{20}^{20}: about 0.901.

n_D^{20}: about 1.471.

bp: about 207 °C.

Methyl γ-linolenate. $C_{19}H_{32}O_2$. (M_r 292.5). *1158400.* [16326-32-2]. Methyl (6*Z*,9*Z*,12*Z*)-octadeca-6,9,12-trienoate.

Content: minimum 99.0 per cent, determined by gas chromatography.

Methyl margarate. $C_{18}H_{36}O_2$. (M_r 284.5). *1120900.* [1731-92-6]. Methyl heptadecanoate.

White or almost white powder.

mp: 32 °C to 34 °C.

Methyl margarate used in the assay of total fatty acids in Saw palmetto fruit (1848) complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Saw palmetto fruit (1848)*.

Content: minimum 97 per cent, calculated by the normalisation procedure.

Methyl methacrylate. $C_5H_8O_2$. (M_r 100.1). *1054500.* [80-62-6]. Methyl 2-methylprop-2-enoate.

Colourless liquid.

n_D^{20}: about 1.414.

bp: about 100 °C.

mp: about – 48 °C.

It contains a suitable stabilising reagent.

Methyl methanesulfonate. $C_2H_6O_3S$. (M_r 110.1). *1179500.* [66-27-3].

Clear, colourless or slightly yellow liquid.

Content: minimum 99.0 per cent.

Density: about 1.3 g/cm^3 (25 °C).

n_D^{20}: about 1.414.

bp: about 202 °C.

Methyl 2-methoxybenzoate. $C_9H_{10}O_3$. (M_r 166.2). *1206300.* [606-45-1].

Colourless liquid.

Methyl 4-methoxybenzoate. $C_9H_{10}O_3$. (M_r 166.2). *1206400.* [121-98-2].

White or almost white powder.

Methyl *N*-methylanthranilate. $C_9H_{11}NO_2$. (M_r 165.2). *1164600.* [85-91-6]. Methyl 2-(methylamino)benzoate.

Pale yellow liquid.

d_4^{20}: about 1.128.

n_D^{20}: about 1.579.

bp: 255 °C to 258 °C.

Methyl N-methylanthranilate used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Mandarin oil (2355)*.

Test solution. The substance to be examined.

Content: minimum 97 per cent, calculated by the normalisation procedure.

Methyl myristate. $C_{15}H_{30}O_2$. (M_r 242.4). *1054600.* [124-10-7]. Methyl tetradecanoate.

Content: minimum 98.0 per cent, determined by gas chromatography (*2.4.22*).

Colourless or slightly yellow liquid, soluble in ethanol (96 per cent) and in light petroleum.

d_{20}^{20}: about 0.87.

n_D^{20}: about 1.437.

mp: about 20 °C.

Methyl nervonate. *1144800.* [2733-88-2].

See *Tetracos-15-enoic acid methyl ester R*.

Methyl oleate. $C_{19}H_{36}O_2$. (M_r 296.4). *1054700.* [112-62-9]. Methyl (9*Z*)-octadec-9-enoate.

Content: minimum 98.0 per cent, determined by gas chromatography (*2.4.22*).

Colourless or slightly yellow liquid, soluble in ethanol (96 per cent) and in light petroleum.

d_{20}^{20}: about 0.88.

n_D^{20}: about 1.452.

Methylophiopogonanone A. $C_{19}H_{18}O_6$. (M_r 342.3). *1206500.* [74805-92-8]. (3*R*)-3-[(1,3-Benzodioxol-5-yl)methyl]-2,3-dihydro-5,7-dihydroxy-6,8-dimethyl-4*H*-1-benzopyran-4-one.

Methyl orange. $C_{14}H_{14}N_3NaO_3S$. (M_r 327.3). *1054800.* [547-58-0].

Schultz No. 176.

Colour Index No. 13025.

Sodium 4′-(dimethylamino)azobenzene-4-sulfonate.

Orange-yellow, crystalline powder, slightly soluble in water, practically insoluble in ethanol (96 per cent).

Methyl orange mixed solution. *1054801.*

Dissolve 20 mg of *methyl orange R* and 0.1 g of *bromocresol green R* in 1 mL of *0.2 M sodium hydroxide* and dilute to 100 mL with *water R*.

Colour change: pH 3.0 (orange) to pH 4.4 (olive-green).

Methyl orange solution. *1054802.*

Dissolve 0.1 g of *methyl orange R* in 80 mL of *water R* and dilute to 100 mL with *ethanol (96 per cent) R*.

Test for sensitivity. A mixture of 0.1 mL of the methyl orange solution and 100 mL of *carbon dioxide-free water R* is yellow. Not more than 0.1 mL of *1 M hydrochloric acid* is required to change the colour to red.

Colour change: pH 3.0 (red) to pH 4.4 (yellow).

Methyl palmitate. $C_{17}H_{34}O_2$. (M_r 270.5). *1054900.* [112-39-0]. Methyl hexadecanoate.

Content: minimum 98.0 per cent, determined by gas chromatography (*2.4.22*).

White or yellow, crystalline mass, soluble in ethanol (96 per cent) and in light petroleum.

mp: about 30 °C.

Methyl palmitoleate. $C_{17}H_{32}O_2$. (M_r 268.4). *1121000.* [1120-25-8]. Methyl (9*Z*)-hexadec-9-enoate.

d_{20}^{20}: about 0.876.

n_D^{20}: about 1.451.

Methyl parahydroxybenzoate. *1055000.* [99-76-3].

See *Methyl parahydroxybenzoate (0409)*.

Methyl pelargonate. $C_{10}H_{20}O_2$. (M_r 172.3). *1143500.* [1731-84-6]. Methyl nonanoate.

Clear, colourless liquid.

d_4^{20}: about 0.873.

n_D^{20}: about 1.422.

bp: 91 °C to 92 °C.

Methyl pelargonate used in the assay of total fatty acids in Saw palmetto fruit (1848) complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Saw palmetto fruit (1848)*.

Content: minimum 98 per cent, calculated by the normalisation procedure.

2-Methylpentane. C_6H_{14}. (M_r 86.2). *1180400.* [107-83-5].
Isohexane.
d_{20}^{20}: about 0.653.
bp: about 60.0 °C.
Colourless, flammable liquid, practically insoluble in water, miscible with anhydrous ethanol.

3-Methylpentan-2-one. $C_6H_{12}O$. (M_r 100.2). *1141100.* [565-61-7].
Colourless, flammable liquid.
d_{20}^{20}: about 0.815.
n_D^{20}: about 1.400.
bp: about 118 °C

4-Methylpentan-2-ol. $C_6H_{14}O$. (M_r 102.2). *1114300.* [108-11-2].
Clear, colourless, volatile liquid.
d_4^{20}: about 0.802.
n_D^{20}: about 1.411.
bp: about 132 °C.

Methylphenyloxazolylbenzene. $C_{26}H_{20}N_2O_2$. (M_r 392.5). *1056200.* [3073-87-8]. 1,4-Bis[2-(4-methyl-5-phenyl)-oxazolyl]benzene.
Fine, greenish-yellow powder with a blue fluorescence or small crystals, soluble in ethanol (96 per cent), sparingly soluble in xylene.
mp: about 233 °C.
Methylphenyloxazolylbenzene used for liquid scintillation is of a suitable analytical grade.

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine. $C_{12}H_{15}N$. (M_r 173.3). *1137100.* [28289-54-5]. MPTP.
White or almost white, crystalline powder, slightly soluble in water.
mp: about 41 °C.

Methylpiperazine. $C_5H_{12}N_2$. (M_r 100.2). *1056300.* [109-01-3]. 1-Methylpiperazine.
Colourless liquid, miscible with water and with ethanol (96 per cent).
d_{20}^{20}: about 0.90.
n_D^{20}: about 1.466.
bp: about 138 °C.

4-(4-Methylpiperidin-1-yl)pyridine. $C_{11}H_{16}N_2$. (M_r 176.3). *1114400.* [80965-30-6].
Clear liquid.
n_D^{20}: about 1.565.

Methylpolysiloxane. *1066800.*
Polysiloxane substituted with 100 per cent of methyl groups.

Methylprednisolone. $C_{22}H_{30}O_5$. (M_r 374.5). *1193400.* [83-43-2]. 11β,17,21-Trihydroxy-6α-methylpregna-1,4-diene-3,20-dione.
White or almost white, crystalline powder.

2-Methylpropanol. $C_4H_{10}O$. (M_r 74.1). *1056400.* [78-83-1]. Isobutyl alcohol. 2-Methylpropan-1-ol.
Clear colourless liquid, soluble in water, miscible with ethanol (96 per cent).
d_{20}^{20}: about 0.80.
n_D^{15}: 1.397 to 1.399.
bp: about 107 °C.
Distillation range (*2.2.11*). Not less than 96 per cent distils between 107 °C and 109 °C.

2-Methyl-2-propanol. $C_4H_{10}O$. (M_r 74.1). *1056500.* [75-65-0]. 1,1-Dimethyl ethyl alcohol. *tert*-Butyl alcohol.
Clear, colourless liquid or crystalline mass, soluble in water, miscible with ethanol (96 per cent).
Freezing point (*2.2.18*): about 25 °C.
Distillation range (*2.2.11*). Not less than 95 per cent distils between 81 °C and 83 °C.

(15*R*)-15-Methylprostaglandin $F_{2\alpha}$. $C_{21}H_{36}O_5$. (M_r 368.5). *1159900.* [35864-81-4]. (5*Z*)-7-[(1*R*,2*R*,3*R*,5*S*)-3,5-Dihydroxy-2-[(1*E*)-(3*R*)-3-hydroxy-3-methyloct-1-enyl]cyclopentyl]hept-5-enoic acid.
Available as a 10 g/L solution in *methyl acetate R*.
Storage: at a temperature below − 15 °C.

5-Methylpyridin-2-amine. $C_6H_8N_2$. (M_r 108.1). *1193500.* [1603-41-4]. 6-Amino-3-picoline.
White or yellow crystals or crystalline powder.
mp: about 76 °C.

5-Methylpyridin-2(1*H*)-one. C_6H_7NO. (M_r 109.1). *1193600.* [1003-68-5].
White or almost white powder, soluble in anhydrous ethanol and in methanol.
mp: about 181 °C.
Storage: at a temperature of 2 °C to 8 °C.

***N*-Methylpyrrolidine.** $C_5H_{11}N$. (M_r 85.2). *1164700.* [120-94-5].
Content: minimum 97.0 per cent.
bp: about 80 °C.

***N*-Methylpyrrolidone.** C_5H_9NO. (M_r 99.1). *1164800.* [872-50-4]. 1-Methylpyrrolidin-2-one.
d_{20}^{20}: about 1.028.
bp: about 202 °C.
mp: about − 24 °C.

Methyl red. $C_{15}H_{15}N_3O_2$. (M_r 269.3). *1055100.* [493-52-7].
Schultz No. 250.
Colour Index No. 13020.
2-(4-Dimethylamino-phenylazo)benzoic acid.
Dark-red powder or violet crystals, practically insoluble in water, soluble in ethanol (96 per cent).

Methyl red mixed solution. *1055101.*
Dissolve 0.1 g of *methyl red R* and 50 mg of *methylene blue R* in 100 mL of *ethanol (96 per cent) R*.
Colour change: pH 5.2 (red-violet) to pH 5.6 (green).

Methyl red solution. *1055102.*
Dissolve 50 mg of *methyl red R* in a mixture of 1.86 mL of *0.1 M sodium hydroxide* and 50 mL of *ethanol (96 per cent) R* and dilute to 100 mL with *water R*.
Test for sensitivity. To 0.1 mL of the methyl red solution add 100 mL of *carbon dioxide-free water R* and 0.05 mL of *0.02 M hydrochloric acid*. The solution is red. Not more than 0.1 mL of *0.02 M sodium hydroxide* is required to change the colour to yellow.
Colour change: pH 4.4 (red) to pH 6.0 (yellow).

Methyl salicylate. *1146200.* [119-36-8].
See *Methyl salicylate (0230)*

Methyl stearate. $C_{19}H_{38}O_2$. (M_r 298.5). *1055200.* [112-61-8]. Methyl octadecanoate.
Content: minimum 98.0 per cent, determined by gas chromatography (*2.4.22*).
White or yellow, crystalline mass, soluble in ethanol (96 per cent) and in light petroleum.
mp: about 38 °C.

Methylthymol blue. $C_{37}H_{40}N_2Na_4O_{13}S$. ($M_r$ 845). *1158500.* [1945-77-3]. Tetrasodium 2,2′,2″,2‴-[3*H*-2,1-benzoxathiol-3-ylidenebis[[6-hydroxy-2-methyl-5-(1-methylethyl)-3,1-phenylene]methylenenitrilo]]tetraacetate *S*,*S*-dioxide.

Produces a blue colour with calcium in alkaline solution.

Methylthymol blue mixture. *1158501.*

A mixture of 1 part of *methylthymol blue R* and 100 parts of *potassium nitrate R*.

Methyl toluenesulfonate. $C_8H_{10}O_3S$. (M_r 186.2). *1191200.* [80-48-8]. Methyl 4-methylbenzenesulfonate. Methyl tosilate.

Content: minimum 97.0 per cent.

Density: about 1.234 g/mL (25 °C).

bp: about 292 °C.

mp: 25 °C to 28 °C.

***N*-Methyl-*m*-toluidine.** $C_8H_{11}N$. (M_r 121.2). *1175200.* [696-44-6]. *N*,3-Dimethylaniline. *N*,3-Dimethylbenzenamine. Methyl-*m*-tolylamine.

Content: minimum 97 per cent.

Methyl tricosanoate. $C_{24}H_{48}O_2$. (M_r 368.6). *1111500.* [2433-97-8]. Tricosanoic acid methyl ester.

Content: minimum 99.0 per cent.

White or almost white crystals, practically insoluble in water, soluble in hexane.

mp: 55 °C to 56 °C.

Methyl tridecanoate. $C_{14}H_{28}O_2$. (M_r 228.4). *1121100.* [1731-88-0].

Colourless or slightly yellow liquid, soluble in ethanol (96 per cent) and in light petroleum.

d_{20}^{20}: about 0.86.

n_D^{20}: about 1.441.

mp: about 6 °C.

Methyl 3,4,5-trimethoxybenzoate. $C_{11}H_{14}O_5$. (M_r 226.23). *1177200.* [1916-07-0].

***N*-Methyltrimethylsilyl-trifluoroacetamide.** $C_6H_{12}F_3NOSi$. (M_r 199.3). *1129600.* [24589-78-4]. 2,2,2-Trifluoro-*N*-methyl-*N*-(trimethylsilyl)acetamide.

n_D^{20}: about 1.380.

bp: 130 °C to 132 °C.

Minocycline hydrochloride. *1146300.*

See *Minocycline hydrochloride (1030)*.

Molecular sieve. *1056600.*

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.

Molecular sieve for chromatography. *1129700.*

Molecular sieve composed of sodium aluminosilicate. The pore size is indicated after the name of the reagent in the tests where it is used. If necessary, the particle size is also indicated.

Molybdovanadic reagent. *1056700.*

In a 150 mL beaker, mix 4 g of finely powdered *ammonium molybdate R* and 0.1 g of finely powdered *ammonium vanadate R*. Add 70 mL of *water R* and grind the particles using a glass rod. A clear solution is obtained within a few minutes. Add 20 mL of *nitric acid R* and dilute to 100 mL with *water R*.

Monodocosahexaenoin. $C_{25}H_{38}O_4$. (M_r 402.6). *1143600.* [124516-13-8]. Monoglyceride of docosahexaenoic acid (C22:6). Glycerol monodocosahexaenoate. (*all-Z*)-Docosa-4,7,10,13,16,19-hexaenoic acid, monoester with propane-1,2,3-triol.

Mordant black 11. $C_{20}H_{12}N_3NaO_7S$. (M_r 461.4). *1056800.* [1787-61-7].

Schultz No. 241.

Colour Index No. 14645.

Sodium 2-hydroxy-1-[(1-hydroxynaphth-2-yl)azo]-6-nitronaph-thalene-4-sulfonate. Eriochrome black.

Brownish-black powder, soluble in water and in ethanol (96 per cent).

Storage: in an airtight container, protected from light.

Mordant black 11 triturate. *1056801.*

Mix 1 g of *mordant black 11 R* with 99 g of *sodium chloride R*.

Test for sensitivity. Dissolve 50 mg in 100 mL of *water R*. The solution is brownish-violet. On addition of 0.3 mL of *dilute ammonia R1* the solution turns blue. On the subsequent addition of 0.1 mL of a 10 g/L solution of *magnesium sulfate R*, it turns violet.

Storage: in an airtight container, protected from light.

Mordant black 11 triturate R1. *1056802.*

Mix 1.0 g of *mordant black 11 R*, 0.4 g of *methyl orange R* and 100 g of *sodium chloride R*.

Morphine hydrochloride. *1056900.*

See *Morphine hydrochloride (0097)*.

Morpholine. C_4H_9NO. (M_r 87.1). *1057000.* [110-91-8]. Tetrahydro-1,4-oxazine.

Colourless, hygroscopic liquid, flammable, soluble in water and in ethanol (96 per cent).

d_{20}^{20}: about 1.01.

Distillation range (*2.2.11*). Not less than 95 per cent distils between 126 °C and 130 °C.

Storage: in an airtight container.

Morpholine for chromatography. *1057001.*

Complies with the requirements prescribed for *morpholine R* with the following additional requirement.

Content: minimum 99.5 per cent.

2-[*N*-Morpholino]ethanesulfonic acid. $C_6H_{13}NO_4S$. (M_r 195.2). *1186500.* [4432-31-9]. 2-(Morpholin-4-yl)sulfonic acid. MES.

White or almost white, crystalline powder, soluble in water.

mp: about 300 °C.

Murexide. $C_8H_8N_6O_6,H_2O$. (M_r 302.2). *1137200.* 5,5′-Nitrilobis(pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione) monoammonium salt.

Brownish-red crystalline powder, sparingly soluble in cold water, soluble in hot water, practically insoluble in ethanol (96 per cent), soluble in solutions of potassium hydroxide or sodium hydroxide giving a blue colour.

Myosmine. $C_9H_{10}N_2$. (M_r 146.2). *1121200.* [532-12-7]. 3-(4,5-Dihydro-3*H*-pyrrol-2-yl)pyridine.

Colourless crystals.

mp: about 45 °C.

β-Myrcene. $C_{10}H_{16}$. (M_r 136.2). *1114500.* [123-35-3]. 7-Methyl-3-methylenocta-1,6-diene.

Oily liquid with a pleasant odour, practically insoluble in water, miscible with ethanol (96 per cent), soluble in glacial acetic acid. It dissolves in solutions of alkali hydroxides.

d_4^{20}: about 0.794.

n_D^{20}: about 1.470.

β-Myrcene used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Peppermint oil (0405)*.

Test solution. The substance to be examined.

Content: minimum 90.0 per cent, calculated by the normalisation procedure.

Myristic acid. $C_{14}H_{28}O_2$. (M_r 228.4). *1143700.* [544-63-8]. Tetradecanoic acid.

Colourless or white or almost white flakes.

mp: about 58.5 °C.

Myristic acid used in the assay of total fatty acids in Saw palmetto fruit (1848) complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Saw palmetto fruit (1848)*.

Content: minimum 97 per cent, calculated by the normalisation procedure.

Myristicine. $C_{11}H_{12}O_3$. (M_r 192.2). *1099600.* [607-91-0]. 5-Allyl-1-methoxy-2,3-methylenedioxybenzene. 4-Methoxy-6-(prop-2-enyl)-1,3-benzodioxole.

Oily colourless liquid, practically insoluble in water, slightly soluble in anhydrous ethanol, miscible with toluene and with xylene.

d_{20}^{20}: about 1.144.

n_D^{20}: about 1.540.

bp: 276 °C to 277 °C.

mp: about 173 °C.

Chromatography. Thin-layer chromatography (*2.2.27*) as prescribed in the monograph *Star anise (1153)*; the chromatogram shows only one principal spot.

Myristicine used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Nutmeg oil (1552)*.

Content: minimum 95.0 per cent, calculated by the normalisation procedure.

Storage: protected from light.

Myristyl alcohol. $C_{14}H_{30}O$. (M_r 214.4). *1121300.* [112-72-1]. Tetradecan-1-ol.

d_{20}^{20}: about 0.823.

mp: 38 °C to 40 °C.

Myrtillin. $C_{21}H_{21}ClO_{12}$. (M_r 500.8). *1172300.* [6906-38-3]. Delphinidin 3-*O*-glucoside chloride.

Naphthalene. $C_{10}H_8$. (M_r 128.2). *1057100.* [91-20-3].

White or almost white crystals, practically insoluble in water, soluble in ethanol (96 per cent).

mp: about 80 °C.

Naphthalene used for liquid scintillation is of a suitable analytical grade.

2,3-Naphthalenediamine. $C_{10}H_{10}N_2$. (M_r 158.2). *1199700.* [771-97-1]. Naphthalene-2,3-diamine. 2,3-Diaminonaphthalene.

Brownish-yellow crystalline powder, slightly soluble in ethanol (96 per cent), practically insoluble in acetone.

mp: 195 °C to 198 °C.

Naphtharson. $C_{16}H_{11}AsN_2Na_2O_{10}S_2$. ($M_r$ 576.3). *1121400.* [3688-92-4]. Thorin. Disodium 4-[(2-arsonophenyl)azo]-3-hydroxynaphthalene-2,7-disulfonate.

Red powder, soluble in water.

Naphtharson solution. *1121401.*

A 0.58 g/L solution of *naphtharson R*.

Test for sensitivity. To 50 mL of *ethanol (96 per cent) R*, add 20 mL of *water R*, 1 mL of *dilute sulfuric acid R1* and 1 mL of the naphtharson solution. Titrate with *0.025 M barium perchlorate*; the colour changes from orange-yellow to orange-pink.

Storage: protected from light; use within 1 week.

Naphtharson solution R1. *1121402.*

A 1 g/L solution in *deionised distilled water R*.

Test for sensitivity. To 50 mL of *ethanol (96 per cent) R*, add 20 mL of *water R*, 1 mL of *dilute sulfuric acid R1* and 1 mL of *naphtharson solution R1*. Titrate with *0.025 M barium perchlorate*; the colour changes from orange-yellow to orange-pink.

Storage: protected from light; use within 1 week.

α-Naphthol. $C_{10}H_8O$. (M_r 144.2). *1057300.* [90-15-3]. 1-Naphthol.

White or almost white, crystalline powder or colourless or white or almost white crystals, darkening on exposure to light, slightly soluble in water, freely soluble in ethanol (96 per cent).

mp: about 95 °C.

Storage: protected from light.

α-Naphthol solution. *1057301.*

Dissolve 0.10 g of *α-naphthol R* in 3 mL of a 150 g/L solution of *sodium hydroxide R* and dilute to 100 mL with *water R*. Prepare immediately before use.

β-Naphthol. $C_{10}H_8O$. (M_r 144.2). *1057400.* [135-19-3]. 2-Naphthol.

White or slightly pink plates or crystals, very slightly soluble in water, very soluble in ethanol (96 per cent).

mp: about 122 °C.

Storage: protected from light.

β-Naphthol solution. *1057401.*

Dissolve 5 g of freshly recrystallised *β-naphthol R* in 40 mL of *dilute sodium hydroxide solution R* and dilute to 100 mL with *water R*. Prepare immediately before use.

β-Naphthol solution R1. *1057402.*

Dissolve 3.0 mg of *β-naphthol R* in 50 mL of *sulfuric acid R* and dilute to 100.0 mL with the same acid. Use the recently prepared solution.

Naphtholbenzein. $C_{27}H_{18}O_2$. (M_r 374.4). *1057600.* [145-50-6]. α-Naphtholbenzein. 4-[(4-Hydroxynaphthalen-1-yl)(phenyl)methylidene] naphthalen-1(4*H*)-one.

Brownish-red powder or shiny brownish-black crystals, practically insoluble in water, soluble in ethanol (96 per cent) and in glacial acetic acid.

Naphtholbenzein solution. *1057601.*

A 2 g/L solution of *naphtholbenzein R* in *anhydrous acetic acid R*.

Test for sensitivity. To 50 mL of *glacial acetic acid R* add 0.25 mL of the naphtholbenzein solution. The solution is brownish-yellow. Not more than 0.05 mL of *0.1 M perchloric acid* is required to change the colour to green.

Naphthol yellow. $C_{10}H_5N_2NaO_5$. (M_r 256.2). *1136600.* 2,4-Dinitro-1-naphthol, sodium salt.

Orange-yellow powder or crystals, freely soluble in water, slightly soluble in ethanol (96 per cent).

Naphthol yellow S. $C_{10}H_4N_2Na_2O_8S$. (M_r 358.2). *1143800.* [846-70-8].

Colour Index No. 10316.
8-Hydroxy-5,7-dinitro-2-naphthalenesulfonic acid disodium salt. Disodium 5,7-dinitro-8-oxidonaphthalene-2-sulfonate.

Yellow or orange-yellow powder, freely soluble in water.

1-Naphthylacetic acid. $C_{12}H_{10}O_2$. (M_r 186.2). *1148400.* [86-87-3]. (Naphthalen-1-yl)acetic acid.

White or yellow crystalline powder, very slightly soluble in water, freely soluble in acetone.

mp: about 135 °C.

Naphthylamine. $C_{10}H_9N$. (M_r 143.2). *1057700.* [134-32-7]. 1-Naphthylamine.

White or almost white, crystalline powder, turning pink on exposure to light and air, slightly soluble in water, freely soluble in ethanol (96 per cent).

mp: about 51 °C.

Storage: protected from light.

Naphthylethylenediamine dihydrochloride. $C_{12}H_{16}Cl_2N_2$. (M_r 259.2). *1057800.* [1465-25-4]. *N*-(1-Naphthyl)ethylene-diamine dihydrochloride.

It may contain methanol of crystallisation.

White or yellowish-white powder, soluble in water, slightly soluble in ethanol (96 per cent).

Naphthylethylenediamine dihydrochloride solution. *1057801.*

Dissolve 0.1 g of *naphthylethylenediamine dihydrochloride R* in *water R* and dilute to 100 mL with the same solvent. Prepare immediately before use.

Naringin. $C_{27}H_{32}O_{14}$. (M_r 580.5). *1137300.* [10236-47-2]. 7-[[2-*O*-(6-Deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-5-hydroxy-2-(4-hydroxyphenyl)-2,3-dihydro-4*H*-chromen-4-one.

White or almost white crystalline powder, slightly soluble in water, soluble in methanol and in dimethylformamide.

mp: about 171 °C.

Absorbance (*2.2.25*). Naringin dissolved in a 5 g/L solution of *dimethylformamide R* in *methanol R* shows an absorption maximum at 283 nm.

Neohesperidin. $C_{28}H_{34}O_{15}$. (M_r 610.6). *1182200.* [13241-33-3]. Hesperetin-7-neohesperidoside. (2*S*)-7-[[2-*O*-(6-Deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-2,3-dihydro-4*H*-1-benzopyran-4-one.

***trans*-Nerolidol.** $C_{15}H_{26}O$. (M_r 222.4). *1107900.* [40716-66-3]. 3,7,11-Trimethyldodeca-1,6,10-trien-3-ol.

Slightly yellow liquid, slight odour of lily and lily of the valley, practically insoluble in water and in glycerol, miscible with ethanol (96 per cent).

d_{20}^{20}: about 0.876.

n_D^{20}: about 1.479.

bp_{12}: 145 °C to 146 °C.

trans-Nerolidol used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Bitter-orange-flower oil (1175)*.

Test solution. The substance to be examined.

Content: minimum 90.0 per cent, calculated by the normalisation procedure.

Neryl acetate. $C_{12}H_{20}O_2$. (M_r 196.3). *1108000.* [141-12-8]. (*Z*)-3,7-Dimethylocta-2,6-dienyl acetate.

Colourless, oily liquid.

d_{20}^{20}: about 0.907.

n_D^{20}: about 1.460.

bp_{25}: 134 °C.

Neryl acetate used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Bitter-orange-flower oil (1175)*.

Test solution. The substance to be examined.

Content: minimum 93.0 per cent, calculated by the normalisation procedure.

Nickel-aluminium alloy. *1058100.*

Contains 48 per cent to 52 per cent of aluminium (Al; A_r 26.98) and 48 per cent to 52 per cent of nickel (Ni; A_r 58.70).

Before use, reduce to a fine powder (180) (*2.9.12*).

It is practically insoluble in water and soluble in mineral acids.

Nickel-aluminium alloy (halogen-free). *1118100.*

Contains 48 per cent to 52 per cent of aluminium (Al; A_r 26.98) and 48 per cent to 52 per cent of nickel (Ni; A_r 58.71).

Fine, grey powder, practically insoluble in water, soluble in mineral acids with formation of salts.
Chlorides: maximum 10 ppm.

Dissolve 0.400 g in 40 mL of a mixture of 67 volumes of *sulfuric acid R* and 33 volumes of *dilute nitric acid R*. Evaporate the solution nearly to dryness, dissolve the residue in *water R* and dilute to 20.0 mL with the same solvent. To one half-aliquot of the solution, add 1.0 mL of *0.1 M silver nitrate*. Filter after 15 min and add 0.2 mL of sodium chloride solution (containing 10 µg of chlorides per millilitre) to the filtrate. After 5 min the solution is more opalescent than a mixture of the second half-aliquot of the solution with 1.0 mL of *0.1 M silver nitrate*.

Nickel chloride. $NiCl_2$. (M_r 129.6). *1057900.* [7718-54-9]. Nickel chloride, anhydrous.

Yellow, crystalline powder, very soluble in water, soluble in ethanol (96 per cent). It sublimes in the absence of air and readily absorbs ammonia. The aqueous solution is acid.

Nickel nitrate hexahydrate. $Ni(NO_3)_2,6H_2O$. (M_r 290.8). *1175300.* [13478-00-7].

Nickel sulfate. $NiSO_4,7H_2O$. (M_r 280.9). *1058000.* [10101-98-1]. Nickel sulfate heptahydrate.

Green, crystalline powder or crystals, freely soluble in water, slightly soluble in ethanol (96 per cent).

Nicotinamide-adenine dinucleotide. $C_{21}H_{27}N_7O_{14}P_2$. (M_r 663). *1108100.* [-84-9]. NAD^+.

White or almost white powder, very hygroscopic, freely soluble in water.

Nicotinamide-adenine dinucleotide solution. *1108101.*

Dissolve 40 mg of *nicotinamide-adenine dinucleotide R* in *water R* and dilute to 10 mL with the same solvent. Prepare immediately before use.

Nicotinic acid. *1158600.* [59-67-6].

See *Nicotinic acid (0459)*.

Nicotinoyl hydrazide. $C_6H_7N_3O$. (M_r 137.1). *1202400.* [553-53-7]. Pyridine-3-carbohydrazide.

White or almost white powder or crystalline powder, soluble in water.

mp: about 160 °C.

Nile blue A. $C_{20}H_{21}N_3O_5S$. (M_r 415.5). *1058200.* [3625-57-8].

Schultz No. 1029.

Colour Index No. 51180.
5-Amino-9-(diethylamino)benzo[*a*]phenoxazinylium hydrogen sulfate.

Green, crystalline powder with a bronze lustre, sparingly soluble in ethanol (96 per cent), in glacial acetic acid and in pyridine.

Absorbance (*2.2.25*). A 0.005 g/L solution in *ethanol (50 per cent V/V) R* shows an absorption maximum at 640 nm.

Nile blue A solution. *1058201.*

A 10 g/L solution of *Nile blue A R* in *anhydrous acetic acid R*.

Test for sensitivity. To 50 mL of *anhydrous acetic acid R* add 0.25 mL of the Nile blue A solution. The solution is blue. On the addition of 0.1 mL of *0.1 M perchloric acid*, the colour changes to blue-green.

Colour change: pH 9.0 (blue) to pH 13.0 (red).

Ninhydrin. $C_9H_4O_3,H_2O$. (M_r 178.1). *1058300.* [485-47-2]. 1,2,3-Indanetrione monohydrate.

White or very pale yellow, crystalline powder, soluble in water and in ethanol (96 per cent).

Storage: protected from light.

Ninhydrin and stannous chloride reagent. *1058301.*

Dissolve 0.2 g of *ninhydrin R* in 4 mL of hot *water R*, add 5 mL of a 1.6 g/L solution of *stannous chloride R*, allow to stand for 30 min, then filter and store at a temperature of 2 °C to 8 °C. Immediately before use dilute 2.5 mL of the solution with 5 mL of *water R* and 45 mL of *2-propanol R*.

Ninhydrin solution. *1058303.*

A 2 g/L solution of *Ninhydrin R* in a mixture of 5 volumes of *dilute acetic acid R* and 95 volumes of *butanol R*.

Ninhydrin solution R1. *1058304.*

Dissolve 1.0 g of *ninhydrin R* in 50 mL of *ethanol (96 per cent) R* and add 10 mL of *glacial acetic acid R*.

Ninhydrin solution R2. *1058305.*

Dissolve 3 g of *ninhydrin R* in 100 mL of a 45.5 g/L solution of *sodium metabisulfite R*.

Ninhydrin solution R3. *1058306.*

A 4 g/L solution in a mixture of 5 volumes of *anhydrous acetic acid R* and 95 volumes of *butanol R*.

Ninhydrin solution R4. *1058307.*

A 3 g/L solution of *ninhydrin R* in a mixture of 5 volumes of *glacial acetic acid R* and 95 volumes of *2-propanol R*.

Nitrazepam. *1143900.* [146-22-5].

See *Nitrazepam (0415)*.

Nitric acid. HNO_3. (M_r 63.0). *1058400.* [7697-37-2].

Content: 63.0 per cent *m/m* to 70.0 per cent *m/m*.

Clear, colourless or almost colourless liquid, miscible with water.

d_{20}^{20}: 1.384 to 1.416.

A 10 g/L solution is strongly acid and gives the reaction of nitrates (*2.3.1*).

Appearance. Nitric acid is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Chlorides (*2.4.4*): maximum 0.5 ppm.

To 5 g add 10 mL of *water R* and 0.3 mL of *silver nitrate solution R2* and allow to stand for 2 min protected from light. Any opalescence is not more intense than that of a standard prepared in the same manner using 13 mL of *water R*, 0.5 mL of *nitric acid R*, 0.5 mL of *chloride standard solution (5 ppm Cl) R* and 0.3 mL of *silver nitrate solution R2*.

Sulfates (*2.4.13*): maximum 2 ppm.

Evaporate 10 g to dryness with 0.2 g of *sodium carbonate R*. Dissolve the residue in 15 mL of *distilled water R*. Prepare the standard using a mixture of 2 mL of *sulfate standard solution (10 ppm SO_4) R* and 13 mL of *distilled water R*.

Arsenic (*2.4.2, Method A*): maximum 0.02 ppm.

Gently heat 50 g with 0.5 mL of *sulfuric acid R* until white fumes begin to evolve. To the residue add 1 mL of a 100 g/L solution of *hydroxylamine hydrochloride R* and dilute to 2 mL with *water R*. Prepare the standard using 1.0 mL of *arsenic standard solution (1 ppm As) R*.

Iron (*2.4.9*): maximum 1 ppm.

Dissolve the residue from the determination of sulfated ash in 1 mL of *dilute hydrochloric acid R* and dilute to 50 mL with *water R*. Dilute 5 mL of this solution to 10 mL with *water R*.

Heavy metals (*2.4.8*): maximum 2 ppm.

Dilute 10 mL of the solution prepared for the limit test for iron to 20 mL with *water R*. 12 mL of the solution complies with test A. Prepare the reference solution using *lead standard solution (2 ppm Pb) R*.

Sulfated ash: maximum 0.001 per cent.

Carefully evaporate 100 g to dryness. Moisten the residue with a few drops of *sulfuric acid R* and heat to dull red.

Assay. To 1.50 g add about 50 mL of *water R* and titrate with *1 M sodium hydroxide*, using 0.1 mL of *methyl red solution R* as indicator.

1 mL of *1 M sodium hydroxide* is equivalent to 63.0 mg of HNO_3.

Storage: protected from light.

Nitric acid, cadmium- and lead-free. *1058401.*

Complies with the requirements prescribed for *nitric acid R* and with the following additional test.

Test solution. To 100 g add 0.1 g of *anhydrous sodium carbonate R* and evaporate to dryness. Dissolve the residue in *water R* heating slightly, and dilute to 50.0 mL with the same solvent.

Cadmium: maximum 0.1 ppm.

Atomic absorption spectrometry (*2.2.23, Method II*).

Source: cadmium hollow-cathode lamp.

Wavelength: 228.8 nm.

Atomisation device: air-acetylene or air-propane flame.

Lead: maximum 0.1 ppm.

Atomic absorption spectrometry (*2.2.23, Method II*).

Source: lead hollow-cathode lamp.

Wavelength: 283.3 nm or 217.0 nm.

Atomisation device: air-acetylene flame.

Nitric acid, dilute. *1058402.*

Contains about 125 g/L of HNO_3 (M_r 63.0).

Dilute 20 g of *nitric acid R* to 100 mL with *water R*.

Nitric acid, dilute R1. *1058407.*

Dilute 40 g of *nitric acid R* to 100 mL with *water R*.

Nitric acid, dilute R2. *1058409.*

Dilute 30 g of *nitric acid R* to 100 mL with *water R*.

Nitric acid, dilute, heavy metal-free. *1058410.*

Complies with the requirements prescribed for *dilute nitric acid R* with the following maximum contents of heavy metals.

As: 0.005 ppm.
Cd: 0.005 ppm.
Cu: 0.001 ppm.
Fe: 0.02 ppm.
Hg: 0.002 ppm.
Ni: 0.005 ppm.
Pb: 0.001 ppm.
Zn: 0.01 ppm.

Nitric acid, heavy metal-free. *1058404.*

Complies with the requirements prescribed for *nitric acid R* with the following maximum contents of heavy metals.

As: 0.005 ppm.
Cd: 0.005 ppm.
Cu: 0.001 ppm.
Fe: 0.02 ppm.
Hg: 0.002 ppm.

Ni: 0.005 ppm.

Pb: 0.001 ppm.

Zn: 0.01 ppm.

Nitric acid, lead-free. *1058403.*

Complies with the requirements prescribed for *Nitric acid R* with the following additional test.

Lead: maximum 0.1 ppm.

Atomic absorption spectrometry (*2.2.23, Method II*).

Test solution. To 100 g add 0.1 g of *anhydrous sodium carbonate R* and evaporate to dryness. Dissolve the residue in *water R*, heating slightly, and dilute to 50.0 mL with the same solvent.

Source: lead hollow-cathode lamp.

Wavelength: 283.3 nm or 217.0 nm.

Atomisation device: air-acetylene flame.

Nitric acid, lead-free R1. *1058405.*

Nitric acid R containing not more than 1 µg/kg of lead.

Nitric acid, lead-free, dilute. *1058406.*

Dilute 5 g of *lead-free nitric acid R1* to 100 mL with *deionised distilled water R*.

Nitric acid, nickel-free. *1058408.*

Complies with the requirements prescribed for *nitric acid R* with the following additional requirement.

Nickel: maximum 0.005 ppm.

Nitric acid, fuming. *1058500.* [7697-37-2].

Clear, slightly yellowish liquid, fuming on contact with air.

d_{20}^{20}: about 1.5.

Nitrilotriacetic acid. $C_6H_9NO_6$. (M_r 191.1). *1137400.* [139-13-9].

White or almost white crystalline powder, practically insoluble in water and in most organic solvents.

mp: about 240 °C, with decomposition.

Nitroaniline. $C_6H_6N_2O_2$. (M_r 138.1). *1058600.* [100-01-6]. 4-Nitroaniline.

Bright yellow, crystalline powder, very slightly soluble in water, sparingly soluble in boiling water, soluble in ethanol (96 per cent), forms water-soluble salts with strong mineral acids.

mp: about 147 °C.

Nitrobenzaldehyde. $C_7H_5NO_3$. (M_r 151.1). *1058700.* [552-89-6]. 2-Nitrobenzaldehyde.

Yellow needles, slightly soluble in water, freely soluble in ethanol (96 per cent), volatile in steam.

mp: about 42 °C.

Nitrobenzaldehyde paper. *1058701.*

Dissolve 0.2 g of *nitrobenzaldehyde R* in 10 mL of a 200 g/L solution of *sodium hydroxide R*. Use the solution within 1 h. Immerse the lower half of a slow filter paper strip 10 cm long and 0.8-1 cm wide. Absorb the excess reagent between two sheets of filter paper. Use within a few minutes of preparation.

Nitrobenzaldehyde solution. *1058702.*

Add 0.12 g of powdered *nitrobenzaldehyde R* to 10 mL of *dilute sodium hydroxide solution R*; allow to stand for 10 min shaking frequently and filter. Prepare immediately before use.

4-Nitrobenzaldehyde. $C_7H_5NO_3$. (M_r 151.1). *1198700.* [555-16-8].

Nitrobenzene. $C_6H_5NO_2$. (M_r 123.1). *1058800.* [98-95-3].

Colourless or very slightly yellow liquid, practically insoluble in water, miscible with ethanol (96 per cent).

bp: about 211 °C.

Dinitrobenzene. To 0.1 mL add 5 mL of *acetone R*, 5 mL of *water R* and 5 mL of *strong sodium hydroxide solution R*. Shake and allow to stand. The upper layer is almost colourless.

4-Nitrobenzoic acid. $C_7H_5NO_4$. (M_r 167.1). *1144000.* [62-23-7].

Yellow crystals.

mp: about 240 °C.

Nitrobenzoyl chloride. $C_7H_4ClNO_3$. (M_r 185.6). *1058900.* [122-04-3]. 4-Nitrobenzoyl chloride.

Yellow crystals or a crystalline mass, decomposing in moist air, completely soluble in sodium hydroxide solution giving a yellowish-orange colour.

mp: about 72 °C.

Nitrobenzyl chloride. $C_7H_6ClNO_2$. (M_r 171.6). *1059000.* [100-14-1]. 4-Nitrobenzyl chloride.

Pale-yellow crystals, lachrymatory, practically insoluble in water, very soluble in ethanol (96 per cent).

4-(4-Nitrobenzyl)pyridine. $C_{12}H_{10}N_2O_2$. (M_r 214.2). *1101900.* [1083-48-3].

Yellow powder.

mp: about 70 °C.

Nitroethane. $C_2H_5NO_2$. (M_r 75.1). *1059200.* [79-24-3].

Clear, oily, colourless liquid.

bp: about 114 °C.

Nitrofurantoin. *1099700.* [67-20-9].

See *Nitrofurantoin (0101)*.

Nitrogen. N_2. (M_r 28.01). *1059300.* [7727-37-9].

Nitrogen, washed and dried.

Nitrogen gas mixture. *1136900.*

Nitrogen R containing 1 per cent *V/V* of each of the following gases: *carbon dioxide R2*, *carbon monoxide R1* and *oxygen R1*.

Nitrogen, oxygen-free. *1059600.*

Nitrogen R which has been freed from oxygen by passing it through *alkaline pyrogallol solution R*.

Nitrogen R1. N_2. (M_r 28.01). *1059400.* [7727-37-9].

Content: minimum 99.999 per cent *V/V*.

Carbon monoxide: less than 5 ppm.

Oxygen: less than 5 ppm.

Nitrogen dioxide. NO_2. (M_r 46.01). *1179600.* [10102-44-0].

Content: minimum 98.0 per cent *V/V*.

Nitrogen for chromatography. N_2. (M_r 28.01). *1059500.* [7727-37-9].

Content: minimum 99.95 per cent *V/V*.

Nitrogen monoxide. NO. (M_r 30.01). *1108300.*

Content: minimum 98.0 per cent *V/V*.

Nitromethane. CH_3NO_2. (M_r 61.0). *1059700.* [75-52-5].

Clear, colourless, oily liquid, slightly soluble in water, miscible with ethanol (96 per cent).

d_{20}^{20}: 1.132 to 1.134.

n_D^{20}: 1.381 to 1.383.

Distillation range (*2.2.11*). Not less than 95 per cent distils between 100 °C and 103 °C.

Nitro-molybdovanadic reagent. *1060100.*

Solution A. Dissolve 10 g of *ammonium molybdate R* in *water R*, add 1 mL of *ammonia R* and dilute to 100 mL with *water R*.

Solution B. Dissolve 2.5 g of *ammonium vanadate R* in hot *water R*, add 14 mL of *nitric acid R* and dilute to 500 mL with *water R*.

To 96 mL of *nitric acid R* add 100 mL of solution A and 100 mL of solution B and dilute to 500 mL with *water R*.

4-Nitrophenol. $C_6H_5NO_3$. (M_r 139.1). *1146400.* [100-02-7]. *p*-Nitrophenol.

Content: minimum 95 per cent.

Colourless or slightly yellow powder, sparingly soluble in water and in methanol.

mp: about 114 °C.

3-Nitrosalicylic acid. $C_7H_5NO_5$. (M_r 183.1). *1184300.* [85-38-1]. 2-Hydroxy-3-nitrobenzoic acid.

Yellowish crystals, slightly soluble in water, freely soluble in ethanol (96 per cent).

mp: 142 °C to 147 °C.

***N*-Nitrosodiethanolamine.** $C_4H_{10}N_2O_3$. (M_r 134.1). *1129800.* [1116-54-7]. 2,2′-(Nitrosoimino)diethanol.

Yellow liquid, miscible with anhydrous ethanol.

n_D^{20}: about 1.485.

bp: about 125 °C.

***N*-Nitrosodiisopropanolamine.** $C_6H_{14}N_2O_3$. (M_r 162.2). *1176500.* [53609-64-6]. 1,1′-(Nitrosoimino)bispropan-2-ol.

bp: 122-124 °C.

Nitrosodipropylamine. $C_6H_{14}N_2O$. (M_r 130.2). *1099900.* [621-64-7]. Dipropylnitrosamine.

Liquid, soluble in anhydrous ethanol and in strong acids.

d_{20}^{20}: about 0.915.

bp: about 78 °C.

Appropriate grade for chemiluminescence determination.

Nitrosodipropylamine solution. *1099901.*

Inject 78.62 g of *anhydrous ethanol R* through the septum of a vial containing *nitrosodipropylamine R*. Dilute 1/100 in *anhydrous ethanol R* and place 0.5 mL aliquots in crimp-sealed vials.

Storage: in the dark at 5 °C.

Nitrotetrazolium blue. $C_{40}H_{30}Cl_2N_{10}O_6$. ($M_r$ 818). *1060000.* [298-83-9]. 3,3′-(3,3′-Dimethoxy-4,4′-diphenylene)di[2-(4-nitrophenyl)-5-phenyl-2*H*-tetrazolium] dichloride. *p*-Nitro-tetrazolium blue.

Crystals, soluble in methanol, giving a clear, yellow solution.

mp: about 189 °C, with decomposition.

Nitrous oxide. N_2O. (M_r 44.01). *1108500.*

Content: minimum 99.99 per cent *V/V*.

Nitrogen monoxide: less than 1 ppm.

Carbon monoxide: less than 1 ppm.

Nonivamide. $C_{17}H_{27}NO_3$. (M_r 293.4). *1148500.* [2444-46-4]. *N*-[(4-Hydroxy-3-methoxyphenyl)methyl]nonanamide.

White or almost white, crystalline powder, practically insoluble in cold water, freely soluble in anhydrous ethanol.

Nonivamide used in the test for nonivamide in the monograph Capsicum (1859) complies with the following additional test.

Assay. Liquid chromatography (*2.2.29*) as prescribed in the monograph *Capsicum (1859)*.

Content: minimum 98.0 per cent, calculated by the normalisation procedure.

Nonylamine. $C_9H_{21}N$. (M_r 143.3). *1139800.* [112-20-9]. Nonan-1-amine. 1-Aminononane.

Corrosive, colourless, clear liquid.

d_4^{20}: about 0.788.

n_D^{20}: about 1.433.

Nordazepam. $C_{15}H_{11}ClN_2O$. (M_r 270.7). *1060200.* [1088-11-5]. 7-Chloro-2,3-dihydro-5-phenyl-1*H*-1,4-benzodiazepin-2-one.

White or pale yellow, crystalline powder, practically insoluble in water, slightly soluble in ethanol (96 per cent).

mp: about 216 °C.

DL-Norleucine. $C_6H_{13}NO_2$. (M_r 131.2). *1060300.* [616-06-8]. (*RS*)-2-Aminohexanoic acid.

Shiny crystals, sparingly soluble in water and in ethanol (96 per cent), soluble in acids.

Noscapine hydrochloride. *1060500.* [912-60-7].

See *Noscapine hydrochloride (0515)*.

Ochratoxin A solution. *1175700.*

50 µg/mL solution of (2*S*)-2-([[(3*R*)-5-chloro-8-hydroxy-3-methyl-1-oxo-3,4-dihydro-1*H*-2-benzopyran-7-yl]carbonyl]amino)-3-phenylpropanoic acid (ochratoxin A) in a mixture of 1 volume of *acetic acid R* and 99 volumes of *benzene R*.

Octadecyl [3-[3,5-bis(1,1-dimethylethyl)-4-hydroxyphenyl]-propionate]. $C_{35}H_{62}O_3$. (M_r 530.9). *1060600.* [2082-79-3]. Octadecyl 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propionate.

White or slightly yellowish, crystalline powder, practically insoluble in water, very soluble in acetone and in hexane, slightly soluble in methanol.

mp: 49 °C to 55 °C.

Octanal. $C_8H_{16}O$. (M_r 128.2). *1150400.* [124-13-0]. Octyl aldehyde.

Oily, colourless liquid. Practically insoluble in water.

Octanal used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Sweet orange oil (1811)*.

Content: minimum 99 per cent, calculated by the normalisation procedure.

Octane. C_8H_{18}. (M_r 114.2). *1166500.* [111-65-9]. *n*-Octane.

Content: minimum 99 per cent.

Octanol. $C_8H_{18}O$. (M_r 130.2). *1060700.* [111-87-5]. Octan-1-ol. Caprylic alcohol.

Colourless liquid, practically insoluble in water, miscible with ethanol (96 per cent).

d_{20}^{20}: about 0.828.

bp: about 195 °C.

3-Octanone. $C_8H_{16}O$. (M_r 128.2). *1114600.* [106-68-3]. Octan-3-one. Ethylpentylketone.

Colourless liquid with a characteristic odour.

d_{20}^{20}: about 0.822.

n_D^{20}: about 1.415.

bp: about 167 °C.

3-Octanone used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Lavender oil (1338)*.

Test solution. The substance to be examined.

Content: minimum 98.0 per cent, calculated by the normalisation procedure.

Octoxinol 10. $C_{34}H_{62}O_{11}$ (average). (M_r 647). *1060800.* [9002-93-1]. α-[4-(1,1,3,3-Tetramethylbutyl)phenyl]-ω-hydroxypoly-(oxyethylene).

Clear, pale-yellow, viscous liquid, miscible with water, with acetone and with ethanol (96 per cent), soluble in toluene.

Storage: in an airtight container.

Octreotide acetate. $C_{49}H_{66}N_{10}O_{10}S_2,xC_2H_4O_2$. *1182900.* [79517-01-4]. (Acetate-free peptide: M_r 1019. [83150-76-9]). D-Phenylalanyl-L-cysteinyl-L-phenylalanyl-D-tryptophyl-L-lysyl-L-threonyl-*N*-[(1*R*,2*R*)-2-hydroxy-1-(hydroxymethyl)propyl]-L-cysteinamide cyclic (2→7)-disulfide acetate. It contains a variable quantity of acetic acid.
White or almost white powder, freely soluble in water and acetic acid.

Content: minimum 96.0 per cent.

Octylamine. $C_8H_{19}N$. (M_r 129.2). *1150500.* [111-86-4]. Octan-1-amine.

Colourless liquid.

d_{20}^{20}: about 0.782.

bp: 175 °C to 179 °C.

Oleamide. $C_{18}H_{35}NO$. (M_r 281.5). *1060900.* (9*Z*)-Octadec-9-enoamide.

Yellowish or white powder or granules, practically insoluble in water, very soluble in methylene chloride, soluble in anhydrous ethanol.

mp: about 80 °C.

Oleanolic acid. $C_{30}H_{48}O_3$. (M_r 456.7). *1183000.* [508-02-1]. 3β-Hydroxyolean-12-en-28-oic acid. Astrantiagenin C.

Oleic acid. $C_{18}H_{34}O_2$. (M_r 282.5). *1144100.* [112-80-1]. (9*Z*)-Octadec-9-enoic acid.

Clear, colourless liquid, practically insoluble in water.

d_4^{20}: about 0.891.

n_D^{20}: about 1.459.

mp: 13 °C to 14 °C.

Oleic acid used in the assay of total fatty acids in the monograph Saw palmetto fruit (1848) complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Saw palmetto fruit (1848)*.

Content: minimum 98 per cent, calculated by the normalisation procedure.

Oleuropein. $C_{25}H_{32}O_{13}$. (M_r 540.5). *1152900.* [32619-42-4]. 2-(3,4-Dihydroxyphenyl)ethyl[(2*S*,3*E*,4*S*)-3-ethylidene-2-(β-D-glucopyranosyloxy)-5-(methoxycarbonyl)-3,4-dihydro-2*H*-pyran-4-yl]acetate.

Powder, soluble in methanol.

Oleuropein used in Olive leaf (1878) complies with the following test.

Assay. Liquid chromatography (*2.2.29*) as prescribed in the monograph *Olive leaf (1878)*.

Content: minimum 80 per cent, calculated by the normalisation procedure.

Oleyl alcohol. $C_{18}H_{36}O$. (M_r 268.5). *1156000.* [143-28-2]. (9*Z*)-Octadec-9-en-1-ol.

bp: about 207 °C.

n_D^{20}: 1.460.

Content: minimum 85 per cent.

Olive oil. *1061000.* [8001-25-0].

See *Olive oil, virgin (0518)*.

Orcinol. $C_7H_8O_2,H_2O$. (M_r 142.2). *1108700.* [6153-39-5]. 5-Methylbenzene-1,3-diol monohydrate.

Crystalline powder, sensitive to light.

bp: about 290 °C.

mp: 58 °C to 61 °C.

Organosilica polymer, amorphous, octadecylsilyl. *1144200.*

Synthetic, spherical hybrid particles, containing both inorganic (silica) and organic (organosiloxanes) components, chemically modified at the surface by trifunctionally bonded octadecylsilyl groups.

Organosilica polymer, amorphous, polar-embedded octadecylsilyl, end-capped. *1150600.*

Synthetic, spherical hybrid particles containing both inorganic (silica) and organic (organosiloxanes) components, chemically modified at the surface by the bonding of polar-embedded octadecylsilyl groups. To minimise any interaction with basic compounds, they are carefully end-capped to cover most of the remaining silanol groups.

Organosilica polymer, amorphous, propyl-2-phenylsilyl, end-capped. *1178100.*

Synthetic, spherical hybrid particles containing both inorganic (silica) and organic (organosiloxanes) components, chemically modified at the surface by the bonding of propyl-2-phenylsilyl groups. To minimise any interaction with basic compounds, they are carefully end-capped to cover most of the remaining silanol groups.

Organosilica polymer compatible with 100 per cent aqueous mobile phases, octadecylsilyl, solid core, end-capped. *1201700.*

Silica gel with spherical silica particles containing a solid non-porous silica core surrounded by a thin outer organosilica polymer coating with octadecylsilyl groups, suitable for use with highly aqueous mobile phases including 100 per cent aqueous phases. To minimise any interaction with basic compounds, it is carefully end-capped to cover most of the remaining silanol groups.

Organosilica polymer for chromatography, amorphous, octadecylsilyl, end-capped. *1164900.*

Synthetic, spherical hybrid particles containing both inorganic (silica) and organic (organosiloxanes) components, chemically modified at the surface by the bonding of octadecylsilyl groups. To minimise any interaction with basic compounds, they are carefully end-capped to cover most of the remaining silanol groups.

Organosilica polymer, multi-layered, octadecylsilyl, end-capped. *1202500.*

Synthetic, spherical hybrid particles, multi-layered, containing both inorganic (silica) and organic (organosiloxanes) components, chemically modified at the surface by the bonding of octadecylsilyl groups. To minimise any interaction with basic compounds, they are carefully end-capped to cover most of the remaining silanol groups.

Osthole. $C_{15}H_{16}O_3$. (M_r 244.3). *1180500.* [484-12-8]. 7-Methoxy-8-(3-methylbut-2-enyl)-2*H*-1-benzopyran-2-one. 7-Methoxy-8-isopentenylcoumarin.

Oxalic acid. $C_2H_2O_4,2H_2O$. (M_r 126.1). *1061400.* [6153-56-6]. Ethanedioic acid dihydrate.

White or almost white crystals, soluble in water, freely soluble in ethanol (96 per cent).

Oxalic acid and sulfuric acid solution. *1061401.*

A 50 g/L solution of *oxalic acid R* in a cooled mixture of equal volumes of *sulfuric acid R* and *water R*.

Oxazepam. *1144300.* [604-75-1].

See *Oxazepam (0778)*.

Ox brain, acetone-dried. *1061300.*

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 this 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 h or until the odour of acetone is no longer present.

2,2′-Oxybis(*N*,*N*-dimethylethylamine). $C_8H_{20}N_2O$. (M_r 160.3). *1141200.* [3033-62-3]. Bis(2-dimethylaminoethyl) ether.

Colourless, corrosive liquid.

d_{20}^{20}: about 0.85.

n_D^{20}: about 1.430.

Oxygen. O_2. (M_r 32.00). *1108800.*

Content: minimum 99.99 per cent *V/V*.

Nitrogen and argon: less than 100 ppm.

Carbon dioxide: less than 10 ppm.

Carbon monoxide: less than 5 ppm.

Oxygen R1. O_2. (M_r 32.00). *1137600.*

Content: minimum 99 per cent *V/V*.

Oxytetracycline hydrochloride. *1146500.*

See *Oxytetracycline hydrochloride (0198)*.

Paeoniflorin. $C_{23}H_{28}O_{11}$. (M_r 480.5). *1197300.* [23180-57-6]. [(1*R*,2*S*,3*R*,5*R*,6*R*,8*S*)-3-(β-D-Glucopyranosyloxy)6-hydroxy-8-methyl-9,10-dioxatetracyclo[4.3.1.$0^{2,5}$.$0^{3,8}$]decan-2-yl]methyl benzoate.

Paeonol. $C_9H_{10}O_3$. (M_r 166.2). *1197400.* [552-41-0]. 1-(2-Hydroxy-4-methoxyphenyl)ethan-1-one. 2′-Hydroxy-4′-methoxyacetophenone.

Palladium. Pd. (A_r 106.4). *1114700.* [7440-05-3].

Grey white metal, soluble in hydrochloric acid.

Palladium chloride. $PdCl_2$. (M_r 177.3). *1061500.* [7647-10-1].

Red crystals.

mp: 678 °C to 680 °C.

Palladium chloride solution. *1061501.*

Dissolve 1 g of *palladium chloride R* in 10 mL of warm *hydrochloric acid R*. Dilute the solution to 250 mL with a mixture of equal volumes of *dilute hydrochloric acid R* and *water R*. Dilute this solution immediately before use with 2 volumes of *water R*.

Palmatine. $C_{21}H_{22}NO_4^+$. (M_r 352.4). *1198800.* [3486-67-7]. 2,3,9,10-Tetramethoxy-5,6-dihydro-7λ^5-isoquinolino-[3,2-*a*]isoquinolin-7-ylium. 7,8,13,13a-Tetradehydro-2,3,9,10-tetramethoxyberbinium.

Palmitic acid. $C_{16}H_{32}O_2$. (M_r 256.4). *1061600.* [57-10-3]. Hexadecanoic acid.

White or almost white, crystalline scales, practically insoluble in water, freely soluble in hot ethanol (96 per cent).

mp: about 63 °C.

Chromatography. Thin-layer chromatography (*2.2.27*) as prescribed in the monograph *Chloramphenicol palmitate (0473)*; the chromatogram shows only one principal spot.

Palmitic acid used in the assay of total fatty acids in the monograph Saw palmetto fruit (1848) complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Saw palmetto fruit (1848)*.

Content: minimum 98 per cent, calculated by the normalisation procedure.

Palmitoleic acid. $C_{16}H_{30}O_2$. (M_r 254.4). *1144400.* [373-49-9]. (9*Z*)-Hexadec-9-enoic acid.

Clear, colourless liquid.

bp: about 162 °C.

Palmitoleic acid used in the assay of total fatty acids in Saw palmetto fruit (1848) complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Saw palmetto fruit (1848)*.

Content: minimum 98 per cent, calculated by the normalisation procedure.

Palmityl alcohol. $C_{16}H_{34}O$. (M_r 242.4). *1156100.* [36653-82-4]. Hexadecan-1-ol. Cetyl alcohol.

mp: about 48 °C.

Content: minimum 96 per cent.

Pancreas powder. *1061700.*

See *Pancreas powder (0350)*.

Papain. *1150700.* [9001-73-4].

A proteolytic enzyme obtained from the latex of the green fruit and leaves of *Carica papaya* L.

Papaverine hydrochloride. *1061800.* [61-25-6].

See *Papaverine hydrochloride (0102)*.

Paper chromatography performance test solutions. *1150800.*

Test solution (A). Sodium pertechnetate (^{99m}Tc) injection (fission) (0124) or *Sodium pertechnetate (^{99m}Tc) injection (non-fission) (0283)*.

Test solution (B). In a closed vial mix 100 µL of a 5 g/L solution of *stannous chloride R* in *0.05 M hydrochloric acid* and 100 MBq to 200 MBq of *Sodium pertechnetate (^{99m}Tc) injection (fission) (0124)* or *Sodium pertechnetate (^{99m}Tc) injection (non-fission) (0283)* in a volume not exceeding 2 mL.

Paper for chromatography. *1150900.*

Pure cellulose grade thin paper with a smooth surface and a thickness of about 0.2 mm.

Chromatographic separation. To 2 strips of *paper for chromatography R* apply separately 2-5 µL of test solution (a) and test solution (b) of *paper chromatography performance test solutions R*. Develop over a pathlength of 3/4 of the paper height, using a mixture of equal volumes of *methanol R* and *water R*. Allow to dry and determine the distribution of radioactivity using a suitable detector. The paper is not satisfactory, unless the chromatogram obtained with test solution (a) shows a single radioactivity spot with an R_F value in the range 0.8-1.0 and the chromatogram obtained with test solution (b) shows a single radioactivity spot at the application point (R_F value in the range 0.0-0.1).

Paracetamol. *1061900.* [103-90-2].

See *Paracetamol (0049)*.

Paracetamol, 4-aminophenol-free. *1061901.*

Recrystallise *paracetamol R* from *water R* and dry *in vacuo* at 70 °C; repeat the procedure until the product complies with the following test: dissolve 5 g of the dried substance in a mixture of equal volumes of *methanol R* and *water R* and dilute to 100 mL with the same mixture of solvents. Add 1 mL of a freshly prepared solution containing 10 g/L of *sodium nitroprusside R* and 10 g/L of *anhydrous sodium carbonate R*, mix and allow to stand for 30 min protected from light. No blue or green colour is produced.

Paraffin, liquid. *1062000.* [8042-47-5].

See *Liquid paraffin (0239)*.

Paraffin, white soft. *1062100.*

A semi-liquid mixture of hydrocarbons obtained from petroleum and bleached, practically insoluble in water and in ethanol (96 per cent), soluble in *light petroleum R1*, the solution sometimes showing a slight opalescence.

Paraldehyde. *1151000.* [123-63-7].

See *Paraldehyde (0351)*.

Pararosaniline hydrochloride. $C_{19}H_{18}ClN_3$. (M_r 323.8). *1062200.* [569-61-9].

Schultz No. 779.

Colour Index No. 42500.

4-[Bis(4-aminophenyl)methylene]cyclohexa-2,5-dieniminium chloride.

Bluish-red, crystalline powder, slightly soluble in water, soluble in anhydrous ethanol. Solutions in water and anhydrous ethanol are deep-red; solutions in sulfuric acid and in hydrochloric acid are yellow.

mp: about 270 °C, with decomposition.

Decolorised pararosaniline solution. *1062201.*

To 0.1 g of *pararosaniline hydrochloride R* in a ground-glass-stoppered flask add 60 mL of *water R* and a solution of 1.0 g of *anhydrous sodium sulfite R* or 2.0 g of *sodium sulfite heptahydrate R* or 0.75 g of *sodium metabisulfite R* in 10 mL of *water R*. Slowly and with stirring add 6 mL of *dilute hydrochloric acid R*, stopper the flask and continue stirring until dissolution is complete. Dilute to 100 mL with *water R*. Allow to stand for 12 h before use.

Storage: protected from light.

Parthenolide. $C_{15}H_{20}O_3$. (M_r 248.3). *1129900.* [20554-84-1]. (4*E*)-(1a*R*,7a*S*,10a*S*,10b*S*)-1a,5-Dimethyl-8-methylene-2,3,6,7,7a,8,10a,10b-octahydro-oxireno[9,10]cyclodeca[1,2-*b*]furan-9(1a*H*)-one. (*E*)-(5*S*,6*S*)-4,5-Epoxygermacra-1(10),11(13)-dieno-12(6)-lactone.

White or almost white, crystalline powder, very slightly soluble in water, very soluble in methylene chloride, soluble in methanol.

$[\alpha]_D^{22}$: – 71.4, determined on a 2.2 g/L solution in *methylene chloride R*.

mp: 115 °C to 116 °C.

Absorbance (*2.2.25*). A 0.01 g/L solution in *ethanol (96 per cent) R* shows an absorption maximum at 214 nm.

Assay. Liquid chromatography (*2.2.29*) as prescribed in the monograph *Feverfew (1516)*, at the concentration of the reference solution.

Content: minimum 90 per cent, calculated by the normalisation procedure.

L-Penicillamine coated silica gel for chiral separations. *1200500.*

A very finely divided silica gel for chromatography coated with L-penicillamine.

Penicillinase solution. *1062300.*

Dissolve 10 g of casein hydrolysate, 2.72 g of *potassium dihydrogen phosphate R* and 5.88 g of *sodium citrate R* in 200 mL of *water R*, adjust to pH 7.2 with a 200 g/L solution of *sodium hydroxide R* and dilute to 1000 mL with *water R*. Dissolve 0.41 g of *magnesium sulfate R* in 5 mL of *water R* and add 1 mL of a 1.6 g/L solution of *ferrous ammonium sulfate R* and sufficient *water R* to produce 10 mL. Sterilise both solutions by heating in an autoclave, cool, mix, distribute in shallow layers in conical flasks and inoculate with *Bacillus cereus* (NCTC 9946). Allow the flasks to stand at 18 °C to 37 °C until growth is apparent and then maintain at 35 °C to 37 °C for 16 h, shaking constantly to ensure maximum aeration. Centrifuge and sterilise the supernatant by filtration through a membrane filter. 1.0 mL of penicillinase solution contains not less than 0.4 microkatals (corresponding to the hydrolysis of not less than 500 mg of benzylpenicillin to benzylpenicilloic acid per hour) at 30 °C and pH 7, provided that the concentration of benzylpenicillin does not fall below the level necessary for enzyme saturation.

The Michaelis constant for benzylpenicillin of the penicillinase in penicillinase solution is approximately 12 µg/mL.

Sterility (*2.6.1*). It complies with the test for sterility.

Storage: at a temperature between 0 °C and 2 °C for 2 to 3 days. When freeze-dried and kept in sealed ampoules, it may be stored for several months.

Pentaerythrityl tetrakis[3-(3,5-di(1,1-dimethylethyl)-4-hydroxyphenyl)propionate]. $C_{73}H_{108}O_{12}$. (M_r 1178). *1062400.* [6683-19-8]. Pentaerythrityl tetrakis[3-(3,5-di-*tert*-butyl-4-hydroxyphenyl) propionate]. 2,2′-Bis(hydroxymethyl)propane-1,3-diol tetrakis[3-[3,5-di(1,1-dimethylethyl)-4-hydroxyphenyl]]propionate.

White or slightly yellow, crystalline powder, practically insoluble in water, very soluble in acetone, soluble in methanol, slightly soluble in hexane.

mp: 110 °C to 125 °C.

α-form: 120 °C to 125 °C.

β-form: 110 °C to 115 °C.

Pentafluoropropanoic acid. $C_3HF_5O_2$. (M_r 164.0). *1151100.* [422-64-0].

Clear, colourless liquid.

d_{20}^{20}: about 1.561.

n_D^{20}: about 1.284.

bp: about 97 °C.

Pentafluoropropionic anhydride. $C_6F_{10}O_3$. (M_r 310.0). *1177300.* [356-42-3]. Pentafluoropropanoic anhydride.

Pentane. C_5H_{12}. (M_r 72.2). *1062500.* [109-66-0].

Clear, colourless, flammable liquid, very slightly soluble in water, miscible with acetone and with anhydrous ethanol.

d_{20}^{20}: about 0.63.

n_D^{20}: about 1.359.

bp: about 36 °C.

Pentane used in spectrophotometry complies with the following additional test.

Absorbance (*2.2.25*): maximum 0.70 at 200 nm, 0.30 at 210 nm, 0.07 at 220 nm, 0.03 at 230 nm, 0.01 at 240 nm, determined using *water R* as compensation liquid.

1,2-Pentanediol. $C_5H_{12}O_2$. (M_r 104.2). *1155800.* [5343-92-0]. (2*RS*)-Pentane-1,2-diol.

d_4^{20}: about 0.971.

n_D^{20}: about 1.439.

bp: about 201 °C.

Pentanol. $C_5H_{12}O$. (M_r 88.1). *1062600.* [71-41-0]. Pentan-1-ol.

Colourless liquid, sparingly soluble in water, miscible with ethanol (96 per cent).

n_D^{20}: about 1.410.

bp: about 137 °C.

3-Pentanone. $C_5H_{10}O$. (M_r 86.13). *1173600.* [96-22-0]. Pentan-3-one. Diethyl ketone.

***tert*-Pentyl alcohol.** $C_5H_{12}O$. (M_r 88.1). *1062700.* [75-85-4]. *tert*-Amyl alcohol. 2-Methyl-2-butanol.

Volatile, flammable liquid, freely soluble in water, miscible with ethanol (96 per cent) and with glycerol.

d_{20}^{20}: about 0.81.

Distillation range (*2.2.11*). Not less than 95 per cent distils between 100 °C and 104 °C.

Storage: protected from light.

Pentetic acid. $C_{14}H_{23}N_3O_{10}$. (M_r 393.3). *1183100.* [67-43-6]. [[(Carboxymethyl)imino]bis(ethylenenitrilo)]tetraacetic acid.
White or almost white powder, slightly soluble in water.
mp: 219 °C to 220 °C, with decomposition.

Pepsin powder. *1062800.* [9001-75-6].
See *Pepsin powder (0682)*.

Peptide *N*-glycosidase F. *1186600.* [83534-39-8].
Peptide-N^4-(*N*-acetyl-β-glucosaminyl)asparagine amidase (EC 3.5.1.52). PNGase F.

Perchloric acid. $HClO_4$. (M_r 100.5). *1062900.* [7601-90-3].
Content: 70.0 per cent *m/m* to 73.0 per cent *m/m*.
Clear, colourless liquid, miscible with water.
d_{20}^{20}: about 1.7.
Assay. To 2.50 g add 50 mL of *water R* and titrate with *1 M sodium hydroxide*, using 0.1 mL of *methyl red solution R* as indicator.
1 mL of *1 M sodium hydroxide* is equivalent to 100.5 mg of $HClO_4$.

Perchloric acid solution. *1062901.*
Dilute 8.5 mL of *perchloric acid R* to 100 mL with *water R*.

Perfluoroheptanoic acid. $C_7HF_{13}O_2$. (M_r 364.1). *1207400.* [375-85-9]. Tridecafluoroheptanoic acid.

Periodic acetic acid solution. *1063000.*
Dissolve 0.446 g of *sodium periodate R* in 2.5 mL of a 25 per cent *V/V* solution of *sulfuric acid R*. Dilute to 100.0 mL with *glacial acetic acid R*.

Periodic acid. H_5IO_6. (M_r 227.9). *1108900.* [10450-60-9].
Crystals, freely soluble in water and soluble in ethanol (96 per cent).
mp: about 122 °C.

Permethrin. $C_{21}H_{20}Cl_2O_3$. (M_r 391.3). *1130000.* [52645-53-1].
mp: 34 °C to 35 °C.
A suitable certified reference solution (10 ng/µL in cyclohexane) may be used.

Peroxide test strips. *1147800.*
Use commercial test strips with a suitable scale in the range from 0 ppm to 25 ppm peroxide.

Perylene. $C_{20}H_{12}$. (M_r 252.3). *1130100.* [198-55-0]. Dibenz[*de*,*kl*]anthracene.
Orange powder.
mp: about 279 °C.

Petroleum, light. *1063100.* [8032-32-4]. Petroleum ether 50-70 °C.
Clear, colourless, flammable liquid without fluorescence, practically insoluble in water, miscible with ethanol (96 per cent).
d_{20}^{20}: 0.661 to 0.664.
Distillation range (*2.2.11*): 50 °C to 70 °C.

Petroleum, light R1. *1063101.* Petroleum ether 40-60 °C.
Complies with the requirements prescribed for *light petroleum R*, with the following modifications.
d_{20}^{20}: 0.630 to 0.656.
Distillation range (*2.2.11*): 40 °C to 60 °C. It does not become cloudy at 0 °C.

Petroleum, light R2. *1063102.* Petroleum ether 30-40 °C.
Complies with the requirements prescribed for *light petroleum R*, with the following modifications.
d_{20}^{20}: 0.620 to 0.630.
Distillation range (*2.2.11*): 30 °C to 40 °C. It does not become cloudy at 0 °C.

Petroleum, light R3. *1063103.* Petroleum ether 100-120 °C.
Complies with the requirements prescribed for *light petroleum R*, with the following modifications.
d_{20}^{20}: about 0.720.
Distillation range (*2.2.11*): 100 °C to 120 °C.
Water (*2.5.12*): maximum 0.03 per cent.

Petroleum, light R4. *1063104.* Petroleum ether 80-100 °C.
Complies with the requirements prescribed for *light petroleum R*, with the following modifications.
d_{20}^{20}: about 0.70.
Distillation range (*2.2.11*): 80 °C to 100 °C.

pH indicator strip. *1178900.*
Paper strip, or plastic strip containing multiple segments of different dye-impregnated papers, allowing visual determination of pH in the prescribed range, by comparison with the corresponding master chart.

α-Phellandrene. $C_{10}H_{16}$. (M_r 136.2). *1130400.* [4221-98-1]. (*R*)-5-Isopropyl-2-methyl-cyclohexa-1,3-diene. (–)-*p*-Mentha-1,5-diene.
n_D^{20}: about 1.471.
bp: 171 °C to 174 °C.
α-Phellandrene used in gas chromatography complies with the following additional test.
Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Eucalyptus oil (0390)*.
Test solution. The substance to be examined.
Content: 95.0 per cent, calculated by the normalisation procedure.

Phenanthrene. $C_{14}H_{10}$. (M_r 178.2). *1063200.* [85-01-8].
White or almost white crystals, practically insoluble in water, sparingly soluble in ethanol (96 per cent).
mp: about 100 °C.

Phenanthroline hydrochloride. $C_{12}H_9ClN_2,H_2O$. (M_r 234.7). *1063300.* [18851-33-7]. 1,10-Phenanthroline hydrochloride monohydrate.
White or almost white, crystalline powder, freely soluble in water, soluble in ethanol (96 per cent).
mp: about 215 °C, with decomposition.

Phenazone. *1063400.* [60-80-0].
See *Phenazone (0421)*.

Phenol. *1063500.* [108-95-2].
See *Phenol (0631)*.

Phenolphthalein. $C_{20}H_{14}O_4$. (M_r 318.3). *1063700.* [77-09-8]. 3,3-Bis(4-hydroxyphenyl)-3*H*-isobenzofuran-1-one.
White or yellowish-white powder, practically insoluble in water, soluble in ethanol (96 per cent).

Phenolphthalein paper. *1063704.*
Immerse strips of filter paper for a few minutes in *phenolphthalein solution R*. Allow to dry.

Phenolphthalein solution. *1063702.*
Dissolve 0.1 g of *phenolphthalein R* in 80 mL of *ethanol (96 per cent) R* and dilute to 100 mL with *water R*.
Test for sensitivity. 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 solution R1. *1063703.*
A 10 g/L solution of *phenolphthalein R* in *ethanol (96 per cent) R*.

Phenol red. *1063600.* [143-74-8].

Bright red or dark red, crystalline powder, very slightly soluble in water, slightly soluble in ethanol (96 per cent).

Phenol red solution. *1063601.*

Dissolve 0.1 g of *phenol red R* in a mixture of 2.82 mL of *0.1 M sodium hydroxide* and 20 mL of *ethanol (96 per cent) R* 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 *0.02 M sodium hydroxide* is required to change the colour to reddish-violet.

Colour change: pH 6.8 (yellow) to pH 8.4 (reddish-violet).

Phenol red solution R2. *1063603.*

Solution A. Dissolve 33 mg of *phenol red R* in 1.5 mL of *dilute sodium hydroxide solution R* and dilute to 100 mL with *water R*.

Solution B. Dissolve 25 mg of *ammonium sulfate R* in 235 mL of *water R*; add 105 mL of *dilute sodium hydroxide solution R* and 135 mL of *dilute acetic acid R*.

Add 25 mL of solution A to solution B. If necessary, adjust the pH of the mixture to 4.7.

Phenol red solution R3. *1063604.*

Solution A. Dissolve 33 mg of *phenol red R* in 1.5 mL of *dilute sodium hydroxide solution R* and dilute to 50 mL with *water R*.

Solution B. Dissolve 50 mg of *ammonium sulfate R* in 235 mL of *water R*; add 105 mL of *dilute sodium hydroxide solution R* and 135 mL of *dilute acetic acid R*.

Add 25 mL of solution A to solution B; if necessary, adjust the pH of the mixture to 4.7.

Phenoxyacetic acid. $C_8H_8O_3$. (M_r 152.1). *1063800.* [122-59-8]. 2-Phenoxyethanoic acid.

Almost white crystals, sparingly soluble in water, freely soluble in ethanol (96 per cent), and in glacial acetic acid.

mp: about 98 °C.

Chromatography. Thin-layer chromatography (*2.2.27*) as prescribed in the monograph *Phenoxymethylpenicillin (0148)*; the chromatogram shows only one principal spot.

2-Phenoxyaniline. $C_{12}H_{11}NO$. (M_r 185.2). *1165500.* [2688-84-8]. 2-Phenoxybenzenamine. 2-Aminophenyl phenyl ether.

Phenoxyethanol. $C_8H_{10}O_2$. (M_r 138.2). *1064000.* [122-99-6]. 2-Phenoxyethanol.

Clear, colourless, oily liquid, slightly soluble in water, freely soluble in ethanol (96 per cent).

d_{20}^{20}: about 1.11.

n_D^{20}: about 1.537.

Freezing point (*2.2.18*): minimum 12 °C.

Phenylacetic acid. $C_8H_8O_2$. (M_r 136.2). *1160000.* [103-82-2].

White or almost white powder, soluble in water.

bp: about 265 °C.

mp: about 75 °C.

Phenylalanine. *1064100.* [63-91-2].

See *Phenylalanine (0782)*.

***p*-Phenylenediamine dihydrochloride.** $C_6H_{10}Cl_2N_2$. (M_r 181.1). *1064200.* [615-28-1]. 1,4-Diaminobenzene dihydrochloride.

Crystalline powder or white or slightly coloured crystals, turning reddish on exposure to air, freely soluble in water, slightly soluble in ethanol (96 per cent).

α-Phenylglycine. $C_8H_9NO_2$. (M_r 151.2). *1064300.* [2835-06-5]. (*RS*)-2-Amino-2-phenylacetic acid.

D-Phenylglycine. $C_8H_9NO_2$. (M_r 151.2). *1144500.* [875-74-1]. (2*R*)-2-Amino-2-phenylacetic acid.

Content: minimum 99 per cent.

White or almost white, crystalline powder.

Phenylhydrazine. $C_6H_8N_2$. (M_r 108.1). *1190800.* [100-63-0].

White or almost white, crystalline powder, becoming yellow or dark red on exposure to air, melting at room temperature giving an oily liquid, miscible with anhydrous ethanol, sparingly soluble in water.

bp: about 244 °C, with decomposition.

mp: about 20 °C.

Phenylhydrazine hydrochloride. $C_6H_9ClN_2$. (M_r 144.6). *1064500.* [59-88-1].

White or almost white, crystalline powder, becoming brown on exposure to air, soluble in water and in ethanol (96 per cent).

mp: about 245 °C, with decomposition.

Storage: protected from light.

Phenylhydrazine hydrochloride solution. *1064501.*

Dissolve 0.9 g of *phenylhydrazine hydrochloride R* in 50 mL of *water R*. Decolorise with *activated charcoal R* and filter. To the filtrate add 30 mL of *hydrochloric acid R* and dilute to 250 mL with *water R*.

Phenylhydrazine-sulfuric acid solution. *1064502.*

Dissolve 65 mg of *phenylhydrazine hydrochloride R*, previously recrystallised from *ethanol (85 per cent V/V) R*, in a mixture of 80 volumes of *water R* and 170 volumes of *sulfuric acid R* and dilute to 100 mL with the same mixture of solvents. Prepare immediately before use.

Phenyl isothiocyanate. C_7H_5NS. (M_r 135.2). *1121500.* [103-72-0].

Liquid, insoluble in water, soluble in ethanol (96 per cent).

d_{20}^{20}: about 1.13.

n_D^{20}: about 1.65.

bp: about 221 °C.

mp: about − 21 °C.

Use a grade suitable for protein sequencing.

Phenyl(5)methyl(95)polysiloxane. *1066900.*

Polysiloxane substituted with 5 per cent of phenyl groups and 95 per cent of methyl groups.

Phenyl(5)methyl(95)polysiloxane, base-deactivated. *1176600.*

Base-deactivated polysiloxane substituted with 5 per cent of phenyl groups and 95 per cent of methyl groups.

Phenyl(50)methyl(50)polysiloxane. *1067900.*

Polysiloxane substituted with 50 per cent of phenyl groups and 50 per cent of methyl groups.

1-Phenylpiperazine. $C_{10}H_{14}N_2$. (M_r 162.2). *1130500.* [92-54-6].

Slightly viscous, yellow liquid, not miscible with water.

d_4^{20}: about 1.07.

n_D^{20}: about 1.588.

1-Phenylpropan-2-ol. $C_9H_{12}O$. (M_r 136.2). *1205200.* [698-87-3]. (2*RS*)-1-Phenylpropan-2-ol.

mp: 65 °C to 67 °C.

1-Phenyl-1,2,3,4-tetrahydroisoquinoline. $C_{15}H_{15}N$. (M_r 209.3). *1193700.* [22990-19-8].

Phloroglucide. $C_{12}H_{10}O_5$. (M_r 234.2). *1177400.* [491-45-2]. 2,3′,4,5′,6-Biphenylpentol.

White or almost white powder, hygroscopic, light sensitive. Slowly discolours on exposure to light.

Phloroglucinol. $C_6H_6O_3,2H_2O$. (M_r 162.1). *1064600.* [6099-90-7]. Benzene-1,3,5-triol.

White or yellowish crystals, slightly soluble in water, soluble in ethanol (96 per cent).

mp: about 223 °C (instantaneous method).

Phloroglucinol solution. *1064601.*

To 1 mL of a 100 g/L solution of *phloroglucinol R* in *ethanol (96 per cent) R*, add 9 mL of *hydrochloric acid R*.

Storage: protected from light.

Phosalone. $C_{12}H_{15}ClNO_4PS_2$. (M_r 367.8). *1130200.* [2310-17-0].

mp: 45 °C to 48 °C

A suitable certified reference solution (10 ng/µL in iso-octane) may be used.

Phosphomolybdic acid. $12MoO_3,H_3PO_4,xH_2O$. *1064900.* [51429-74-4].

Orange-yellow, fine crystals, freely soluble in water, soluble in ethanol (96 per cent).

Phosphomolybdic acid solution. *1064901.*

Dissolve 4 g of *phosphomolybdic acid R* in *water R* and dilute to 40 mL with the same solvent. Add cautiously and with cooling 60 mL of *sulfuric acid R*. Prepare immediately before use.

Phosphomolybdotungstic reagent. *1065000.*

Dissolve 100 g of *sodium tungstate R* and 25 g of *sodium molybdate R* in 700 mL of *water R*. Add 100 mL of *hydrochloric acid R* and 50 mL of *phosphoric acid R*. Heat the mixture under a reflux condenser in a glass apparatus for 10 h. Add 150 g of *lithium sulfate R*, 50 mL of *water R* and a few drops of *bromine R*. Boil to remove the excess of bromine (15 min), allow to cool, dilute to 1000 mL with *water R* and filter. The reagent should be yellow in colour. If it acquires a greenish tint, it is unsatisfactory for use but may be regenerated by boiling with a few drops of *bromine R*. Care must be taken to remove the excess of bromine by boiling.

Storage: at 2 °C to 8 °C.

Phosphomolybdotungstic reagent, dilute. *1065001.*

To 1 volume of *phosphomolybdotungstic reagent R* add 2 volumes of *water R*.

Phosphoric acid. *1065100.* [7664-38-2].

See *Concentrated phosphoric acid (0004)*.

Phosphoric acid, dilute. *1065101.*

See *Dilute phosphoric acid (0005)*.

Phosphoric acid, dilute R1. *1065102.*

Dilute 93 mL of *dilute phosphoric acid R* to 1000 mL with *water R*.

Phosphorous acid. H_3PO_3. (M_r 82.0). *1130600.* [13598-36-2].

White or almost white, very hygroscopic and deliquescent crystalline mass; slowly oxidised by oxygen (air) to H_3PO_4.

Unstable, orthorhombic crystals, soluble in water, in ethanol (96 per cent) and in a mixture of 3 volumes of ether and 1 volume of ethanol (96 per cent).

d_4^{21}: 1.651.

mp: about 73 °C.

Phosphotungstic acid solution. *1065200.*

Heat under a reflux condenser for 3 h, 10 g of *sodium tungstate R* with 8 mL of *phosphoric acid R* and 75 mL of *water R*. Allow to cool and dilute to 100 mL with *water R*.

Phthalaldehyde. $C_8H_6O_2$. (M_r 134.1). *1065300.* [643-79-8]. Benzene-1,2-dicarboxaldehyde.

Yellow, crystalline powder.

mp: about 55 °C.

Storage: protected from light and air.

Phthalaldehyde reagent. *1065301.*

Dissolve 2.47 g of *boric acid R* in 75 mL of *water R*, adjust to pH 10.4 using a 450 g/L solution of *potassium hydroxide R* and dilute to 100 mL with *water R*. Dissolve 1.0 g of *phthalaldehyde R* in 5 mL of *methanol R*, add 95 mL of the boric acid solution and 2 mL of *thioglycollic acid R* and adjust to pH 10.4 with a 450 g/L solution of *potassium hydroxide R*.

Storage: protected from light; use within 3 days.

Phthalazine. $C_8H_6N_2$. (M_r 130.1). *1065400.* [253-52-1].

Pale yellow crystals, freely soluble in water, soluble in anhydrous ethanol, in ethyl acetate and in methanol.

mp: 89 °C to 92 °C.

Phthalein purple. $C_{32}H_{32}N_2O_{12},xH_2O$. ($M_r$ 637, anhydrous substance). *1065500.* [2411-89-4]. Metalphthalein. 2,2′,2″,2‴-[*o*-Cresolphthalein-3′,3″-bis(methylenenitrilo)]tetra-acetic acid. (1,3-Dihydro-3-oxo-isobenzofuran-1-ylidene)bis[(6-hydroxy-5-methyl-3,1-phenylene)bis(methyleneimino)diacetic acid].

Yellowish-white or brownish powder, practically insoluble in water, soluble in ethanol (96 per cent). The product may be found in commerce in the form of the sodium salt: a yellowish-white to pink powder, soluble in water, practically insoluble in ethanol (96 per cent).

Test for sensitivity. Dissolve 10 mg in 1 mL of *concentrated ammonia R* and dilute to 100 mL with *water R*. To 5 mL of the solution add 95 mL of *water R*, 4 mL of *concentrated ammonia R*, 50 mL of *ethanol (96 per cent) R* and 0.1 mL of *0.1 M barium chloride*. The solution is blue-violet. Add 0.15 mL of *0.1 M sodium edetate*. The solution becomes colourless.

Phthalic acid. $C_8H_6O_4$. (M_r 166.1). *1065600.* [88-99-3]. Benzene-1,2-dicarboxylic acid.

White or almost white, crystalline powder, soluble in hot water and in ethanol (96 per cent).

Phthalic anhydride. $C_8H_4O_3$. (M_r 148.1). *1065700.* [85-44-9]. Isobenzofuran-1,3-dione.

Content: minimum 99.0 per cent.

White or almost white flakes.

mp: 130 °C to 132 °C.

Assay. Dissolve 2.000 g in 100 mL of *water R* and boil under a reflux condenser for 30 min. Cool and titrate with *1 M sodium hydroxide*, using *phenolphthalein solution R* as indicator.

1 mL of *1 M sodium hydroxide* is equivalent to 74.05 mg of $C_8H_4O_3$.

Phthalic anhydride solution. *1065701.*

Dissolve 42 g of *phthalic anhydride R* in 300 mL of *anhydrous pyridine R*. Allow to stand for 16 h.

Storage: protected from light; use within 1 week.

Picein. $C_{14}H_{18}O_7$. (M_r 298.3). *1130700.* [530-14-3]. 1-[4-(β-D-Glucopyranosyloxy)phenyl]ethanone. *p*-(Acetylphenyl)-β-D-glucopyranoside.

mp: 194 °C to 195 °C.

Picric acid. $C_6H_3N_3O_7$. (M_r 229.1). *1065800.* [88-89-1]. 2,4,6-Trinitrophenol.

Yellow prisms or plates, soluble in water and in ethanol (96 per cent).

Storage: moistened with *water R*.

Picric acid solution. *1065801.*

A 10 g/L solution of *picric acid R*.

Picric acid solution R1. *1065802.*

Prepare 100 mL of a saturated solution of *picric acid R* and add 0.25 mL of *strong sodium hydroxide solution R*.

Picrotin. $C_{15}H_{18}O_7$. (M_r 310.3). *1188100.* [21416-53-5]. (1*R*,3*R*,5*S*,8*S*,9*R*,12*S*,13*R*,14*S*)-1-hydroxy-14-(2-hydroxypropan-2-yl)-13-methyl-4,7,10-trioxapentacyclo[6.4.1.1.^{9,12}.0^{3,5}.0^{5,13}]tetradecane-6,11-dione.

White or colourless crystalline powder or crystals, soluble in boiling water and in ethanol (96 per cent), practically insoluble in methylene chloride.

mp: 248 °C to 250 °C.

Picrotoxinin. $C_{15}H_{16}O_6$. (M_r 292.2). *1188200.* [17617-45-7]. (1*R*,3*R*,5*S*,8*S*,9*R*,12*S*,13*R*,14*R*)-1-hydroxy-13-methyl-14-(prop-1-en-2-yl)-4,7,10-trioxapentacyclo[6.4.1.1^{9,12}.0^{3,5}.0^{5,13}]tetradecane-6,11-dione.

White or colourless crystalline powder or crystals, soluble in methylene chloride, in ethanol (96 per cent) and in alkaline solutions.

mp: 207 to 210 °C.

α-Pinene. $C_{10}H_{16}$. (M_r 136.2). *1130800.* [7785-70-8]. (1*R*,5*R*)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene.

Liquid not miscible with water.

d_{20}^{20}: about 0.859.

n_D^{20}: about 1.466.

bp: 154 °C to 156 °C.

α-Pinene used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Bitter-orange-flower oil (1175)*.

Test solution. The substance to be examined.

Content: minimum 99.0 per cent, calculated by the normalisation procedure.

β-Pinene. $C_{10}H_{16}$. (M_r 136.2). *1109000.* [127-91-3]. 6,6-Dimethyl-2-methylenebicyclo[3.1.1]heptane.

Colourless, oily liquid, odour reminiscent of turpentine, practically insoluble in water, miscible with ethanol (96 per cent).

β-Pinene used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Bitter-orange-flower oil (1175)*.

Test solution. The substance to be examined.

Content: minimum 95.0 per cent.

1,4-Piperazinediethanesulfonic acid. $C_8H_{18}N_2O_6S_2$. (M_r 302.4). *1186700.* [5625-37-6]. Piperazine-1,4-bis(2-ethanesulfonic acid). 2,2′-(Piperazine-1,4-diyl)bis(ethanesulfonic acid). Piperazine-*N*,*N*′-bis(2-ethanesulfonic acid). PIPES.

Content: minimum 99 per cent.

White, crystalline powder.

Piperazine hydrate. *1065900.* [142-63-2].

See *Piperazine hydrate (0425)*.

Piperidine. $C_5H_{11}N$. (M_r 85.2). *1066000.* [110-89-4]. Hexahydropyridine.

Colourless to slightly yellow, alkaline liquid, miscible with water, with ethanol (96 per cent) and with light petroleum.

bp: about 106 °C.

Piperine. $C_{17}H_{19}NO_3$. (M_r 285.3). *1183200.* [94-62-2]. (2*E*,4*E*)-1-(Piperidin-1-yl)-5-(1,3-benzodioxol-5-yl)penta-2,4-dien-1-one. 1-Piperoyl-piperidine. 1-[(2*E*,4*E*)-5-(3,4-Methylenedioxyphenyl)-1-oxo-2,4-pentadienyl]piperidine.

Piperitone. $C_{10}H_{16}O$. (M_r 152.2). *1151200.* [89-81-6]. 6-Isopropyl-3-methyl-cyclohex-2-en-1-one.

Pirimiphos-ethyl. $C_{13}H_{24}N_3O_3PS$. (M_r 333.4). *1130300.* [23505-41-1].

mp: 15 °C to 18 °C.

A suitable certified reference solution (10 ng/µL in cyclohexane) may be used.

Plasma, platelet-poor. *1066100.*

Withdraw 45 mL of human blood into a 50 mL plastic syringe containing 5 mL of a sterile 38 g/L solution of *sodium citrate R*. Without delay, centrifuge at 1500 *g* at 4 °C for 30 min. Remove the upper two-thirds of the supernatant plasma using a plastic syringe and without delay centrifuge at 3500 *g* at 4 °C for 30 min. Remove the upper two-thirds of the liquid and freeze it rapidly in suitable amounts in plastic tubes at or below − 40 °C. Use plastic or silicone-treated equipment.

Plasma substrate. *1066200.*

Separate the plasma from human or bovine blood collected into one-ninth its volume of a 38 g/L solution of *sodium citrate R*, or into two-sevenths its volume of a solution containing 20 g/L of *disodium hydrogen citrate R* and 25 g/L of *glucose R*. With the former, prepare the substrate on the day of collection of the blood. With the latter, prepare within two days of collection of the blood.

Storage: at − 20 °C.

Plasma substrate R1. *1066201.*

Use water-repellent equipment (made from materials such as suitable plastics or suitably silicone-treated glass) for taking and handling blood.

Collect a suitable volume of blood from each of at least five sheep; a 285 mL volume of blood collected into 15 mL of anticoagulant solution is suitable but smaller volumes may be collected, taking the blood, either from a live animal or at the time of slaughter, using a needle attached to a suitable cannula which is long enough to reach the bottom of the collecting vessel. Discarding the first few millilitres and collecting 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* per 100 mL of *water R* 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 flask and cool to 10-15 °C. When cold, pool the contents of all the flasks with the exception of any that show obvious haemolysis or clots and keep the pooled blood at 10-15 °C.

As soon as possible and within 4 h of collection, centrifuge the pooled blood at 1000-2000 *g* at 10-15 °C for 30 min. Separate the supernatant and centrifuge it at 5000 *g* for 30 min. (Faster centrifugation, for example 20 000 *g* for 30 min, may be used if necessary to clarify the plasma, but filtration procedures should not be used.) Separate the supernatant and, without delay, mix thoroughly and distribute the plasma substrate into small stoppered containers in portions sufficient for a complete heparin assay (for example 10 mL to 30 mL). Without delay, rapidly cool to a temperature below − 70 °C (for example by immersing the containers into liquid nitrogen) and store at a temperature below − 30 °C.

The plasma is suitable for use as plasma substrate 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.

When required for use, thaw a portion of the plasma substrate in a water-bath at 37 °C, gently swirling until thawing is complete; once thawed it should be kept at

10-20 °C and used without delay. The thawed plasma substrate may be lightly centrifuged if necessary; filtration procedures should not be used.

Plasma substrate R2. *1066202.*

Prepare from human blood containing less than 1 per cent of the normal amount of factor IX. Collect the blood into one-ninth its volume of a 38 g/L solution of *sodium citrate R*.

Storage: in small amounts in plastic tubes at a temperature of – 30 °C or lower.

Plasma substrate R3. *1066203.*

Prepare from human blood containing less than 1 per cent of the normal amount of factor XI. Collect the blood into one-ninth its volume of a 38 g/L solution of *sodium citrate R*.

Storage: in small amounts in plastic tubes at a temperature of – 30 °C or lower.

Plasma substrate deficient in factor V. *1066300.*

Use preferably a plasma which is congenitally deficient, or prepare it as follows: separate the plasma from human blood collected into one tenth of its volume of a 13.4 g/L solution of *sodium oxalate R*. Incubate at 37 °C for 24 h to 36 h. The coagulation time determined by the method prescribed for *coagulation factor V solution R* should be 70 s to 100 s. If the coagulation time is less than 70 s, incubate again for 12 h to 24 h.

Storage: in small quantities at a temperature of – 20 °C or lower.

Plasminogen, human. *1109100.* [9001-91-6].

A substance present in blood that may be activated to plasmin, an enzyme that lyses fibrin in blood clots.

Plutonium-242 spiking solution. *1167400.*

Contains 50 Bq/L ^{242}Pu and a 134 mg/L solution of *lanthanum chloride heptahydrate R* in a 284 g/L solution of *nitric acid R*.

Poloxamer 188. *1186800.*

See *Poloxamers (1464)*.

Polyamine grafted poly(vinyl alcohol) copolymer. *1188300.*

Copolymer beads of poly(vinyl alcohol) to which polyamine is covalently bonded. The size range of the beads is specified after the name of the reagent in the tests where it is used.

Polydatin. $C_{20}H_{22}O_8$. (M_r 390.4). *1197500.* [65914-17-2]. 3-Hydroxy-5-[2-(4-hydroxyphenyl)eth-1-en-1-yl]phenyl β-D-glucopyranoside. Resveratrol-3-β-mono-D-glucoside.

Polyether hydroxylated gel for chromatography. *1067000.*

Gel with a small particle size having a hydrophilic surface with hydroxyl groups. It has an exclusion limit for dextran of relative molecular mass 2×10^5 to 2.5×10^6.

Polyethyleneglycol adipate. $(C_8H_{12}O_4)_n$. (M_r $(172.2)_n$). *1067700.*

White or almost white, wax-like mass, practically insoluble in water.

mp: about 43 °C.

Polyethyleneglycol succinate. $(C_6H_8O_4)_n$. (M_r $(144.1)_n$). *1067800.*

White or almost white, crystalline powder, practically insoluble in water.

mp: about 102 °C.

Polymethacrylate gel. *1181100.*

A methacrylate-based size-exclusion stationary phase for water-soluble samples.

Polymethacrylate gel, hydroxylated. *1151300.*

Stationary phase for size-exclusion chromatography.

Gel based on hydroxylated methacrylic acid polymer.

Polyorganosiloxane for oxygen-containing compounds. *1200600.*

Combination of suitable polyorganosiloxanes with high affinity for oxygen-containing compounds.

Polyoxyethylated castor oil. *1068200.*

Light yellow liquid. It becomes clear above 26 °C.

Polysorbate 20. *1068300.* [9005-64-5].

See *Polysorbate 20 (0426)*.

Polysorbate 65. *1196200.* [9005-71-4].

Polysorbate 80. *1068400.* [9005-65-6].

See *Polysorbate 80 (0428)*.

Polystyrene 900-1000. *1112200.* [9003-53-6].

Organic standard used for calibration in gas chromatography.

M_w: about 950.

M_w/M_n: 1.10.

Potassium acetate. *1175900.* [127-08-2].

See *Potassium acetate (1139)*.

Potassium bicarbonate. *1069900.* [298-14-6].

See *Potassium hydrogen carbonate R*.

Potassium bicarbonate solution, saturated methanolic. *1069901.*

See *potassium hydrogen carbonate solution, saturated methanolic R*.

Potassium bromate. $KBrO_3$. (M_r 167.0). *1068700.* [7758-01-2].

White or almost white granular powder or crystals, soluble in water, slightly soluble in ethanol (96 per cent).

Potassium bromide. *1068800.* [7758-02-3].

See *Potassium bromide (0184)*.

Potassium bromide used for infrared absorption spectrophotometry (2.2.24) also complies with the following additional test.

A disc 2 mm thick prepared from the substance previously dried at 250 °C for 1 h, has a substantially flat baseline over the range 4000 cm^{-1} to 620 cm^{-1}. It exhibits no maxima with absorbance greater than 0.02 above the baseline, except maxima for water at 3440 cm^{-1} and 1630 cm^{-1}.

Potassium carbonate. K_2CO_3. (M_r 138.2). *1068900.* [584-08-7]. Dipotassium carbonate.

White or almost white, granular powder, hygroscopic, very soluble in water, practically insoluble in anhydrous ethanol.

Storage: in an airtight container.

Potassium chlorate. $KClO_3$. (M_r 122.6). *1069000.* [3811-04-9].

A white or almost white powder, granules or crystals, soluble in water.

Potassium chloride. *1069100.* [7447-40-7].

See *Potassium chloride (0185)*.

Potassium chloride used for infrared absorption spectrophotometry (2.2.24) also complies with the following additional test.

A disc 2 mm thick, prepared from the substance previously dried at 250 °C for 1 h, has a substantially flat baseline over the range 4000 cm^{-1} to 620 cm^{-1}. It exhibits no maxima with absorbance greater than 0.02 above the baseline, except maxima for water at 3440 cm^{-1} and 1630 cm^{-1}.

Potassium chloride, 0.1 M. *1069101.*

A solution of *potassium chloride R* containing the equivalent of 7.46 g of KCl in 1000.0 mL.

Potassium chromate. K_2CrO_4. (M_r 194.2). *1069200.* [7789-00-6]. Dipotassium chromate.

Yellow crystals, freely soluble in water.

Potassium chromate solution. *1069201.*

A 50 g/L solution of *potassium chromate R*.

Potassium citrate. *1069300.* [6100-05-6].

See *Potassium citrate (0400)*.

Potassium cyanide. KCN. (M_r 65.1). *1069400.* [151-50-8].

White or almost white, crystalline powder or white or almost white mass or granules, freely soluble in water, slightly soluble in ethanol (96 per cent).

Potassium cyanide solution. *1069401.*

A 100 g/L solution of *potassium cyanide R*.

Potassium cyanide solution, lead-free. *1069402.*

Dissolve 10 g of *potassium cyanide R* in 90 mL of *water R*, add 2 mL of *strong hydrogen peroxide solution R* diluted 1 to 5. Allow to stand for 24 h, dilute to 100 mL with *water R* and filter.

The solution complies with the following test: take 10 mL of the solution, add 10 mL of *water R* and 10 mL of *hydrogen sulfide solution R*. No colour is evolved even after addition of 5 mL of *dilute hydrochloric acid R*.

Potassium dichromate. $K_2Cr_2O_7$. (M_r 294.2). *1069500.* [7778-50-9]. Dipotassium dichromate.

Potassium dichromate used for the calibration of spectrophotometers (*2.2.25*) contains not less than 99.9 per cent of $K_2Cr_2O_7$, calculated with reference to the substance dried at 130 °C.

Orange-red crystals, soluble in water, practically insoluble in ethanol (96 per cent).

Assay. Dissolve 1.000 g in *water R* and dilute to 250.0 mL with the same solvent. To 50.0 mL of this solution add a freshly prepared solution of 4 g of *potassium iodide R*, 2 g of *sodium hydrogen carbonate R* and 6 mL of *hydrochloric acid R* in 100 mL of *water R* in a 500 mL flask. Stopper the flask and allow to stand protected from light for 5 min. Titrate with *0.1 M sodium thiosulfate*, using 1 mL of *iodide-free starch solution R* as indicator.

1 mL of *0.1 M sodium thiosulfate* is equivalent to 4.903 mg of $K_2Cr_2O_7$.

Potassium dichromate solution. *1069501.*

A 106 g/L solution of *potassium dichromate R*.

Potassium dichromate solution R1. *1069502.*

A 5 g/L solution of *potassium dichromate R*.

Potassium dihydrogen phosphate. *1069600.* [7778-77-0].

See *Potassium dihydrogen phosphate (0920)*.

Potassium dihydrogen phosphate, 0.2 M. *1069601.*

A solution of *potassium dihydrogen phosphate R* containing the equivalent of 27.22 g of KH_2PO_4 in 1000.0 mL.

Potassium ferricyanide. $K_3[Fe(CN)_6]$. (M_r 329.3). *1069700.* [13746-66-2]. Potassium hexacyanoferrate(III).

Red crystals, freely soluble in water.

Potassium ferricyanide solution. *1069701.*

Wash 5 g of *potassium ferricyanide R* with a little *water R*, dissolve and dilute to 100 mL with *water R*. Prepare immediately before use.

Potassium ferriperiodate solution. *1070801.*

Dissolve 1 g of *potassium periodate R* in 5 mL of a freshly prepared 120 g/L solution of *potassium hydroxide R*. Add 20 mL of *water R* and 1.5 mL of *ferric chloride solution R1*. Dilute to 50 mL with a freshly prepared 120 g/L solution of *potassium hydroxide R*.

Potassium ferrocyanide. $K_4[Fe(CN)_6],3H_2O$. (M_r 422.4). *1069800.* [14459-95-1]. Potassium hexacyanoferrate(II).

Transparent yellow crystals, freely soluble in water, practically insoluble in ethanol (96 per cent).

Potassium ferrocyanide solution. *1069801.*

A 53 g/L solution of *potassium ferrocyanide R*.

Potassium fluoride. KF. (M_r 58.1). *1137800.* [7789-23-3].

Colourless crystals or white or almost white crystalline powder, deliquescent, soluble in water, practically insoluble in ethanol (96 per cent).

Potassium hydrogen carbonate. $KHCO_3$. (M_r 100.1). *1069900.* [298-14-6]. Potassium bicarbonate.

Transparent, colourless crystals, freely soluble in water, practically insoluble in ethanol (96 per cent).

Potassium hydrogen carbonate solution, saturated methanolic. *1069901.*

Dissolve 0.1 g of *potassium hydrogen carbonate R* in 0.4 mL of *water R*, heating on water-bath. Add 25 mL of *methanol R* and swirl, keeping the solution on the water-bath until dissolution is complete. Use a freshly prepared solution.

Potassium hydrogen phthalate. $C_8H_5KO_4$. (M_r 204.2). *1070000.* [877-24-7]. Potassium hydrogen benzene-1,2-dicarboxylate.

White or almost white crystals, soluble in water, slightly soluble in ethanol (96 per cent).

Potassium hydrogen phthalate, 0.2 M. *1070001.*

A solution of *potassium hydrogen phthalate R* containing the equivalent of 40.84 g of $C_8H_5KO_4$ in 1000.0 mL.

Potassium hydrogen sulfate. $KHSO_4$. (M_r 136.2). *1070100.* [7646-93-7].

Colourless, transparent, hygroscopic crystals, freely soluble in water giving a strongly acid solution.

Storage: in an airtight container.

Potassium hydrogen tartrate. $C_4H_5KO_6$. (M_r 188.2). *1070200.* [868-14-4]. Potassium hydrogen (2*R*,3*R*)-2,3-dihydroxybutane-1,4-dioate.

White or almost white, crystalline powder or colourless, slightly opaque crystals, slightly soluble in water, soluble in boiling water, practically insoluble in ethanol (96 per cent).

Potassium hydroxide. *1070300.* [1310-58-3].

See *Potassium hydroxide (0840)*.

Potassium hydroxide, alcoholic, 2 M. *1070301.*

Dissolve 12 g of *potassium hydroxide R* in 10 mL of *water R* and dilute to 100 mL with *ethanol (96 per cent) R*.

Potassium hydroxide in alcohol (10 per cent *V/V*), 0.5 M. *1070302.*

Dissolve 28 g of *potassium hydroxide R* in 100 mL of *ethanol (96 per cent) R* and dilute to 1000 mL with *water R*.

Potassium hydroxide solution, alcoholic. *1070303.*

Dissolve 3 g of *potassium hydroxide R* in 5 mL of *water R* and dilute to 100 mL with *aldehyde-free alcohol R*. Decant the clear solution. The solution should be almost colourless.

Potassium hydroxide solution, alcoholic R1. *1070304.*

Dissolve 6.6 g of *potassium hydroxide R* in 50 mL of *water R* and dilute to 1000 mL with *anhydrous ethanol R*.

Potassium iodate. KIO_3. (M_r 214.0). *1070400.* [7758-05-6].
White or almost white, crystalline powder, soluble in water.

Potassium iodide. *1070500.* [7681-11-0].
See *Potassium iodide (0186)*.

Potassium iodide and starch solution. *1070501.*
Dissolve 0.75 g of *potassium iodide R* in 100 mL of *water R*. Heat to boiling and add whilst stirring a solution of 0.5 g of *soluble starch R* in 35 mL of *water R*. Boil for 2 min and allow to cool.
Test for sensitivity. A mixture of 15 mL of the potassium iodide and starch solution, 0.05 mL of *glacial acetic acid R* and 0.3 mL of *iodine solution R2* is blue.

Potassium iodide solution. *1070502.*
A 166 g/L solution of *potassium iodide R*.

Potassium iodide solution, iodinated. *1070503.*
Dissolve 2 g of *iodine R* and 4 g of *potassium iodide R* in 10 mL of *water R*. When solution is complete dilute to 100 mL with *water R*.

Potassium iodide solution, iodinated R1. *1070505.*
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 solution, saturated. *1070504.*
A saturated solution of *potassium iodide R* in *carbon dioxide-free water R*. Make sure the solution remains saturated as indicated by the presence of undissolved crystals.
Test by adding to 0.5 mL of the saturated potassium iodide solution 30 mL of a mixture of 2 volumes of *chloroform R* and 3 volumes of *glacial acetic acid R*, as well as 0.1 mL of *starch solution R*. Any blue colour formed should be discharged by the addition of 0.05 mL of *0.1 M sodium thiosulfate*.
Storage: protected from light.

Potassium iodobismuthate solution. *1070600.*
To 0.85 g of *bismuth subnitrate R* add 40 mL of *water R*, 10 mL of *glacial acetic acid R* and 20 mL of a 400 g/L solution of *potassium iodide R*.

Potassium iodobismuthate solution, dilute. *1070603.*
Dissolve 100 g of *tartaric acid R* in 500 mL of *water R* and add 50 mL of *potassium iodobismuthate solution R1*.
Storage: protected from light.

Potassium iodobismuthate solution R1. *1070601.*
Dissolve 100 g of *tartaric acid R* in 400 mL of *water R* and add 8.5 g of *bismuth subnitrate R*. Shake for 1 h, add 200 mL of a 400 g/L solution of *potassium iodide R* and shake well. Allow to stand for 24 h and filter.
Storage: protected from light.

Potassium iodobismuthate solution R2. *1070602.*
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 a 400 g/L solution of *potassium iodide R* and stir for 1 h. Filter. The solution may be kept for several days in brown bottles.
Spray solution. Mix immediately before use 5 mL of the stock solution with 15 mL of *water R*.

Potassium iodobismuthate solution R3. *1070604.*
Dissolve 0.17 g of *bismuth subnitrate R* in a mixture of 2 mL of *glacial acetic acid R* and 18 mL of *water R*. Add 4 g of *potassium iodide R*, 1 g of *iodine R* and dilute to 100 mL with *dilute sulfuric acid R*.

Potassium iodobismuthate solution R4. *1070605.*
Dissolve 1.7 g of *bismuth subnitrate R* in 20 mL of *glacial acetic acid R*. Add 80 mL of *distilled water R*, 100 mL of a 400 g/L solution of *potassium iodide R*, 200 mL of *glacial acetic acid R* and dilute to 1000 mL with *distilled water R*. Mix 2 volumes of this solution with 1 volume of a 200 g/L solution of *barium chloride R*.

Potassium iodobismuthate solution R5. *1070606.*
To 0.85 g of *bismuth subnitrate R* add 10 mL of *glacial acetic acid R* and gently heat until completely dissolved. Add 40 mL of *water R* and allow to cool. To 5 mL of this solution, add 5 mL of a 400 g/L solution of *potassium iodide R*, 20 mL of *glacial acetic acid R* and 70 mL of *water R*.

Potassium nitrate. KNO_3. (M_r 101.1). *1070700.* [7757-79-1].
Colourless crystals, very soluble in water.

Potassium periodate. KIO_4. (M_r 230.0). *1070800.* [7790-21-8].
White or almost white, crystalline powder or colourless crystals, soluble in water.

Potassium permanganate. *1070900.* [7722-64-7].
See *Potassium permanganate (0121)*.

Potassium permanganate and phosphoric acid solution. *1070901.*
Dissolve 3 g of *potassium permanganate R* in a mixture of 15 mL of *phosphoric acid R* and 70 mL of *water R*. Dilute to 100 mL with *water R*.

Potassium permanganate solution. *1070902.*
A 30 g/L solution of *potassium permanganate R*.

Potassium perrhenate. $KReO_4$. (M_r 289.3). *1071000.* [10466-65-6].
White or almost white, crystalline powder, soluble in water, slightly soluble in ethanol (96 per cent), in methanol and in propylene glycol.

Potassium persulfate. $K_2S_2O_8$. (M_r 270.3). *1071100.* [7727-21-1]. Dipotassium peroxodisulfate.
Colourless crystals or white or almost white, crystalline powder, sparingly soluble in water, practically insoluble in ethanol (96 per cent). Aqueous solutions decompose at room temperature and more rapidly on warming.

Potassium plumbite solution. *1071200.*
Dissolve 1.7 g of *lead acetate R*, 3.4 g of *potassium citrate R* and 50 g of *potassium hydroxide R* in *water R* and dilute to 100 mL with the same solvent.

Potassium pyroantimonate. $KSb(OH)_6$. (M_r 262.9). *1071300.* [12208-13-8]. Potassium hexahydroxoantimoniate.
White or almost white, crystals or crystalline powder, sparingly soluble in water.

Potassium pyroantimonate solution. *1071301.*
Dissolve 2 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 solution R*. Allow to stand for 24 h, filter and dilute to 150 mL with *water R*.

Potassium pyroantimonate solution R1. *1071302.*
Dissolve 2.0 g of *potassium pyroantimonate R* in 100 mL of hot *water R*. Boil for about 5 min, cool quickly and add 10 mL of a 150 g/L solution of *potassium hydroxide R*. Allow to stand for 24 h and filter.

Potassium 4-sulfobenzoate. $C_7H_5KO_5S$. (M_r 240.3). *1190000.* [5399-63-3]. 4-Sulfobenzoic acid potassium salt. Potassium 4-carboxybenzenesulfonate.
White, crystalline powder.

Potassium tartrate. $C_4H_4K_2O_6,{}^1/_2H_2O$. (M_r 235.3). *1071400.* [921-53-9]. Dipotassium (2*R*,3*R*)-2,3-dihydroxybutane-1,4-dioate hemihydrate.
White or almost white, granular powder or crystals, very soluble in water, very slightly soluble in ethanol (96 per cent).

Potassium tetraiodomercurate solution. *1071500.*
Dissolve 1.35 g of *mercuric chloride R* in 50 mL of *water R*. Add 5 g of *potassium iodide R* and dilute to 100 mL with *water R*.

Potassium tetraiodomercurate solution, alkaline. *1071600.*
Dissolve 11 g of *potassium iodide R* and 15 g of *mercuric iodide R* in *water R* and dilute to 100 mL with the same solvent. Immediately before use, mix 1 volume of this solution with an equal volume of a 250 g/L solution of *sodium hydroxide R*.

Potassium tetroxalate. $C_4H_3KO_8,2H_2O$. (M_r 254.2). *1071700.* [6100-20-5].
White or almost white, crystalline powder, sparingly soluble in water, soluble in boiling water, slightly soluble in ethanol (96 per cent).

Potassium thiocyanate. KSCN. (M_r 97.2). *1071800.* [333-20-0].
Colourless crystals, deliquescent, very soluble in water and in ethanol (96 per cent).
Storage: in an airtight container.

Potassium thiocyanate solution. *1071801.*
A 97 g/L solution of *potassium thiocyanate R*.

Povidone. *1068500.* [9003-39-8].
See *Povidone (0685)*.

Procaine hydrochloride. *1109400.*
See *Procaine hydrochloride (0050)*.

Proline. *1152200.* [147-85-3].
See *Proline (0785)*.

Propane. C_3H_8. (M_r 44.10). *1190100.* [74-98-6].
Content: minimum 99.0 per cent *V/V*.

Propane-1,3-diol. $C_3H_8O_2$. (M_r 76.1). *1185100.* [504-63-2]. 1,3-Dihydroxypropane.
Colourless, viscous liquid.
bp: about 214 °C.
mp: about − 27 °C.

Propanol. C_3H_8O. (M_r 60.1). *1072000.* [71-23-8]. Propan-1-ol.
Clear colourless liquid, miscible with water and with ethanol (96 per cent).
d_{20}^{20}: about 0.802 to 0.806.
bp: about 97.2 °C.
Distillation range (*2.2.11*). Not less than 95 per cent distils between 96 °C and 99 °C.

Propanol R1. *1184400.* [71-23-8].
See *Propanol (2036)*.

2-Propanol. C_3H_8O. (M_r 60.1). *1072100.* [67-63-0]. Propan-2-ol. Isopropyl alcohol.
Clear, colourless, flammable liquid, miscible with water and with ethanol (96 per cent).
d_{20}^{20}: about 0.785.
bp: 81 °C to 83 °C.

2-Propanol R1. *1072101.*
Complies with the requirements prescribed for *2-propanol R* with the following additional requirements.
n_D^{20}: about 1.378.
Water (*2.5.12*): maximum 0.05 per cent, determined on 10 g.
Absorbance (*2.2.25*): maximum 0.60 at 210 nm, 0.26 at 220 nm, 0.13 at 230 nm, 0.02 at 250 nm, 0.01 at 260 nm, determined using *water R* as compensation liquid.

2-Propanol R2. *1184900.* [67-63-0].
See *Isopropyl alcohol (0970)*.

Propetamphos. $C_{10}H_{20}NO_4PS$. (M_r 281.3). *1130900.* [31218-83-4].
A suitable certified reference solution (10 ng/µL in cyclohexane) may be used.

Propidium iodide. $C_{27}H_{34}I_2N_4$. (M_r 668.4). *1154200.* [25535-16-4]. 3,8-Diamino-5-[3(diethylmethylammonio)-propyl]-6-phenylphenanthridinium diiodide.
Dark red solid.

Propionaldehyde. C_3H_6O. (M_r 58.1). *1072300.* [123-38-6]. Propanal.
Liquid freely soluble in water, miscible with ethanol (96 per cent).
d_{20}^{20}: about 0.81.
n_D^{20}: about 1.365.
bp: about 49 °C.
mp: about − 81 °C.

Propionic acid. $C_3H_6O_2$. (M_r 74.1). *1072400.* [79-09-4].
Oily liquid, soluble in ethanol (96 per cent), miscible with water.
d_{20}^{20}: about 0.993.
n_D^{20}: about 1.387.
bp: about 141 °C.
mp: about − 21 °C.

Propionic anhydride. $C_6H_{10}O_3$. (M_r 130.1). *1072500.* [123-62-6].
Clear, colourless liquid, soluble in ethanol (96 per cent).
d_{20}^{20}: about 1.01.
bp: about 167 °C.

Propionic anhydride reagent. *1072501.*
Dissolve 1 g of *toluenesulfonic acid R* in 30 mL of *glacial acetic acid R*, add 5 mL of *propionic anhydride R* and allow to stand for at least 15 min before use.
Storage: use within 24 h.

Propyl acetate. $C_5H_{10}O_2$. (M_r 102.1). *1072600.* [109-60-4].
d_{20}^{20}: about 0.888.
bp: about 102 °C.
mp: about − 95 °C.

Propyl parahydroxybenzoate. *1072700.* [94-13-3].
See *Propyl parahydroxybenzoate (0431)*.

D-Prolyl-L-phenylalanyl-L-arginine 4-nitroanilide dihydrochloride. $C_{26}H_{36}Cl_2N_8O_5$. (M_r 612). *1072800.*

Propylene glycol. *1072900.* [57-55-6].
See *Propylene glycol (0430)*.

Propylene oxide. C_3H_6O. (M_r 58.1). *1121800.* [75-56-9].
Colourless liquid, miscible with ethanol (96 per cent).

Protamine sulfate. *1073000.* [53597-25-4 (salmine) 9007-31-2 (clupeine)].
See *Protamine sulfate (0569)*.

Protopine hydrochloride. $C_{20}H_{20}ClNO_5$. (M_r 389.8). *1163500.* [6164-47-2].
5-Methyl-4,6,7,14-tetrahydrobis[1,3]benzodioxolo[4,5-*c*:5′,6′-*g*]azecin-13(5*H*)-one hydrochloride.

Pteroic acid. $C_{14}H_{12}N_6O_3$. (M_r 312.3). *1144600.* [119-24-4]. 4-[[(2-Amino-4-oxo-1,4-dihydropteridin-6-yl)methyl]amino]benzoic acid.

Crystals, soluble in solutions of alkali hydroxides.

Puerarin. $C_{21}H_{20}O_9$. (M_r 416.4). *1180600.* [3681-99-0]. 7,4′-Dihydroxy-8-*C*-glucosyliso-haloprone. 8-β-D-Glucopyranosyl-7-hydroxy-3-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one.

Pulegone. $C_{10}H_{16}O$. (M_r 152.2). *1073100.* [89-82-7]. (*R*)-2-Isopropylidene-5-methylcyclohexanone. (+)-*p*-Menth-4-en-3-one.

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

d_{15}^{20}: about 0.936.

n_D^{20}: 1.485 to 1.489.

bp: 222 °C to 224 °C.

Pulegone used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Peppermint oil (0405)*.

Test solution. The substance to be examined.

Content: minimum 98.0 per cent, calculated by the normalisation procedure.

Pullulanase. *1190200.* [9075-68-7]. Pullulan-6-glucanohydrolase obtained from *Klebsiella pneumoniae*.

Content: minimum 30 units/mg of protein.

One unit represents the enzymatic activity required to produce 1.0 µmol of maltotriose from pullulan per minute at pH 5.0 at 30 °C.

DETERMINATION OF PULLULANASE ACTIVITY

Substrate. Dissolve 0.250 g of pullulan in 20.0 mL of *water R*, adding pullulan to the water.

Buffer solution A. 21 g/L solution of *citric acid monohydrate R* adjusted to pH 5.0 with a 27 g/L solution of *disodium hydrogen phosphate dodecahydrate R*.

Buffer solution B. Prepare a 136 g/L solution of *sodium acetate R* adjusted to pH 6.0 with *dilute acetic acid R*. Dilute 1 mL of this solution to 100 mL with *water R*.

Somogyi reagent. To 28 g of *anhydrous disodium hydrogen phosphate R* and 40 g of *sodium potassium tartrate R* add about 700 mL of *water R*. Add 100 mL of a 42 g/L solution of *sodium hydroxide R* and mix. Add 80 mL of a 100 g/L solution of *copper sulfate pentahydrate R*. Heat until complete dissolution. Add 180 g of *anhydrous sodium sulfate R* and adjust the volume to 1 L with *water R*. Allow to stand at room temperature for 1 or 2 days to let insoluble matter precipitate. Filter the solution and keep the filtrate in a brown-glass bottle with a ground-glass stopper.

Nelson reagent. Dissolve 50 g of *ammonium molybdate R* in 900 mL of *water R*. Add 42 g of *sulfuric acid R* and mix. Dissolve 6 g of *disodium arsenate R* in 50 mL of *water R*. Add the latter solution to the 1st solution, and allow to stand in a brown-glass bottle with a ground-glass stopper at 37 °C for 1 or 2 days.

Glucose standard solution. Dry *glucose R* at a pressure less than 6 kPa at 60 °C for 5 h, and calculate the water content. Transfer 10.00 g of dried glucose to a volumetric flask, dissolve with *water R*, dilute to 1.0 L with the same solvent, and mix. Transfer 10.0 mL of this solution to a volumetric flask and dilute to 1.0 L with *water R*. Each millilitre contains 100 µg of glucose.

Pullulanase diluent. Dilute *pullulanase R* with buffer solution B to prepare a solution with an enzyme activity of about 0.2 units/mL. The measurement range is between 0.1 and 0.4 units/mL. Record the dilution factor (*D*). This diluent is used as a diluted enzyme solution.

Procedure. Transfer 4.0 mL of substrate to a test tube and add 0.5 mL of buffer solution A, mix, and incubate at 30 °C. Add 0.5 mL of pullulanase diluent and mix thoroughly. After 30 s, transfer 1.0 mL of this solution to a test tube labelled "pullulan test solution 1", add 2.0 mL of Somogyi reagent, and mix. After 30.5 min, transfer 1.0 mL of the mixture of substrate and pullulanase diluent to a second test tube labelled "pullulan test solution 2", add 2.0 mL of Somogyi reagent, and mix. In a third test tube labelled "standard blank", mix 2.0 mL of Somogyi reagent and 1.0 mL of *water R*. In a fourth test tube labelled "glucose standard solution", mix 2.0 mL of Somogyi reagent and 1.0 mL of glucose standard solution, and add 1.0 mL of *water R*. Incubate the fourth test tube in a water-bath for exactly 10 min. Remove the tube and allow it to cool under running water. Add 2.0 mL of Nelson reagent, mix well, and allow the solution to stand for at least 15 min. Add 5.0 mL of *water R* to each of the 4 test tubes and mix thoroughly. Determine the absorbance at 520 nm of the standard blank (A_{blank}), the glucose standard solution (A_{Std}), pullulan test solution 1 (A_0) and pullulan test solution 2 (A_{30}), using *water R* as the blank. One unit is defined as the enzymatic activity required to produce 1 µmol of maltotriose (measured as glucose) from pullulan per minute. Calculate the pullulanase activity, *P*, in units/mL, using the following expression:

$$[(A_{30} - A_0) / (A_{Std} - A_{blank})] \times 0.185 \times D$$

MEASUREMENT OF PROTEIN CONTENT (MEASURED AS ALBUMINOID CONTENT) FOR THE CALCULATION OF SPECIFIC ACTIVITY

Reagent A. Prepare a solution having known concentrations of about 4 g/L of *sodium hydroxide R* and about 21 g/L of *anhydrous sodium carbonate R*.

Reagent B. Transfer 0.5 g of *copper sulfate pentahydrate R* and 1.0 g of *sodium citrate R* to a volumetric flask, dissolve in and dilute with *water R* to 100.0 mL, and mix.

Lowry solution. Mix 50 volumes of reagent A and 1 volume of reagent B.

Diluted Folin-Ciocalteu's phenol reagent (for albuminoid quantification). Prepare a two-fold dilution of the commercially available 2 N Folin-Ciocalteu's phenol reagent or prepare a solution by making an appropriate dilution of *phosphomolybdotungstic reagent R*.

Bovine albumin standard stock solution. Transfer 50.0 mg of *bovine albumin R* to a volumetric flask, dissolve in and dilute with *water R* to 500.0 mL, and mix. It contains 100 µg/mL of bovine albumin.

Standard solutions. Using appropriate dilutions of bovine albumin standard stock solution in *water R*, prepare 5 standard solutions having concentrations equally spaced between 5 µg/mL and 100 µg/mL of bovine albumin.

Test solution. Dilute *pullulanase R* with buffer solution B in order to obtain a solution having a concentration of 60-70 µg/mL of albuminoid. Water may be used as diluent. Record the dilution factor, D_f.

Procedure. Introduce into separate tubes 0.3 mL of each of the standard solutions, the test solution and *water R*. Add 3.0 mL of Lowry solution to each tube and mix. Incubate at room temperature for 10 min. Add 0.3 mL of diluted Folin-Ciocalteu's phenol reagent to each tube, mix immediately, and allow to stand at room temperature for 60 min. Determine the absorbances of the standard solutions and the test solution at the wavelength of maximum absorbance, about 750 nm, using *water R* as the blank.

Calculation. The relationship of absorbance to protein concentration is non-linear; however, if the standard curve concentration range is sufficiently small, it will approach linearity. Using linear regression method, plot the absorbances of the standard solutions versus the protein (bovine albumin) concentrations, in µg/mL. Using the plot, determine the concentration of protein (albuminoid content), $C_{albuminoid}$,

in µg/mL, in the test solution. Calculate the albuminoid concentration, in mg/mL, in *pullulanase R* using the following expression:

$$C_{protein} = (C_{albuminoid} \times D_f) / 1000$$

Calculate the specific activity, in units/mg, of pullulanase using the formula:

$$P / C_{protein}$$

P = pullulanase activity in units/mL.

Putrescine. $C_4H_{12}N_2$. (M_r 88.15). *1137900.* [110-60-1]. 1,4-Butanediamine. Tetramethylenediamine.

Colourless oily liquid, very soluble in water. Strong piperidine-like odour.

bp: about 159 °C.

mp: about 23 °C.

Pyrazine-2-carbonitrile. $C_5H_3N_3$. (M_r 105.1). *1183300.* [19847-12-2]. 2-Cyanopyrazine.

Clear, pale yellow liquid.

Content: minimum 99 per cent.

Pyridin-2-amine. $C_5H_6N_2$. (M_r 94.1). *1073400.* [504-29-0]. 2-Aminopyridine.

Large crystals soluble in water and in ethanol (96 per cent).

bp: about 210 °C.

mp: about 58 °C.

Pyridine. C_5H_5N. (M_r 79.1). *1073200.* [110-86-1].

Clear, colourless liquid, hygroscopic, miscible with water and with ethanol (96 per cent).

bp: about 115 °C.

Storage: in an airtight container.

Pyridine, anhydrous. *1073300.*

Dry *pyridine R* over *anhydrous sodium carbonate R*. Filter and distil.

Water (*2.5.12*): maximum 0.01 per cent *m/m*.

Pyridine-4-carbonitrile. $C_6H_4N_2$. (M_r 104.1). *1190300.* [100-48-1]. 4-Cyanopyridine.

White or almost white crystalline powder.

bp: 194 °C to 196 °C.

mp: 76 °C to 79 °C.

Pyridinium hydrobromide perbromide. $C_5H_6Br_3N$. (M_r 319.8). *1166100.* [39416-48-3]. Pyridinium tribromide(1-).

Red crystals.

Pyridylazonaphthol. $C_{15}H_{11}N_3O$. (M_r 249.3). *1073500.* [85-85-8]. 1-(2-Pyridylazo)-2-naphthol.

Brick-red powder, practically insoluble in water, soluble in ethanol (96 per cent), in methanol and in hot dilute alkali solutions.

mp: about 138 °C.

Pyridylazonaphthol solution. *1073501.*

A 1 g/L solution of *pyridylazonaphthol R* in *anhydrous ethanol R*.

Test for sensitivity. To 50 mL of *water R* add 10 mL of *acetate buffer solution pH 4.4 R*, 0.10 mL of *0.02 M sodium edetate* and 0.25 mL of the pyridylazonaphthol solution. After addition of 0.15 mL of a 5 g/L solution of *copper sulfate pentahydrate R*, the colour changes from light yellow to violet.

4-(2-Pyridylazo)resorcinol monosodium salt. $C_{11}H_8N_3NaO_2$, H_2O. (M_r 255.2). *1131500.* [16593-81-0].

Orange crystalline powder.

Pyrocatechol. $C_6H_6O_2$. (M_r 110.1). *1073600.* [120-80-9]. Benzene-1,2-diol.

Colourless or slightly yellow crystals, soluble in water, in acetone and in ethanol (96 per cent).

mp: about 102 °C.

Storage: protected from light.

Pyrogallol. $C_6H_6O_3$. (M_r 126.1). *1073700.* [87-66-1]. Benzene-1,2,3-triol.

White or almost white crystals, becoming brownish on exposure to air and light, very soluble in water and in ethanol (96 per cent), slightly soluble in carbon disulfide. On exposure to air, aqueous solutions, and more rapidly alkaline solutions, become brown owing to the absorption of oxygen.

mp: about 131 °C.

Storage: protected from light.

Pyrogallol solution, alkaline. *1073701.*

Dissolve 0.5 g of *pyrogallol R* in 2 mL of *carbon dioxide-free water R*. Dissolve 12 g of *potassium hydroxide R* in 8 mL of *carbon dioxide-free water R*. Mix the two solutions immediately before use.

Pyrrolidine. C_4H_9N. (M_r 71.1). *1165000.* [123-75-1].

Content: minimum 99 per cent.

bp: 87 °C to 88 °C.

2-Pyrrolidone. C_4H_7NO. (M_r 85.1). *1138000.* [616-45-5]. Pyrrolidin-2-one.

Liquid above 25 °C, miscible with water, with anhydrous ethanol and with ethyl acetate.

d_4^{25}: 1.116.

Water (*2.5.12*): maximum 0.2 per cent determined on 2.00 g.

Assay. Gas chromatography (*2.2.28*): use the normalisation procedure.

Test solution. Dissolve 1.0 g in *methanol R* and dilute to 10.0 mL with the same solvent.

Column:

- *material*: glass;
- *size*: *l* = 30 m; Ø = 0.53 mm;
- *stationary phase*: *macrogol 20 000 R* (1.0 µm).

Carrier gas: *helium for chromatography R*.

Flow rate: adjusted so that the retention time of 2-pyrrolidone is about 10 min.

Split ratio: 1:20.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 1	80
	1 - 12	80 → 190
	12 - 32	190
Injection port		200

Detection: flame ionisation.

Injection: 1 µL of the test solution.

Content: minimum 98.0 per cent.

Pyruvic acid. $C_3H_4O_3$. (M_r 88.1). *1109300.* [127-17-3]. 2-Oxopropanoic acid.

Yellowish liquid, miscible with water and with anhydrous ethanol.

d_{20}^{20}: about 1.267.

n_D^{20}: about 1.413.

bp: about 165 °C.

Quercetin dihydrate. $C_{15}H_{10}O_7,2H_2O$. (M_r 338.2). *1138100.* 2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-1-benzopyran-4-one.

Yellow crystals or yellowish powder, practically insoluble in water, soluble in acetone and in methanol.

Water (*2.5.12*): maximum 12.0 per cent, determined on 0.100 g.

Assay. Liquid chromatography (*2.2.29*) as prescribed in the monograph *Ginkgo leaf (1828)*.

Content:minimum 90 per cent (anhydrous substance) calculated by the normalisation procedure.

Storage: protected from light.

Quercitrin. $C_{21}H_{20}O_{11}$. (M_r 448.4). *1138200.* [522-12-3]. Quercetin 3-L-rhamnopyranoside. 3-[(6-Deoxy-α-L-mannopyranosyl)oxy]-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4*H*-1-benzopyran-4-one. Quercitroside.

Yellow crystals, practically insoluble in cold water, soluble in ethanol (96 per cent).

mp: 176 °C to 179 °C.

Chromatography. Thin-layer chromatography (*2.2.27*) as prescribed in the monograph *Goldenrod (1892)*: apply 20 µL of the solution; after spraying, the chromatogram shows a yellowish-brown fluorescent zone with an R_F of about 0.6.

Storage: at a temperature of 2 °C to 8 °C.

Quillaia saponins, purified. *1184500.*

A mixture of related saponins obtained from the bark of *Quillaja saponaria* Molina *s.l.*

Chromatography. Thin-layer chromatography (*2.2.27*) as prescribed in the monograph *Quillaia bark (1843)*: apply 5 µL of the solution; after treating with a 10 per cent *V/V* solution of *sulfuric acid R* in *methanol R*, heat at 120 °C for 5 min and examine in daylight; the chromatogram shows 3 principal zones in the upper part of the middle third.

Quinaldine red. $C_{21}H_{23}IN_2$. (M_r 430.3). *1073800.* [117-92-0]. 2-[2-[4-(Dimethylamino)phenyl]ethenyl]-1-ethylquinolinium iodide.

Dark bluish-black powder, sparingly soluble in water, freely soluble in ethanol (96 per cent).

Quinaldine red solution. *1073801.*

Dissolve 0.1 g of *quinaldine red R* in *methanol R* and dilute to 100 mL with the same solvent.

Colour change: pH 1.4 (colourless) to pH 3.2 (red).

Quinhydrone. $C_{12}H_{10}O_4$. (M_r 218.2). *1073900.* [106-34-3]. Equimolecular compound of 1,4-benzoquinone and hydroquinone.

Dark green, lustrous crystals or a crystalline powder, slightly soluble in water, sparingly soluble in hot water, soluble in ethanol (96 per cent) and in concentrated ammonia.

mp: about 170 °C.

Quinidine. $C_{20}H_{24}N_2O_2$. (M_r 324.4). *1074000.* [56-54-2]. (*S*)-(6-Methoxyquinol-4-yl)[(2*R*,4*S*,5*R*)-5-vinylquinuclidin-2-yl]methanol.

White or almost white crystals, very slightly soluble in water, sparingly soluble in ethanol (96 per cent), slightly soluble in methanol.

$[\alpha]_D^{20}$: about + 260, determined on a 10 g/L solution in *anhydrous ethanol R*.

mp: about 172 °C.

Storage: protected from light.

Quinidine sulfate. *1109500.* [6591-63-5].

See *Quinidine sulfate (0017)*.

Quinine. $C_{20}H_{24}N_2O_2$. (M_r 324.4). *1074100.* [130-95-0]. (*R*)-(6-Methoxyquinol-4-yl)[(2*S*,4*S*,5*R*)-5-vinylquinuclidin-2-yl]methanol.

White or almost white, microcrystalline powder, very slightly soluble in water, slightly soluble in boiling water, very soluble in anhydrous ethanol.

$[\alpha]_D^{20}$: about − 167, determined on a 10 g/L solution in *anhydrous ethanol R*.

mp: about 175 °C.

Storage: protected from light.

Quinine hydrochloride. *1074200.* [6119-47-7].

See *Quinine hydrochloride (0018)*.

Quinine sulfate. *1074300.* [6119-70-6].

See *Quinine sulfate (0019)*.

3-Quinuclidinol. $C_7H_{13}NO$. (M_r 127.2). *1193800.* [1619-34-7]. (3*R*)-1-Azabicyclo[2.2.2]octan-3-ol.

Content: minimum 99 per cent.

Light yellow powder.

Rabbit erythrocyte suspension. *1074500.*

Prepare a 1.6 per cent *V/V* suspension of rabbit erythrocytes as follows: defibrinate 15 mL of freshly drawn rabbit blood by shaking with glass beads, centrifuge at 2000 *g* for 10 min and wash the erythrocytes with three quantities, each of 30 mL, of a 9 g/L solution of *sodium chloride R*. Dilute 1.6 mL of the suspension of erythrocytes to 100 mL with a mixture of 1 volume of *phosphate buffer solution pH 7.2 R* and 9 volumes of a 9 g/L solution of *sodium chloride R*.

Raclopride tartrate. $C_{19}H_{26}Cl_2N_2O_9$. (M_r 497.3). *1144700.* [98185-20-7]. Raclopride L-tartrate.

White or almost white solid, sensitive to light, soluble in water.

$[\alpha]_D^{25}$: + 0.3, determined on a 3 g/L solution.

mp: about 141 °C.

Raffinose pentahydrate. $C_{18}H_{32}O_{16},5H_2O$. (M_r 594.5). *1201800.* [17629-30-0]. β-D-Fructofuranosyl α-D-galactopyranosyl-(1→6)-α-D-glucopyranoside pentahydrate.

Content: minimum 98.0 per cent.

Crystalline powder.

mp: about 80 °C.

Raltegravir potassium. $C_{20}H_{20}FKN_6O_5$. *1202600.* [871038-72-1].

See *Raltegravir potassium (2887)*.

Rapeseed oil. *1074600.*

See *Rapeseed oil, refined (1369)*.

Reducing mixture. *1074700.*

Grind the substances added in the following order to obtain a homogeneous mixture: 20 mg of *potassium bromide R*, 0.5 g of *hydrazine sulfate R* and 5 g of *sodium chloride R*.

Reichstein's substance S. $C_{21}H_{30}O_4$. (M_r 346.5). *1175400.* [152-58-9].

Content: minimum 95.0 per cent.

mp: about 208 °C.

Resin for hydrophobic interaction chromatography. *1202700.*

Non-porous resin consisting of spherical polymethacrylate particles bonded with butyl groups.

pH limits of use: 2 to 12.

Resin for reversed-phase ion chromatography. *1131100.*

A neutral, macroporous, high specific surface area with a non-polar character resin consisting of polymer lattice of polystyrene cross-linked with divinylbenzene.

Resin, weak cationic. *1096000.*

See *weak cationic resin R*.

Resorcinol. *1074800.* [108-46-3].

See *Resorcinol (0290)*.

Resorcinol reagent. *1074801.*

To 80 mL of *hydrochloric acid R1* add 10 mL of a 20 g/L solution of *resorcinol R* and 0.25 mL of a 25 g/L solution of *copper sulfate pentahydrate R* and dilute to 100.0 mL with *water R*. Prepare the solution at least 4 h before use.

Storage: at 2 °C to 8 °C for 1 week.

Resveratrol. $C_{14}H_{12}O_3$. (M_r 228.2). *1186900.* [501-36-0]. 3,4′,5-Stilbenetriol. 5-[(*E*)-2-(4-Hydroxyphenyl)ethenyl]benzene-1,3-diol.

Rhamnose. $C_6H_{12}O_5,H_2O$. (M_r 182.2). *1074900.* [6155-35-7]. (2*R*,3*R*,4*R*,5*R*,6*S*)-6-Methyltetrahydro-2*H*-pyran-2,3,4,5-tetrol monohydrate. 6-Deoxy-α-L-mannopyranose monohydrate. α-L-Rhamnopyranose monohydrate. L-(+)-Rhamnose monohydrate.

White or almost white, crystalline powder, freely soluble in water.

$[\alpha]_D^{20}$: + 7.8 to + 8.3, determined on a 50 g/L solution in *water R* containing about 0.05 per cent of NH_3.

Rhaponticin. $C_{21}H_{24}O_9$. (M_r 420.4). *1075000.* [155-58-8]. 3-Hydroxy-5-[2-(3-hydroxy-4-methoxyphenyl)ethenyl]phenyl β-D-glucopyranoside.

Yellowish-grey, crystalline powder, soluble in ethanol (96 per cent) and in methanol.

Chromatography. Thin-layer chromatography (*2.2.27*) as prescribed in the monograph *Rhubarb (0291)*; the chromatogram shows only one principal spot.

Rhein. $C_{15}H_8O_6$. (M_r 284.2). *1197700.* [478-43-3]. 4,5-Dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylic acid. 1,8-Dihydroxy-3-carboxyanthraquinone.

Rhodamine 6 G. $C_{28}H_{31}ClN_2O_3$. (M_r 479.0). *1153300.* [989-38-8].

Colour Index No. 45160.

9-[2-(Ethoxycarbonyl)phenyl]-3,6-bis(ethylamino)-2,7-dimethylxanthenylium chloride.

Brownish-red powder.

Rhodamine B. $C_{28}H_{31}ClN_2O_3$. (M_r 479.0). *1075100.* [81-88-9].

Schultz No. 864.

Colour Index No. 45170.

[9-(2-Carboxyphen-yl)-6-(diethylamino)-3*H*-xanthen-3-ylidene]diethylammonium chloride.

Green crystals or reddish-violet powder, very soluble in water and in ethanol (96 per cent).

Rhynchophylline. $C_{22}H_{28}N_2O_4$. (M_r 384.5). *1197800.* [76-66-4]. Methyl (16*E*)-17-methoxy-2-oxo-16,17-didehydro-7β,20α-corynoxan-16-carboxylate. Methyl (16*E*)-16-(methoxymethylidene)-2-oxo-7β,20α-corynoxan-17-oate.

Ribose. $C_5H_{10}O_5$. (M_r 150.1). *1109600.* [50-69-1]. D-Ribose.

Soluble in water, slightly soluble in ethanol (96 per cent).

mp: 88 °C to 92 °C.

Ricinoleic acid. $C_{18}H_{34}O_3$. (M_r 298.5). *1100100.* [141-22-0]. (9*Z*,12*R*)-12-Hydroxyoctadec-9-enoic acid. 12-Hydroxyoleic acid.

Yellow or yellowish-brown viscous liquid, consisting of a mixture of fatty acids obtained by the hydrolysis of castor oil, practically insoluble in water, very soluble in anhydrous ethanol.

d_{20}^{20}: about 0.942.

n_D^{20}: about 1.472.

mp: about 285 °C, with decomposition.

Rosmarinic acid. $C_{18}H_{16}O_8$. (M_r 360.3). *1138300.* [20283-92-5].

mp: 170 °C to 174 °C.

Rutecarpine. $C_{18}H_{13}N_3O$. (M_r 287.3). *1199500.* [84-26-4]. 8,13-Dihydroindolo[2′,3′:3,4]pyrido[2,1-*b*]quinazolin-5(7*H*)-one.

Ruthenium red. $[(NH_3)_5RuORu(NH_3)_4ORu(NH_3)_5]Cl_6,4H_2O$. ($M_r$ 858). *1075200.* [11103-72-3].

Brownish-red powder, soluble in water.

Ruthenium red solution. *1075201.*

A 0.8 g/L solution of *ruthenium red R* in *lead acetate solution R*.

Rutin. *1075300.* [250249-75-3].

See *Rutoside trihydrate R*.

Rutoside trihydrate. *1075300.* [250249-75-3].

See *Rutoside trihydrate (1795)*.

Sabinene. $C_{10}H_{16}$. (M_r 136.2). *1109700.* [3387-41-5]. Thuj-4(10)-ene. 4-Methylene-1-isopropylbicyclo[3.1.0]hexane.

A colourless, oily liquid.

Sabinene used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Bitter-orange-flower oil (1175)*.

Test solution. The substance to be examined.

Content: minimum 95.0 per cent, calculated by the normalisation procedure.

Saccharin sodium. *1131400.* [128-44-9].

See *Saccharin sodium (0787)*.

Safrole. $C_{10}H_{10}O_2$. (M_r 162.2). *1131200.* [94-59-7]. 5-(Prop-2-enyl)-1,3-benzodioxole. 4-Allyl-1,2-(methylenedioxy)benzene.

Colourless or slightly yellow, oily liquid, with the odour of sassafras, insoluble in water, very soluble in ethanol (96 per cent), miscible with hexane.

d_{20}^{20}: 1.095 to 1.096.

n_D^{20}: 1.537 to 1.538.

bp: 232 °C to 234 °C.

Freezing point: about 11 °C.

Safrole used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Cinnamon bark oil, Ceylon (1501)*.

Content: minimum 96.0 per cent, calculated by the normalisation procedure.

Saikosaponin A. $C_{42}H_{68}O_{13}$. (M_r 781). *1201900.* [20736-09-8]. 13,28-Epoxy-16β,23-dihydroxy-4α-olean-11-en-3β-yl 6-deoxy-3-*O*-β-D-glucopyranosyl-β-D-galactopyranoside.

Saikosaponin D. $C_{42}H_{68}O_{13}$. (M_r 781). *1201200.* [20874-52-6]. 13,28-Epoxy-16α,23-dihydroxy-4α-olean-11-en-3β-yl 6-deoxy-3-*O*-β-D-glucopyranosyl-β-D-galactopyranoside.

Salicin. $C_{13}H_{18}O_7$. (M_r 286.3). *1131300.* [138-52-3]. 2-(Hydroxymethyl)phenyl-β-D-glucopyranoside. Salicoside.

$[\alpha]_D^{20}$: − 62.5 ± 2.

mp: 199 °C to 201 °C.

Assay. Liquid chromatography (*2.2.29*) as prescribed in the monograph *Willow bark (1583)* at the concentration of the reference solution.

Content: minimum 99.0 per cent, calculated by the normalisation procedure.

Salicylaldehyde. $C_7H_6O_2$. (M_r 122.1). *1075400.* [90-02-8]. 2-Hydroxybenzaldehyde.

Clear, colourless, oily liquid.

d_{20}^{20}: about 1.167.

n_D^{20}: about 1.574.

bp: about 196 °C.

mp: about − 7 °C.

Salicylaldehyde azine. $C_{14}H_{12}N_2O_2$. (M_r 240.3). *1075500.* [959-36-4]. 2,2′-Azinodimethyldiphenol.

Dissolve 0.30 g of *hydrazine sulfate R* in 5 mL of *water R*, add 1 mL of *glacial acetic acid R* and 2 mL of a freshly prepared 20 per cent *V/V* solution of *salicylaldehyde R* in *2-propanol R*. Mix, allow to stand until a yellow precipate is formed. Shake with two quantities, each of 15 mL, of *methylene chloride R*. Combine the organic layers and dry over *anhydrous sodium sulfate R*. Decant or filter the solution and evaporate to dryness. Recrystallise from a mixture of 40 volumes of *methanol R* and 60 volumes of *toluene R* with cooling. Dry the crystals *in vacuo*.

mp: about 213 °C.

Chromatography. Thin-layer chromatography (*2.2.27*) as prescribed in the test for hydrazine in the monograph *Povidone (0685)*; the chromatogram shows only one principal spot.

Salicylic acid. *1075600.* [69-72-7].

See *Salicylic acid (0366)*.

Salvianolic acid B. $C_{36}H_{30}O_{16}$. (M_r 719). *1184600.* [121521-90-2]. (2*R*)-2-[[(2*E*)-3-[(2*S*,3*S*)-3-[[(1*R*)-1-Carboxy-2-(3,4-dihydroxyphenyl)ethoxy]carbonyl]-2-(3,4-dihydroxyphenyl)-7-hydroxy-2,3-dihydrobenzofuran-4-yl]prop-2-enoyl]oxy]-3-(3,4-dihydroxyphenyl)propanoic acid.

Sand. *1075800.*

White or slightly greyish grains of silica with a particle size between 150 µm and 300 µm.

Sarafloxacin hydrochloride. $C_{20}H_{18}ClF_2N_3O_3$. (M_r 421.8). *1181400.* [91296-87-6]. 6-Fluoro-1-(4-fluorophenyl)-4-oxo-7-piperazin-1-yl-1,4-dihydroquinoline-3-carboxylic acid hydrochloride.

Schisandrin. $C_{24}H_{32}O_7$. (M_r 432.5). *1173800.* [7432-28-2]. Schisandrol A. Wuweizichun A. (6*S*,7*S*,12aR_a)-5,6,7,8-Tetrahydro-1,2,3,10,11,12-hexamethoxy-6,7-dimethyldibenzo[*a,c*]cyclooctan-6-ol.

White or almost white, crystalline powder.

Schisandrin used in the assay in the monograph *Schisandra fruit (2428)* complies with the following additional test.

Assay. Liquid chromatography (*2.2.29*) as prescribed in the monograph *Schisandra fruit (2428)*.

Content: minimum 95 per cent, calculated by the normalisation procedure.

Storage: in an airtight container, at − 20 °C or below.

γ-Schisandrin. $C_{23}H_{28}O_6$. (M_r 400.5). *1173900.* [61281-37-6]. Schisandrin B. Wuweizisu B. *rac*-(6*R*,7*S*,13aR_a)-1,2,3,13-Tetramethoxy-6,7-dimethyl-5,6,7,8-tetrahydrobenzo[3,4]cycloocta[1,2-*f*][1,3]benzodioxole.

White or almost white, crystalline powder.

Storage: in an airtight container, at − 20 °C or below.

Sclareol. $C_{20}H_{36}O_2$. (M_r 308.5). *1139900.* [515-03-7]. (1*R*,2*R*,4a*S*,8a*S*)-1-[(3*R*)-3-Hydroxy-3-methylpent-4-enyl]-2,5,5,8a-tetramethyldecahydronaphthalen-2-ol.

Odourless crystals.

$[\alpha]_D^{20}$: 6.7, determined with a solution in anhydrous ethanol.

$bp_{19\ mm}$: 218 °C to 220 °C.

mp: 96 °C to 98 °C.

Sclareol used in the chromatographic profile test in the monograph Clary sage oil (1850) complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Clary sage oil (1850)*.

Content: minimum 97 per cent, calculated by the normalisation procedure.

Scopoletin. $C_{10}H_8O_4$. (M_r 192.2). *1158700.* [92-61-5]. 7-Hydroxy-6-methoxy-2*H*-1-benzopyran-2-one. 7-Hydroxy-6-methoxycoumarin.

Faintly beige, fine crystals.

mp: 202 °C to 208 °C.

SDS-PAGE running buffer. *1114900.*

Dissolve 151.4 g of *tris(hydroxymethyl)aminomethane R*, 721.0 g of *glycine R* and 50.0 g of *sodium laurilsulfate R* in *water R* and dilute to 5000 mL with the same solvent. Immediately before use, dilute to 10 times its volume with *water R* and mix. Measure the pH (*2.2.3*) of the diluted solution. The pH is between 8.1 and 8.8.

SDS-PAGE sample buffer (concentrated). *1115000.*

Dissolve 1.89 g of *tris(hydroxymethyl)aminomethane R*, 5.0 g of *sodium laurilsulfate R* and 50 mg of *bromophenol blue R* in *water R*. Add 25.0 mL of *glycerol R* and dilute to 100 mL with *water R*. Adjust the pH to 6.8 with *hydrochloric acid R*, and dilute to 125 mL with *water R*.

SDS-PAGE sample buffer for reducing conditions (concentrated). *1122100.*

Dissolve 3.78 g of *tris(hydroxymethyl)aminomethane R*, 10.0 g of *sodium dodecyl sulfate R* and 100 mg of *bromophenol blue R* in *water R*. Add 50.0 mL of *glycerol R* and dilute to 200 mL with *water R*. Add 25.0 mL of *2-mercaptoethanol R*. Adjust to pH 6.8 with *hydrochloric acid R*, and dilute to 250.0 mL with *water R*.

Alternatively, dithiothreitol may be used as reducing agent instead of 2-mercaptoethanol. In this case prepare the sample buffer as follows: dissolve 3.78 g of *tris(hydroxymethyl)aminomethane R*, 10.0 g of *sodium dodecyl sulfate R* and 100 mg of *bromophenol blue R* in *water R*. Add 50.0 mL of *glycerol R* and dilute to 200 mL with *water R*. Adjust to pH 6.8 with *hydrochloric acid R*, and dilute to 250.0 mL with *water R*. Immediately before use, add *dithiothreitol R* to a final concentration of 100 mM.

Selenious acid. H_2SeO_3. (M_r 129.0). *1100200.* [7783-00-8].

Deliquescent crystals, freely soluble in water.

Storage: in an airtight container.

Selenium. Se. (A_r 79.0). *1075900.* [7782-49-2].

Brown-red or black powder or granules, practically insoluble in water and in ethanol (96 per cent), soluble in nitric acid.

mp: about 220 °C.

Sennoside B. $C_{42}H_{38}O_{20}$. (M_r 863). *1190400.* [128-57-4]. (9*R*,9′*S*)-5,5′-Bis(β-D-glucopyranosyloxy)-4,4′-dihydroxy-10,10′-dioxo-9,9′,10,10′-tetrahydro-9,9′-bianthracene-2,2′-dicarboxylic acid.

Pale yellow crystals, practically insoluble in water, very slightly soluble in ethanol (96 per cent), soluble in dilute solutions of alkali hydroxides.

mp: 180 °C to 186 °C.

Serine. *1076000.* [56-45-1].

See *Serine (0788)*.

Sialic acid. *1001100.* [131-48-6].

See *N-acetylneuraminic acid R*.

Silibinin. $C_{25}H_{22}O_{10}$. (M_r 482.4). *1151400.* [22888-70-6]. Silybin. (2*R*,3*R*)-3,5,7-Trihydroxy-2-[(2*R*,3*R*)-3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-2,3-dihydro-1,4-benzodioxin-6-yl]-2,3-dihydro-4*H*-1-benzopyran-4-one.

White or yellowish powder, practically insoluble in water, soluble in acetone and in methanol.

Silibinin used in the assay of Milk thistle fruit (1860) complies with the following additional test.

Assay. Liquid chromatography (*2.2.29*) as prescribed in the monograph *Milk thistle fruit (1860)*.

Test solution. Dissolve 5.0 mg of silibinin, dried *in vacuo*, in *methanol R* and dilute to 50.0 mL with the same solvent.

Silibinin A and silibinin B content: minimum 95.0 per cent, calculated by the normalisation procedure.

Silica for chromatography, porous. *1207800.*

Porous silica with porous layer open tubular (PLOT) design.

Silica gel π-acceptor/π-donor for chiral separations. *1160100.*

A very finely divided silica gel for chromatography consisting of spherical particles to which 1-(3,5-dinitrobenzamido)-1,2,3,4-tetrahydrophenantrene has been covalently bound, showing both π-electron acceptor and π-electron donor characteristics.

Silica gel AGP for chiral chromatography. *1148700.*

See *α1-Acid-glycoprotein silica gel for chiral separation R*.

Silica gel, anhydrous. *1076100.* [112926-00-8].

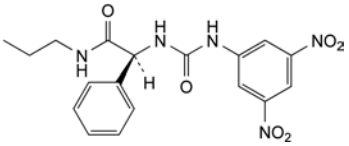
Partly dehydrated polymerised, amorphous silicic acid, absorbing at 20 °C about 30 per cent of its mass of water. Practically insoluble in water, partly soluble in solutions of sodium hydroxide. It contains a suitable indicator for detection of the humidity status, for which the colour change from the hydrated to anhydrous form is given on the label.

Silica gel BC for chiral chromatography. *1161300.*

A very finely divided silica gel for chromatography (5 µm) coated with β-cyclodextrin. Higher selectivity may be obtained when cyclodextrin has been derivatized with propylene oxide.

Silica gel for chiral chromatography, urea type. *1181000.*

A very finely divided silica gel (5 µm) coated with the following derivative:



Silica gel for chiral separation, amylose derivative of. *1171700.*

Substituted amylose coated on a very finely divided silica gel for chromatography.

Silica gel for chiral separation, cellulose derivative of. *1110300.*

Substituted cellulose coated on a very finely divided silica gel for chromatography.

Silica gel for chiral separation, human albumin coated. *1138500.*

A very finely divided silica gel, chemically modified at the surface by the bonding of human albumin.

Silica gel for chiral separation, protein derivative of. *1196300.*

A very finely divided silica gel for chromatography consisting of spherical particles coated with a protein derivative.

Silica gel for chiral separation, vancomycin-bonded. *1205300.*

High-purity silica gel chemically modified by the bonding of vancomycin through multiple covalent linkages.

Silica gel for chromatography. *1076900.*

A very finely divided silica gel.

Silica gel for chromatography, alkyl-bonded for use with highly aqueous mobile phases. *1160200.*

A very finely divided silica gel with bonded alkyl groups suitable for use with highly aqueous mobile phases.

Silica gel for chromatography, alkyl-bonded for use with highly aqueous mobile phases, end-capped. *1176900.*

A very finely divided silica gel with bonded alkyl groups suitable for use with highly aqueous mobile phases. To minimise any interaction with basic compounds it is carefully end-capped to cover most of the remaining silanol groups.

Silica gel for chromatography, alkylsilyl, solid core, end-capped. *1194300.*

Silica gel with spherical silica particles containing a non-porous solid silica core surrounded by a thin outer porous silica coating with alkylsilyl groups. To minimise any interaction with basic compounds, it is carefully end-capped to cover most of the remaining silanol groups.

Silica gel for chromatography, amidoalkylsilyl. *1205400.*

A very finely divided silica gel, chemically modified at the surface by the bonding of amidoalkylsilyl groups.

Silica gel for chromatography, amidohexadecylsilyl. *1170400.*

A very finely divided silica gel with a fine particle size, chemically modified at the surface by the bonding of amidohexadecylsilyl groups.

Silica gel for chromatography, amidohexadecylsilyl, end-capped. *1201100.*

A very finely divided silica gel, chemically modified at the surface by the bonding of amidohexadecylsilyl groups. To minimise any interaction with basic compounds, it is carefully end-capped to cover most of the remaining silanol groups.

Silica gel for chromatography, aminopropylmethylsilyl. *1102400.*

Silica gel with a fine particle size, chemically modified by bonding aminopropylmethylsilyl groups on the surface.

Silica gel for chromatography, aminopropylsilyl. *1077000.*

Silica gel with a fine particle size, chemically modified by bonding aminopropylsilyl groups on the surface.

Silica gel for chromatography, aminopropylsilyl R1. *1077001.*

Silica gel with a particle size of about 55 µm, chemically modified by bonding aminopropylsilyl groups on the surface.

Silica gel for chromatography, amylose derivative of. *1109800.*

A very finely divided (10 µm) silica gel, chemically modified at the surface by the bonding of an amylose derivative.

Silica gel for chromatography, butylsilyl. *1076200.*

A very finely divided silica gel, chemically modified at the surface by the bonding of butylsilyl groups.

Silica gel for chromatography, butylsilyl, end-capped. *1170500.*

A very finely divided silica, chemically modified at the surface by the bonding of butylsilyl groups. To minimise any interaction with basic compounds, it is carefully end-capped to cover most of the remaining silanol groups.

Silica gel for chromatography compatible with 100 per cent aqueous mobile phases, octadecylsilyl. *1203900.*

A very finely divided silica gel with bonded octadecylsilyl groups suitable for use with highly aqueous mobile phases including 100 per cent aqueous phases.

Silica gel for chromatography compatible with 100 per cent aqueous mobile phases, octadecylsilyl, end-capped. *1188400.*

A very finely divided silica gel with bonded octadecylsilyl groups suitable for use with highly aqueous mobile phases including 100 per cent aqueous phases. To minimise any interaction with basic compounds it is carefully end-capped to cover most of the remaining silanol groups.

Silica gel for chromatography compatible with highly aqueous mobile phases, octadecylsilyl diol, end-capped. *1207500.*

A very finely divided silica gel, chemically modified at the surface by the bonding of octadecylsilyl groups and end-capping. Free diol groups are also present. For use with highly aqueous mobile phases.

Silica gel for chromatography, cyanopropylsilyl, end-capped, base-deactivated. *1194200.*

A very finely divided silica gel, pre-treated before the bonding of cyanopropylsilyl groups by washing and hydrolysing most of the superficial siloxane bridges. To minimise any interaction with basic compounds, it is carefully end-capped to cover most of the remaining silanol groups.

Silica gel for chromatography, cyanosilyl. *1109900.*

A very finely divided silica gel chemically modified at the surface by the bonding of cyanosilyl groups.

Silica gel for chromatography, cyanosilyl, end-capped. *1195000.*

A very finely divided silica gel chemically modified at the surface by the bonding of cyanosilyl groups. To minimise any interaction with basic compounds it is carefully end-capped to cover most of the remaining silanol groups.

Silica gel for chromatography, di-isobutyloctadecylsilyl. *1140000.*

A very finely divided silica gel chemically modified at the surface by the bonding of di-isobutyloctadecylsilyl groups.

Silica gel for chromatography, diisopropylcyanosilyl. *1168100.*

A very finely divided silica gel chemically modified at the surface by the bonding of diisopropylcyanosilyl groups.

Silica gel for chromatography, 4-dimethylaminobenzylcarbamidesilyl. *1204000.*

A very finely divided silica gel, chemically modified at the surface by the bonding of 4-dimethylaminobenzylcarbamide groups.

Silica gel for chromatography, dimethyloctadecylsilyl. *1115100.*

A very finely divided silica gel, chemically modified at the surface by the bonding of dimethyloctadecylsilyl groups.

Specific surface area: 300 m^2/g.

Silica gel for chromatography, diol. *1110000.*

Spherical silica particles to which dihydroxypropyl groups are bonded. Pore size 10 nm.

Silica gel for chromatography, dodecylsilyl, end-capped. *1179700.*

A very finely divided silica gel, chemically modified at the surface by the introduction of dodecylsilyl groups. To minimise any interaction with basic compounds, it is carefully end-capped to cover most of the remaining silanol groups.

Silica gel for chromatography, hexadecylamidylsilyl. *1162500.*

A very finely divided (5 µm) silica gel, chemically modified at the surface by the introduction of hexadecylcarboxamidopropyldimethylsilyl groups.

Silica gel for chromatography, hexadecylamidylsilyl, end-capped. *1172400.*

A very finely divided (5 µm) silica gel, chemically modified at the surface by the introduction of hexadecylcarboxamidopropyldimethylsilyl groups. To minimise any interaction with basic compounds it is carefully end-capped to cover most of the remaining silanol groups.

Silica gel for chromatography, hexylsilyl. *1077100.*

A very finely divided silica gel, chemically modified at the surface by the bonding of hexylsilyl groups.

Silica gel for chromatography, hexylsilyl, end-capped. *1174400.*

A very finely divided silica gel, chemically modified at the surface by the bonding of hexylsilyl groups. To minimise any interaction with basic compounds it is carefully end-capped to cover most of the remaining silanol groups.

Silica gel for chromatography, human albumin coated. *1138500.*

A very finely divided silica gel, chemically modified at the surface by the bonding of human albumin.

Silica gel for chromatography (hybrid material), octadecylsilyl, ethylene-bridged, charged surface, end-capped. *1202800.*

Synthetic, spherical ethylene-bridged hybrid particles with a charged surface, containing both inorganic (silica) and organic (organosiloxanes) components, chemically modified at the surface by the bonding of octadecylsilyl groups. To minimise any interaction with basic compounds they are carefully end-capped to cover most of the remaining silanol groups.

Silica gel for chromatography (hybrid material), octadecylsilyl, ethylene-bridged, end-capped. *1190500.*

Synthetic, spherical, ethylene-bridged hybrid particles, containing both inorganic (silica) and organic (organosiloxanes) components, chemically modified at the surface by the bonding of octadecylsilyl groups. To minimise any interaction with basic compounds, they are carefully end-capped to cover most of the remaining silanol groups.

Silica gel for chromatography (hybrid material), phenylhexylsilyl, ethylene-bridged, charged surface, end-capped. *1204100.*

Synthetic, spherical ethylene-bridged hybrid particles with a charged surface, containing both inorganic (silica) and organic (organosiloxanes) components, chemically modified at the surface by the bonding of phenylhexylsilyl groups. To minimise any interaction with basic compounds they are carefully end-capped to cover most of the remaining silanol groups.

Silica gel for chromatography (hybrid material), phenylsilyl, ethylene-bridged, end-capped. *1200700.*

Synthetic, spherical, ethylene-bridged hybrid particles, containing both inorganic (silica) and organic (organosiloxanes) components, chemically modified at the surface by the bonding of phenylsilyl groups. To minimise any interaction with basic compounds, they are carefully end-capped to cover most of the remaining silanol groups.

Silica gel for chromatography (hybrid material), polar-embedded, octadecylsilyl, ethylene-bridged, end-capped. *1200800.*

Synthetic, spherical, ethylene-bridged hybrid particles containing both inorganic (silica) and organic (organosiloxanes) components, chemically modified at the surface by the bonding of polar-embedded octadecylsilyl groups. To minimise any interaction with basic compounds, they are carefully end-capped to cover most of the remaining silanol groups.

Silica gel for chromatography, hydrophilic. *1077200.*

A very finely divided silica gel whose surface has been modified to provide hydrophilic characteristics.

Silica gel for chromatography, nitrile. *1077300.*

A very finely divided silica gel, chemically modified at the surface by the bonding of cyanopropylsilyl groups.

Silica gel for chromatography, nitrile R1. *1077400.*

A very finely divided silica gel consisting of porous, spherical particles with chemically bonded nitrile groups.

Silica gel for chromatography, nitrile R2. *1119500.*

Ultrapure silica gel, chemically modified at the surface by the introduction of cyanopropylsilyl groups. Less than 20 ppm of metals.

Silica gel for chromatography, nitrile, end-capped. *1174500.*

A very finely divided silica gel, chemically modified at the surface by the bonding of cyanopropylsilyl groups. To minimise any interaction with basic components it is carefully end-capped to cover most of the remaining silanol groups.

Silica gel for chromatography, 4-nitrophenylcarbamidesilyl. *1185200.*

A very finely divided silica gel, chemically modified at the surface by bonding of 4-nitrophenylcarbamide groups.

Silica gel for chromatography, octadecanoylaminopropylsilyl. *1115200.*

A very finely divided silica gel, chemically modified at the surface by the bonding of aminopropylsilyl groups which are acylated with octadecanoyl groups.

Silica gel for chromatography, octadecylphenylsilyl, end-capped. *1199300.*

A very finely divided silica gel, chemically modified at the surface by bonding of octadecylphenylsilyl groups. To minimise any interaction with basic compounds it is carefully end-capped to cover most of the remaining silanol groups.

Silica gel for chromatography, octadecylsilyl. *1077500.*

A very finely divided silica gel, chemically modified at the surface by the bonding of octadecylsilyl groups.

Silica gel for chromatography, octadecylsilyl R1. *1110100.*

A very finely divided ultrapure silica gel, chemically modified at the surface by the bonding of octadecylsilyl groups. Less than 20 ppm of metals.

Silica gel for chromatography, octadecylsilyl R2. *1115300.*

A very finely divided (15 nm pore size) ultrapure silica gel, chemically modified at the surface by the bonding of octadecylsilyl groups (20 per cent carbon load), optimised for the analysis of polycyclic aromatic hydrocarbons.

Silica gel for chromatography, octadecylsilyl, base-deactivated. *1077600.*

A very finely divided silica gel, pretreated before the bonding of octadecylsilyl groups by careful washing and hydrolysing most of the superficial siloxane bridges to minimise the interaction with basic components.

Silica gel for chromatography, octadecylsilyl, cross-linked, end-capped. *1204200.*

A very finely divided silica gel, chemically modified at the surface by the cross-linking and bonding of octadecylsilyl groups. To minimise any interaction with basic compounds it is carefully end-capped to cover most of the remaining silanol groups.

Silica gel for chromatography, octadecylsilyl, end-capped. *1115400.*

A very finely divided silica gel, chemically modified at the surface by the bonding of octadecylsilyl groups. To minimise any interaction with basic compounds it is carefully end-capped to cover most of the remaining silanol groups.

Silica gel for chromatography, octadecylsilyl, end-capped R1. *1115401.*

A very finely divided ultrapure silica gel, chemically modified at the surface by the bonding of octadecylsilyl groups. To minimise any interaction with basic compounds it is carefully end-capped to cover most of the remaining silanol groups.

Silica gel for chromatography, octadecylsilyl, end-capped, base-deactivated. *1108600.*

A very finely divided silica gel, pre-treated before the bonding of octadecylsilyl groups by washing and hydrolysing most of the superficial siloxane bridges. To further minimise any interaction with basic compounds, it is carefully end-capped to cover most of the remaining silanol groups.

Silica gel for chromatography, octadecylsilyl, extra-dense bonded, end-capped. *1188500.*

A very finely divided silica gel, chemically modified at the surface by the extra-dense bonding of octadecylsilyl groups. To minimise any interaction with basic compounds it is carefully end-capped to cover most of the remaining silanol groups.

Silica gel for chromatography, octadecylsilyl, for separation of polycyclic aromatic hydrocarbons. *1202900.*

A very finely divided ultrapure silica gel, chemically modified at the surface by the bonding of octadecylsilyl groups, optimised for the analysis of polycyclic aromatic hydrocarbons.

Silica gel for chromatography, octadecylsilyl, monolithic, end-capped. *1154500.*

Monolithic rods of highly porous (greater than 80 per cent) metal-free silica with a bimodal pore structure, modified at the surface by the bonding of octadecylsilyl groups. To minimise any interaction with basic compounds, they are carefully end-capped to cover most of the remaining silanol groups.

Silica gel for chromatography, octadecylsilyl, polar-embedded, encapsulated. *1206600.*

Silica gel chemically modified at the surface by the bonding of polar-embedded octadecylsilyl groups. To minimise any interaction with basic compounds, it is carefully encapsulated to cover most of the remaining silanol groups.

Silica gel for chromatography, octadecylsilyl, polar end-capped. *1205500.*

A very finely divided silica gel, chemically modified at the surface by the bonding of octadecylsilyl groups. To minimise any interaction with basic compounds it is carefully polar end-capped to cover most of the remaining silanol groups.

Silica gel for chromatography, octadecylsilyl, solid core. *1205600.* Silica gel with spherical silica particles containing a non-porous solid silica core surrounded by a thin outer porous silica coating with octadecylsilyl groups.

Silica gel for chromatography, octadecylsilyl, solid core, end-capped. *1193900.*

Silica gel with spherical silica particles containing a non-porous solid silica core surrounded by a thin outer porous silica coating with octadecylsilyl groups. To minimise any interaction with basic compounds it is carefully end-capped to cover most of the remaining silanol groups.

Silica gel for chromatography, octadecylsilyl, with embedded polar groups, end-capped. *1177900.*

A very finely divided silica gel. The particles are based on a mixture of silica chemically modified at the surface by the bonding of octadecylsilyl groups and silica chemically modified with a reagent providing a surface with chains having embedded polar groups. Furthermore, the packing material is end-capped.

Silica gel for chromatography, octadecylsilyl, with extended pH range, end-capped. *1196700.*

A very finely divided silica gel, chemically modified at the surface by the bonding of octadecylsilyl groups resistant to bases up to pH 11. To minimise any interaction with basic compounds it is carefully end-capped to cover most of the remaining silanol groups.

Silica gel for chromatography, octadecylsilyl, with polar incorporated groups, end-capped. *1165100.*

A very finely divided silica gel. The particles are based on silica, chemically modified with a reagent providing a surface with chains having polar incorporated groups and terminating octadecyl groups. Furthermore, the packing material is end-capped.

Silica gel for chromatography, octylsilyl. *1077700.*

A very finely divided silica gel, chemically modified at the surface by the bonding of octylsilyl groups.

Silica gel for chromatography, octylsilyl R1. *1077701.*

A very finely divided silica gel, chemically modified at the surface by the bonding of octylsilyl and methyl groups (double bonded phase).

Silica gel for chromatography, octylsilyl R2. *1077702.*

Ultrapure very finely divided (10 nm pore size) silica gel, chemically modified at the surface by the bonding of octylsilyl groups (19 per cent carbon load). Less than 20 ppm of metals.

Silica gel for chromatography, octylsilyl R3. *1155200.*

A very finely divided ultrapure silica gel, chemically modified at the surface by the bonding of octylsilyl groups and sterically protected with branched hydrocarbons at the silanes.

Silica gel for chromatography, octylsilyl, base-deactivated. *1131600.*

A very finely divided silica gel, pretreated before the bonding of octylsilyl groups by careful washing and hydrolysing most of the superficial siloxane bridges to minimise the interaction with basic components.

Silica gel for chromatography, octylsilyl, end-capped. *1119600.*

A very finely divided silica gel, chemically modified at the surface by the bonding of octylsilyl groups. To minimise any interaction with basic compounds, it is carefully end-capped to cover most of the remaining silanol groups.

Silica gel for chromatography, octylsilyl, end-capped, base-deactivated. *1148800.*

A very finely divided silica gel, pre-treated before the bonding of octylsilyl groups by washing and hydrolysing most of the superficial siloxane bridges. To further minimise any interaction with basic compounds it is carefully end-capped to cover most of the remaining silanol groups.

Silica gel for chromatography, octylsilyl, extra-dense bonded, end-capped. *1200900.*

A very finely divided silica gel, chemically modified at the surface by the extra-dense bonding of octylsilyl groups. To minimise any interaction with basic compounds, it is carefully end-capped to cover most of the remaining silanol groups.

Silica gel for chromatography, octylsilyl, with polar incorporated groups, end-capped. *1152600.*

A very finely divided silica gel. The particles are based on silica, chemically modified with a reagent providing a surface with chains having polar incorporated groups and terminating octyl groups. Furthermore, the packing material is end-capped.

Silica gel for chromatography, oxypropionitrilsilyl. *1184700.*

A very finely divided silica gel chemically modified at the surface by the bonding of oxypropionitrilsilyl groups.

Silica gel for chromatography, palmitamidopropylsilyl, end-capped. *1161900.*

A very finely divided silica gel, chemically modified at the surface by the bonding of palmitamidopropyl groups and end-capped with acetamidopropyl groups.

Silica gel for chromatography, pentafluorophenylpropylsilyl, solid core, end-capped. *1207600.*

Silica gel with spherical silica particles containing a non-porous solid silica core surrounded by a thin outer porous silica coating with pentafluorophenylpropylsilyl groups. To minimise any interaction with basic compounds it is carefully end-capped to cover most of the remaining silanol groups.

Silica gel for chromatography, phenylhexylsilyl. *1153900.*

A very finely divided silica gel, chemically modified at the surface by the bonding of phenylhexyl groups.

Silica gel for chromatography, phenylhexylsilyl, end-capped. *1170600.*

A very finely divided silica gel, chemically modified at the surface by the bonding of phenylhexylsilyl groups. To minimise any interaction with basic compounds it is carefully end-capped to cover most of the remaining silanol groups.

Silica gel for chromatography, phenylhexylsilyl, solid core, end-capped. *1198900.*

Silica gel with spherical silica particles containing a non-porous solid silica core surrounded by a thin outer porous silica coating with phenylhexylsilyl groups. To minimise any interaction with basic compounds it is carefully end-capped to cover most of the remaining silanol groups.

Silica gel for chromatography, phenylsilyl. *1110200.*

A very finely divided silica gel, chemically modified at the surface by the bonding of phenyl groups.

Silica gel for chromatography, phenylsilyl, end-capped. *1154900.*

A very finely divided silica gel, chemically modified at the surface by the bonding of phenyl groups. To minimise any interaction with basic compounds it is carefully end-capped to cover most of the remaining silanol groups.

Silica gel for chromatography, phenylsilyl, end-capped, base-deactivated. *1197900.*

A very finely divided silica gel pre-treated before the bonding of phenylsilyl groups by washing and hydrolysing most of the superficial siloxane bridges, chemically modified at the surface by bonding of phenyl groups. To further minimise any interaction with basic compounds it is carefully end-capped to cover most of the remaining silanol groups.

Silica gel for chromatography, phenylsilyl, extra-dense bonded, end-capped. *1207700.*

A very finely divided silica gel, chemically modified at the surface by the extra-dense bonding of phenylsilyl groups. To minimise any interaction with basic compounds, it is carefully end-capped to cover most of the remaining silanol groups.

Silica gel for chromatography, propoxybenzene, end-capped. *1174600.*

A very finely divided silica gel, chemically modified at the surface by the bonding of propoxybenzene groups.

Silica gel for chromatography, propylsilyl. *1170700.*

A very finely divided silica gel, chemically modified at the surface by the bonding of propylsilyl groups.

Silica gel for chromatography, strong-anion-exchange. *1077800.*

A very finely divided silica gel, chemically modified at the surface by the bonding of quaternary ammonium groups.

pH limit of use: 2 to 8.

Silica gel for chromatography, strong cation-exchange. *1161400.*

A very finely divided silica gel, chemically modified at the surface by the bonding of sulfonic acid groups.

Silica gel for chromatography, trimethylsilyl. *1115500.*

A very finely divided silica gel, chemically modified at the surface by the bonding of trimethylsilyl groups.

Silica gel for size-exclusion chromatography. *1077900.*

A very finely divided silica gel (10 µm) with a very hydrophilic surface. The average diameter of the pores is about 30 nm. It is compatible with aqueous solutions between pH 2 and 8 and with organic solvents. It is suitable for the separation of proteins with relative molecular masses of 1×10^3 to 3×10^5.

Silica gel G. *1076300.* [112926-00-8].

Contains about 13 per cent of calcium sulfate hemihydrate. The particle size is about 15 µm.

Calcium sulfate content. Place 0.25 g in a ground-glass stoppered flask, add 3 mL of *dilute hydrochloric acid R* and 100 mL of *water R* and shake vigorously for 30 min. Filter through a sintered-glass filter (*2.1.2*) and wash the residue. Carry out on the combined filtrate and washings the complexometric assay of calcium (*2.5.11*).

1 mL of *0.1 M sodium edetate* is equivalent to 14.51 mg of $CaSO_4,{}^1/_2H_2O$.

pH (*2.2.3*). Shake 1 g for 5 min with 10 mL of *carbon dioxide-free water R*. The pH of the suspension is about 7.

Silica gel GF_{254}. *1076400.* [112926-00-8].

Contains about 13 per cent of calcium sulfate hemihydrate and about 1.5 per cent of a fluorescent indicator having an optimal intensity at 254 nm. The particle size is about 15 µm.

Calcium sulfate content. Determine by the method prescribed for *silica gel G R*.

pH. Complies with the test prescribed for *silica gel G R*.

Fluorescence. Thin-layer chromatography (*2.2.27*) using *silica gel GF_{254} R* as the coating substance. Apply separately to the plate at ten points increasing volumes from 1 µL to 10 µL of a 1 g/L solution of *benzoic acid R* in a mixture of 10 volumes of *anhydrous formic acid R* and 90 volumes of *2-propanol R*. Develop over a path of 10 cm with the same mixture of solvents. After evaporating the solvents examine the chromatogram in ultraviolet light at 254 nm. The benzoic acid appears as dark spots on a fluorescent background in the upper third of the chromatogram for quantities of 2 µg and greater.

Silica gel H. *1076500.* [112926-00-8].

The particle size is of about 15 µm.

pH (2.2.3). Complies with the test prescribed for *silica gel G R*.

Silica gel H, silanised. *1076600.*

Preparation of a thin layer. See *silanised silica gel HF_{254} R*

Chromatographic separation. Complies with the test prescribed for *silanised silica gel HF_{254} R*.

Silica gel HF_{254}. *1076700.*

Contains about 1.5 per cent of a fluorescent indicator having an optimal intensity at 254 nm. The particle size is about 15 µm.

pH. Complies with the test prescribed for *silica gel G R*.

Fluorescence. Complies with the test prescribed for *silica gel GF_{254} R*.

Silica gel HF_{254}, silanised. *1076800.*

Contains about 1.5 per cent of a fluorescent indicator having an optimal intensity at 254 nm.

Preparation of a thin layer. Vigorously shake 30 g for 2 min with 60 mL of a mixture of 1 volume of *methanol R* and 2 volumes of *water R*. Coat carefully cleaned plates with a layer 0.25 mm thick using a spreading device. Allow the coated plates to dry in air and then heat in an oven at 100 °C to 105 °C for 30 min.

Chromatographic separation. Introduce 0.1 g each of *methyl laurate R*, *methyl myristate R*, *methyl palmitate R* and *methyl stearate R* into a 250 mL conical flask. Add 40 mL of *alcoholic potassium hydroxide solution R* and heat under a reflux condenser on a water-bath for 1 h. Allow to cool, transfer the solution to a separating funnel by means of 100 mL of *water R*, acidify (pH 2 to 3) with *dilute hydrochloric acid R* and shake with three quantities, each of 10 mL of *chloroform R*. Dry the combined chloroform extracts over *anhydrous sodium sulfate R*, filter and evaporate to dryness on a water-bath. Dissolve the residue in 50 mL of *chloroform R*. Examine by thin-layer chromatography (*2.2.27*), using silanised silica gel HF_{254} as the coating substance. Apply to the plate at each of three separate points 10 µL of the chloroformic solution. Develop over a path of 14 cm with a mixture of 10 volumes of *glacial acetic acid R*, 25 volumes of *water R* and 65 volumes of *dioxan R*. Dry the plate at 120 °C for 30 min. Allow to cool, spray with a 35 g/L solution of *phosphomolybdic acid R* in *2-propanol R* and heat at 150 °C until the spots become visible. Treat the plate with ammonia vapour until the background is white. The chromatograms show four clearly separated, well-defined spots.

Silicotungstic acid. $H_4SiW_{12}O_{40},xH_2O$. *1078000.* [11130-20-4].

White or yellowish-white crystals, deliquescent, very soluble in water and in ethanol (96 per cent).

Storage: in an airtight container.

Silicristin. $C_{25}H_{22}O_{10}$. (M_r 482.4). *1151500.* [33889-69-9]. (2*R*,3*R*)-3,5,7-Trihydroxy-2-[(2*R*,3*S*)-7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-3-hydroxymethyl-2,3-dihydro-1-benzofuran-5-yl]chroman-4-one.

White or yellowish powder, practically insoluble in water, soluble in acetone and in methanol.

Silidianin. $C_{25}H_{22}O_{10}$. (M_r 482.4). *1151600.* [29782-68-1]. (3*R*,3a*R*,6*R*,7a*R*,8*R*)-7a-Hydroxy-8-(4-hydroxy-3-methoxyphenyl)-4-[(2*R*, 3*R*)-3,5,7-trihydroxy-4-oxochroman-2-yl]-2,3,3a,7a-tetrahydro-3,6-methano-1-benzofuran-7(6a*H*)-one.

White or yellowish powder, practically insoluble in water, soluble in acetone and in methanol.

Silver diethyldithiocarbamate. $C_5H_{10}AgNS_2$. (M_r 256.1). *1110400.* [1470-61-7]. Silver diethylcarbamodithioate.

Pale-yellow or greyish-yellow powder, practically insoluble in water, soluble in pyridine.

Storage below 8 °C is recommended.

It may be prepared as follows. Dissolve 1.7 g of *silver nitrate R* in 100 mL of *water R*. Separately dissolve 2.3 g of *sodium diethyldithiocarbamate R* in 100 mL of *water R*. Cool both solutions to 10 °C, then mix, and while stirring, collect the yellow precipitate on a sintered-glass filter (16) (*2.1.2*) and wash with 200 mL of cold *water R*. Dry the precipitate *in vacuo* for 10 h (*2.2.32*).

Silver diethyldithiocarbamate solution. *1110401.*

Prepare the solution immediately before use. Dissolve 0.100 g of *silver diethyldithiocarbamate R* in *pyridine R* and dilute to 20.0 mL with the same solvent.

Suitability test. The solution is clear (*2.2.1*). The absorbance (*2.2.25*) of the solution is maximum 0.20 at 450 nm, maximum 0.01 at 510 nm and maximum 0.010 at 538 nm.

Silver manganese paper. *1078200.*

Immerse strips of slow filter paper into a solution containing 8.5 g/L of *manganese sulfate R* and 8.5 g/L of *silver nitrate R*. Maintain for a few minutes and allow to dry over an appropriate desiccant, protected from acid and alkaline vapours.

Silver nitrate. *1078300.* [7761-88-8].

See *Silver nitrate (0009)*.

Silver nitrate reagent. *1078305.*

Prepare immediately before use. To a mixture of 3 mL of *concentrated ammonia R* and 40 mL of *1 M sodium hydroxide*, add 8 mL of a 200 g/L solution of *silver nitrate R*, dropwise, with stirring. Dilute to 200 mL with *water R*.

Silver nitrate solution R1. *1078301.*

A 42.5 g/L solution of *silver nitrate R*.

Storage: protected from light.

Silver nitrate solution R2. *1078302.*

A 17 g/L solution of *silver nitrate R*.

Storage: protected from light.

Silver nitrate solution, ammoniacal. *1078303.*

Dissolve 2.5 g of *silver nitrate R* in 80 mL of *water R* and add *dilute ammonia R1* dropwise until the precipitate has dissolved. Dilute to 100 mL with *water R*. Prepare immediately before use.

Silver nitrate solution in pyridine. *1078304.*

An 85 g/L solution of *silver nitrate R* in *pyridine R*.

Storage: protected from light.

Silver oxide. Ag_2O. (M_r 231.7). *1078400.* [20667-12-3]. Disilver oxide.

Brownish-black powder, practically insoluble in water and in ethanol (96 per cent), freely soluble in dilute nitric acid and in ammonia.

Storage: protected from light.

Silver sulfate. Ag_2SO_4. (M_r 311.8). *1201000.* [10294-26-5].

Content: minimum 99.0 per cent.

White or light grey powder, slightly soluble in water.

mp: about 652 °C.

Storage: protected from light.

Sinensetin. $C_{20}H_{20}O_7$. (M_r 372.4). *1110500.* [2306-27-6]. 3′,4′,5,6,7-Pentamethoxyflavone.

White or almost white, crystalline powder, practically insoluble in water, soluble in ethanol (96 per cent).

mp: about 177 °C.

Absorbance (*2.2.25*). A solution in *methanol R* shows 3 absorption maxima, at 243 nm, 268 nm and 330 nm.

Assay. Liquid chromatography (*2.2.29*) as prescribed in the monograph *Java tea (1229)*.

Content: minimum 95 per cent, calculated by the normalisation procedure.

Sinomenine. $C_{19}H_{23}NO_4$. (M_r 329.4). *1183400.* [115-53-7]. 7,8-Didehydro-4-hydroxy-3,7-dimethoxy-17-methyl-9α,13α,14α-morphinan-6-one. Cucoline.

Sirolimus. $C_{51}H_{79}NO_{13}$. (M_r 914). *1205700.* [53123-88-9]. Rapamycin.

mp: 183 °C to 185 °C.

Sitostanol. $C_{29}H_{52}O$. (M_r 416.7). *1140100.* [19466-47-8]. Dihydro-β-sitosterol.

Content: minimum 95.0 per cent.

β-Sitosterol. $C_{29}H_{50}O$. (M_r 414.7). *1140200.* [83-46-5]. Stigmast-5-en-3β-ol. 22,23-Dihydrostigmasterol.

White or almost white powder, practically insoluble in water, sparingly soluble in tetrahydrofuran.

Content: minimum 75.0 per cent *m/m* (dried substance).

Assay. Gas chromatography (*2.2.28*), as prescribed in the monograph *Phytosterol (1911)*.

Test solution. Dissolve 0.100 g of the substance to be examined in *tetrahydrofuran R* and dilute to 10.0 mL with the same solvent. Introduce 100 µL of this solution into a suitable 3 mL flask and evaporate to dryness under *nitrogen R*. To the residue add 100 µL of a freshly prepared mixture of 50 µL of *1-methylimidazole R* and 1.0 mL of *heptafluoro-N-methyl-N-(trimethylsilyl)butanamide R*. Close the flask tightly and heat at 100 °C for 15 min. Allow to cool.

Injection: 1 µL of the test solution.

Sodium. Na. (A_r 22.99). *1078500.* [7440-23-5].

A metal whose freshly cut surface is bright silver-grey. It rapidly tarnishes in contact with air and is oxidised completely to sodium hydroxide and converted to sodium carbonate. It reacts violently with water, yielding hydrogen and a solution of sodium hydroxide; soluble in anhydrous methanol, yielding hydrogen and a solution of sodium methoxide; practically insoluble in light petroleum.

Storage: under light petroleum or liquid paraffin.

Sodium acetate. *1078600.* [6131-90-4].

See *Sodium acetate trihydrate (0411)*.

Sodium acetate, anhydrous. $C_2H_3NaO_2$. (M_r 82.0). *1078700.* [127-09-3].

Colourless crystals or granules, very soluble in water, sparingly soluble in ethanol (96 per cent).

Loss on drying (*2.2.32*). Not more than 2.0 per cent, determined by drying in an oven at 105 °C.

Sodium arsenite. $NaAsO_2$. (M_r 129.9). *1165900.* [7784-46-5]. Sodium metaarsenite.

Sodium arsenite solution. *1165901.*

Dissolve 5.0 g of *sodium arsenite R* in 30 mL of *1 M sodium hydroxide*. Cool to 0 °C and add, while stirring, 65 mL of *dilute hydrochloric acid R*.

Sodium ascorbate solution. *1078800.* [134-03-2].

Dissolve 3.5 g of *ascorbic acid R* in 20 mL of *1 M sodium hydroxide*. Prepare immediately before use.

Sodium azide. NaN_3. (M_r 65.0). *1078900.* [26628-22-8].

White or almost white, crystalline powder or crystals, freely soluble in water, slightly soluble in ethanol (96 per cent).

Sodium benzenesulfonate. $C_6H_5SO_3Na$. (M_r 180.16). *1196600.* [515-42-4].

White crystalline powder, soluble in water.

Sodium bicarbonate. *1081300.* [144-55-8].

See *sodium hydrogen carbonate R*.

Sodium bismuthate. $NaBiO_3$. (M_r 280.0). *1079000.* [12232-99-4].

Content: minimum 85.0 per cent.

Yellow or yellowish-brown powder, slowly decomposing when moist or at a high temperature, practically insoluble in cold water.

Assay. Suspend 0.200 g in 10 mL of a 200 g/L solution of *potassium iodide R* and add 20 mL of *dilute sulfuric acid R*. Using 1 mL of *starch solution R* as indicator, titrate with *0.1 M sodium thiosulfate* until an orange colour is obtained.

1 mL of *0.1 M sodium thiosulfate* is equivalent to 14.00 mg of $NaBiO_3$.

Sodium bromide. *1154300.* [7647-15-6].

See *Sodium bromide (0190)*.

Sodium butanesulfonate. $C_4H_9NaO_3S$. (M_r 160.2). *1115600.* [2386-54-1].

White or almost white, crystalline powder, soluble in water.

mp: greater than 300 °C.

Sodium calcium edetate. *1174000.* [62-33-9].

See *sodium calcium edetate (0231)*.

Sodium carbonate. *1079200.* [6132-02-1].

See *Sodium carbonate decahydrate (0191)*.

Sodium carbonate, anhydrous. Na_2CO_3. (M_r 106.0). *1079300.* [497-19-8]. Disodium carbonate.

White or almost white powder, hygroscopic, freely soluble in water.

When heated to about 300 °C it loses not more than 1 per cent of its mass.

Storage: in an airtight container.

Sodium carbonate solution. *1079301.*

A 106 g/L solution of *anhydrous sodium carbonate R*.

Sodium carbonate solution R1. *1079302.*

A 20 g/L solution of *anhydrous sodium carbonate R* in *0.1 M sodium hydroxide*.

Sodium carbonate solution R2. *1079303.*

A 40 g/L solution of *anhydrous sodium carbonate R* in *0.2 M sodium hydroxide*.

Sodium carbonate monohydrate. *1131700.* [5968-11-6].

See *Sodium carbonate monohydrate (0192)*.

Sodium cetostearyl sulfate. *1079400.*

See *Sodium cetostearyl sulfate (0847)*.

Sodium chloride. *1079500.* [7647-14-5].

See *Sodium chloride (0193)*.

Sodium chloride solution. *1079502.*

A 20 per cent *m/m* solution of *sodium chloride R*.

Sodium chloride solution, saturated. *1079503.*

Mix 1 part of *sodium chloride R* with 2 parts of *water R*, shake from time to time and allow to stand. Before use, decant the solution from any undissolved substance and filter, if necessary.

Sodium citrate. *1079600.* [6132-04-3].

See *Sodium citrate (0412)*.

Sodium cobaltinitrite. $Na_3[Co(NO_2)_6]$. (M_r 403.9). *1079700.* [13600-98-1]. Trisodium hexanitrocobaltate(III).

Orange-yellow powder, freely soluble in water, slightly soluble in ethanol (96 per cent).

Sodium cobaltinitrite solution. *1079701.*

A 100 g/L solution of *sodium cobaltinitrite R*. Prepare immediately before use.

Sodium decanesulfonate. $C_{10}H_{21}NaO_3S$. (M_r 244.3). *1079800.* [13419-61-9].

Crystalline powder or flakes, white or almost white, freely soluble in water, soluble in methanol.

Sodium decyl sulfate. $C_{10}H_{21}NaO_4S$. (M_r 260.3). *1138600.* [142-87-0].

Content: minimum 95.0 per cent.

White or almost white powder, freely soluble in water.

Sodium deoxycholate. $C_{24}H_{39}NaO_4$. (M_r 414.6). *1131800.* [302-95-4]. Sodium 3α,12α-dihydroxy-5β-cholan-24-oate.

Sodium deoxyribonucleate. (About 85 per cent has a relative molecular mass of 2×10^7 or greater). *1079900.* [73049-39-5].

White or almost white, fibrous preparation obtained from calf thymus.

Test for suitability. Dissolve 10 mg in *imidazole buffer solution pH 6.5 R* and dilute to 10.0 mL with the same buffer solution (solution A). Dilute 2.0 mL of solution A to 50.0 mL with *imidazole buffer solution pH 6.5 R*. The absorbance (*2.2.25*) of the solution, measured at 260 nm, is 0.4 to 0.8.

To 0.5 mL of solution A add 0.5 mL of *imidazole buffer solution pH 6.5 R* and 3 mL of perchloric acid (25 g/L $HClO_4$). A precipitate is formed. Centrifuge. The absorbance of the supernatant, measured at 260 nm using a mixture of 1 mL of *imidazole buffer solution pH 6.5 R* and 3 mL of perchloric acid (25 g/L $HClO_4$) as compensation liquid, is not greater than 0.3.

In each of two tubes, place 0.5 mL of solution A and 0.5 mL of a solution of a reference preparation of streptodornase containing 10 IU/mL in *imidazole buffer solution pH 6.5 R*. To one tube add immediately 3 mL of perchloric acid (25 g/L $HClO_4$). A precipitate is formed. Centrifuge and collect supernatant A. Heat the other tube at 37 °C for 15 min and add 3 mL of perchloric acid (25 g/L $HClO_4$). Centrifuge and collect supernatant B. The absorbance of supernatant B, measured at 260 nm with reference to supernatant A is not less than 0.15.

Sodium diethyldithiocarbamate. $C_5H_{10}NNaS_2,3H_2O$. (M_r 225.3). *1080000.* [20624-25-3].

White or almost white or colourless crystals, freely soluble in water, soluble in ethanol (96 per cent). The aqueous solution is colourless.

Sodium dihydrogen phosphate. *1080100.* [13472-35-0].

See *Sodium dihydrogen phosphate dihydrate (0194)*.

Sodium dihydrogen phosphate, anhydrous. NaH_2PO_4. (M_r 120.0). *1080200.* [7558-80-7].

White or almost white powder, hygroscopic.

Storage: in an airtight container.

Sodium dihydrogen phosphate monohydrate. NaH_2PO4,H_2O. (M_r 138.0). *1080300.* [10049-21-5].

White or almost white, slightly deliquescent crystals or granules, freely soluble in water, practically insoluble in ethanol (96 per cent).

Storage: in an airtight container.

Sodium dioctyl sulfosuccinate. $C_{20}H_{37}NaO_7S$. (M_r 444.6). *1170800.* [577-11-7]. Sodium 1,4-bis[(2-ethylhexyl)oxy]-1,4-dioxobutane-2-sulfonate. 1,4-Bis(2-ethylhexyl) sulfobutanedioate sodium salt.
White or almost white, waxy solid.

Sodium dithionite. $Na_2S_2O_4$. (M_r 174.1). *1080400.* [7775-14-6].
White or greyish-white, crystalline powder, oxidises in air, very soluble in water, slightly soluble in ethanol (96 per cent).
Storage: in an airtight container.

Sodium dodecyl sulfate. *1080500.* [151-21-3].
See *Sodium laurilsulfate (0098)*.
Content: minimum 99.0 per cent.

Sodium edetate. *1080600.* [6381-92-6].
See *Disodium edetate (0232)*.

Sodium fluoresceinate. $C_{20}H_{10}Na_2O_5$. (M_r 376.3). *1080700.* [518-47-8].
Schultz No. 880.
Colour Index No. 45350.
Fluorescein sodium. Disodium 2-(3-oxo-6-oxido-3H-xanthen-9-yl)benzoate.
Orange-red powder, freely soluble in water. Aqueous solutions display an intense yellowish-green fluorescence.

Sodium fluoride. *1080800.* [7681-49-4].
See *Sodium fluoride (0514)*.

Sodium formate. $CHNaO_2$. (M_r 68.0). *1122200.* [141-53-7]. Sodium methanoate.
White or almost white, crystalline powder or deliquescent granules, soluble in water and in glycerol, slightly soluble in ethanol (96 per cent).
mp: about 253 °C.

Sodium glucuronate. $C_6H_9NaO_7,H_2O$. (M_r 234.1). *1080900.* Sodium D-glucuronate monohydrate.
$[\alpha]_D^{20}$: about + 21.5, determined on a 20 g/L solution.

Sodium glycocholate. $C_{26}H_{42}NNaO_6,2H_2O$. (M_r 523.6). *1155500.* [207300-80-9]. Sodium [(3,7,12-trihydroxy-5-cholan-24-oyl)amino]acetate dihydrate. *N*-[(3,5,7,12)-3,7,12-Trihydroxy-24-oxocholan-24-yl]glycine monosodium salt dihydrate.
Content: minimum 97 per cent of $C_{26}H_{42}NNaO_6,2H_2O$.

Sodium heptanesulfonate. $C_7H_{15}NaO_3S$. (M_r 202.3). *1081000.* [22767-50-6].
White or almost white, crystalline mass, freely soluble in water, soluble in methanol.

Sodium heptanesulfonate monohydrate. $C_7H_{15}NaO_3S,H_2O$. (M_r 220.3). *1081100.*
Content: minimum 96 per cent (anhydrous substance).
White or almost white, crystalline powder, soluble in water, very slightly soluble in anhydrous ethanol.
Water (*2.5.12*): maximum 8 per cent, determined on 0.300 g.
Assay. Dissolve 0.150 g in 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).
1 mL of *0.1 M perchloric acid* is equivalent to 20.22 mg of $C_7H_{15}NaO_3S$.

Sodium hexanesulfonate. $C_6H_{13}NaO_3S$. (M_r 188.2). *1081200.* [2832-45-3].
White or almost white powder, freely soluble in water.

Sodium hexanesulfonate monohydrate. $C_6H_{13}NaO_3S,H_2O$. (M_r 206.2). *1161500.* [207300-91-2].
White or almost white powder, soluble in water.

Sodium hexanesulfonate monohydrate for ion-pair chromatography. $C_6H_{13}NaO_3S,H_2O$. (M_r 206.2). *1182300.* [207300-91-2].
Content: minimum 99.0 per cent.

Sodium hydrogen carbonate. *1081300.* [144-55-8].
See *Sodium hydrogen carbonate (0195)*.

Sodium hydrogen carbonate solution. *1081301.*
A 42 g/L solution of *sodium hydrogen carbonate R*.

Sodium hydrogen sulfate. $NaHSO_4$. (M_r 120.1). *1131900.* [7681-38-1]. Sodium bisulfate.
Freely soluble in water, very soluble in boiling water. It decomposes in ethanol (96 per cent) into sodium sulfate and free sulfuric acid.
mp: about 315 °C.

Sodium hydrogensulfite. $NaHO_3S$. (M_r 104.1). *1115700.* [7631-90-5].
White or almost white, crystalline powder, freely soluble in water, sparingly soluble in ethanol (96 per cent).
On exposure to air, some sulfur dioxide is lost and the substance is gradually oxidated to sulfate.

Sodium hydroxide. *1081400.* [1310-73-2].
See *Sodium hydroxide (0677)*.

2 M Sodium hydroxide. *3009800.*
Dissolve 84 g of *sodium hydroxide R* in *carbon dioxide-free water R* and dilute to 1000.0 mL with the same solvent.

4 M Sodium hydroxide. *1081407.*
Dissolve 168 g of *sodium hydroxide R* in *carbon dioxide-free water R* and dilute to 1.0 L with the same solvent.

Sodium hydroxide solution. *1081401.*
Dissolve 20.0 g of *sodium hydroxide R* in *water R* and dilute to 100.0 mL with the same solvent. Verify the concentration by titration with *1 M hydrochloric acid*, using *methyl orange solution R* as indicator, and adjust if necessary to 200 g/L.

Sodium hydroxide solution, carbonate-free. *1081406.*
Dissolve *sodium hydroxide R* in *carbon dioxide-free water R* to give a concentration of 500 g/L and allow to stand. Decant the clear supernatant, taking precautions to avoid the introduction of carbon dioxide.

Sodium hydroxide solution, dilute. *1081402.*
Dissolve 8.5 g of *sodium hydroxide R* in *water R* and dilute to 100 mL with the same solvent.

Sodium hydroxide solution, methanolic. *1081403.*
Dissolve 40 mg of *sodium hydroxide R* in 50 mL of *water R*. Cool and add 50 mL of *methanol R*.

Sodium hydroxide solution, methanolic R1. *1081405.*
Dissolve 200 mg of *sodium hydroxide R* in 50 mL of *water R*. Cool and add 50 mL of *methanol R*.

Sodium hydroxide solution, strong. *1081404.*
Dissolve 42 g of *sodium hydroxide R* in *water R* and dilute to 100 mL with the same solvent.

Sodium 2-hydroxybutyrate. $C_4H_7NaO_3$. (M_r 126.1). *1158800.* [19054-57-0]. Sodium (2*RS*)-2-hydroxybutanoate.

Sodium hypobromite solution. *1081500.*
In a bath of iced water mix 20 mL of *strong sodium hydroxide solution R* and 500 mL of *water R*, add 5 mL of *bromine solution R* and stir gently until solution is complete. Prepare immediately before use.

Sodium hypochlorite solution, strong. *1081600.*
Content: 25 g/L to 30 g/L of active chlorine.

Yellowish liquid with an alkaline reaction.

Assay. Introduce into a flask, successively, 50 mL of *water R*, 1 g of *potassium iodide R* and 12.5 mL of *dilute acetic acid R*. Dilute 10.0 mL of the substance to be examined to 100.0 mL with *water R*. Introduce 10.0 mL of this solution into the flask and titrate with *0.1 M sodium thiosulfate*, using 1 mL of *starch solution R* as indicator.

1 mL of *0.1 M sodium thiosulfate* is equivalent to 3.546 mg of active chlorine.

Storage: protected from light.

Sodium hypophosphite. NaH_2PO_2,H_2O. (M_r 106.0). *1081700.* [10039-56-2]. Sodium phosphinate monohydrate.

White or almost white, crystalline powder or colourless crystals, hygroscopic, freely soluble in water, soluble in ethanol (96 per cent).

Storage: in an airtight container.

Sodium iodide. *1081800.* [7681-82-5].

See *Sodium iodide (0196)*.

Sodium laurilsulfate. *1081900.* [151-21-3].

See *Sodium laurilsulfate (0098)*.

Sodium lauryl sulfate. *1081900.* [151-21-3].

See *Sodium laurilsulfate R*.

Sodium laurylsulfonate for chromatography. $C_{12}H_{25}NaO_3S$. (M_r 272.4). *1132000.* [2386-53-0].

White or almost white powder or crystals, freely soluble in water.

Absorbance $A_{1\,cm}^{5\%}$ (*2.2.25*), determined in *water R*: about 0.05 at 210 nm; about 0.03 at 220 nm; about 0.02 at 230 nm; about 0.02 at 500 nm.

Sodium metabisulfite. *1082000.* [7681-57-4].

See *Sodium metabisulfite (0849)*.

Sodium methanesulfonate. CH_3SO_3Na. (M_r 118.1). *1082100.* [2386-57-4].

White or almost white, crystalline powder, hygroscopic.

Storage: in an airtight container.

Sodium molybdate. $Na_2MoO_4,2H_2O$. (M_r 242.0). *1082200.* [10102-40-6]. Disodium molybdate dihydrate.

White or almost white, crystalline powder or colourless crystals, freely soluble in water.

Sodium naphthoquinonesulfonate. $C_{10}H_5NaO_5S$. (M_r 260.2). *1082300.* [521-24-4]. Sodium 1,2-naphthoquinone-4-sulfonate.

Yellow or orange-yellow, crystalline powder, freely soluble in water, practically insoluble in ethanol (96 per cent).

Sodium nitrate. $NaNO_3$. (M_r 85.0). *1082400.* [7631-99-4].

White or almost white powder or granules or colourless, transparent crystals, deliquescent in moist air, freely soluble in water, slightly soluble in ethanol (96 per cent).

Storage: in an airtight container.

Sodium nitrite. $NaNO_2$. (M_r 69.0). *1082500.* [7632-00-0].

Content: minimum 97.0 per cent.

White or almost white, granular powder or a slightly yellow, crystalline powder, freely soluble in water.

Assay. Dissolve 0.100 g in 50 mL of *water R*. Add 50.0 mL of *0.02 M potassium permanganate* and 15 mL of *dilute sulfuric acid R*. Add 3 g of *potassium iodide R*. Titrate with *0.1 M sodium thiosulfate*, using 1.0 mL of *starch solution R* added towards the end of the titration as indicator.

1 mL of *0.02 M potassium permanganate* is equivalent to 3.450 mg of $NaNO_2$.

Sodium nitrite solution. *1082501.*

A 100 g/L solution of *sodium nitrite R*. Prepare immediately before use.

Sodium nitroprusside. $Na_2[Fe(CN)_5(NO)],2H_2O$. (M_r 298.0). *1082600.* [13755-38-9]. Sodium pentacyano-nitrosylferrate(III) dihydrate.

Reddish-brown powder or crystals, freely soluble in water, slightly soluble in ethanol (96 per cent).

Sodium octanesulfonate. $C_8H_{17}NaO_3S$. (M_r 216.3). *1082700.* [5324-84-5].

Content: minimum 98.0 per cent.

White or almost white, crystalline powder or flakes, freely soluble in water, soluble in methanol.

Absorbance (*2.2.25*): maximum 0.10, determined at 200 nm and maximum 0.01, determined at 250 nm using a 54 g/L solution.

Sodium octanesulfonate monohydrate. $C_8H_{17}NaO_3S,H_2O$. (M_r 234.3). *1176700.* [207596-29-0].

White or almost white powder.

Sodium octyl sulfate. $C_8H_{17}NaO_4S$. (M_r 232.3). *1082800.* [142-31-4].

White or almost white, crystalline powder or flakes, freely soluble in water, soluble in methanol.

Sodium oxalate. $C_2Na_2O_4$. (M_r 134.0). *1082900.* [62-76-0].

White or almost white, crystalline powder, soluble in water, practically insoluble in ethanol (96 per cent).

Sodium oxidronate. $CH_4Na_2O_7P_2$. (M_r 236.0). *1194000.* [14255-61-9]. Sodium hydroxymethylenediphosphonate.

White or almost white powder or colourless crystals, very soluble in water, very slightly soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

Sodium pentanesulfonate. $C_5H_{11}NaO_3S$. (M_r 174.2). *1083000.* [22767-49-3].

White or almost white, crystalline solid, soluble in water.

Sodium pentanesulfonate monohydrate. $C_5H_{11}NaO_3S,H_2O$. (M_r 192.2). *1132100.* [207605-40-1].

White or almost white crystalline solid, soluble in water.

Sodium pentanesulfonate monohydrate R1. $C_5H_{11}NaO_3S,H_2O$. (M_r 192.2). *1172500.* [207605-40-1].

Content: minimum 99 per cent of $C_5H_{11}NaO_3S,H_2O$.

Sodium perchlorate. $NaClO_4,H_2O$. (M_r 140.5). *1083100.* [7791-07-3].

Content: minimum 99.0 per cent of $NaClO_4,H_2O$.

White or almost white, deliquescent crystals, very soluble in water.

Storage: in a well-closed container.

Sodium periodate. $NaIO_4$. (M_r 213.9). *1083200.* [7790-28-5]. Sodium metaperiodate.

Content: minimum 99.0 per cent.

White or almost white, crystalline powder or crystals, soluble in water and in mineral acids.

Sodium periodate solution. *1083201.*

Dissolve 1.07 g of *sodium periodate R* in *water R*, add 5 mL of *dilute sulfuric acid R* and dilute to 100.0 mL with *water R*. Use a freshly prepared solution.

Sodium phosphite pentahydrate. $Na_2HPO_3,5H_2O$. (M_r 216.0). *1132200.* [13517-23-2].

White or almost white, crystalline powder, hygroscopic, freely soluble in water.

Storage: in an airtight container.

Sodium picrate solution, alkaline. *1083300.*

Mix 20 mL of *picric acid solution R* and 10 mL of a 50 g/L solution of *sodium hydroxide R* and dilute to 100 mL with *water R*.

Storage: use within 2 days.

Sodium potassium tartrate. $C_4H_4KNaO_6,4H_2O$. (M_r 282.2). *1083500.* [6381-59-5].

Colourless, prismatic crystals, very soluble in water.

Sodium 1-propanesulfonate. $C_3H_9SO_4Na$. (M_r 164.2). *1197600.* [304672-01-3]. Sodium propane-1-sulfonate monohydrate.

mp: about 250 °C.

Sodium pyrophosphate. $Na_4P_2O_7,10H_2O$. (M_r 446.1). *1083600.* [13472-36-1]. Tetrasodium diphosphate decahydrate.

Colourless, slightly efflorescent crystals, freely soluble in water.

Sodium pyruvate. $C_3H_3NaO_3$. (M_r 110.0). *1204300.* [113-24-6]. 2-Oxopropanoic acid sodium salt.

White or faint yellow powder, soluble in water (100 mg/mL).

mp: greater than 300 °C.

Sodium rhodizonate. $C_6Na_2O_6$. (M_r 214.0). *1122300.* [523-21-7]. [(3,4,5,6-Tetraoxocyclohex-1-en-1,2-ylene)dioxy]disodium.

Violet crystals, soluble in water with an orange-yellow colour.

Solutions are unstable and must be prepared on the day of use.

Sodium salicylate. *1083700.* [54-21-7].

See *Sodium salicylate (0413)*.

Sodium stearyl fumarate. $C_{22}H_{39}NaO_4$. *1195100.* [4070-80-8].

See *Sodium stearyl fumarate (1567)*.

Sodium sulfate, anhydrous. *1083800.* [7757-82-6].

Ignite at 600 °C to 700 °C anhydrous sodium sulfate complying with the requirements prescribed in the monograph on *Anhydrous sodium sulfate (0099)*.

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined by drying in an oven at 130 °C.

Sodium sulfate, anhydrous R1. *1083801.*

Complies with the requirements prescribed for *anhydrous sodium sulfate R* with the following maximum contents.

Cl: 20 ppm.

Pb: 10 ppm.

As: 3 ppm.

Ca: 50 ppm.

Fe: 10 ppm.

Mg: 10 ppm.

Sodium sulfate decahydrate. $Na_2SO_4,10H_2O$. (M_r 322.2). *1132300.* [7727-73-3].

See *Sodium sulfate decahydrate (0100)*.

Sodium sulfide. $Na_2S,9H_2O$. (M_r 240.2). *1083900.* [1313-84-4]. Disodium sulfide nonahydrate.

Colourless, rapidly yellowing crystals, deliquescent, very soluble in water.

Storage: in an airtight container.

Sodium sulfide solution. *1083901.*

Dissolve 12 g of *sodium sulfide R* with heating in 45 mL of a mixture of 10 volumes of *water R* and 29 volumes of *glycerol (85 per cent) R*, allow to cool and dilute to 100 mL with the same mixture of solvents.

The solution should be colourless.

Sodium sulfide solution R1. *1083902.*

Prepare by one of the following methods.

– Dissolve 5 g of *sodium sulfide R* in a mixture of 10 mL of *water R* and 30 mL of *glycerol R*.

– Dissolve 5 g of *sodium hydroxide R* in a mixture of 30 mL of *water R* and 90 mL of *glycerol R*. Divide the solution into 2 equal portions. Saturate 1 portion with *hydrogen sulfide R*, with cooling. Mix the 2 portions.

Storage: in a well-filled container, protected from light; use within 3 months.

Sodium sulfite, anhydrous. *1084100.* [7757-83-7].

See *Sodium sulfite (0775)*.

Sodium sulfite heptahydrate. *1084000.* [10102-15-5].

See *Sodium sulfite heptahydrate (0776)*.

Sodium tartrate. $C_4H_4Na_2O_6,2H_2O$. (M_r 230.1). *1084200.* [6106-24-7]. Disodium (2*R*,3*R*)-2,3-dihydroxybutanedioate dihydrate.

White or almost white crystals or granules, very soluble in water, practically insoluble in ethanol (96 per cent).

Sodium taurodeoxycholate. $C_{26}H_{44}NNaO_6S,H_2O$. (M_r 539.7). *1155600.* [110026-03-4]. Sodium 2-[(3,12-dihydroxy-5-cholan-24-oyl)amino]ethanesulfonate monohydrate. 2-[[(3,5,12)-3,12-Dihydroxy-24-oxocholan-24-yl]amino]ethanesulfonic acid monosodium salt monohydrate.

Content: minimum 94 per cent of $C_{26}H_{44}NNaO_6S,H_2O$.

Sodium tetrahydroborate. $NaBH_4$. (M_r 37.8). *1146900.* [16940-66-2]. Sodium borohydride.

Colourless, hygroscopic crystals, freely soluble in water, soluble in anhydrous ethanol, decomposing at higher temperature or in the presence of acids or certain metal salts forming borax and hydrogen.

Storage: in an airtight container.

Sodium tetrahydroborate reducing solution. *1146901.*

Introduce about 100 mL of *water R* into a 500 mL volumetric flask containing a stirring bar. Add 5.0 g of *sodium hydroxide R* in pellets and 2.5 g of *sodium tetrahydroborate R*. Stir until complete dissolution, dilute to 500.0 mL with *water R* and mix. Prepare immediately before use.

Sodium tetraphenylborate. $NaB(C_6H_5)_4$. (M_r 342.2). *1084400.* [143-66-8].

White or slightly yellowish, bulky powder, freely soluble in water and in acetone.

Sodium tetraphenylborate solution. *1084401.*

Filter before use if necessary.

A 10 g/L solution of *sodium tetraphenylborate R*.

Storage: use within 1 week.

Sodium thioglycollate. $C_2H_3NaO_2S$. (M_r 114.1). *1084500.* [367-51-1]. Sodium mercaptoacetate.

White or almost white, granular powder or crystals, hygroscopic, freely soluble in water and in methanol, slightly soluble in ethanol (96 per cent).

Storage: in an airtight container.

Sodium thiosulfate. *1084600.* [10102-17-7].

See *Sodium thiosulfate (0414)*.

Sodium thiosulfate, anhydrous. $Na_2S_2O_3$. (M_r 158.1). *1180700.* [7772-98-7]. Disodium thiosulfate.

Content: minimum 98.0 per cent.

Sodium tungstate. $Na_2WO_4,2H_2O$. (M_r 329.9). *1084700.* [10213-10-2]. Disodium tungstate dihydrate.

White or almost white, crystalline powder or colourless crystals, freely soluble in water forming a clear solution, practically insoluble in ethanol (96 per cent).

Sorbitol. *1084800.* [50-70-4].

See *Sorbitol (0435)*.

Soya bean lecithin. *1196400.* [8030-76-0].

Soya-bean oil, refined. *1201500.*

See *Soya-bean oil, refined (1473)*.

Sphingomyelin from egg yolk. *1199100.* [85187-10-6].

(2*R*,3*S*,4*E*)-2-(Acylamino)-3-hydroxyoctadec-4-en-1-yl 2-(trimethylazaniumyl)ethyl phosphate.

Squalane. $C_{30}H_{62}$. (M_r 422.8). *1084900.* [111-01-3]. (6*Ξ*,10*Ξ*,15*Ξ*,19*Ξ*)-2,6,10,15,19,23-Hexamethyltetracosane. Perhydrosqualene.

Colourless, oily liquid, freely soluble in fatty oils, slightly soluble in acetone, in ethanol (96 per cent), in glacial acetic acid and in methanol.

d_{20}^{20}: 0.811 to 0.813.

n_D^{20}: 1.451 to 1.453.

Stannous chloride. $SnCl_2,2H_2O$. (M_r 225.6). *1085000.* [10025-69-1]. Tin dichloride dihydrate.

Content: minimum 97.0 per cent of $SnCl_2,2H_2O$.

Colourless crystals, very soluble in water, freely soluble in ethanol (96 per cent), in glacial acetic acid and in dilute and concentrated hydrochloric acid.

Assay. Dissolve 0.500 g in 15 mL of *hydrochloric acid R* in a ground-glass-stoppered flask. Add 10 mL of *water R* and 5 mL of *chloroform R*. Titrate rapidly with *0.05 M potassium iodate* until the chloroform layer is colourless.

1 mL of *0.05 M potassium iodate* is equivalent to 22.56 mg of $SnCl_2,2H_2O$.

Stannous chloride solution. *1085001.*

Heat 20 g of *tin R* with 85 mL of *hydrochloric acid R* until no more hydrogen is released. Allow to cool.

Storage: over an excess of *tin R*, protected from air.

Stannous chloride solution R1. *1085002.*

Immediately before use, dilute 1 volume of *stannous chloride solution R* with 10 volumes of *dilute hydrochloric acid R*.

Stannous chloride solution R2. *1085003.*

To 8 g of *stannous chloride R* add 100 mL of a 20 per cent *V/V* solution of *hydrochloric acid R*. Shake until dissolved, heating, if necessary, on a water-bath at 50 °C. Pass a current of *nitrogen R* for 15 min. Prepare immediately before use.

Stanolone. $C_{19}H_{30}O_2$. (M_r 290.4). *1154400.* [521-18-6]. 17β-Hydroxy-5α-androstan-3-one.

White or almost white powder.

mp: about 180 °C.

Standard solution for the micro determination of water. *1147300.*

Commercially available standard solution for the coulometric titration of water, containing a certified content of water in a suitable solvent.

***Staphylococcus aureus* strain V8 protease, type XVII-B.** *1115800.* [66676-43-5].

Microbial extracellular proteolytic enzyme. A lyophilised powder containing 500 units to 1000 units per milligram of solid.

Starch, soluble. *1085100.* [9005-84-9].

White or almost white powder.

Starch iodate paper. *1085101.*

Immerse strips of filter paper in 100 mL of *iodide-free starch solution R* containing 0.1 g of *potassium iodate R*. Drain and allow to dry protected from light.

Starch iodide paper. *1085106.*

Immerse strips of filter paper in 100 mL of *potassium iodide and starch solution R*. Drain and allow to dry protected from light.

Test for sensitivity. Mix 0.05 mL of *0.1 M sodium nitrite* with 4 mL of *hydrochloric acid R* and dilute to 100 mL with *water R*. Apply one drop of the solution to starch iodide paper; a blue spot appears.

Starch solution. *1085103.*

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 R1*. The solution is blue.

Starch solution, iodide-free. *1085104.*

Prepare the solution as prescribed for *starch solution R* omitting the mercuric iodide. Prepare immediately before use.

Starch solution R1. *1085105.*

Mix 1 g of *soluble starch R* and a small amount of cold *water R*. Add this mixture, while stirring, to 200 mL of boiling *water R*. Add 0.25 g of *salicylic acid R* and boil for 3 min. Immediately remove from the heat and cool.

Storage: if long storage is required, the solution shall be stored at 4 °C to 10 °C. A fresh starch solution shall be prepared when the end-point of the titration from blue to colourless fails to be sharp. If stored under refrigeration, the starch solution is stable for about 2 to 3 weeks.

Test for sensitivity. A mixture of 2 mL of *starch solution R1*, 20 mL of *water R*, about 50 mg of *potassium iodide R* and 0.05 mL of *iodine solution R1* is blue.

Starch solution R2. *1085107.*

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*. Use a freshly prepared solution.

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 R1*. The solution is blue.

Stavudine. *1187000.* [3056-17-5].

See *Stavudine (2130)*.

Stearic acid. $C_{18}H_{36}O_2$. (M_r 284.5). *1085200.* [57-11-4]. Octadecanoic acid.

White or almost white powder or flakes, greasy to the touch, practically insoluble in water, soluble in hot ethanol (96 per cent).

mp: about 70 °C.

Stearic acid used in the assay of total fatty acids in Saw palmetto fruit (1848) complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Saw palmetto fruit (1848)*.

Content: minimum 98 per cent, calculated by the normalisation procedure.

Stearyl alcohol. $C_{18}H_{38}O$. (M_r 270.5). *1156400.* [112-92-5]. Octadecan-1-ol.

mp: about 60 °C.

Content: minimum 95 per cent.

Stigmasterol. $C_{29}H_{48}O$. (M_r 412.7). *1141400.* [83-48-7]. (22*E*)-Stigmasta-5,22-dien-3β-ol. (22*E*)-24-Ethylcholesta-5,22-dien-3β-ol.

White or almost white powder, insoluble in water.

mp: about 170 °C.

$[\alpha]_D^{22}$: about – 51, determined with a 20 g/L solution in *chloroform R*.

Streptomycin sulfate. *1085300.* [3810-74-0].

See *Streptomycin sulfate (0053)*.

Strongly acidic ion-exchange resin. *1085400.*

See *ion-exchange resin, strongly acidic R*.

Strontium carbonate. $SrCO_3$. (M_r 147.6). *1122700.* [1633-05-2].

White or almost white, crystalline powder.

Content: minimum 99.5 per cent.

Strontium chloride hexahydrate. $SrCl_2,6H_2O$. (M_r 266.6). *1167000.* [10025-70-4].

White or almost white crystals, very soluble in water.

mp: about 115 °C (loss of water) and 872 °C.

Strontium selective extraction resin. *1167100.*

Commercially available resin prepared by loading a suspension of 4,4′(5′)-di-*tert*-butylcyclohexano-18-crown-6 (crown ether) in octanol onto an inert chromatographic support. The bed density of this resin is approximately 0.35 g/mL.

Strontium-85 spiking solution. *1166800.*

Dilute *strontium-85 standard solution R* to a radioactivity concentration of approximately 10 kBq/mL with a 0.27 g/L solution of *strontium chloride hexahydrate R* in a 1.03 g/L solution of *hydrochloric acid R*.

Strontium-85 standard solution. *1166900.*

A solution of strontium-85 in the form of Sr^{2+} ions in a 51.5 g/L solution of *hydrochloric acid R*.

Strychnine. $C_{21}H_{22}N_2O_2$. (M_r 334.4). *1190600.* [57-24-9]. (4a*R*,4b*R*,5a*S*,8a*R*,13a*S*,15a*S*)-2,4a,4b,5,5a,7,8,13a,15,15a-Decahydro-4,6-methano-6*H*-indolo[3,2,1-*ij*]oxepino[2,3,4-*de*]pyrrolo[2,3-*h*]quinolin-14-one. Strychnidin-10-one.

White or almost white, crystalline powder, sparingly soluble in water.

mp: about 285 °C.

Styrene. C_8H_8. (M_r 104.2). *1151700.* [100-42-5]. Ethenylbenzene.

bp: about 145 °C.

Colourless, oily liquid, very slightly soluble in water.

Styrene-divinylbenzene copolymer. *1085500.*

Porous, rigid, cross-linked polymer beads. Several grades are available with different sizes of beads. The size range of the beads is specified after the name of the reagent in the tests where it is used.

Succinic acid. $C_4H_6O_4$. (M_r 118.1). *1085600.* [110-15-6]. Butanedioic acid.

White or almost white, crystalline powder or colourless crystals, soluble in water and in ethanol (96 per cent).

mp: 184 °C to 187 °C.

Sucrose. *1085700.* [57-50-1].

See *Sucrose (0204)*.

Sudan orange. $C_{16}H_{12}N_2O$. (M_r 248.3). *1110700.* [842-07-9].

Colour Index No. 12055.

1-(Phenylazo)naphthalen-2-ol. Sudan I.

Orange-red powder, practically insoluble in water, soluble in methylene chloride.

mp: about 131 °C.

Sudan red G. $C_{17}H_{14}N_2O_2$. (M_r 278.3). *1085800.*

Schultz No. 149.

Colour Index No. 12150.

Solvent Red 1. 1-[(2-Methoxyphenyl)azo]naphtalen-2-ol.

Reddish-brown powder, practically insoluble in water.

Chromatography. Thin-layer chromatography (*2.2.27*) using *silica gel G R* as the coating substance: apply 10 µL of a 0.1 g/L solution in *methylene chloride R* and develop over a path of 10 cm with the same solvent; the chromatogram shows only one principal spot.

Sulfanilamide. $C_6H_8N_2O_2S$. (M_r 172.2). *1086100.* [63-74-1]. 4-Aminobenzenesulfonamide.

White or almost white powder, slightly soluble in water, freely soluble in boiling water, in acetone, in dilute acids and in solutions of the alkali hydroxides, sparingly soluble in ethanol (96 per cent) and in light petroleum.

mp: about 165 °C.

Sulfathiazole. $C_9H_9N_3O_2S_2$. (M_r 255.3). *1086300.* [72-14-0]. 4-Amino-*N*-(thiazol-2-yl)benzenesulfonamide.

White or yellowish-white powder or crystals, very slightly soluble in water, soluble in acetone, slightly soluble in ethanol (96 per cent). It dissolves in dilute mineral acids and in solutions of alkali hydroxides and carbonates.

mp: about 200 °C.

Sulfamic acid. H_3NO_3S. (M_r 97.1). *1085900.* [5329-14-6].

White or almost white crystalline powder or crystals, freely soluble in water, sparingly soluble in acetone, in ethanol (96 per cent) and in methanol.

mp: about 205 °C, with decomposition.

Sulfan blue. $C_{27}H_{31}N_2NaO_6S_2$. (M_r 566.6). *1086000.* [129-17-9].

Schultz No. 769.

Colour Index No. 42045.

Acid Blue 1. Patent Blue VF. Disulfine blue. Blue VS. Sodium [[[(4-diethylamino)phenyl](2,4-disulfonatophenyl)-methylene]cyclohexa-2,5-dien-1-ylidene]diethylammonium.

Violet powder, soluble in water. Dilute solutions are blue and turn yellow on the addition of concentrated hydrochloric acid.

Sulfanilic acid. $C_6H_7NO_3S$. (M_r 173.2). *1086200.* [121-57-3]. 4-Aminobenzenesulfonic acid.

Colourless crystals, sparingly soluble in water, practically insoluble in ethanol (96 per cent).

Sulfanilic acid solution. *1086203.*

Dissolve 0.33 g of *sulfanilic acid R* in 75 mL of *water R* heating gently if necessary and dilute to 100 mL with *glacial acetic acid R*.

Sulfanilic acid solution R1. *1086201.*

Dissolve 0.5 g of *sulfanilic acid R* in a mixture of 75 mL of *dilute acetic acid R* and 75 mL of *water R*.

Sulfanilic acid solution, diazotised. *1086202.*

Dissolve, with warming, 0.9 g of *sulfanilic acid R* in 9 mL of *hydrochloric acid R*, and dilute to 100 mL with *water R*. Cool 10 mL of this solution in iced water and add 10 mL of an ice-cold 45 g/L solution of *sodium nitrite R*. Allow to

stand at 0 °C for 15 min (if stored at this temperature, the solution is stable for 3 days) and immediately before use add 20 mL of a 100 g/L solution of *sodium carbonate R*.

Sulfomolybdic reagent R2. *1086400.*

Dissolve about 50 mg of *ammonium molybdate R* in 10 mL of *sulfuric acid R*.

Sulfomolybdic reagent R3. *1086500.*

Dissolve with heating 2.5 g of *ammonium molybdate R* in 20 mL of *water R*. Dilute 28 mL of *sulfuric acid R* in 50 mL of *water R*, then cool. Mix the two solutions and dilute to 100 mL with *water R*.

Storage: in a polyethylene container.

Sulfosalicylic acid. $C_7H_6O_6S,2H_2O$. (M_r 254.2). *1086600.* [5965-83-3]. 2-Hydroxy-5-sulfobenzoic acid.

White or almost white, crystalline powder or crystals, very soluble in water and in ethanol (96 per cent).

mp: about 109 °C.

Sulfur. *1110800.* [7704-34-9].

See *Sulfur for external use (0953)*.

Sulfur dioxide. SO_2. (M_r 64.1). *1086700.* [7446-09-5]. Sulfurous anhydride.

A colourless gas. When compressed it is a colourless liquid.

Sulfur dioxide R1. SO_2. (M_r 64.1). *1110900.* [7446-09-5].

Content: minimum 99.9 per cent *V/V*.

Sulfuric acid. H_2SO_4. (M_r 98.1). *1086800.* [7664-93-9].

Content: 95.0 per cent *m/m* to 97.0 per cent *m/m*.

Colourless, caustic liquid with an oily consistency, highly hygroscopic, miscible with water and with ethanol (96 per cent) producing intense heat.

d_{20}^{20}: 1.834 to 1.837.

A 10 g/L solution is strongly acid and gives the reactions of sulfates (*2.3.1*).

Appearance. It is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Oxidisable substances. Pour 20 g cautiously, with cooling, into 40 mL of *water R*. Add 0.5 mL of *0.002 M potassium permanganate*. The violet colour persists for at least 5 min.

Chlorides: maximum 0.5 ppm.

Pour 10 g, carefully and while cooling, into 10 mL of *water R* and after cooling dilute to 20 mL with the same solvent. Add 0.5 mL of *silver nitrate solution R2*. Allow to stand for 2 min protected from bright light. The solution is not more opalescent than a standard prepared at the same time using a mixture of 1 mL of *chloride standard solution (5 ppm Cl) R*, 19 mL of *water R* and 0.5 mL of *silver nitrate solution R2*.

Nitrates: maximum 0.5 ppm.

Pour 50 g or 27.2 mL, carefully and while cooling, into 15 mL of *water R*. Add 0.2 mL of a freshly prepared 50 g/L solution of *brucine R* in *glacial acetic acid R*. After 5 min any colour is less intense than that of a reference mixture prepared in the same manner and containing 12.5 mL of *water R*, 50 g of *nitrogen-free sulfuric acid R*, 2.5 mL of *nitrate standard solution (10 ppm NO_3) R* and 0.2 mL of a 50 g/L solution of *brucine R* in *glacial acetic acid R*.

Ammonium: maximum 2 ppm.

Pour 2.5 g, carefully and while cooling, into *water R* and dilute to 20 mL with the same solvent. Cool, and add dropwise 10 mL of a 200 g/L solution of *sodium hydroxide R*, followed by 1 mL of *alkaline potassium tetraiodomercurate solution R*. The colour of the solution is less intense than that of a mixture of 5 mL of *ammonium standard solution (1 ppm NH_4) R*, 15 mL of *water R*, 10 mL of a 200 g/L solution of *sodium hydroxide R* and 1 mL of *alkaline potassium tetraiodomercurate solution R*.

Arsenic (*2.4.2, Method A*): maximum 0.02 ppm.

To 50 g add 3 mL of *nitric acid R* and evaporate carefully until the volume is reduced to about 10 mL. Cool, add to the residue 20 mL of *water R* and concentrate to 5 mL. Prepare the standard using 1.0 mL of *arsenic standard solution (1 ppm As) R*.

Iron (*2.4.9*): maximum 1 ppm.

Dissolve the residue on ignition with slight heating in 1 mL of *dilute hydrochloric acid R* and dilute to 50.0 mL with *water R*. Dilute 5 mL of this solution to 10 mL with *water R*.

Heavy metals (*2.4.8*): maximum 2 ppm.

Dilute 10 mL of the solution obtained in the test for iron to 20 mL with *water R*. 12 mL of the solution complies with test A. Prepare the reference solution using *lead standard solution (2 ppm Pb) R*.

Residue on ignition: maximum 0.001 per cent, determined on 100 g by evaporating cautiously in a small crucible over a naked flame and igniting the residue to redness.

Assay. Weigh accurately a ground-glass-stoppered flask containing 30 mL of *water R*, introduce 0.8 mL of the sulfuric acid, cool and weigh again. Titrate with *1 M sodium hydroxide*, using 0.1 mL of *methyl red solution R* as indicator.

1 mL of *1 M sodium hydroxide* is equivalent to 49.04 mg of H_2SO_4.

Storage: in a ground-glass-stoppered container made of glass or other inert material.

5 M Sulfuric acid. *1086809.*

Dilute 28 mL of *sulfuric acid R* to 100 mL with *water R*.

Sulfuric acid, alcoholic, 2.5 M. *1086801.*

Carefully and with constant cooling, stir 14 mL of *sulfuric acid R* into 60 mL of *anhydrous ethanol R*. Allow to cool and dilute to 100 mL with *anhydrous ethanol R*. Prepare immediately before use.

Sulfuric acid, alcoholic, 0.25 M. *1086802.*

Dilute 10 mL of *2.5 M alcoholic sulfuric acid R* to 100 mL with *anhydrous ethanol R*. Prepare immediately before use.

Sulfuric acid, alcoholic solution of. *1086803.*

Carefully and with constant cooling, stir 20 mL of *sulfuric acid R* into 60 mL of *ethanol (96 per cent) R*. Allow to cool and dilute to 100 mL with *ethanol (96 per cent) R*. Prepare immediately before use.

Sulfuric acid, dilute. *1086804.*

Contains 98 g/L of H_2SO_4.

Add 5.5 mL of *sulfuric acid R* to 60 mL of *water R*, allow to cool and dilute to 100 mL with the same solvent.

Assay. Into a ground-glass-stoppered flask containing 30 mL of *water R*, introduce 10.0 mL of the dilute sulfuric acid. Titrate with *1 M sodium hydroxide*, using 0.1 mL of *methyl red solution R* as indicator.

1 mL of *1 M sodium hydroxide* is equivalent to 49.04 mg of H_2SO_4.

Sulfuric acid, dilute R1. *1086810.*

Contains 4.9 g/L of H_2SO_4.

Prepared from *sulfuric acid R*.

Sulfuric acid-formaldehyde reagent. *1086805.*

Mix 2 mL of *formaldehyde solution R* with 100 mL of *sulfuric acid R*.

Sulfuric acid, heavy metal-free. *1086807.*

Complies with the requirements prescribed for *sulfuric acid R* with the following maximum contents of heavy metals.

As: 0.005 ppm.

Cd: 0.002 ppm.

Cu: 0.001 ppm.

Fe: 0.05 ppm.
Hg: 0.005 ppm.
Ni: 0.002 ppm.
Pb: 0.001 ppm.
Zn: 0.005 ppm.

Sulfuric acid, nitrogen-free. *1086806.*
Complies with the requirements prescribed for *sulfuric acid R* with the following additional test.
Nitrates. To 5 mL of *water R* add carefully 45 mL of the sulfuric acid, allow to cool to 40 °C and add 8 mg of *diphenylbenzidine R*. The solution is colourless or very pale blue.

Sulfuric acid, nitrogen-free R1. *1086808.*
Complies with the requirements prescribed for *nitrogen-free sulfuric acid R*.
Content: 95.0 per cent *m/m* to 95.5 per cent *m/m*.

Sulfuric acid R1. H_2SO_4. (M_r 98.1). *1190900.* [7664-93-9].
Content: 75 per cent *V/V*.

Sunflower oil. *1086900.*
See *Sunflower oil, refined (1371)*.

Swertiamarin. $C_{16}H_{22}O_{10}$. (M_r 374.3). *1163600.* [17388-39-5]. Swertiamaroside. (4*R*,5*R*,6*S*)-5-Ethenyl-6-(β-D-glucopyranosyloxy)-4a-hydroxy-4,4a,5,6-tetrahydro-1*H*,3*H*-pyrano[3,4-*c*]pyran-1-one.

Tagatose. $C_6H_{12}O_6$. (M_r 180.16). *1111000.* [87-81-0]. D-*lyxo*-Hexulose.
White or almost white powder.
$[\alpha]_D^{20}$: – 2.3 determined on a 21.9 g/L solution.
mp: 134 °C to 135 °C.

Talc. *1087000.* [14807-96-6].
See *Talc (0438)*.

Tannic acid. *1087100.* [1401-55-4].
Yellowish or light-brown, glistening scales or amorphous powder, very soluble in water, freely soluble in ethanol (96 per cent), soluble in acetone.
Storage: protected from light.

Tanshinone II_A. $C_{19}H_{18}O_3$. (M_r 294.3). *1184800.* [568-72-9].
1,6,6-Trimethyl-6,7,8,9-tetrahydrophenanthro[1,2-*b*]furan-10,11-dione.

Tartaric acid. *1087200.* [87-69-4].
See *Tartaric acid (0460)*.

Taxifolin. $C_{15}H_{12}O_7$. (M_r 304.3). *1151800.* [480-18-2].
(2*R*,3*R*)-2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-2,3-dihydro-4*H*-1-benzopyran-4-one.
White or almost white powder, slightly soluble in anhydrous ethanol.
Absorbance (*2.2.25*). A solution in *anhydrous ethanol R* shows an absorption maximum at 290 nm.

Tecnazene. $C_6HCl_4NO_2$. (M_r 260.9). *1132400.* [117-18-0].
bp: about 304 °C.
mp: 99 °C to 100 °C.
A suitable certified reference solution (10 ng/µL in cyclohexane) may be used.

***trans*-Terpin.** $C_{10}H_{20}O_2$. (M_r 172.3). *1205800.* [565-50-4].
(1*r*,4*r*)-4-(2-Hydroxypropan-2-yl)-1-methylcyclohexan-1-ol.
p-Menthane-1,8-diol.
mp: about 116 °C.

α-Terpinene. $C_{10}H_{16}$. (M_r 136.2). *1140300.* [99-86-5].
1-Isopropyl-4-methylcyclohexa-1,3-diene.
Clear, almost colourless liquid.
d_4^{20}: about 0.837.
n_D^{20}: about 1.478.
bp: about 174 °C.
α-Terpinene used in gas chromatography complies with the following additional test.
Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Tea tree oil (1837)*.
Content: minimum 90 per cent, calculated by the normalisation procedure.

γ-Terpinene. $C_{10}H_{16}$. (M_r 136.2). *1115900.* [99-85-4].
1-Isopropyl-4-methylcyclohexa-1,4-diene.
Oily liquid.
γ-Terpinene used in gas chromatography complies with the following additional test.
Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Peppermint oil (0405)*.
Test solution. The substance to be examined.
Content: minimum 93.0 per cent, calculated by the normalisation procedure.

Terpinen-4-ol. $C_{10}H_{18}O$. (M_r 154.2). *1116000.* [562-74-3].
4-Methyl-1-(1-methylethyl)cyclohex-3-en-1-ol.
p-Menth-1-en-4-ol.
Oily, colourless liquid.
Terpinen-4-ol used in gas chromatography complies with the following additional test.
Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Lavender oil (1338)*.
Test solution. The substance to be examined.
Content: minimum 90.0 per cent, calculated by the normalisation procedure.

α-Terpineol. $C_{10}H_{18}O$. (M_r 154.2). *1087300.* [98-55-5].
(*RS*)-2-(4-Methylcyclohex-3-enyl)-2-propanol.
Colourless crystals, practically insoluble in water, soluble in ethanol (96 per cent).
d_{20}^{20}: about 0.935.
n_D^{20}: about 1.483.
$[\alpha]_D^{20}$: about 92.5.
mp: about 35 °C.
It may contain 1 to 3 per cent of β-terpineol.
α-Terpineol used in gas chromatography complies with the following test.
Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Anise oil (0804)*.
Test solution. A 100 g/L solution in *hexane R*.
Content: minimum 97.0 per cent, calculated by the normalisation procedure.

Terpinolene. $C_{10}H_{16}$. (M_r 136.2). *1140400.* [586-62-9].
p-Mentha-1,4(8)-diene. 4-Isopropylidene-1-methylcyclohexene.
Clear, almost colourless liquid.
d_4^{20}: about 0.863.
n_D^{20}: about 1.488.
bp: about 184 °C.
Terpinolene used in gas chromatography complies with the following additional test.
Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Tea tree oil (1837)*.
Content: minimum 90 per cent, calculated by the normalisation procedure.

Testosterone. *1116100.* [58-22-0].
See *Testosterone (1373)*.

Testosterone propionate. *1087400.* [57-85-2].

See *Testosterone propionate (0297)*.

1,2,3,4-Tetra-*O*-acetyl-β-D-glucopyranose. $C_{14}H_{20}O_{10}$. (M_r 348.3). *1172600.* [13100-46-4].

White or almost white powder, soluble in water with gentle heating.

$[\alpha]_D^{20}$: + 11, determined on a 6 g/L solution in *chloroform R*.

mp: 126 °C to 128 °C.

1,3,4,6-Tetra-*O*-acetyl-β-D-mannopyranose. $C_{14}H_{20}O_{10}$. (M_r 348.3). *1174100.* [18968-05-3].

Colourless or white powder or crystals.

mp: 160 °C to 161 °C.

$[\alpha]_D^{20}$: − 68, determined on a 7 g/L solution in *methylene chloride R*.

Tetrabutylammonium bromide. $C_{16}H_{36}BrN$. (M_r 322.4). *1087500.* [1643-19-2].

White or almost white crystals.

mp: 102 °C to 104 °C.

Tetrabutylammonium dihydrogen phosphate. $C_{16}H_{38}NO_4P$. (M_r 339.5). *1087600.* [5574-97-0].

White or almost white powder, hygroscopic.

pH (*2.2.3*): about 7.5 for a 170 g/L solution.

Absorbance (*2.2.25*): about 0.10 determined at 210 nm using a 170 g/L solution.

Storage: in an airtight container.

Tetrabutylammonium dihydrogen phosphate solution. *1087601.*

A 1.0 M solution of *tetrabutylammonium dihydrogen phosphate R*. This solution is commercially available.

Tetrabutylammonium hydrogen sulfate. $C_{16}H_{37}NO_4S$. (M_r 339.5). *1087700.* [32503-27-8].

Crystalline powder or colourless crystals, freely soluble in water and in methanol.

mp: 169 °C to 173 °C.

Absorbance (*2.2.25*): maximum 0.05, determined between 240 nm and 300 nm using a 50 g/L solution.

Tetrabutylammonium hydrogen sulfate R1. *1087701.*

Complies with the requirements prescribed for *tetrabutylammonium hydrogen sulfate R* with the following additional requirement.

Absorbance (*2.2.25*): maximum 0.02, determined between 215 nm and 300 nm using a 50 g/L solution.

Tetrabutylammonium hydroxide. $C_{16}H_{37}NO,30H_2O$. (M_r 800). *1087800.* [147741-30-8].

Content: minimum 98.0 per cent of $C_{16}H_{37}NO,30H_2O$.

White or almost white crystals, soluble in water.

Assay. Dissolve 1.000 g in 100 mL of *water R*. Titrate immediately with *0.1 M hydrochloric acid* determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

1 mL of *0.1 M hydrochloric acid* is equivalent to 80.0 mg of $C_{16}H_{37}NO,30H_2O$.

Tetrabutylammonium hydroxide solution (104 g/L). *1087801.*

A solution containing 104 g/L of $C_{16}H_{37}NO$ (M_r 259.5), prepared by dilution of a suitable reagent grade.

Tetrabutylammonium hydroxide solution (400 g/L). *1087802.*

A solution containing 400 g/L of $C_{16}H_{37}NO$ (M_r 259.5) of a suitable grade.

Tetrabutylammonium iodide. $C_{16}H_{36}IN$. (M_r 369.4). *1087900.* [311-28-4].

Content: minimum 98.0 per cent.

White or slightly coloured, crystalline powder or crystals, soluble in ethanol (96 per cent).

Sulfated ash (*2.4.14*): maximum 0.02 per cent.

Assay. Dissolve 1.200 g in 30 mL of *water R*. Add 50.0 mL of *0.1 M silver nitrate* and 5 mL of *dilute nitric acid R*. Titrate the excess of silver nitrate with *0.1 M ammonium thiocyanate*, using 2 mL of *ferric ammonium sulfate solution R2* as indicator.

1 mL of *0.1 M silver nitrate* is equivalent to 36.94 mg of $C_{16}H_{36}IN$.

Tetrachloroethane. $C_2H_2Cl_4$. (M_r 167.9). *1088000.* [79-34-5]. 1,1,2,2-Tetrachloroethane.

Clear, colourless liquid, slightly soluble in water, miscible with ethanol (96 per cent).

d_{20}^{20}: about 1.59.

n_D^{20}: about 1.495.

Distillation range (*2.2.11*). Not less than 95 per cent distils between 145 °C and 147 °C.

Tetrachlorvinphos. $C_{10}H_9Cl_4O_4P$. (M_r 366.0). *1132500.* [22248-79-9].

mp: about 95 °C.

A suitable certified reference solution (10 ng/µL in iso-octane) may be used.

Tetracos-15-enoic acid methyl ester. $C_{25}H_{48}O_2$. (M_r 380.7). *1144800.* [2733-88-2]. 15-Tetracosaenoic acid methyl ester. Methyl tetracos-15-enoate. Nervonic acid methyl ester.

Content: minimum 99.0 per cent, determined by gas chromatography.

Liquid.

Tetracycline hydrochloride. *1147000.*

See *Tetracycline hydrochloride (0210)*.

Tetradecane. $C_{14}H_{30}$. (M_r 198.4). *1088200.* [629-59-4]. *n*-Tetradecane.

Content: minimum 99.5 per cent *m/m*.

A colourless liquid.

d_{20}^{20}: about 0.76.

n_D^{20}: about 1.429.

bp: about 252 °C.

mp: about − 5 °C.

Tetradecylammonium bromide. $C_{40}H_{84}BrN$. (M_r 659). *1088300.* [14937-42-9]. Tetrakis(decyl)ammonium bromide.

White or slightly coloured, crystalline powder or crystals.

mp: 88 °C to 89 °C.

Tetraethylammonium hydrogen sulfate. $C_8H_{21}NO_4S$. (M_r 227.3). *1116200.* [16873-13-5].

Hygroscopic powder.

mp: about 245 °C.

Tetraethylammonium hydroxide solution. $C_8H_{21}NO$. (M_r 147.3). *1100300.* [77-98-5].

A 200 g/L solution.

Colourless liquid, strongly alkaline.

d_{20}^{20}: about 1.01.

n_D^{20}: about 1.372.

HPLC grade.

Tetraethylene pentamine. $C_8H_{23}N_5$. (M_r 189.3). *1102000.* [112-57-2]. 3,6,9-Triazaundecan-1,11-diamine.

Colourless liquid, soluble in acetone.

n_D^{20}: about1.506.

Storage: protected from humidity and heat.

Tetraheptylammonium bromide. $C_{28}H_{60}BrN$. (M_r 490.7). *1088400.* [4368-51-8].

White or slightly coloured, crystalline powder or crystals.

mp: 89 °C to 91 °C.

Tetrahexylammonium bromide. $C_{24}H_{52}BrN$. (M_r 434.6). *1152500.* [4328-13-6]. *N,N,N*-Trihexylhexan-1-aminium bromide.

White or almost white, crystalline powder, hygroscopic.

mp: about 100 °C.

Tetrahexylammonium hydrogen sulfate. $C_{24}H_{53}NO_4S$. (M_r 451.8). *1116300.* [32503-34-7]. *N,N,N*-Trihexylhexan-1-aminium hydrogen sulfate.

White or almost white crystals.

mp: 100 °C to 102 °C.

Tetrahydrofuran. C_4H_8O. (M_r 72.1). *1088500.* [109-99-9]. Tetramethylene oxide.

Clear, colourless, flammable liquid, miscible with water, with ethanol (96 per cent).

d_{20}^{20}: about 0.89.

Do not distil if the tetrahydrofuran does not comply with the test for peroxides.

Peroxides. Place 8 mL of *potassium iodide and starch solution R* in a 12 mL ground-glass-stoppered cylinder about 1.5 cm in diameter. Fill completely with the substance to be examined, shake vigorously and allow to stand protected from light for 30 min. No colour is produced.

Tetrahydrofuran used in spectrophotometry complies with the following additional test.

Absorbance (*2.2.25*): maximum 0.70 at 255 nm, 0.10 at 270 nm, 0.01 at 310 nm, determined using *water R* as compensation liquid.

Tetrahydrofuran for chromatography. *1147100.*

Complies with the requirements prescribed for *tetrahydrofuran R* with the following additional requirements:

d_4^{20} = 0.8892.

bp: about 66 °C.

Content: minimum 99.8 per cent of C_4H_8O.

Tetrahydropalmatine. $C_{21}H_{25}NO_4$. (M_r 355.4). *1205900.* [2934-97-6]. (13a*RS*)-5,8,13,13a-Tetrahydro-2,3,9,10-tetramethoxy-6*H*-dibenzo[*a*,*g*]quinolizine.

α-Tetralone. $C_{10}H_{10}O$. (M_r 146.2). *1171800.* [529-34-0]. 1-Oxotetraline. 3,4-Dihydronaphthalen-1(2*H*)-one.

bp: about 115 °C.

mp: about 5 °C.

Tetramethylammonium bromide. $C_4H_{12}BrN$. (M_r 154.1). *1156600.* [64-20-0]. *N,N,N*-Trimethylmethanaminium bromide.

White or slightly yellow crystals, freely soluble in water.

mp: about 285 °C, with decomposition.

Tetramethylammonium chloride. $C_4H_{12}ClN$. (M_r 109.6). *1100400.* [75-57-0].

Colourless crystals, soluble in water and in ethanol (96 per cent).

mp: about 300 °C, with decomposition.

Tetramethylammonium hydrogen sulfate. $C_4H_{13}NO_4S$. (M_r 171.2). *1116400.* [80526-82-5].

Hygroscopic powder.

mp: about 295 °C.

Tetramethylammonium hydroxide. $C_4H_{13}NO,5H_2O$. (M_r 181.2). *1122800.* [10424-65-4]. Tetramethylammonium hydroxide pentahydrate.

Suitable grade for HPLC.

Tetramethylammonium hydroxide solution. *1088600.* [75-59-2].

Content: minimum 10.0 per cent *m/m* of $C_4H_{13}NO$. (M_r 91.2).

Clear, colourless or very pale yellow liquid, miscible with water and with ethanol (96 per cent).

Assay. To 1.000 g add 50 mL of *water R* and titrate with *0.05 M sulfuric acid*, using 0.1 mL of *methyl red solution R* as indicator.

1 mL of *0.05 M sulfuric acid* is equivalent to 9.12 mg of $C_4H_{13}NO$.

Tetramethylammonium hydroxide solution, dilute. *1088601.*

Dilute 10 mL of *tetramethylammonium hydroxide solution R* to 100 mL with *aldehyde-free alcohol R*. Prepare immediately before use.

Tetramethylbenzidine. $C_{16}H_{20}N_2$. (M_r 240.3). *1132600.* [54827-17-7]. 3,3′,5,5′-Tetramethylbiphenyl-4,4′-diamine.

Powder, practically insoluble in water, very soluble in methanol.

mp: about 169 °C.

1,1,3,3-Tetramethylbutylamine. $C_8H_{19}N$. (M_r 129.3). *1141500.* [107-45-9]. 2-Amino-2,4,4-trimethylpentane.

Clear, colourless liquid.

d_{20}^{20}: about 0.805.

n_D^{20}: about 1.424.

bp: about 140 °C.

Tetramethyldiaminodiphenylmethane. $C_{17}H_{22}N_2$. (M_r 254.4). *1088700.* [101-61-1]. 4,4′-Methylenebis-(*N,N*-dimethylaniline).

White or bluish-white crystals or leaflets, practically insoluble in water, slightly soluble in ethanol (96 per cent), soluble in mineral acids.

mp: about 90 °C.

Tetramethyldiaminodiphenylmethane reagent. *1088701.*

Solution A. Dissolve 2.5 g of *tetramethyldiaminodiphenylmethane R* in 10 mL of *glacial acetic acid R* and add 50 mL of *water R*.

Solution B. Dissolve 5 g of *potassium iodide R* in 100 mL of *water R*.

Solution C. Dissolve 0.30 g of *ninhydrin R* in 10 mL of *glacial acetic acid R* and add 90 mL of *water R*.

Mix solution A, solution B and 1.5 mL of solution C.

Tetramethylethylenediamine. $C_6H_{16}N_2$. (M_r 116.2). *1088800.* [110-18-9]. *N,N,N′,N′*-Tetramethylethylenediamine.

Colourless liquid, miscible with water and with ethanol (96 per cent).

d_{20}^{20}: about 0.78.

n_D^{20}: about 1.418.

bp: about 121 °C.

Tetramethylsilane. $C_4H_{12}Si$. (M_r 88.2). *1088900.* [75-76-3]. TMS.

Clear, colourless liquid, very slightly soluble in water, soluble in acetone and in ethanol (96 per cent).

d_{20}^{20}: about 0.64.

n_D^{20}: about 1.358.

bp: about 26 °C.

Tetramethylsilane used in nuclear magnetic resonance spectrometry complies with the following additional test.

In the nuclear magnetic resonance spectrum of an approximately 10 per cent *V/V* solution of the tetramethylsilane in *deuterated chloroform R*, the intensity of any foreign signal, excluding those due to spinning side bands

and to chloroform, is not greater than the intensity of the C-13 satellite signals located at a distance of 59.1 Hz on each side of the principal signal of tetramethylsilane.

Tetrandrine. $C_{38}H_{42}N_2O_6$. (M_r 623). *1178500.* [518-34-3].

Tetrapropylammonium chloride. $C_{12}H_{28}ClN$. (M_r 221.8). *1151900.* [5810-42-4].

White or almost white, crystalline powder, sparingly soluble in water.

mp: about 241 °C.

Tetrapropylammonium hydrogen sulfate. $C_{12}H_{29}NO_4S$. (M_r 283.4). *1191300.* [56211-70-2]. *N,N,N*-Tripropylpropan-1-aminium hydrogen sulfate.

White or almost white, crystalline, hygroscopic powder.

Tetrazolium blue. $C_{40}H_{32}Cl_2N_8O_2$. (M_r 728). *1089000.* [1871-22-3]. 3,3′-(3,3′-Dimethoxy[1,1′-biphenyl]-4,4′-diyl)bis[2,5-diphenyl-2*H*-tetrazolium] dichloride.

Yellow crystals, slightly soluble in water, freely soluble in ethanol (96 per cent) and in methanol, practically insoluble in acetone.

mp: about 245 °C, with decomposition.

Tetrazolium bromide. $C_{18}H_{16}BrN_5S$. (M_r 414.3). *1152700.* [298-93-1]. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide. MTT.

Tetrazolium salt. $C_{20}H_{17}N_5O_6S_2$. (M_r 487.5). *1174200.* [138169-43-4]. 5-(3-Carboxymethoxyphenyl)-3-(4,5-dimethylthiazol-2-yl)-2-(4-sulfophenyl)-2*H*-tetrazolium, inner salt. MTS.

Thallous sulfate. Tl_2SO_4. (M_r 504.8). *1089100.* [7446-18-6]. Dithallium sulfate.

White or almost white, rhomboid prisms, slightly soluble in water, practically insoluble in ethanol (96 per cent).

Thebaine. $C_{19}H_{21}NO_3$. (M_r 311.4). *1089200.* [115-37-7]. (5*R*,9*R*,13*S*)-4,5-Epoxy-3,6-dimethoxy-9a-methylmorphina-6,8-diene.

White or pale yellow, crystalline powder, very slightly soluble in water, soluble in hot anhydrous ethanol and in toluene.

mp: about 193 °C.

Chromatography (*2.2.27*). Thin-layer chromatography (*2.2.27*) as prescribed in identification test B in the monograph *Raw opium (0777)*: apply to the plate as a band (20 mm × 3 mm) 20 µL of a 0.5 g/L solution; the chromatogram shows an orange-red or red principal band with an R_F of about 0.5.

Theobromine. *1138800.* [83-67-0].

See *Theobromine (0298)*.

Theophylline. *1089300.* [58-55-9].

See *Theophylline (0299)*.

Thiamazole. $C_4H_6N_2S$. (M_r 114.2). *1089400.* [60-56-0]. Methimazole. 1-Methyl-1*H*-imidazole-2-thiol.

White or almost white, crystalline powder, freely soluble in water, soluble in ethanol (96 per cent) and in methylene chloride.

mp: about 145 °C.

2-(2-Thienyl)acetic acid. $C_6H_6O_2S$. (M_r 142.1). *1089500.* [1918-77-0].

Brown powder.

mp: about 65 °C.

Thioacetamide. C_2H_5NS. (M_r 75.1). *1089600.* [62-55-5].

Crystalline powder or colourless crystals, freely soluble in water and in ethanol (96 per cent).

mp: about 113 °C.

Thioacetamide reagent. *1089601.*

To 0.2 mL of *thioacetamide solution R* add 1 mL of a mixture of 5 mL of *water R*, 15 mL of *1 M sodium hydroxide* and 20 mL of *glycerol (85 per cent) R*. Heat in a water-bath for 20 s. Prepare immediately before use.

Thioacetamide solution. *1089602.*

A 40 g/L solution of *thioacetamide R*.

Thiobarbituric acid. $C_4H_4N_2O_2S$. (M_r 144.2). *1111200.* [504-17-6]. 4,6-Dihydroxy-2-sulfanylpyrimidine.

Thiodiethylene glycol. $C_4H_{10}O_2S$. (M_r 122.2). *1122900.* [111-48-8]. Di(2-hydroxyethyl) sulfide.

Colourless or yellow, viscous liquid.

Content: minimum 99.0 per cent.

d_{20}^{20}: about 1.18.

Thioglycollic acid. $C_2H_4O_2S$. (M_r 92.1). *1089700.* [68-11-1]. 2-Mercaptoacetic acid.

Colourless liquid, miscible with water, soluble in ethanol (96 per cent).

Thiomalic acid. $C_4H_6O_4S$. (M_r 150.2). *1161600.* [70-49-5]. (2*RS*)-2-Sulfanylbutanedioic acid.

mp: 150 °C to 152 °C.

Thiomersal. $C_9H_9HgNaO_2S$. (M_r 404.8). *1089800.* [54-64-8]. Sodium mercurothiolate. Sodium 2-[(ethylmercurio)thio]benzoate.

Light, yellowish-white, crystalline powder, very soluble in water, freely soluble in ethanol (96 per cent).

Thiourea. CH_4N_2S. (M_r 76.1). *1089900.* [62-56-6].

White or almost white, crystalline powder or crystals, soluble in water and in ethanol (96 per cent).

mp: about 178 °C.

Threonine. *1090000.* [72-19-5].

See *Threonine (1049)*.

Thrombin, bovine. *1090200.* [9002-04-4].

A preparation of the enzyme, obtained from bovine plasma, that converts fibrinogen into fibrin.

A yellowish-white powder.

Storage: at a temperature below 0 °C.

Thrombin, human. *1090100.* [9002-04-4].

Dried human thrombin. A preparation of the enzyme which converts human fibrinogen into fibrin. It is obtained from liquid human plasma and may be prepared by precipitation with suitable salts and organic solvents under controlled conditions of pH, ionic strength and temperature.

Yellowish-white powder, freely soluble in a 9 g/L solution of sodium chloride forming a cloudy, pale yellow solution.

Storage: in a sealed, sterile container under nitrogen, protected from light, at a temperature below 25 °C.

Thrombin solution, human. *1090101.*

Reconstitute *human thrombin R* as directed by the manufacturer and dilute to 5 IU/mL with *tris(hydroxymethyl)aminomethane sodium chloride buffer solution pH 7.4 R*.

Thrombin solution, human R1. *1090102.*

Reconstitute *human thrombin R* as directed by the manufacturer and dilute to 2.5 IU/mL with *phosphate buffer solution pH 6.5 R*.

Thrombin solution, human R2. *1090103.*

Reconstitute *human thrombin R* as directed by the manufacturer and dilute to 5 IU/mL with *tris(hydroxymethyl)aminomethane-EDTA buffer solution pH 8.4 R1*.

Thromboplastin. *1090300.*

A preparation containing the membrane glycoprotein tissue factor and phospholipid, either purified from animal brain (usually rabbit) or human placenta or manufactured using recombinant DNA technology with added phospholipid. The preparation is formulated for routine use in the prothrombin time test and may contain calcium.

Thujone. $C_{10}H_{16}O$. (M_r 152.2). *1116500.* [76231-76-0]. 4-Methyl-1-(1-methylethyl)bicyclo[3.1.0]hexan-3-one.

Colourless or almost colourless liquid, practically insoluble in water, soluble in ethanol (96 per cent) and in many other organic solvents.

Thymidine. $C_{10}H_{14}N_2O_5$. (M_r 242.2). *1158900.* 1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione.

Needles, soluble in water, in hot ethanol (96 per cent) and in glacial acetic acid.

Thymine. $C_5H_6N_2O_2$. (M_r 126.1). *1090400.* [65-71-4]. 5-Methylpyrimidine-2,4(1*H*,3*H*)-dione.

Short needles or plates, slightly soluble in cold water, soluble in hot water. It dissolves in dilute solution of alkali hydroxides.

Thymol. *1090500.* [89-83-8]. See *Thymol (0791)*.

Thymol used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Peppermint oil (0405)*.

Test solution. Dissolve 0.1 g in about 10 mL of *acetone R*.

Content: minimum 95.0 per cent, calculated by the normalisation procedure.

Thymol blue. $C_{27}H_{30}O_5S$. (M_r 466.6). *1090600.* [76-61-9]. Thymolsulfonphthalein. 4,4′-(3*H*-2,1-Benzoxathiol-3-ylidene)bis(2-isopropyl-5-methylphenol) *S,S*-dioxide.

Brownish-green or greenish-blue, crystalline powder, slightly soluble in water, soluble in ethanol (96 per cent) and in dilute solutions of alkali hydroxides.

Thymol blue solution. *1090601.*

Dissolve 0.1 g of *thymol blue R* in a mixture of 2.15 mL of *0.1 M sodium hydroxide* and 20 mL of *ethanol (96 per cent) R* and dilute to 100 mL with *water R*.

Test for sensitivity. To 0.1 mL of the thymol blue solution add 100 mL of *carbon dioxide-free water R* and 0.2 mL of *0.02 M sodium hydroxide*. The solution is blue. Not more than 0.15 mL of *0.02 M hydrochloric acid* is required to change the colour to yellow.

Colour change: pH 1.2 (red) to pH 2.8 (yellow); pH 8.0 (olive-green) to pH 9.6 (blue).

Thymolphthalein. $C_{28}H_{30}O_4$. (M_r 430.5). *1090700.* [125-20-2]. 3,3-Bis(4-hydroxy-5-isopropyl-2-methylphenyl)-3*H*-isobenzo-furan-1-one.

White or yellowish-white powder, practically insoluble in water, soluble in ethanol (96 per cent) and in dilute solutions of alkali hydroxides.

Thymolphthalein solution. *1090701.*

A 1 g/L solution of *thymolphthalein R* in *ethanol (96 per cent) R*.

Test for sensitivity. To 0.2 mL of the thymolphthalein solution add 100 mL of *carbon dioxide-free water R*. The solution is colourless. Not more than 0.05 mL of *0.1 M sodium hydroxide* is required to change the colour to blue.

Colour change: pH 9.3 (colourless) to pH 10.5 (blue).

Tin. Sn. (A_r 118.7). *1090800.* [7440-31-5].

Silvery-white granules, soluble in hydrochloric acid with release of hydrogen.

Arsenic (*2.4.2, Method A*): maximum 10 ppm, determined on 0.1 g.

Tin test kit, semi-quantitative. *1194100.*

Commercially available set of reagents consisting of tin test strips and a reagent mixture for the determination of tin in aqueous solutions, in a range of 10-200 µg/mL.

Titan yellow. $C_{28}H_{19}N_5Na_2O_6S_4$. ($M_r$ 696). *1090900.* [1829-00-1].

Schultz No. 280.

Colour Index No. 19540.
Thiazol yellow. Disodium 2,2′-[(1-triazene-1,3-diyl)di-4,1-phenylene]bis-[6-methylbenzothiazole-7-sulfonate].

A yellowish-brown powder, freely soluble in water and in ethanol (96 per cent).

Titan yellow paper. *1090901.*

Immerse strips of filter paper in *titan yellow solution R* and leave for a few minutes. Allow to dry at room temperature.

Titan yellow solution. *1090902.*

A 0.5 g/L solution of *titan yellow R*.

Test for sensitivity. To 0.1 mL of the titan yellow solution add 10 mL of *water R*, 0.2 mL of *magnesium standard solution (10 ppm Mg) R* and 1.0 mL of *1 M sodium hydroxide*. A distinct pink colour is visible by comparison with a reference solution prepared in a similar manner omitting the magnesium.

Titanium. Ti. (A_r 47.88). *1091000.* [7440-32-6].

Content: minimum 99 per cent.

Metal powder, fine wire (diameter not more than 0.5 mm), sponge.

mp: about 1668 °C.

Density: about 4.507 g/cm^3.

Titanium dioxide. *1117900.* [13463-67-7].

See *Titanium dioxide (0150)*.

Titanium trichloride. $TiCl_3$. (M_r 154.3). *1091200.* [7705-07-9]. Titanium(III) chloride.

Reddish-violet crystals, deliquescent, soluble in water and in ethanol (96 per cent).

mp: about 440 °C.

Storage: in an airtight container.

Titanium trichloride solution. *1091201.*

d_{20}^{20}: about 1.19.

A 150 g/L solution of *titanium trichloride R* in hydrochloric acid (100 g/L HCl).

Titanium trichloride-sulfuric acid reagent. *1091202.*

Carefully mix 20 mL of *titanium trichloride solution R* with 13 mL of *sulfuric acid R*. Add sufficient *strong hydrogen peroxide solution R* to give a yellow colour. Heat until white fumes are evolved. Allow to cool. Dilute with *water R* and repeat the evaporation and addition of *water R* until a colourless solution is obtained. Dilute to 100 mL with *water R*.

TLC aluminium oxide G plate. *1165200.*

Support of metal, glass or plastic, coated with a layer of aluminium oxide (particle size 5-40 µm) containing about 10 per cent of calcium sulfate hemihydrate as a binder.

TLC cellulose plate. *1191400.*

Support of glass, metal or plastic, coated with a layer of cellulose.

TLC octadecylsilyl silica gel plate. *1148600.*

Support of glass, metal or plastic coated with a layer of octadecylsilyl silica gel. The plate may contain an organic binder.

TLC octadecylsilyl silica gel F_{254} plate. *1146600.*

Support of glass, metal or plastic coated with a layer of octadecylsilyl silica gel.

It contains a fluorescent indicator having a maximum absorbance in ultraviolet light at 254 nm.

TLC performance test solution. *1116600.*

Prepare a mixture of 1.0 mL of each of the following solutions and dilute to 10.0 mL with *acetone R*: a 0.5 g/L solution of *Sudan red G R* in *toluene R*, a 0.5 g/L solution of *methyl orange R* in *ethanol R* prepared immediately before use, a 0.5 g/L solution of *bromocresol green R* in *acetone R* and a 0.25 g/L solution of *methyl red R* in *acetone R*.

TLC silica gel plate. *1116700.*

Support of glass, metal or plastic, coated with a layer of silica gel of a suitable thickness and particle size (usually 2 µm to 10 µm for fine particle size [High Performance Thin-Layer Chromatography, HPTLC] plates and 5 µm to 40 µm for normal TLC plates). If necessary, the particle size is indicated after the name of the reagent in the tests where it is used.

The plate may contain an organic binder.

Chromatographic separation. Apply to the plate an appropriate volume (10 µL for a normal TLC plate and 1 µL to 2 µL for a fine particle size plate) of *TLC performance test solution R*. Develop over a pathlength two-thirds of the plate height, using a mixture of 20 volumes of *methanol R* and 80 volumes of *toluene R*. The plate is not satisfactory, unless the chromatogram shows four clearly separated spots, the spot of bromocresol green with an R_F value less than 0.15, the spot of methyl orange with an R_F value in the range of 0.1 to 0.25, the spot of methyl red with an R_F value in the range of 0.35 to 0.55 and the spot of Sudan red G with an R_F value in the range of 0.75 to 0.98.

TLC silica gel F_{254} plate. *1116800.*

Complies with the requirements prescribed for *TLC silica gel plate R* with the following modification.

It contains a fluorescent indicator having a maximum absorbance at 254 nm.

Fluorescence suppression. Apply separately to the plate at five points increasing volumes (1 µL to 10 µL for normal TLC plates and 0.2 µL to 2 µL for fine particle size plates) of a 1 g/L solution of *benzoic acid R* in a mixture of 15 volumes of *anhydrous ethanol R* and 85 volumes of *cyclohexane R*. Develop over a pathlength half of the plate height with the same mixture of solvents. After evaporating the solvents examine the chromatogram in ultraviolet light at 254 nm. For normal TLC plates the benzoic acid appears as dark spots on a fluorescent background approximately in the middle of the chromatogram for quantities of 2 µg and greater. For fine particle size plates the benzoic acid appears as dark spots on a fluorescent background approximately in the middle of the chromatogram for quantities of 0.2 µg and greater.

TLC silica gel F_{254}, silanised plate. *1117200.*

It complies with the requirements prescribed for *TLC silanised silica gel plate R* with the following modification.

It contains a fluorescent indicator having a maximum absorbance at 254 nm.

TLC silica gel G plate. *1116900.*

Complies with the requirements prescribed for *TLC silica gel plate R* with the following modification.

It contains calcium sulfate hemihydrate as binder.

TLC silica gel GF_{254} plate. *1117000.*

Complies with the requirements prescribed for *TLC silica gel plate R* with the following modifications.

It contains calcium sulfate hemihydrate as binder and a fluorescent indicator having a maximum absorbance at 254 nm.

Fluorescence suppression. Complies with the test prescribed for *TLC silica gel F_{254} plate R*.

TLC silica gel plate for aminopolyether test. *1172700.*

Immerse a *TLC silica gel plate R* in *iodoplatinate reagent R1* for 5-10 s. Dry at room temperature for 12 h, protected from light.

Storage: protected from light, in an open container; use within 30 days after preparation.

TLC silica gel plate for chiral separations, octadecylsilyl. *1137700.*

Support of glass, metal or plastic, coated with a layer of octadecylsilyl silica gel, impregnated with Cu^{2+} ions and enantiomerically pure hydroxyproline. The plate may contain an organic binder.

TLC silica gel, silanised plate. *1117100.*

Support of glass, metal or plastic, coated with a layer of silanised silica gel of a suitable thickness and particle size (usually 2 µm to 10 µm for fine particle size [High Performance Thin-Layer Chromatography, HPTLC] plates and 5 µm to 40 µm for normal TLC plates). If necessary, the particle size is indicated after the name of the reagent in the tests where it is used.

The plate may contain an organic binder.

Chromatographic separation. Introduce 0.1 g each of *methyl laurate R*, *methyl myristate R*, *methyl palmitate R* and *methyl stearate R* into a 250 mL conical flask. Add 40 mL of *alcoholic potassium hydroxide solution R* and heat under a reflux condenser on a water-bath for 1 h. Allow to cool, transfer the solution to a separating funnel by means of 100 mL of *water R*, acidify (pH 2 to 3) with *dilute hydrochloric acid R* and shake with three quantitites each of 10 mL of *methylene chloride R*. Dry the combined methylene chloride extracts over *anhydrous sodium sulfate R*, filter and evaporate to dryness on a water-bath. Dissolve the residue in 50 mL of *methylene chloride R*. Examine by thin-layer chromatography (*2.2.27*), using *TLC silanised silica gel plate R*. Apply an appropriate quantity (about 10 µL for normal TLC plates and about 1 µL to 2 µL for fine particle size plates) of the methylene chloride solution at each of three separate points. Develop over a pathlength two-thirds of the plate height with a mixture of 10 volumes of *glacial acetic acid R*, 25 volumes of *water R* and 65 volumes of *dioxan R*. Dry the plate at 120 °C for 30 min. Allow to cool, spray with a 35 g/L solution of *phosphomolybdic acid R* in *2-propanol R* and heat at 150 °C until the spots become visible. Treat the plate with ammonia vapour until the background is white. The chromatograms show four clearly separated, well-defined spots.

α-Tocopherol. *1152300.* [10191-41-0].

See *all-rac-α-Tocopherol (0692)*.

α-Tocopheryl acetate. *1152400.* [7695-91-2].

See *all-rac-α-Tocopheryl acetate (0439)*.

***o*-Tolidine.** $C_{14}H_{16}N_2$. (M_r 212.3). *1123000.* [119-93-7]. 3,3′-Dimethylbenzidine.

Content: minimum 97.0 per cent.

Light brownish, crystalline power.

mp: about 130 °C.

***o*-Tolidine solution.** *1123001.*

Dissolve 0.16 g of *o-tolidine R* in 30.0 mL of *glacial acetic acid R*, add 1.0 g of *potassium iodide R* and dilute to 500.0 mL with *water R*.

Toluene. C_7H_8. (M_r 92.1). *1091300.* [108-88-3]. Methylbenzene.

Clear, colourless, flammable liquid, very slightly soluble in water, miscible with ethanol (96 per cent).

d_{20}^{20}: 0.865 to 0.870.

bp: about 110 °C.

Toluene, sulfur-free. *1091301.*

Complies with the requirements prescribed for *toluene R* with the following additional requirements.

Sulfur compounds. To 10 mL add 1 mL of *anhydrous ethanol R* and 3 mL of *potassium plumbite solution R* and boil under a reflux condenser for 15 min. Allow to stand for 5 min. No darkening is produced in the aqueous layer.

Thiophen-related substances. Shake 2 mL with 5 mL of *isatin reagent R* for 5 min and allow to stand for 15 min. No blue colour is produced in the lower layer.

Toluenesulfonamide. $C_7H_9NO_2S$. (M_r 171.2). *1091500.* [70-55-3]. 4-Methylbenzenesulfonamide. *p*-Toluenesulfonamide.

Content: minimum 99.0 per cent.

White or almost white, crystalline powder, slightly soluble in water, soluble in ethanol (96 per cent) and in solutions of alkali hydroxides.

mp: about 136 °C.

***o*-Toluenesulfonamide.** $C_7H_9NO_2S$. (M_r 171.2). *1091400.* [88-19-7]. 2-Methylbenzenesulfonamide.

White or almost white, crystalline powder, slightly soluble in water, soluble in ethanol (96 per cent) and in solutions of alkali hydroxides.

mp: about 156 °C.

***p*-Toluenesulfonamide.** *1091500.* [70-55-3].

See *toluenesulfonamide R*.

Toluenesulfonic acid. $C_7H_8O_3S,H_2O$. (M_r 190.2). *1091600.* [6192-52-5]. 4-Methylbenzenesulfonic acid.

Content: minimum 87.0 per cent of $C_7H_8O_3S$.

White or almost white, crystalline powder or crystals, freely soluble in water, soluble in ethanol (96 per cent).

Toluenesulfonylurea. $C_8H_{10}N_2O_3S$. (M_r 214.2). *1177000.* [1694-06-0]. 4-Methylbenzenesulfonylurea. *p*-Toluenesulfonylurea. (4-Methylphenyl)sulfonylurea.

White or almost white, crystalline powder.

mp: 196 to 198 °C.

***o*-Toluidine.** C_7H_9N. (M_r 107.2). *1091700.* [95-53-4]. 2-Methylaniline.

Pale-yellow liquid becoming reddish-brown on exposure to air and light, slightly soluble in water, soluble in ethanol (96 per cent) and in dilute acids.

d_{20}^{20}: about 1.01.

n_D^{20}: about 1.569.

bp: about 200 °C.

Storage: in an airtight container, protected from light.

***o*-Toluidine hydrochloride.** $C_7H_{10}ClN$. (M_r 143.6). *1117300.* [636-21-5]. 2-Methylaniline hydrochloride. 2-Methylbenzenamine hydrochloride.

Content: minimum 98.0 per cent.

mp: 215 °C to 217 °C.

***p*-Toluidine.** C_7H_9N. (M_r 107.2). *1091800.* [106-49-0]. 4-Methylaniline.

Lustrous plates or flakes, slightly soluble in water, freely soluble in acetone and in ethanol (96 per cent).

mp: about 44 °C.

Toluidine blue. $C_{15}H_{16}ClN_3S$. (M_r 305.8). *1091900.* [92-31-9]. Schultz No. 1041.

Colour Index No. 52040.
Toluidine Blue O. 3-Amino-7-dimethylamino-2-methylphenothiazin-5-ium chloride.

Dark-green powder, soluble in water, slightly soluble in ethanol (96 per cent).

Tosylarginine methyl ester hydrochloride. $C_{14}H_{23}ClN_4O_4S$. (M_r 378.9). *1092000.* [1784-03-8]. *N*-Tosyl-L-arginine methyl ester hydrochloride. Methyl (*S*)-5-guanidino-2-(4-methylbenzenesulfonamido)valerate hydrochloride.

$[\alpha]_D^{20}$: − 12 to − 16, determined on a 40 g/L solution.

mp: about 145 °C.

Tosylarginine methyl ester hydrochloride solution. *1092001.*

To 98.5 mg of *tosylarginine methyl ester hydrochloride R* add 5 mL of *tris(hydroxymethyl)aminomethane buffer solution pH 8.1 R* and shake to dissolve. Add 2.5 mL of *methyl red mixed solution R* and dilute to 25.0 mL with *water R*.

Tosyl-lysyl-chloromethane hydrochloride. $C_{14}H_{22}Cl_2N_2O_3S$. (M_r 369.3). *1092100.* [4238-41-9]. *N*-Tosyl-L-lysyl-chloromethane hydrochloride. (3*S*)-7-Amino-1-chloro-3-(4-methylbenzenesulfonamido)heptan-2-one hydrochloride.

$[\alpha]_D^{20}$: − 7 to − 9, determined on a 20 g/L solution.

mp: about 155 °C, with decomposition.

$A_{1\text{ cm}}^{1\%}$: 310 to 340, determined at 230 nm in *water R*.

Tosylphenylalanylchloromethane. $C_{17}H_{18}ClNO_3S$. (M_r 351.9). *1092200.* [402-71-1]. *N*-Tosyl-L-phenylalanylchloromethane.

$[\alpha]_D^{20}$: − 85 to − 89, determined on a 10 g/L solution in *ethanol (96 per cent) R*.

mp: about 105 °C.

$A_{1\text{ cm}}^{1\%}$: 290 to 320, determined at 228.5 nm in *ethanol (96 per cent) R*.

Toxaphene. *1132800.* [8001-35-2].

A mixture of polychloro derivatives.

mp: 65 °C to 90 °C.

A suitable certified reference solution (10 ng/µL in iso-octane) may be used.

Tragacanth. *1092300.* [9000-65-1].

See *Tragacanth (0532)*.

Triacetin. $C_9H_{14}O_6$. (M_r 218.2). *1092400.* [102-76-1]. Propane-1,2,3-triyl triacetate. Glycerol triacetate.

Almost clear, colourless to yellowish liquid, soluble in water, miscible with ethanol (96 per cent).

d_{20}^{20}: about 1.16.

n_D^{20}: about 1.43.

bp: about 260 °C.

Triamcinolone. $C_{21}H_{27}FO_6$. (M_r 394.4). *1111300.* [124-94-7]. 9-Fluoro-11β,16α,17,21-tetrahydroxypregna-1,4-diene-3,20-dione.

A crystalline powder.

mp: 262 °C to 263 °C.

Triamcinolone acetonide. *1133100.* [76-25-5].

See *Triamcinolone acetonide (0533)*.

Tribromophenol. $C_6H_3Br_3O$. (M_r 330.8). *1165300.* [118-79-6]. 2,4,6-Tribromophenol.

Tributyl citrate. $C_{18}H_{32}O_7$. (M_r 360.4). *1152800.* [77-94-1]. Tributyl 2-hydroxypropane-1,2,3-tricarboxylate.

d_4^{20}: about 1.043.

n_D^{20}: about 1.445.

Tributyl phosphate. $C_{12}H_{27}O_4P$. (M_r 266.3). *1179900.* [126-73-8]. Tributoxyphosphine oxide. Tributoxyphosphane oxide.

Colourless liquid, slightly soluble in water, soluble in the usual organic solvents.

d_{25}^{25}: about 0.976.

n_D^{25}: about 1.422.

bp: about 289 °C, with decomposition.

Tributylphosphine. $C_{12}H_{27}P$. (M_r 202.3). *1187100.* [998-40-3].

Clear, colourless liquid.

bp: about 240 °C.

mp: about − 60 °C.

Trichloroacetic acid. $C_2HCl_3O_2$. (M_r 163.4). *1092500.* [76-03-9].

Colourless crystals or a crystalline mass, very deliquescent, very soluble in water and in ethanol (96 per cent).

Storage: in an airtight container.

Trichloroacetic acid solution. *1092501.*

Dissolve 40.0 g of *trichloroacetic acid R* in *water R* and dilute to 1000.0 mL with the same solvent. Verify the concentration by titration with *0.1 M sodium hydroxide* and adjust if necessary to 40 ± 1 g/L.

1,1,1-Trichloroethane. $C_2H_3Cl_3$. (M_r 133.4). *1092600.* [71-55-6]. Methylchloroform.

Non-flammable liquid, practically insoluble in water, soluble in acetone and in methanol.

d_{20}^{20}: about 1.34.

n_D^{20}: about 1.438.

bp: about 74 °C.

Trichloroethylene. C_2HCl_3. (M_r 131.4). *1102100.* [79-01-6].

Colourless liquid, practically insoluble in water, miscible with ethanol (96 per cent).

d_{20}^{20}: about 1.46.

n_D^{20}: about 1.477.

Trichlorotrifluoroethane. $C_2Cl_3F_3$. (M_r 187.4). *1092700.* [76-13-1]. 1,1,2-Trichloro-1,2,2-trifluoroethane.

Colourless, volatile liquid, practically insoluble in water, miscible with acetone.

d_{20}^{20}: about 1.58.

Distillation range (*2.2.11*). Not less than 98 per cent distils between 47 °C and 48 °C.

Tricine. $C_6H_{13}NO_5$. (M_r 179.2). *1138900.* [5704-04-1]. *N*-[2-Hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine.

Use electrophoresis-grade reagent.

mp: about 183 °C.

Tricosane. $C_{23}H_{48}$. (M_r 324.6). *1092800.* [638-67-5].

White or almost white crystals, practically insoluble in water, soluble in hexane.

mp: about 48 °C.

Tridecyl alcohol. $C_{13}H_{28}O$. (M_r 200.4). *1192500.* [112-70-9]. Tridecanol.

Tridocosahexaenoin. $C_{69}H_{98}O_6$. (M_r 1023.5). *1144900.* [124596-98-1]. Triglyceride of docosahexaenoic acid (C22:6). Glycerol tridocosahexaenoate. Propane-1,2,3-triyl tri-(*all-Z*)-docosa-4,7,10,13,16,19-hexaenoate.

The reagent from Nu-Chek Prep, Inc. has been found suitable.

Triethanolamine. *1092900.* [102-71-6].

See *Trolamine (1577)*.

Triethylamine. $C_6H_{15}N$. (M_r 101.2). *1093000.* [121-44-8]. *N,N*-Diethylethanamine.

Colourless liquid, slightly soluble in water at a temperature below 18.7 °C, miscible with ethanol (96 per cent).

d_{20}^{20}: about 0.727.

n_D^{20}: about 1.401.

bp: about 90 °C.

Triethylamine R1. $C_6H_{15}N$. (M_r 101.2). *1093001.* [121-44-8]. *N,N*-Diethylethanamine.

Complies with the requirements prescribed for *triethylamine R* with the following additional requirements.

Content: minimum 99.5 per cent, determined by gas chromatography.

Water: maximum 0.1 per cent.

Use freshly distilled or from a freshly opened container.

Triethylamine R2. $C_6H_{15}N$. (M_r 101.2). *1093002.* [121-44-8]. *N,N*-Diethylethanamine.

Complies with the requirements prescribed for *triethylamine R* and with the following additional requirements.

Content: minimum 99.5 per cent, determined by gas chromatography.

Water: maximum 0.2 per cent.

It is suitable for gradient elution in liquid chromatography.

Use freshly distilled or from a freshly opened container.

Triethylenediamine. $C_6H_{12}N_2$. (M_r 112.2). *1093100.* 1,4-Diazabicyclo[*2.2.2*]octane.

Crystals, very hygroscopic, sublimes readily at room temperature, freely soluble in water, in acetone and in anhydrous ethanol.

bp: about 174 °C.

mp: about 158 °C.

Storage: in an airtight container.

Triethyl phosphonoformate. $C_7H_{15}O_5P$. (M_r 210.2). *1132900.* [1474-78-8]. Ethyl (diethoxyphosphoryl)formate.

Colourless liquid.

$bp_{12\,mm}$: about 135 °C.

Triflumuron. $C_{15}H_{10}ClF_3N_2O_3$. (M_r 358.7). *1180800.* [64628-44-0]. 1-(2-Chlorobenzoyl)-3-(4-triflumoromethoxyphenyl)urea.

White or almost white crystalline powder, practically insoluble in water, sparingly soluble in acetone and in methylene chloride.

Trifluoroacetic acid. $C_2HF_3O_2$. (M_r 114.0). *1093200.* [76-05-1].

Content: minimum 99 per cent.

Liquid, miscible with acetone and with ethanol (96 per cent).

d_{20}^{20}: about 1.53.

bp: about 72 °C.

Use a grade suitable for protein sequencing.

Storage: in an airtight container.

Trifluoroacetic anhydride. $C_4F_6O_3$. (M_r 210.0). *1093300.* [407-25-0].

Colourless liquid.

d_{20}^{20}: about 1.5.

3-Trifluoromethylaniline. $C_7H_6F_3N$. (M_r 161.1). *1171900.* [98-16-8]. 3-(Trifluoromethyl)aniline. α,α,α-Trifluoro-*m*-toluidine. 3-(Trifluoromethyl)benzenamide.

Colourless liquid.

Density: 1.30 g/cm^3 (20 °C).

4-Trifluoromethylphenol. $C_7H_5F_3O$. (M_r 162.1). *1161700.* [402-45-9].

White or light yellow, crystalline solid or powder.

mp: about 46 °C.

Trifluoropropylmethylpolysiloxane. *1171600.*

Polysiloxane substituted with trifluoropropyl groups and methyl groups.

Triglycine. $C_6H_{11}N_3O_4$. (M_r 189.2). *1192600.* [556-33-2]. 2-[[2-[(2-Aminoacetyl)amino]acetyl]amino]acetic acid. Glycylglycylglycine.

Trigonelline hydrochloride. $C_7H_8ClNO_2$. (M_r 173.6). *1117400.* [6138-41-6]. 3-Carboxy-1-methylpyridinium chloride. Nicotinic acid *N*-methylbetaine hydrochloride.

Crystalline powder, very soluble in water, soluble in ethanol (96 per cent).

mp: about 258 °C.

1,2,4-Trimethylbenzene. C_9H_{12}. (M_r 120.2). *1188600.* [95-63-6]. Pseudocumene.

Trimethylpentane. C_8H_{18}. (M_r 114.2). *1093400.* [540-84-1]. Iso-octane. 2,2,4-Trimethylpentane.

Colourless, flammable liquid, practically insoluble in water, soluble in anhydrous ethanol.

d_{20}^{20}: 0.691 to 0.696.

n_D^{20}: 1.391 to 1.393.

Distillation range (*2.2.11*). Not less than 95 per cent distils between 98 °C and 100 °C.

Trimethylpentane used in spectrophotometry complies with the following additional test.

Absorbance (*2.2.25*): maximum 0.01 from 250 nm to 420 nm, determined using *water R* as compensation liquid.

Trimethylpentane R1. *1093401.*

Complies with the requirements prescribed for *trimethylpentane R* with the following modification.

Absorbance (2.2.25). Not more than 0.07 from 220 nm to 360 nm, determined using *water R* as the compensation liquid.

Trimethylpentane for chromatography. *1093402.*

Complies with the requirements prescribed for *trimethylpentane R* with the following additional requirement.

Residue on evaporation: maximum 2 mg/L.

***N,O*-bis(Trimethylsilyl)acetamide.** $C_8H_{21}NOSi_2$. (M_r 203.4). *1093600.* [10416-59-8].

Colourless liquid.

d_{20}^{20}: about 0.83.

***N*-Trimethylsilylimidazole.** $C_6H_{12}N_2Si$. (M_r 140.3). *1100500.* [18156-74-6]. 1-Trimethylsilylimidazole.

Colourless, hygroscopic liquid.

d_{20}^{20}: about 0.96.

n_D^{20}: about 1.48.

Storage: in an airtight container.

***N,O*-bis(Trimethylsilyl)trifluoroacetamide.** $C_8H_{18}F_3NOSi_2$. (M_r 257.4). *1133200.* [25561-30-2]. BSTFA.

Colourless liquid.

d_{20}^{20}: about 0.97.

n_D^{20}: about 1.38.

bp_{12mm}: about 40 °C

Trimethylsulfonium hydroxide. $C_3H_{10}OS$. (M_r 94.2). *1145000.* [17287-03-5].

d_4^{20}: about 0.81.

Trimethyltin chloride. C_3H_9ClSn. (M_r 199.3). *1170900.* [1066-45-1]. Chlorotrimethylstannane.

2,4,6-Trinitrobenzene sulfonic acid. $C_6H_3N_3O_9S,3H_2O$. (M_r 347.2). *1117500.* [2508-19-2].

White or almost white, crystalline powder, soluble in water.

mp: 190 °C to 195 °C.

Triolein. $C_{57}H_{104}O_6$. (M_r 885.4). *1168200.* [122-32-7]. Propane-1,2,3-triyl tris[(9*Z*)-octadec-9-enoate]. *sn*-Glyceryl trioleate. Glycerol trioleate. Oleyl triglyceride.

Content: minimum 99.0 per cent.

Triphenylmethanol. $C_{19}H_{16}O$. (M_r 260.3). *1093700.* [76-84-6]. Triphenylcarbinol.

Colourless crystals, practically insoluble in water, freely soluble in ethanol (96 per cent).

Triphenyltetrazolium chloride. $C_{19}H_{15}ClN_4$. (M_r 334.8). *1093800.* [298-96-4]. 2,3,5-Triphenyl-2*H*-tetrazol-3-ium chloride.

Pale or dull-yellow powder, soluble in water, in acetone and in ethanol (96 per cent).

mp: about 240 °C, with decomposition.

Storage: protected from light.

Triscyanoethoxypropane. $C_{12}H_{17}N_3O_3$. (M_r 251.3). *1093900.* 1,2,3-Tris(2-cyanoethoxy)propane.

Viscous, brown-yellow liquid, soluble in methanol. Used as a stationary phase in gas chromatography.

d_{20}^{20}: about 1.11.

Viscosity (*2.2.9*): about 172 mPa·s.

1,3,5-Tris[3,5-di(1,1-dimethylethyl)-4-hydroxybenzyl]-1,3,5-triazine-2,4,6(1*H*,3*H*,5*H*)-trione. $C_{48}H_{69}O_6N_3$. (M_r 784.1). *1094000.* [27676-62-6].

White or almost white, crystalline powder.

mp: 218 °C to 222 °C.

Tris[2,4-di(1,1-dimethylethyl)phenyl] phosphite. $C_{42}H_{63}O_3P$. (M_r 647). *1094100.* [31570-04-4].

White or almost white powder.

mp: 182 °C to 186 °C.

Tris(hydroxymethyl)aminomethane. *1094200.* [77-86-1].

See *Trometamol (1053)*.

Tris(hydroxymethyl)aminomethane solution. *1094201.*

A solution containing the equivalent of 24.22 g of $C_4H_{11}NO_3$ in 1000.0 mL.

Tris(hydroxymethyl)aminomethane solution R1. *1094202.*

Dissolve 60.6 mg of *tris(hydroxymethyl)aminomethane R* and 0.234 g of *sodium chloride R* in *water R* and dilute to 100 mL with the same solvent.

Storage: at 2 °C to 8 °C; use within 3 days.

Tripotassium phosphate trihydrate. $K_3PO_4,3H_2O$. (M_r 266.3). *1155300.* [22763-03-7].

White or almost white crystalline powder, freely soluble in water.

Trisodium phosphate dodecahydrate. $Na_3PO_4,12H_2O$. (M_r 380.1). *1094300.* [10101-89-0].

Colourless or white or almost white crystals, freely soluble in water.

Trometamol. *1094200.* [77-86-1].

See *Tris(hydroxymethyl)aminomethane R*.

Tropic acid. $C_9H_{10}O_3$. (M_r 166.17). *1172000.* [529-64-6]. (2*RS*)-3-Hydroxy-2-phenylpropanoic acid.

Troxerutin. $C_{33}H_{42}O_{19}$. (M_r 743). *1160300.* [7085-55-4]. Trihydroxyethylrutin. 3′,4′,7-Tris[*O*-(2-hydroxyethyl)]rutin. 2-[3,4-Bis(2-hydroxyethoxy)phenyl]-3-[[6-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-5-hydroxy-7-(2-hydroxyethoxy)-4*H*-1-benzopyran-4-one.

mp: 168 °C to 176 °C.

Trypsin. *1094500.* [9002-07-7].

A proteolytic enzyme obtained by activation of trypsinogen extracted from the pancreas of beef (*Bos taurus* L.).

White or almost white, crystalline or amorphous powder, sparingly soluble in water.

Trypsin for peptide mapping. *1094600.* [9002-07-7].

Trypsin of high purity treated to eliminate chymotryptic activity.

Tryptophan. $C_{11}H_{12}N_2O_2$. (M_r 204.2). *1094700.* [73-22-3].

White or yellowish-white, crystalline powder or colourless crystals, slightly soluble in water, very slightly soluble in ethanol (96 per cent).

$[\alpha]_D^{20}$: about – 30, determined on a 10 g/L solution.

Typhaneoside. $C_{34}H_{42}O_{20}$. (M_r 771). *1206000.* [104472-68-6]. 3-[6-Deoxy-α-L-mannopyranosyl-(1→2)-[6-deoxy-α-L-mannopyranosyl-(1→6)]-β-D-glucopyranosyloxy]-5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-4*H*-1-benzopyran-4-one.

Tyramine. $C_8H_{11}NO$. (M_r 137.2). *1117600.* [51-67-2]. 4-(2-Aminoethyl)phenol.

Crystals, sparingly soluble in water, soluble in boiling anhydrous ethanol.

mp: 164 °C to 165 °C.

Tyrosine. $C_9H_{11}NO_3$. (M_r 181.2). *1094800.* [60-18-4]. 2-Amino-3-(4-hydroxyphenyl)propionic acid.

White or almost white, crystalline powder, or colourless or white or almost white crystals, slightly soluble in water, practically insoluble in acetone and in anhydrous ethanol, soluble in dilute hydrochloric acid and in solutions of alkali hydroxides.

Umbelliferone. $C_9H_6O_3$. (M_r 162.1). *1137500.* [93-35-6]. 7-Hydroxycoumarin. 7-Hydroxy-2*H*-1-benzopyran-2-one.

Needles from water.

mp: 225 °C to 228 °C.

Undecanoic acid. $C_{11}H_{22}O_2$. (M_r 186.29). *1195200.* [112-37-8]. Hendecanoic acid. Undecylic acid.

mp: about 30 °C.

Content: minimum 97.0 per cent of $C_{11}H_{22}O_2$.

Uracil. $C_4H_4N_2O_2$. (M_r 112.1). *1161800.* [66-22-8].

Content: minimum 95.0 per cent.

Urea. *1095000.* [57-13-6].

See *Urea (0743)*.

Uridine. $C_9H_{12}N_2O_6$. (M_r 244.2). *1095100.* [58-96-8]. 1-β-D-Ribofuranosyluracil.

White or almost white, crystalline powder, soluble in water.

mp: about 165 °C.

Ursolic acid. $C_{30}H_{48}O_3$. (M_r 456.7). *1141600.* [77-52-1]. 3β-Hydroxyurs-12-en-28-oic acid.

White or almost white powder, practically insoluble in water, sparingly soluble in methanol, slightly soluble in ethanol (96 per cent).

$[\alpha]_D^{21}$: about 67.50, determined on a 10 g/L solution in a 56.1 g/L solution of *potassium hydroxide R* in *ethanol (96 per cent) R*.

mp: 285 °C to 288 °C.

Valencene. $C_{15}H_{24}$. (M_r 204.4). *1152100.* [4630-07-3]. 4β*H*,5α-Eremophila-1(10),11-diene. (1*R*,7*R*,8a*S*)-1,8a-Dimethyl-7-(1-methylethenyl)-1,2,3,5,6,7,8,8a-octahydronaphthalene.

Oily, colourless or pale yellow liquid, with a characteristic odour, practically insoluble in water, soluble in ethanol (96 per cent).

d_4^{20}: about 0.918.

n_D^{20}: about 1.508.

bp: about 123 °C.

Valencene used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Sweet orange oil (1811)*.

Content: minimum 80 per cent, calculated by the normalisation procedure.

Valerenic acid. $C_{15}H_{22}O_2$. (M_r 234.3). *1165700.* [3569-10-6]. (2*E*)-3-[(4*S*,7*R*,7a*R*)-3,7-Dimethyl-2,4,5,6,7,7a-hexahydro-1*H*-inden-4-yl]-2-methylprop-2-enoic acid.

mp: 134 °C to 138 °C.

Valeric acid. $C_5H_{10}O_2$. (M_r 102.1). *1095200.* [109-52-4]. Pentanoic acid.

Colourless liquid, soluble in water, freely soluble in ethanol (96 per cent).

d_{20}^{20}: about 0.94.

n_D^{20}: about 1.409.

bp: about 186 °C.

Valine. *1185300.* [72-18-4].

See *Valine (0796)*.

Vanillin. *1095300.* [121-33-5].

See *Vanillin (0747)*.

Vanillin reagent. *1095301.*

Carefully add, dropwise, 2 mL of *sulfuric acid R* to 100 mL of a 10 g/L solution of *vanillin R* in *ethanol (96 per cent) R*.

Storage: use within 48 h.

Vanillin solution, phosphoric. *1095302.*

Dissolve 1.0 g of *vanillin R* in 25 mL of *ethanol (96 per cent) R*. Add 25 mL of *water R* and 35 mL of *phosphoric acid R*.

Veratrole. $C_8H_{10}O_2$. (M_r 138.2). *1165400.* [91-16-7]. 1,2-Dimethoxybenzene.

d_4^{20}: 1.085.

n_D^{20}: 1.534.

bp: about 206 °C.

mp: about 22 °C.

Verbenone. $C_{10}H_{14}O$. (M_r 150.2). *1140500.* [1196-01-6]. (1*S*,5*S*)-4,6,6-Trimethylbicyclo[3.1.1]hept-3-en-2-one.

Oil with a characteristic odour, practically insoluble in water, miscible with organic solvents.

d_{20}^{20}: about 0.978.

n_D^{18}: about 1.49.

$[\alpha]_D^{18}$: about + 249.6.

bp: 227 °C to 228 °C.

mp: about 6.5 °C.

Verbenone used in gas chromatography complies with the following additional test.

Assay. Gas chromatography (*2.2.28*) as prescribed in the monograph *Rosemary oil (1846)*.

Content: minimum 99 per cent, calculated by the normalisation procedure.

Vinyl acetate. $C_4H_6O_2$. (M_r 86,10). *1111800.* [108-05-4]. Ethenyl acetate.
d_{20}^{20}: about 0.930.
bp: about 72 °C.

Vinyl chloride. C_2H_3Cl. (M_r 62.5). *1095400.* [75-01-4].
Colourless gas, slightly soluble in organic solvents.

Vinyl(1)phenyl(5)methyl(94)polysiloxane. *1100000.*
Polysiloxane substituted with 1 per cent of vinyl groups, 5 per cent of phenyl groups and 94 per cent of methyl groups.

Vinyl polymer for chromatography, amino alkyl. *1191500.*
Spherical particles (5 µm) of a vinyl alcohol copolymer chemically modified by bonding of amino alkyl groups.

Vinyl polymer for chromatography, octadecyl. *1155400.*
Spherical particles (5 µm) of a vinyl alcohol copolymer chemically modified by bonding of octadecyl groups on the hydroxyl groups.

Vinyl polymer for chromatography, octadecylsilyl. *1121600.*
Spherical particles (5 µm) of a vinyl alcohol copolymer bonded to an octadecylsilane. Carbon content of 17 per cent.

2-Vinylpyridine. C_7H_7N. (M_r 105.1). *1102200.* [100-69-6].
Yellow liquid, miscible in water.
d_{20}^{20}: about 0.97.
n_D^{20}: about 1.549.

4-Vinylpyridine. C_7H_7N. (M_r 105.1). *1187200.* [100-43-6]. 4-Ethenylpyridine.
Clear, deep yellowish-brown liquid.
bp: 58-61 °C.

1-Vinylpyrrolidin-2-one. C_6H_9NO. (M_r 111.1). *1111900.* [88-12-0]. 1-Ethenylpyrrolidin-2-one.
Content: minimum 99.0 per cent.
Clear colourless liquid.
Water (*2.5.12*): maximum 0.1 per cent, determined on 2.5 g. Use as the solvent, a mixture of 50 mL of *anhydrous methanol R* and 10 mL of *butyrolactone R*.
Assay. Gas chromatography (*2.2.28*): use the normalisation procedure.
Column:
– *material*: fused-silica;
– *size*: *l* = 30 m, Ø = 0.5 mm;
– *stationary phase*: *macrogol 20 000 R*.
Carrier gas: *helium for chromatography R*.
Temperature:

	Time (min)	Temperature (°C)
Column	0 - 1	80
	1 - 12	80 → 190
	12 - 27	190
Injection port		190

Detection: flame-ionisation.
Injection: 0.3 µL of the substance to be examined.
Adjust the flow rate of the carrier gas so that the retention time of the peak corresponding to 1-vinylpyrrolidin-2-one is about 17 min.

Vitexin. $C_{21}H_{20}O_{10}$. (M_r 432.4). *1133300.* [3681-93-4]. Apigenin 8-glucoside.
Yellow powder.
Storage: in an airtight container, protected from light.

Water. *1095500.* [7732-18-5].
See *Purified water (0008)*.

Water R1. *1095509.*
Prepared from *distilled water R* by multiple distillation. Remove carbon dioxide by boiling for at least 15 min before use in a boiling flask of fused silica or borosilicate glass and cool. Any other suitable method may be used. The boiling flask has been already used for the test or has been filled with *water R* and kept in an autoclave at 121 °C for at least 1 h prior to first use. When tested immediately before use, *water R1* is neutral to *methyl red solution R*, i.e. it shall produce an orange-red (not a violet-red or yellow) colour corresponding to pH 5.5 ± 0.1 when 0.05 mL of *methyl red solution R* is added to 50 mL of the water to be examined.
Conductivity: maximum 1 µS·cm^{-1}, determined at 25 °C by an in-line conductivity meter (see *Purified water (0008)*).

Water, ammonium-free. *1095501.*
To 100 mL of *water R* add 0.1 mL of *sulfuric acid R*. Distil using the apparatus described for the determination of *Distillation range* (*2.2.11*). Reject the first 10 mL and collect the following 50 mL.

Water, carbon dioxide-free. *1095502.*
Water R which has been boiled for a few minutes and protected from the atmosphere during cooling and storage or deionised *water R* with a resistivity of not less than 0.18 MΩ·m, determined at 25 °C.

Water, distilled. *1095504.*
Water R prepared by distillation.

Water, distilled, deionised. *1095508.*
Deionised *water R* prepared by distillation with a resistivity of not less than 0.18 MΩ·m, determined at 25 °C.

Water for chromatography. *1095503.*
Deionised water with a resistivity of not less than 0.18 MΩ·m, determined at 25 °C, prepared by distillation, ion exchange, reverse osmosis or any other suitable method, using water that complies with the regulations on water intended for human consumption, as laid down by the competent authority.
Its quality is such that no significant interfering peaks or loss of sensitivity are observed when used in chromatography. Isocratic elution with UV detection at low wavelengths (i.e. less than 230 nm), with evaporative detectors (e.g. light scattering detector, particle counter detector, charged aerosol detector) or mass detectors, or gradient elution, may require the use of water with a total organic carbon content of maximum 5 ppb.

Water for injections. *1095505.*
See *Water for injections (0169)*.

Water, nitrate-free. *1095506.*
To 100 mL of *water R* add a few milligrams of *potassium permanganate R* and of *barium hydroxide R*. Distil using the apparatus described for the determination of *Distillation range* (*2.2.11*). Reject the first 10 mL and collect the following 50 mL.

Water, particle-free. *1095507.*
Filter *water R* through a membrane with a pore size of 0.22 µm.

Weak cationic resin. *1096000.*
Polymethacrylic resin, slightly acid, with carboxyl groups present in a protonated form.
Particle size: 75 µm to 160 µm.
pH limits of use: 5 to 14.
Maximum temperature of use: 120 °C.

Wedelolactone. $C_{16}H_{10}O_7$. (M_r 314.3). *1187300.* [524-12-9]. 1,8,9-Trihydroxy-3-methoxy-6*H*-benzofuro[3,2-*c*][1]benzopyran-6-one.

White beeswax. *1196500.*

See *White beeswax (0069)*.

Xanthydrol. $C_{13}H_{10}O_2$. (M_r 198.2). *1096100.* [90-46-0]. 9-Xanthenol.

Content: minimum 90.0 per cent.

White or pale-yellow powder, very slightly soluble in water, soluble in ethanol (96 per cent) and in glacial acetic acid.

It is also available as a methanolic solution containing 90 g/L to 110 g/L of xanthydrol.

mp: about 123 °C.

Assay. In a 250 mL flask dissolve 0.300 g in 3 mL of *methanol R* or use 3.0 mL of solution. Add 50 mL of *glacial acetic acid R* and, dropwise with shaking, 25 mL of a 20 g/L solution of *urea R*. Allow to stand for 12 h, collect the precipitate on a sintered-glass filter (16) (*2.1.2*), wash with 20 mL of *ethanol (96 per cent) R*, dry in an oven at 100 °C to 105 °C and weigh.

1 g of precipitate is equivalent to 0.9429 g of xanthydrol.

Storage: protected from light. If a methanolic solution is used, store in small sealed ampoules and filter before use if necessary.

Xanthydrol R1. *1096101.*

Complies with the requirements prescribed for *xanthydrol R* with the following requirement.

Content: minimum 98.0 per cent of $C_{13}H_{10}O_2$.

Xanthydrol solution. *1096102.*

To 0.1 mL of a 100 g/L solution of *xanthydrol R* in *methanol R* add 100 mL of *anhydrous acetic acid R* and 1 mL of *hydrochloric acid R*. Allow to stand for 24 h before using.

Xylene. C_8H_{10}. (M_r 106.2). *1096200.* [1330-20-7].

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

d_{20}^{20}: about 0.867.

n_D^{20}: about 1.497.

bp: about 138 °C.

***m*-Xylene.** C_8H_{10}. (M_r 106.2). *1117700.* [108-38-3]. 1,3-Dimethylbenzene.

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

d_{20}^{20}: about 0.884.

n_D^{20}: about 1.497.

bp: about 139 °C.

mp: about – 47 °C.

***o*-Xylene.** C_8H_{10}. (M_r 106.2). *1100600.* [95-47-6]. 1,2-Dimethylbenzene.

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

d_{20}^{20}: about 0.881.

n_D^{20}: about 1.505.

bp: about 144 °C.

mp: about – 25 °C.

Xylenol orange. $C_{31}H_{28}N_2Na_4O_{13}S$. ($M_r$ 761). *1096300.* [3618-43-7]. Tetrasodium 3,3′-(3*H*-2,1-benzoxathiol-3-ylidene)bis[(6-hydroxy-5-methyl-3,1-phenylene)methyleneiminobisacetate] *S*,*S*-dioxide.

Reddish-brown crystalline powder, soluble in water.

Xylenol orange solution. *1096302.*

Dissolve 50.8 mg of *xylenol orange R* in *water R* and dilute to 100.0 mL with the same solvent.

Xylenol orange triturate. *1096301.*

Triturate 1 part of *xylenol orange R* with 99 parts of *potassium nitrate R*.

Test for sensitivity. To 50 mL of *water R* add 1 mL of *dilute acetic acid R*, 50 mg of the xylenol orange triturate and 0.05 mL of *lead nitrate solution R*. Add *hexamethylenetetramine R* until the colour changes from yellow to violet-red. After addition of 0.1 mL of *0.1 M sodium edetate* the colour changes to yellow.

Xylitol. $C_5H_{12}O_5$. (M_r 152.1). *1190700.* [87-99-0].

White or almost white, crystalline powder or crystals.

Content: minimum 96.0 per cent.

Xylose. *1096400.* [58-86-6].

See *Xylose (1278)*.

Zinc. Zn. (A_r 65.4). *1096500.* [7440-66-6].

Content: minimum 99.5 per cent.

Silver-white cylinders, granules, pellets or filings with a blue sheen.

Arsenic (*2.4.2, Method A*): maximum 0.2 ppm.

Dissolve 5.0 g in a mixture of the 15 mL of *hydrochloric acid R* and 25 mL of *water R* prescribed.

Zinc, activated. *1096501.*

Place the zinc cylinders or pellets to be activated in a conical flask and add a sufficient quantity of a 50 ppm solution of *chloroplatinic acid R* to cover the metal. Allow the metal to remain in contact with the solution for 10 min, wash, drain and dry immediately.

Arsenic (*2.4.2, Method A*). To 5 g of the activated zinc add 15 mL of *hydrochloric acid R*, 25 mL of *water R*, 0.1 mL of *stannous chloride solution R* and 5 mL of *potassium iodide solution R*. No colour is produced during the test.

Activity. The requirements of the suitability test for arsenic (*2.4.2, Method A*) are met.

Zinc acetate. $(C_2H_3O_2)_2Zn,2H_2O$. (M_r 219.5). *1102300.* [5970-45-6]. Zinc acetate dihydrate.

Bright white or almost white crystals, slightly efflorescent, freely soluble in water, soluble in ethanol (96 per cent). It loses its crystallisation water at 100 °C.

d_{20}^{20}: about 1.735.

mp: about 237 °C.

Zinc acetate solution. *1102301.*

Mix 600 mL of *water R* with 150 mL of *glacial acetic acid R*, 54.9 g of *zinc acetate R* and stir to dissolve. Continue stirring while adding 150 mL of *concentrated ammonia R*. Cool to room temperature and adjust with *ammonia R* to pH 6.4. Dilute the mixture to 1 L with *water R*.

Zinc chloride. *1096600.* [7646-85-7].

See *Zinc chloride (0110)*.

Zinc chloride-formic acid solution. *1096601.*

Dissolve 20 g of *zinc chloride R* in 80 g of an 850 g/L solution of *anhydrous formic acid R*.

Zinc chloride solution, iodinated. *1096602.*

Dissolve 20 g of *zinc chloride R* and 6.5 g of *potassium iodide R* in 10.5 mL of *water R*. Add 0.5 g of *iodine R* and shake for 15 min. Filter if necessary.

Storage: protected from light.

Zinc iodide and starch solution. *1096502.*

To a solution of 2 g of *zinc chloride R* in 10 mL of *water R* add 0.4 g of *soluble starch R* and heat until the starch has dissolved. After cooling to room temperature add 1.0 mL of a colourless solution containing 0.10 g *zinc R* as filings and 0.2 g of *iodine R* in *water R*. Dilute the solution to 100 mL with *water R* and filter.

Storage: protected from light.

Test for sensitivity. Dilute 0.05 mL of *sodium nitrite solution R* to 50 mL with *water R*. To 5 mL of this solution add 0.1 mL of *dilute sulfuric acid R* and 0.05 mL of the zinc iodide and starch solution and mix. The solution becomes blue.

Zinc oxide. *1096700.* [1314-13-2].

See *Zinc oxide (0252)*.

Zinc powder. Zn. (A_r 65.4). *1096800.* [7440-66-6].

Content: minimum 90.0 per cent.

Very fine, grey powder, soluble in *dilute hydrochloric acid R*.

Zinc sulfate. *1097000.* [7446-20-0].

See *Zinc sulfate (0111)*.

Zirconyl nitrate. A basic salt corresponding approximately to the formula $ZrO(NO_3)_2,2H_2O$. *1097200.* [14985-18-3].

A white or almost white powder or crystals, hygroscopic, soluble in water. The aqueous solution is a clear or at most slightly opalescent liquid.

Storage: in an airtight container.

Zirconyl nitrate solution. *1097201.*

A 1 g/L solution in a mixture of 40 mL of *water R* and 60 mL of *hydrochloric acid R*.

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4.1.2. STANDARD SOLUTIONS FOR LIMIT TESTS

Acetaldehyde standard solution (100 ppm C_2H_4O). *5000100.*

Dissolve 1.0 g of *acetaldehyde R* in *2-propanol R* and dilute to 100.0 mL with the same solvent. Dilute 5.0 mL of the solution to 500.0 mL with *2-propanol R*. Prepare immediately before use.

Acetaldehyde standard solution (100 ppm C_2H_4O) R1. *5000101.*

Dissolve 1.0 g of *acetaldehyde R* in *water R* and dilute to 100.0 mL with the same solvent. Dilute 5.0 mL of the solution to 500.0 mL with *water R*. Prepare immediately before use.

Aluminium standard solution (200 ppm Al). *5000200.*

Dissolve in *water R* a quantity of *aluminium potassium sulfate R* equivalent to 0.352 g of $AlK(SO_4)_2,12H_2O$. Add 10 mL of *dilute sulfuric acid R* and dilute to 100.0 mL with *water R*.

Aluminium standard solution (100 ppm Al). *5000203.*

Immediately before use, dilute with *water R* to 10 times its volume a solution containing 8.947 g of *aluminium chloride R* in 1000.0 mL of *water R*.

Aluminium standard solution (10 ppm Al). *5000201.*

Immediately before use, dilute with *water R* to 100 times its volume in a solution containing *aluminium nitrate R* equivalent to 1.39 g of $Al(NO_3)_3,9H_2O$ in 100.0 mL.

Aluminium standard solution (5 ppm Al). *5006600.*

Immediately before use, dilute with *water R* to 100 times its volume in a solution containing *aluminium nitrate R* equivalent to 0.695 g of $Al(NO_3)_3,9H_2O$ in 100.0 mL. Alternatively, use a commercially available standard solution containing a known amount of aluminium (5 ppm Al).

Aluminium standard solution (2 ppm Al). *5000202.*

Immediately before use, dilute with *water R* to 100 times its volume a solution containing *aluminium potassium sulfate R* equivalent to 0.352 g of $AlK(SO_4)_2,12H_2O$ and 10 mL of *dilute sulfuric acid R* in 100.0 mL.

Ammonium standard solution (100 ppm NH_4). *5000300.*

Immediately before use, dilute to 25 mL with *water R* 10 mL of a solution containing *ammonium chloride R* equivalent to 0.741 g of NH_4Cl in 1000 mL.

Ammonium standard solution (3 ppm NH_4). *5006100.*

Immediately before use, dilute with *water R* to 100 times its volume a solution containing *ammonium chloride R* equivalent to 0.889 g of NH_4Cl in 1000.0 mL.

Ammonium standard solution (2.5 ppm NH_4). *5000301.*

Immediately before use, dilute with *water R* to 100 times its volume a solution containing *ammonium chloride R* equivalent to 0.741 g of NH_4Cl in 1000.0 mL.

Ammonium standard solution (1 ppm NH_4). *5000302.*

Immediately before use, dilute *ammonium standard solution (2.5 ppm NH_4) R* to 2.5 times its volume with *water R*.

Antimony standard solution (100 ppm Sb). *5000401.*

Dissolve *antimony potassium tartrate R* equivalent to 0.274 g of $C_8H_4K_2O_{12}Sb_2,3H_2O$ in 500 mL of *1 M hydrochloric acid* and dilute the clear solution to 1000 mL with *water R*.

Antimony standard solution (1 ppm Sb). *5000400.*

Dissolve *antimony potassium tartrate R* equivalent to 0.274 g of $C_8H_4K_2O_{12}Sb_2,3H_2O$ in 20 mL of *hydrochloric acid R1* and dilute the clear solution to 100.0 mL with *water R*. To 10.0 mL of this solution add 200 mL of *hydrochloric acid R1* and dilute to 1000.0 mL with *water R*. To 100.0 mL of this solution add 300 mL of *hydrochloric acid R1* and dilute to 1000.0 mL with *water R*. Prepare the dilute solutions immediately before use.

Arsenic standard solution (10 ppm As). *5000500.*

Immediately before use, dilute with *water R* to 100 times its volume a solution prepared by dissolving *arsenious trioxide R* equivalent to 0.330 g of As_2O_3 in 5 mL of *dilute sodium hydroxide solution R* and diluting to 250.0 mL with *water R*.

Arsenic standard solution (1 ppm As). *5000501.*

Immediately before use, dilute *arsenic standard solution (10 ppm As) R* to 10 times its volume with *water R*.

Barium standard solution (0.1 per cent Ba). *5000601.*

Dissolve *barium chloride R* equivalent to 0.178 g of $BaCl_2,2H_2O$ in *distilled water R* and dilute to 100.0 mL with the same solvent.

Barium standard solution (50 ppm Ba). *5000600.*

Immediately before use, dilute with *distilled water R* to 20 times its volume a solution in *distilled water R* containing *barium chloride R* equivalent to 0.178 g of $BaCl_2,2H_2O$ in 100.0 mL.

Barium standard solution (2 ppm Ba). *5005600.*

Immediately before use, dilute *barium standard solution (50 ppm Ba) R* to 25 times its volume with *distilled water R*.

Bismuth standard solution (100 ppm Bi). *5005300.*

Dissolve *bismuth subnitrate R* equivalent to 0.500 g of Bi in 50 mL of *nitric acid R* and dilute to 500.0 mL with *water R*. Dilute the solution to 10 times its volume with *dilute nitric acid R* immediately before use.

Cadmium standard solution (0.1 per cent Cd). *5000700.*

Dissolve *cadmium R* equivalent to 0.100 g of Cd in the smallest necessary amount of a mixture of equal volumes of *hydrochloric acid R* and *water R* and dilute to 100.0 mL with a 1 per cent *V/V* solution of *hydrochloric acid R*.

Cadmium standard solution (10 ppm Cd) . *5000701.*

Immediately before use, dilute *cadmium standard solution (0.1 per cent Cd) R* to 100 times its volume with a 1 per cent *V/V* solution of *hydrochloric acid R*.

Calcium standard solution (400 ppm Ca). *5000800.*

Immediately before use, dilute with *distilled water R* to 10 times its volume a solution in *distilled water R* containing *calcium carbonate R* equivalent to 1.000 g of $CaCO_3$ and 23 mL of *1 M hydrochloric acid* in 100.0 mL.

Calcium standard solution (100 ppm Ca). *5000801.*

Immediately before use, dilute with *distilled water R* to 10 times its volume a solution in *distilled water R* containing *calcium carbonate R* equivalent to 0.624 g of $CaCO_3$ and 3 mL of *acetic acid R* in 250.0 mL.

Calcium standard solution (100 ppm Ca) R1. *5000804.*

Immediately before use, dilute with *water R* to 10 times its volume a solution containing *anhydrous calcium chloride R* equivalent to 2.769 g of $CaCl_2$ in 1000.0 mL of *dilute hydrochloric acid R*.

Calcium standard solution (100 ppm Ca), alcoholic. *5000802.*

Immediately before use, dilute with *ethanol (96 per cent) R* to 10 times its volume a solution in *distilled water R* containing *calcium carbonate R* equivalent to 2.50 g of $CaCO_3$ and 12 mL of *acetic acid R* in 1000.0 mL.

Calcium standard solution (10 ppm Ca). *5000803.*

Immediately before use, dilute with *distilled water R* to 100 times its volume a solution in *distilled water R* containing *calcium carbonate R* equivalent to 0.624 g of $CaCO_3$ and 3 mL of *acetic acid R* in 250.0 mL.

Chloride standard solution (50 ppm Cl). *5004100.*

Immediately before use, dilute with *water R* to 10 times its volume a solution containing *sodium chloride R* equivalent to 0.824 g of NaCl in 1000.0 mL.

Chloride standard solution (8 ppm Cl). *5000900.*

Immediately before use, dilute with *water R* to 100 times its volume a solution containing *sodium chloride R* equivalent to 1.32 g of NaCl in 1000.0 mL.

Chloride standard solution (5 ppm Cl). *5000901.*

Immediately before use, dilute with *water R* to 100 times its volume a solution containing *sodium chloride R* equivalent to 0.824 g of NaCl in 1000.0 mL.

Chromium liposoluble standard solution (1000 ppm Cr). *5004600.*

A chromium (metal) organic compound in an oil.

Chromium standard solution (0.1 per cent Cr). *5001002.*

Dissolve *potassium dichromate R* equivalent to 2.83 g of $K_2Cr_2O_7$ in *water R* and dilute to 1000.0 mL with the same solvent.

Chromium standard solution (100 ppm Cr). *5001000.*

Dissolve *potassium dichromate R* equivalent to 0.283 g of $K_2Cr_2O_7$ in *water R* and dilute to 1000.0 mL with the same solvent.

Chromium standard solution (0.1 ppm Cr). *5001001.*

Immediately before use, dilute *chromium standard solution (100 ppm Cr) R* to 1000 times its volume with *water R*.

Cobalt standard solution (100 ppm Co). *5004300.*

Dissolve *cobalt nitrate R* equivalent to 0.494 g of $Co(NO_3)_2,6H_2O$ in 500 mL of *1 M nitric acid* and dilute the clear solution to 1000 mL with *water R*.

Copper liposoluble standard solution (1000 ppm Cu). *5004700.*

A copper (metal) organic compound in an oil.

Copper standard solution (0.1 per cent Cu). *5001100.*

Dissolve *copper sulfate pentahydrate R* equivalent to 0.393 g of $CuSO_4,5H_2O$ in *water R* and dilute to 100.0 mL with the same solvent.

Copper standard solution (0.1 per cent Cu) for ICP. *5006300.*

A copper standard solution (1000 mg/L) suitable for inductively coupled plasma (ICP) applications and traceable to national or international standards.

Copper standard solution (10 ppm Cu). *5001101.*

Immediately before use, dilute *copper standard solution (0.1 per cent Cu) R* to 100 times its volume with *water R*.

Copper standard solution (0.1 ppm Cu). *5001102.*

Immediately before use, dilute *copper standard solution (10 ppm Cu) R* to 100 times its volume with *water R*.

Ferrocyanide standard solution (100 ppm $Fe(CN)_6$). *5001200.*

Immediately before use, dilute with *water R* to 10 times its volume a solution containing *potassium ferrocyanide R* equivalent to 0.20 g of $K_4Fe(CN)_6,3H_2O$ in 100.0 mL.

Ferricyanide standard solution (50 ppm $Fe(CN)_6$). *5001300.*

Immediately before use, dilute with *water R* to 100 times its volume a solution containing *potassium ferricyanide R* equivalent to 0.78 g of $K_3Fe(CN)_6$ in 100.0 mL.

Fluoride standard solution (10 ppm F). *5001400.*

Dissolve in *water R sodium fluoride R* previously dried at 300 °C for 12 h, equivalent to 0.442 g of NaF, and dilute to 1000.0 mL with the same solvent (1 mL = 0.2 mg F). Store in a polyethylene container. Immediately before use, dilute the solution to 20 times its volume with *water R*.

Fluoride standard solution (1 ppm F). *5001401.*

Immediately before use, dilute *fluoride standard solution (10 ppm F) R* to 10 times its volume with *water R*.

Formaldehyde standard solution (5 ppm CH_2O). *5001500.*

Immediately before use, dilute with *water R* to 200 times its volume a solution containing 1.0 g of CH_2O per litre prepared from *formaldehyde solution R*.

Germanium standard solution (100 ppm Ge). *5004400.*

Dissolve *ammonium hexafluorogermanate(IV) R* equivalent to 0.307 g of $(NH_4)_2GeF_6$ in a 0.01 per cent *V/V* solution of *hydrofluoric acid R*. Dilute the clear solution to 1000 mL with *water R*.

Glyoxal standard solution (20 ppm $C_2H_2O_2$). *5003700.*

In a 100 mL graduated flask weigh a quantity of *glyoxal solution R* corresponding to 0.200 g of $C_2H_2O_2$ and make up to volume with *anhydrous ethanol R*. Immediately before use dilute the solution to 100 times its volume with the same solvent.

Glyoxal standard solution (2 ppm $C_2H_2O_2$). *5003701.*

Immediately before use, dilute *glyoxal standard solution (20 ppm $C_2H_2O_2$) R* to 10 times its volume with *anhydrous ethanol R*.

Hydrogen peroxide standard solution (2 ppm H_2O_2). *5005200.*

Dilute 10.0 mL of *dilute hydrogen peroxide solution R* to 300.0 mL with *water R*. Dilute 2.0 mL of this solution to 1000.0 mL with *water R*. Prepare immediately before use.

Iodide standard solution (10 ppm I). *5003800.*

Immediately before use, dilute with *water R* to 100 times its volume a solution containing *potassium iodide R* equivalent to 0.131 g of KI in 100.0 mL.

Iron standard solution (0.1 per cent Fe). *5001605.*

Dissolve 0.100 g of Fe in the smallest amount necessary of a mixture of equal volumes of *hydrochloric acid R* and *water R* and dilute to 100.0 mL with *water R*.

Iron standard solution (250 ppm Fe). *5001606.*

Immediately before use, dilute with *water R* to 40 times its volume a solution containing 4.840 g of *ferric chloride R* in a 150 g/L solution of *hydrochloric acid R* diluted to 100.0 mL.

Iron standard solution (20 ppm Fe). *5001600.*

Immediately before use, dilute with *water R* to 10 times its volume a solution containing *ferric ammonium sulfate R* equivalent to 0.863 g of $FeNH_4(SO_4)_2,12H_2O$ and 25 mL of *dilute sulfuric acid R* in 500.0 mL.

Iron standard solution (10 ppm Fe). *5001601.*

Immediately before use, dilute with *water R* to 100 times its volume a solution containing *ferrous ammonium sulfate R* equivalent to 7.022 g of $Fe(NH_4)_2(SO_4)_2,6H_2O$ and 25 mL of *dilute sulfuric acid R* in 1000.0 mL.

Iron standard solution (8 ppm Fe). *5001602.*

Immediately before use, dilute with *water R* to 10 times its volume a solution containing 80 mg of *iron R* and 50 mL of *hydrochloric acid R* (220 g/L HCl) in 1000.0 mL.

Iron standard solution (2 ppm Fe). *5001603.*

Immediately before use, dilute *iron standard solution (20 ppm Fe) R* to 10 times its volume with *water R*.

Iron standard solution (1 ppm Fe). *5001604.*

Immediately before use, dilute *iron standard solution (20 ppm Fe) R* to 20 times its volume with *water R*.

Lead liposoluble standard solution (1000 ppm Pb). *5004800.*

A lead (metal) organic compound in an oil.

Lead standard solution (0.1 per cent Pb). *5001700.*

Dissolve *lead nitrate R* equivalent to 0.400 g of $Pb(NO_3)_2$ in *water R* and dilute to 250.0 mL with the same solvent.

Lead standard solution (100 ppm Pb). *5001701.*

Immediately before use, dilute *lead standard solution (0.1 per cent Pb) R* to 10 times its volume with *water R*.

Lead standard solution (10 ppm Pb). *5001702.*

Immediately before use, dilute *lead standard solution (100 ppm Pb) R* to 10 times its volume with *water R*.

Lead standard solution (10 ppm Pb) R1. *5001706.*

Immediately before use, dilute with *water R* to 10 times its volume a solution containing 0.160 g of *lead nitrate R* in 100 mL of *water R*, to which is added 1 mL of *lead-free nitric acid R* and dilute to 1000.0 mL.

Lead standard solution (2 ppm Pb). *5001703.*

Immediately before use, dilute *lead standard solution (10 ppm Pb) R* to 5 times its volume with *water R*.

Lead standard solution (1 ppm Pb). *5001704.*

Immediately before use, dilute *lead standard solution (10 ppm Pb) R* to 10 times its volume with *water R*.

Lead standard solution (0.25 ppm Pb). *5006000.*

Immediately before use, dilute *lead standard solution (1 ppm Pb) R* to 4 times its volume with *water R*.

Lead standard solution (0.1 ppm Pb). *5001705.*

Immediately before use, dilute *lead standard solution (1 ppm Pb) R* to 10 times its volume with *water R*.

Lutetium standard solution (20 ppm Lu). *5006500.*

Immediately before use, dissolve 0.445 g of *lutetium chloride hexahydrate R* in a mixture of equal volumes of *heavy metal-free nitric acid R* and *water R*, and dilute to 100.0 mL with the same mixture of solvents.

Dilute 1.0 mL of this solution to 100.0 mL with *water R*.

Magnesium standard solution (0.1 per cent Mg). *5001803.*

Dissolve *magnesium sulfate R* equivalent to 1.010 g of $MgSO_4,7H_2O$ in *distilled water R* and dilute to 100.0 mL with the same solvent.

Magnesium standard solution (1000 ppm Mg). *5006200.*

Dissolve 5.275 g of *magnesium nitrate R* in 16 mL of *dilute nitric acid R* and dilute to 500.0 mL with *water R*.

Standardisation: carry out the determination of magnesium by complexometry (*2.5.11*).

Magnesium standard solution (100 ppm Mg). *5001800.*

Immediately before use, dilute with *water R* to 10 times its volume a solution containing *magnesium sulfate R* equivalent to 1.010 g of $MgSO_4,7H_2O$ in 100.0 mL.

Magnesium standard solution (10 ppm Mg). *5001801.*

Immediately before use, dilute *magnesium standard solution (100 ppm Mg) R* to 10 times its volume with *water R*.

Magnesium standard solution (10 ppm Mg) R1. *5001802.*

Immediately before use, dilute with *water R* to 100 times its volume a solution containing 8.365 g of *magnesium chloride R* in 1000.0 mL of *dilute hydrochloric acid R*.

Manganese standard solution (1000 ppm Mn). *5005800.*

Dissolve *manganese sulfate R* equivalent to 3.08 g of $MnSO_4,H_2O$ in 500 mL of *1 M nitric acid* and dilute the solution to 1000 mL with *water R*.

Manganese standard solution (100 ppm Mn). *5004500.*

Dissolve *manganese sulfate R* equivalent to 0.308 g of $MnSO_4,H_2O$ in 500 mL of *1 M nitric acid* and dilute the clear solution to 1000 mL with *water R*.

Mercury standard solution (1000 ppm Hg). *5001900.*

Dissolve *mercuric chloride R* equivalent to 1.354 g of $HgCl_2$ in 50 mL of *dilute nitric acid R* and dilute to 1000.0 mL with *water R*.

Mercury standard solution (10 ppm Hg). *5001901.*

Immediately before use, dilute with water to 100 times its volume a solution containing *mercuric chloride R* equivalent to 0.338 g of $HgCl_2$ in 250.0 mL.

Nickel liposoluble standard solution (1000 ppm Ni). *5004900.*

A nickel (metal) organic compound in an oil.

Nickel standard solution (10 ppm Ni). *5002000.*

Immediately before use, dilute with *water R* to 100 times its volume a solution containing *nickel sulfate R* equivalent to 4.78 g of $NiSO_4,7H_2O$ in 1000.0 mL.

Nickel standard solution (5 ppm Ni). *5005900.*

Immediately before use dilute *nickel standard solution (10 ppm Ni) R* to twice its volume with *water for chromatography R*.

Nickel standard solution (0.2 ppm Ni). *5002002.*

Immediately before use, dilute *nickel standard solution (10 ppm Ni) R* to 50 times its volume with *water R*.

Nickel standard solution (0.1 ppm Ni). *5002001.*

Immediately before use, dilute *nickel standard solution (10 ppm Ni) R* to 100 times its volume with *water R*.

Nitrate standard solution (100 ppm NO_3). *5002100.*

Immediately before use, dilute with *water R* to 10 times its volume a solution containing *potassium nitrate R* equivalent to 0.815 g of KNO_3 in 500.0 mL.

Nitrate standard solution (10 ppm NO_3). *5002101.*

Immediately before use, dilute *nitrate standard solution (100 ppm NO_3) R* to 10 times its volume with *water R*.

Nitrate standard solution (2 ppm NO_3). *5002102.*

Immediately before use, dilute *nitrate standard solution (10 ppm NO_3) R* to 5 times its volume with *water R*.

Palladium standard solution (500 ppm Pd). *5003600.*

Dissolve 50.0 mg of *palladium R* in 9 mL of *hydrochloric acid R* and dilute to 100.0 mL with *water R*.

Palladium standard solution (20 ppm Pd). *5003602.*

Dissolve 0.333 g of *palladium chloride R* in 2 mL of warm *hydrochloric acid R*. Dilute the solution to 1000.0 mL with a mixture of equal volumes of *dilute hydrochloric acid R* and *water R*. Immediately before use dilute to 10 times its volume with *water R*.

Palladium standard solution (0.5 ppm Pd). *5003601.*
Dilute 1 mL of *palladium standard solution (500 ppm Pd) R* to 1000 mL with a mixture of 0.3 volumes of *nitric acid R* and 99.7 volumes of *water R*.

Phosphate standard solution (200 ppm PO_4). *5004200.*
Dissolve *potassium dihydrogen phosphate R* equivalent to 0.286 g of KH_2PO_4 in *water R* and dilute to 1000.0 mL with the same solvent.

Phosphate standard solution (5 ppm PO_4). *5002200.*
Immediately before use, dilute with *water R* to 100 times its volume a solution containing *potassium dihydrogen phosphate R* equivalent to 0.716 g of KH_2PO_4 in 1000.0 mL.

Platinum standard solution (30 ppm Pt). *5002300.*
Immediately before use, dilute with *1 M hydrochloric acid* to 10 times its volume a solution containing 80 mg of *chloroplatinic acid R* in 100.0 mL of *1 M hydrochloric acid*.

Potassium standard solution (0.2 per cent K). *5002402.*
Dissolve *dipotassium sulfate R* equivalent to 0.446 g of K_2SO_4 in *distilled water R* and dilute to 100.0 mL with the same solvent.

Potassium standard solution (600 ppm K). *5005100.*
Immediately before use, dilute with *water R* to 20 times its volume a solution containing *dipotassium sulfate R* equivalent to 2.676 g of K_2SO_4 in 100.0 mL.

Potassium standard solution (100 ppm K). *5002400.*
Immediately before use, dilute with *water R* to 20 times its volume a solution containing *dipotassium sulfate R* equivalent to 0.446 g of K_2SO_4 in 100.0 mL.

Potassium standard solution (20 ppm K). *5002401.*
Immediately before use, dilute *potassium standard solution (100 ppm K) R* to 5 times its volume with *water R*.

Scandium standard solution (0.1 per cent Sc) for ICP. *5006400.*
A scandium standard solution (1000 mg/L) suitable for inductively coupled plasma (ICP) applications and traceable to national or international standards.

Selenium standard solution (100 ppm Se). *5002500.*
Dissolve 0.100 g of *selenium R* in 2 mL of *nitric acid R*. Evaporate to dryness. Take up the residue in 2 mL of *water R* and evaporate to dryness; carry out three times. Dissolve the residue in 50 mL of *dilute hydrochloric acid R* and dilute to 1000.0 mL with the same acid.

Selenium standard solution (1 ppm Se). *5002501.*
Immediately before use, dilute with *water R* to 40 times its volume a solution containing *selenious acid R* equivalent to 6.54 mg of H_2SeO_3 in 100.0 mL.

Silver standard solution (5 ppm Ag). *5002600.*
Immediately before use, dilute with *water R* to 100 times its volume a solution containing *silver nitrate R* equivalent to 0.790 g of $AgNO_3$ in 1000.0 mL.

Sodium standard solution (1000 ppm Na). *5005700.*
Dissolve a quantity of *anhydrous sodium carbonate R* equivalent to 2.305 g of Na_2CO_3 in a mixture of 25 mL of *water R* and 25 mL of *nitric acid R* and dilute to 1000.0 mL with *water R*.

Sodium standard solution (200 ppm Na). *5002700.*
Immediately before use, dilute with *water R* to 10 times its volume a solution containing *sodium chloride R* equivalent to 0.509 g of NaCl in 100.0 mL.

Sodium standard solution (50 ppm Na). *5002701.*
Dilute the *sodium standard solution (200 ppm Na) R* to four times its volume with *water R*.

Strontium standard solution (1.0 per cent Sr). *5003900.*
Cover with *water R*, *strontium carbonate R* equivalent to 1.6849 g of $SrCO_3$. Cautiously add *hydrochloric acid R* until all the solid has dissolved and there is no sign of further effervescence. Dilute to 100.0 mL with *water R*.

Sulfate standard solution (100 ppm SO_4). *5002802.*
Immediately before use, dilute with *distilled water R* to 10 times its volume a solution in *distilled water R* containing *dipotassium sulfate R* equivalent to 0.181 g of K_2SO_4 in 100.0 mL.

Sulfate standard solution (10 ppm SO_4). *5002800.*
Immediately before use, dilute with *distilled water R* to 100 times its volume a solution in *distilled water R* containing *dipotassium sulfate R* equivalent to 0.181 g of K_2SO_4 in 100.0 mL.

Sulfate standard solution (10 ppm SO_4) R1. *5002801.*
Immediately before use, dilute with *ethanol (30 per cent V/V) R* to 100 times its volume a solution containing *dipotassium sulfate R* equivalent to 0.181 g of K_2SO_4 in 100.0 mL of *ethanol (30 per cent V/V) R*.

Sulfite standard solution (80 ppm SO_2). *5005500.*
Dissolve 3.150 g of *anhydrous sodium sulfite R* in freshly prepared *distilled water R* and dilute to 100.0 mL with the same solvent. Dilute 0.5 mL to 100.0 mL with freshly prepared *distilled water R*.

Sulfite standard solution (1.5 ppm SO_2). *5002900.*
Dissolve *sodium metabisulfite R* equivalent to 0.152 g of $Na_2S_2O_5$ in *water R* and dilute to 100.0 mL with the same solvent. Dilute 5.0 mL of this solution to 100.0 mL with *water R*. To 3.0 mL of the resulting solution, add 4.0 mL of *0.1 M sodium hydroxide* and dilute to 100.0 mL with *water R*.

Thallium standard solution (10 ppm Tl). *5003000.*
Dissolve *thallous sulfate R* equivalent to 0.1235 g of Tl_2SO_4 in a 9 g/L solution of *sodium chloride R* and dilute to 1000.0 mL with the same solution. Dilute 10.0 mL of the solution to 100.0 mL with the 9 g/L solution of *sodium chloride R*.

Tin liposoluble standard solution (1000 ppm Sn). *5005000.*
A tin (metal) organic compound in an oil.

Tin standard solution (5 ppm Sn). *5003100.*
Dissolve *tin R* equivalent to 0.500 g of Sn in a mixture of 5 mL of *water R* and 25 mL of *hydrochloric acid R* and dilute to 1000.0 mL with *water R*. Dilute the solution to 100 times its volume with a 2.5 per cent *V/V* solution of *hydrochloric acid R* immediately before use.

Tin standard solution (0.1 ppm Sn). *5003101.*
Immediately before use, dilute *tin standard solution (5 ppm Sn) R* to 50 times its volume with *water R*.

Titanium standard solution (100 ppm Ti). *5003200.*
Dissolve 100.0 mg of *titanium R* in 100 mL of *hydrochloric acid R* diluted to 150 mL with *water R*, heating if necessary. Allow to cool and dilute to 1000 mL with *water R*.

Vanadium standard solution (1 g/L V). *5003300.*
Dissolve in *water R ammonium vanadate R* equivalent to 0.230 g of NH_4VO_3 and dilute to 100.0 mL with the same solvent.

Zinc standard solution (5 mg/mL Zn). *5003400.*
Dissolve 3.15 g of *zinc oxide R* in 15 mL of *hydrochloric acid R* and dilute to 500.0 mL with *water R*.

Zinc standard solution (100 ppm Zn). *5003401.*
Immediately before use, dilute with *water R* to 10 times its volume a solution containing *zinc sulfate R* equivalent to 0.440 g of $ZnSO_4$,$7H_2O$ and 1 mL of *acetic acid R* in 100.0 mL.

Zinc standard solution (10 ppm Zn). *5003402.*

Immediately before use, dilute *zinc standard solution (100 ppm Zn) R* to 10 times its volume with *water R*.

Zinc standard solution (5 ppm Zn). *5003403.*

Immediately before use, dilute *zinc standard solution (100 ppm Zn) R* to 20 times its volume with *water R*.

Zirconium standard solution (1 g/L Zr). *5003500.*

Dissolve *zirconyl nitrate R* equivalent to 0.293 g of $ZrO(NO_3)_2,2H_2O$ in a mixture of 2 volumes of *hydrochloric acid R* and 8 volumes of *water R* and dilute to 100.0 mL with the same mixture of solvents.

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4.1.3. BUFFER SOLUTIONS

Buffered acetone solution. *4000100.*

Dissolve 8.15 g of *sodium acetate R* and 42 g of *sodium chloride R* in *water R*, add 68 mL of *0.1 M hydrochloric acid* and 150 mL of *acetone R* and dilute to 500 mL with *water R*.

Buffer solution pH 2.0. *4000200.*

Dissolve 6.57 g of *potassium chloride R* in *water R* and add 119.0 mL of *0.1 M hydrochloric acid*. Dilute to 1000.0 mL with *water R*.

0.125 M Phosphate buffer solution pH 2.0. *4015600.*

Dissolve 17.0 g of *potassium dihydrogen phosphate R* and 17.8 g of *anhydrous disodium hydrogen phosphate R* in *water R* and dilute to 1.0 L with the same solvent. If necessary adjust the pH with *phosphoric acid R*.

Phosphate buffer solution pH 2.0. *4007900.*

Dissolve 8.95 g of *disodium hydrogen phosphate dodecahydrate R* and 3.40 g of *potassium dihydrogen phosphate R* in *water R* and dilute to 1000.0 mL with the same solvent. If necessary adjust the pH with *phosphoric acid R*.

Sulfate buffer solution pH 2.0. *4008900.*

Dissolve 132.1 g of *ammonium sulfate R* in *water R* and dilute to 500.0 mL with the same solvent (Solution A). Carefully and with constant cooling stir 14 mL of *sulfuric acid R* into about 400 mL of *water R*; allow to cool and dilute to 500.0 mL with *water R* (Solution B). Mix equal volumes of solutions A and B. Adjust the pH if necessary.

Buffer solution pH 2.2. *4010500.*

Mix 6.7 mL of *phosphoric acid R* with 55.0 mL of a 40 g/L solution of *sodium hydroxide R* and dilute to 1000.0 mL with *water R*.

Buffer solution pH 2.5. *4000300.*

Dissolve 100 g of *potassium dihydrogen phosphate R* in 800 mL of *water R*; adjust to pH 2.5 with *hydrochloric acid R* and dilute to 1000.0 mL with *water R*.

Buffer solution pH 2.5 R1. *4000400.*

To 4.9 g of *dilute phosphoric acid R* add 250 mL of *water R*. Adjust the pH with *dilute sodium hydroxide solution R* and dilute to 500.0 mL with *water R*.

0.2 M Phosphate buffer solution pH 2.5. *4014100.*

Dissolve 27.2 g of *potassium dihydrogen phosphate R* in about 900 mL of *water R*, adjust to pH 2.5 with *phosphoric acid R* and dilute to 1.0 L with *water R*.

Phosphate buffer solution pH 2.8. *4010600.*

Dissolve 7.8 g of *sodium dihydrogen phosphate R* in 900 mL of *water R*, adjust to pH 2.8 with *phosphoric acid R* and dilute to 1000 mL with the same solvent.

Buffer solution pH 3.0. *4008000.*

Dissolve 21.0 g of *citric acid monohydrate R* in 200 mL of *1 M sodium hydroxide* and dilute to 1000 mL with *water R*. Dilute 40.3 mL of this solution to 100.0 mL with *0.1 M hydrochloric acid*.

0.25 M Citrate buffer solution pH 3.0. *4012600.*

Dissolve 5.3 g of *citric acid monohydrate R* in 80 mL of *water R*. Adjust the pH with *1 M sodium hydroxide* and dilute to 100.0 mL with *water R*.

0.1 M Phosphate buffer solution pH 3.0. *4011500.*

Dissolve 12.0 g of *anhydrous sodium dihydrogen phosphate R* in *water R*, adjust the pH with *dilute phosphoric acid R1* and dilute to 1000 mL with *water R*.

Phosphate buffer solution pH 3.0. *4000500.*

Mix 0.7 mL of *phosphoric acid R* with 100 mL of *water R*. Dilute to 900 mL with the same solvent. Adjust to pH 3.0 with *strong sodium hydroxide solution R* and dilute to 1000 mL with *water R*.

Phosphate buffer solution pH 3.0 R1. *4010000.*

Dissolve 3.40 g of *potassium dihydrogen phosphate R* in 900 mL of *water R*. Adjust to pH 3.0 with *phosphoric acid R* and dilute to 1000.0 mL with *water R*.

Phosphate buffer solution pH 3.2. *4008100.*

To 900 mL of a 4 g/L solution of *sodium dihydrogen phosphate R*, add 100 mL of a 2.5 g/L solution of *phosphoric acid R*. Adjust the pH if necessary.

Phosphate buffer solution pH 3.2 R1. *4008500.*

Adjust a 35.8 g/L solution of *disodium hydrogen phosphate dodecahydrate R* to pH 3.2 with *dilute phosphoric acid R*. Dilute 100.0 mL of the solution to 2000.0 mL with *water R*.

Phosphate buffer solution pH 3.25. *4014900.*

Dissolve about 1.36 g of *potassium dihydrogen phosphate R* in 1000 mL of *water R* and adjust to pH 3.25 ± 0.05 with *dilute phosphoric acid R*. Filter through a membrane filter (nominal pore size 0.45 µm or finer).

Phosphate buffer solution pH 3.4. *4015800.*

Dissolve 68.0 g of *potassium dihydrogen phosphate R* in *water R* and dilute to 1000.0 mL with the same solvent. Adjust the pH with *phosphoric acid R*.

Buffer solution pH 3.5. *4000600.*

Dissolve 25.0 g of *ammonium acetate R* in 25 mL of *water R* and add 38.0 mL of *hydrochloric acid R1*. Adjust the pH if necessary with *dilute hydrochloric acid R* or *dilute ammonia R1*. Dilute to 100.0 mL with *water R*.

Phosphate buffer solution pH 3.5. *4000700.*

Dissolve 68.0 g of *potassium dihydrogen phosphate R* in *water R* and dilute to 1000.0 mL with the same solvent. Adjust the pH with *phosphoric acid R*.

Buffer solution pH 3.6. *4000800.*

To 250.0 mL of *0.2 M potassium hydrogen phthalate R* add 11.94 mL of *0.2 M hydrochloric acid*. Dilute to 1000.0 mL with *water R*.

Buffer solution pH 3.7. *4000900.*

To 15.0 mL of *acetic acid R* add 60 mL of *ethanol (96 per cent) R* and 20 mL of *water R*. Adjust to pH 3.7 by the addition of *ammonia R*. Dilute to 100.0 mL with *water R*.

Buffered copper sulfate solution pH 4.0. *4001000.*

Dissolve 0.25 g of *copper sulfate pentahydrate R* and 4.5 g of *ammonium acetate R* in *dilute acetic acid R* and dilute to 100.0 mL with the same solvent.

0.1 M Sodium acetate buffer solution pH 4.0. *4013800.*

Dissolve 822 mg of *sodium acetate R* in 100 mL of *water R* (solution A). Dilute 1.44 mL of *glacial acetic acid R* in 250 mL of *water R* (solution B). Titrate 100 mL of solution B using about 20 mL of solution A.

Acetate buffer solution pH 4.4. *4001100.*

Dissolve 136 g of *sodium acetate R* and 77 g of *ammonium acetate R* in *water R* and dilute to 1000.0 mL with the same solvent; add 250.0 mL of *glacial acetic acid R* and mix.

Phthalate buffer solution pH 4.4. *4001200.*

Dissolve 2.042 g of *potassium hydrogen phthalate R* in 50 mL of *water R*, add 7.5 mL of *0.2 M sodium hydroxide* and dilute to 200.0 mL with *water R*.

Acetate buffer solution pH 4.5. *4012500.*

Dissolve 77.1 g of *ammonium acetate R* in *water R*. Add 70 mL of *glacial acetic acid R* and dilute to 1000.0 mL with *water R*.

0.5 M Ammonium acetate buffer solution pH 4.5. *4014200.*

Mix 14.3 mL of *glacial acetic acid R* and 470 mL of *water R* and adjust to pH 4.5 with *concentrated ammonia R*. Dilute to 500.0 mL with *water R*.

0.05 M Phosphate buffer solution pH 4.5. *4009000.*

Dissolve 6.80 g of *potassium dihydrogen phosphate R* in 1000.0 mL of *water R*. The pH of the solution is 4.5.

Sodium acetate buffer solution pH 4.5. *4010100.*

Dissolve 63 g of *anhydrous sodium acetate R* in *water R*, add 90 mL *acetic acid R* and adjust to pH 4.5, and dilute to 1000 mL with *water R*.

Acetate buffer solution pH 4.6. *4001400.*

Dissolve 5.4 g of *sodium acetate R* in 50 mL of *water R*, add 2.4 g of *glacial acetic acid R* and dilute to 100.0 mL with *water R*. Adjust the pH if necessary.

Succinate buffer solution pH 4.6. *4001500.*

Disssolve 11.8 g of *succinic acid R* in a mixture of 600 mL of *water R* and 82 mL of *1 M sodium hydroxide* and dilute to 1000.0 mL with *water R*.

Acetate buffer solution pH 4.7. *4001600.*

Dissolve 136.1 g of *sodium acetate R* in 500 mL of *water R*. Mix 250 mL of this solution with 250 mL of *dilute acetic acid R*. Shake twice with a freshly prepared, filtered, 0.1 g/L solution of *dithizone R* in *chloroform R*. Shake with *carbon tetrachloride R* until the extract is colourless. Filter the aqueous layer to remove traces of carbon tetrachloride.

Acetate buffer solution pH 4.7 R1. *4013600.*

Dissolve 136.1 g of *sodium acetate R* in 500 mL of *water R*. Mix 250 mL of this solution with 250 mL of *dilute acetic acid R*.

Acetate buffer solution pH 5.0. *4009100.*

To 120 mL of a 6 g/L solution of *glacial acetic acid R* add 100 mL of *0.1 M potassium hydroxide* and about 250 mL of *water R*. Mix. Adjust the pH to 5.0 with a 6 g/L solution of *acetic acid R* or with *0.1 M potassium hydroxide* and dilute to 1000.0 mL with *water R*.

Citrate buffer solution pH 5.0. *4010700.*

Prepare a solution containing 20.1 g/L of *citric acid monohydrate R* and 8.0 g/L of *sodium hydroxide R*. Adjust the pH with *dilute hydrochloric acid R*.

0.2 M Deuterated sodium phosphate buffer solution pH 5.0. *4013900.*

Dissolve 2.76 g of *sodium dihydrogen phosphate monohydrate R* in 90 mL of *deuterium oxide R*, adjust the pH with a deuterated solution of *phosphoric acid R* or a deuterated 1 M solution of *sodium hydroxide R*, dilute to 100 mL with *deuterium oxide R* and mix.

Phosphate buffer solution pH 5.0. *4011300.*

Dissolve 2.72 g of *potassium dihydrogen phosphate R* in 800 mL of *water R*. Adjust the pH with a 1 M potassium hydroxide solution prepared from *potassium hydroxide R* and dilute to 1000 mL with *water R*.

Sodium acetate buffer solution pH 5.0. *4015500.*

Dissolve 50.0 g of *sodium acetate R* in 10.0 mL of *glacial acetic acid R* and add *water R*. Adjust to pH 5.0 with a 4.2 g/L solution of *sodium hydroxide R* or with *glacial acetic acid R* and dilute to 1000.0 mL with *water R*.

Buffer solution pH 5.2. *4001700.*

Dissolve 1.02 g of *potassium hydrogen phthalate R* in 30.0 mL of *0.1 M sodium hydroxide*. Dilute to 100.0 mL with *water R*.

0.067 M Phosphate buffer solution pH 5.4. *4012000.*

Mix appropriate volumes of a 23.99 g/L solution of *disodium hydrogen phosphate dodecahydrate R* with a 9.12 g/L solution of *sodium dihydrogen phosphate monohydrate R* to obtain pH 5.4.

Acetate-edetate buffer solution pH 5.5. *4001900.*

Dissolve 250 g of *ammonium acetate R* and 15 g *sodium edetate R* in 400 mL of *water R* and add 125 mL of *glacial acetic acid R*.

Buffer solution pH 5.5. *4001800.*

Dissolve 54.4 g of *sodium acetate R* in 50 mL of *water R*, heating to 35 °C if necessary. After cooling, slowly add 10 mL of *anhydrous acetic acid R*. Shake and dilute to 100.0 mL with *water R*.

Phosphate buffer solution pH 5.5. *4002000.*

Dissolve 13.61 g of *potassium dihydrogen phosphate R* in *water R* and dilute to 1000.0 mL with the same solvent (solution A). Dissolve 35.81 g of *disodium hydrogen phosphate dodecahydrate R* in *water R* and dilute to 1000.0 mL with the same solvent (solution B). Mix 96.4 mL of solution A and 3.6 mL of solution B.

Phosphate-citrate buffer solution pH 5.5. *4008700.*

Mix 56.85 mL of a 28.4 g/L solution of *anhydrous disodium hydrogen phosphate R* and 43.15 mL of a 21 g/L solution of *citric acid monohydrate R*.

Phosphate buffer solution pH 5.6. *4011200.*

Dissolve 0.908 g of *potassium dihydrogen phosphate R* in *water R* and dilute to 100.0 mL with the same solvent (solution A). Dissolve 1.161 g of *dipotassium hydrogen phosphate R* in *water R* and dilute to 100.0 mL with the same solvent (solution B). Mix 94.4 mL of solution A and 5.6 mL of solution B. If necessary, adjust to pH 5.6 using solution A or solution B.

Phosphate buffer solution pH 5.8. *4002100.*

Dissolve 1.19 g of *disodium hydrogen phosphate dihydrate R* and 8.25 g of *potassium dihydrogen phosphate R* in *water R* and dilute to 1000.0 mL with the same solvent.

Acetate buffer solution pH 6.0. *4002200.*

Dissolve 100 g of *ammonium acetate R* in 300 mL of *water R*, add 4.1 mL of *glacial acetic acid R*, adjust the pH if necessary using *ammonia R* or *acetic acid R* and dilute to 500.0 mL with *water R*.

Diethylammonium phosphate buffer solution pH 6.0. *4002300.*

Dilute 68 mL of *phosphoric acid R* to 500 mL with *water R*. To 25 mL of this solution add 450 mL of *water R* and 6 mL of *diethylamine R*, adjust to pH 6 ± 0.05, if necessary, using *diethylamine R* or *phosphoric acid R* and dilute to 500.0 mL with *water R*.

1 M Morpholinoethanesulfonate buffer solution pH 6.0. *4015900.*

Dissolve 48.8 g of *2-[N-morpholino]ethanesulfonic acid R* in 160 mL of *water R* and add 25 mL of *2 M sodium hydroxide R*. Adjust to pH 6.0 with *2 M sodium hydroxide R*. Dilute to almost 250 mL with *water R*. Adjust the pH, if necessary, with *2 M sodium hydroxide R* and dilute to 250.0 mL with *water R*.

Phosphate buffer solution pH 6.0. *4002400.*

Mix 63.2 mL of a 71.5 g/L solution of *disodium hydrogen phosphate dodecahydrate R* and 36.8 mL of a 21 g/L solution of *citric acid monohydrate R*.

Phosphate buffer solution pH 6.0 R1. *4002500.*

Dissolve 6.8 g of *sodium dihydrogen phosphate R* in *water R* and dilute to 1000.0 mL with *water R*. Adjust the pH with *strong sodium hydroxide solution R*.

Phosphate buffer solution pH 6.0 R2. *4002600.*

To 250.0 mL of *0.2 M potassium dihydrogen phosphate R* add 28.5 mL of *0.2 M sodium hydroxide* and dilute to 1000.0 mL with *water R*.

Phosphate buffer solution pH 6.4. *4002800.*

Dissolve 2.5 g of *disodium hydrogen phosphate dodecahydrate R*, 2.5 g of *sodium dihydrogen phosphate R* and 8.2 g of *sodium chloride R* in 950 mL of *water R*. Adjust the pH of the solution to 6.4 with *1 M sodium hydroxide* or *1 M hydrochloric acid*, if necessary. Dilute to 1000.0 mL with *water R*.

0.5 M Phthalate buffer solution pH 6.4. *4009200.*

Dissolve 100 g of *potassium hydrogen phthalate R* in *water R* and dilute to 1000.0 mL with the same solvent. Adjust the pH if necessary, using *strong sodium hydroxide solution R*.

Buffer solution pH 6.5. *4002900.*

Dissolve 60.5 g of *disodium hydrogen phosphate dodecahydrate R* and 46 g of *potassium dihydrogen phosphate R* in *water R*. Add 100 mL of *0.02 M sodium edetate* and 20 mg of *mercuric chloride R* and dilute to 1000.0 mL with *water R*.

Imidazole buffer solution pH 6.5. *4003000.*

Dissolve 6.81 g of *imidazole R*, 1.23 g of *magnesium sulfate R* and 0.73 g of *calcium sulfate R* in 752 mL of *0.1 M hydrochloric acid*. Adjust the pH if necessary and dilute to 1000.0 mL with *water R*.

0.1 M phosphate buffer solution pH 6.5. *4010800.*

Dissolve 13.80 g of *sodium dihydrogen phosphate monohydrate R* in 900 mL of *distilled water R*. Adjust the pH using a 400 g/L solution of *sodium hydroxide R*. Dilute to 1000 mL with *distilled water R*.

Phosphate buffer solution pH 6.5. *4012800.*

Dissolve 2.75 g of *sodium dihydrogen phosphate R* and 4.5 g of *sodium chloride R* in 500 mL of *water R*. Adjust the pH with *phosphate buffer solution pH 8.5 R*.

Buffer solution pH 6.6. *4003100.*

To 250.0 mL of *0.2 M potassium dihydrogen phosphate R* add 89.0 mL of *0.2 M sodium hydroxide*. Dilute to 1000.0 mL with *water R*.

0.1 M Phosphate buffer solution pH 6.7. *4014300.*

Dissolve 15.6 g of *sodium dihydrogen phosphate R* in *water R* and dilute to 1.0 L with the same solvent. Dissolve 17.8 g of *disodium hydrogen phosphate dihydrate R* in *water R* and dilute to 1.0 L with the same solvent. Mix the solutions, check the pH and if necessary adjust to pH 6.7.

Phosphate buffered saline pH 6.8. *4003200.*

Dissolve 1.0 g of *potassium dihydrogen phosphate R*, 2.0 g of *dipotassium hydrogen phosphate R* and 8.5 g of *sodium chloride R* in 900 mL of *water R*, adjust the pH if necessary and dilute to 1000.0 mL with the same solvent.

Phosphate buffer solution pH 6.8. *4003300.*

Mix 77.3 mL of a 71.5 g/L solution of *disodium hydrogen phosphate dodecahydrate R* with 22.7 mL of a 21 g/L solution of *citric acid monohydrate R*.

Phosphate buffer solution pH 6.8 R1. *4003400.*

To 51.0 mL of a 27.2 g/L solution of *potassium dihydrogen phosphate R* add 49.0 mL of a 71.6 g/L solution of *disodium hydrogen phosphate dodecahydrate R*. Adjust the pH if necessary.

Storage: at 2 °C to 8 °C.

1 M Tris-hydrochloride buffer solution pH 6.8. *4009300.*

Dissolve 60.6 g of *tris(hydroxymethyl)aminomethane R* in 400 mL of *water R*. Adjust the pH with *hydrochloric acid R* and dilute to 500.0 mL with *water R*.

Buffer solution pH 7.0. *4003500.*

To 1000 mL of a solution containing 18 g/L of *disodium hydrogen phosphate dodecahydrate R* and 23 g/L of *sodium chloride R* add sufficient (about 280 mL) of a solution containing 7.8 g/L of *sodium dihydrogen phosphate R* and 23 g/L of *sodium chloride R* to adjust the pH. Dissolve in the solution sufficient *sodium azide R* to give a 0.2 g/L solution.

Maleate buffer solution pH 7.0. *4003600.*

Dissolve 10.0 g of *sodium chloride R*, 6.06 g of *tris(hydroxymethyl)aminomethane R* and 4.90 g of *maleic anhydride R* in 900 mL of *water R*. Adjust the pH using a 170 g/L solution of *sodium hydroxide R*. Dilute to 1000.0 mL with *water R*.

Storage: at 2 °C to 8 °C; use within 3 days.

0.025 M Phosphate buffer solution pH 7.0. *4009400.*

Mix 1 volume of *0.063 M phosphate buffer solution pH 7.0 R* with 1.5 volumes of *water R*.

0.03 M Phosphate buffer solution pH 7.0. *4010300.*

Dissolve 5.2 g of *dipotassium hydrogen phosphate R* in 900 mL of *water for chromatography R*. Adjust the solution to pH 7.0 ± 0.1 using *phosphoric acid R* and dilute to 1000 mL with *water for chromatography R*.

0.05 M Phosphate buffer solution pH 7.0. *4012400.*

Mix 34 mL of *water R* and 100 mL of *0.067 M phosphate buffer solution pH 7.0 R*.

0.063 M Phosphate buffer solution pH 7.0. *4009500.*

Dissolve 5.18 g of *anhydrous disodium hydrogen phosphate R* and 3.65 g of *sodium dihydrogen phosphate monohydrate R* in 950 mL of *water R* and adjust the pH with *phosphoric acid R*; dilute to 1000.0 mL with *water R*.

0.067 M Phosphate buffer solution pH 7.0. *4003800.*

Dissolve 0.908 g of *potassium dihydrogen phosphate R* in *water R* and dilute to 100.0 mL with the same solvent (solution A). Dissolve 2.38 g of *disodium hydrogen phosphate dodecahydrate R* in *water R* and dilute to 100.0 mL with the same solvent (solution B). Mix 38.9 mL of solution A and 61.1 mL of solution B. Adjust the pH if necessary.

0.1 M Phosphate buffer solution pH 7.0. *4008200.*

Dissolve 1.361 g of *potassium dihydrogen phosphate R* in *water R* and dilute to 100.0 mL with the same solvent. Adjust the pH using a 35 g/L solution of *disodium hydrogen phosphate dodecahydrate R*.

Phosphate buffer solution pH 7.0. *4003700.*

Mix 82.4 mL of a 71.5 g/L solution of *disodium hydrogen phosphate dodecahydrate R* with 17.6 mL of a 21 g/L solution of *citric acid monohydrate R*.

Phosphate buffer solution pH 7.0 R1. *4003900.*

Mix 250.0 mL of *0.2 M potassium dihydrogen phosphate R* and 148.2 mL of a 8 g/L solution of *sodium hydroxide R*, adjust the pH if necessary. Dilute to 1000.0 mL with *water R*.

Phosphate buffer solution pH 7.0 R2. *4004000.*

Mix 50.0 mL of a 136 g/L solution of *potassium dihydrogen phosphate R* with 29.5 mL of *1 M sodium hydroxide* and dilute to 100.0 mL with *water R*. Adjust the pH to 7.0 ± 0.1.

Phosphate buffer solution pH 7.0 R3. *4008600.*

Dissolve 5 g of *potassium dihydrogen phosphate R* and 11 g of *dipotassium hydrogen phosphate R* in 900 mL of *water R*. Adjust to pH 7.0 with *dilute phosphoric acid R* or *dilute sodium hydroxide solution R*. Dilute to 1000 mL with *water R* and mix.

Phosphate buffer solution pH 7.0 R4. *4010200.*

Dissolve 28.4 g of *anhydrous disodium hydrogen phosphate R* and 18.2 g of *potassium dihydrogen phosphate R* in *water R* and dilute to 500 mL with the same solvent.

Phosphate buffer solution pH 7.0 R5. *4011400.*

Dissolve 28.4 g of *anhydrous disodium hydrogen phosphate R* in 800 mL of *water R*. Adjust the pH using a 30 per cent *m/m* solution of *phosphoric acid R* and dilute to 1000 mL with *water R*.

Phosphate buffer solution pH 7.0 R6. *4015300.*

Dissolve 3.56 g of *disodium hydrogen phosphate dihydrate R* in 950 mL of *water for chromatography R*. Adjust the pH with *phosphoric acid R* and dilute to 1.0 L with *water for chromatography R*.

Phosphate buffer solution pH 7.0 R7. *4015700.*

Dissolve 35 g of *dipotassium hydrogen phosphate R* in 900 mL of *water R*, adjust to pH 7.0 with *dilute phosphoric acid R* and dilute to 1.0 L with *water R*.

Potassium phosphate buffer solution pH 7.0. *4014700.*

Dissolve 10 mg of *bovine albumin R* and 68 mg of *potassium dihydrogen phosphate R* in 30 mL of *water R*. If necessary, adjust to pH 7.0 with *potassium hydroxide R*. Dilute to 50 mL with *water R* and filter.

Sodium/calcium acetate buffer solution pH 7.0. *4014800.*

Dissolve 10 mg of *bovine albumin R* and 32 mg of *calcium acetate R* in 60 mL of *water R*. Add 580 µL of *glacial acetic acid R* and adjust to pH 7.0 with *2 M sodium hydroxide R*. Dilute to 100 mL with *water R* and filter.

Tetrabutylammonium buffer solution pH 7.0. *4010900.*

Dissolve 6.16 g of *ammonium acetate R* in a mixture of 15 mL of *tetrabutylammonium hydroxide solution (400 g/L) R* and 185 mL of *water R*. Adjust the pH with *nitric acid R*.

Buffered salt solution pH 7.2. *4004300.*

Dissolve in *water R* 8.0 g of *sodium chloride R*, 0.2 g of *potassium chloride R*, 0.1 g of *anhydrous calcium chloride R*, 0.1 g of *magnesium chloride R*, 3.18 g of *disodium hydrogen phosphate dodecahydrate R* and 0.2 g of *potassium dihydrogen phosphate R* and dilute to 1000.0 mL with *water R*.

Buffer solution pH 7.2. *4004100.*

To 250.0 mL of *0.2 M potassium dihydrogen phosphate R* add 175.0 mL of *0.2 M sodium hydroxide*. Dilute to 1000.0 mL with *water R*. Adjust the pH if necessary.

Phosphate-albumin buffered saline pH 7.2. *4004400.*

Dissolve 10.75 g of *disodium hydrogen phosphate dodecahydrate R*, 7.6 g of *sodium chloride R* and 10 g of *bovine albumin R* in *water R* and dilute to 1000.0 mL with the same solvent. Immediately before use adjust the pH using *dilute sodium hydroxide solution R* or *dilute phosphoric acid R*.

Phosphate-albumin buffered saline pH 7.2 R1. *4009600.*

Dissolve 10.75 g of *disodium hydrogen phosphate dodecahydrate R*, 7.6 g of *sodium chloride R* and 1 g of *bovine albumin R* in *water R* and dilute to 1000.0 mL with the same solvent. Immediately before use adjust the pH using *dilute sodium hydroxide solution R* or *dilute phosphoric acid R*.

Phosphate buffer solution pH 7.2. *4004200.*

Mix 87.0 mL of a 71.5 g/L solution of *disodium hydrogen phosphate dodecahydrate R* with 13.0 mL of a 21 g/L solution of *citric acid monohydrate R*.

Imidazole buffer solution pH 7.3. *4004500.*

Dissolve 3.4 g of *imidazole R* and 5.8 g of *sodium chloride R* in *water R*, add 18.6 mL of *1 M hydrochloric acid* and dilute to 1000.0 mL with *water R*. Adjust the pH if necessary.

Barbital buffer solution pH 7.4. *4004700.*

Mix 50 mL of a solution in *water R* containing 19.44 g/L of *sodium acetate R* and 29.46 g/L of *barbital sodium R* with 50.5 mL of *0.1 M hydrochloric acid*, add 20 mL of an 85 g/L of *sodium chloride R* and dilute to 250 mL with *water R*.

Buffer solution pH 7.4. *4004600.*

Dissolve 0.6 g of *potassium dihydrogen phosphate R*, 6.4 g of *disodium hydrogen phosphate dodecahydrate R* and 5.85 g of *sodium chloride R* in *water R*, and dilute to 1000.0 mL with the same solvent. Adjust the pH if necessary.

Phosphate buffered saline pH 7.4. *4005000.*

Dissolve 2.38 g of *disodium hydrogen phosphate dodecahydrate R*, 0.19 g of *potassium dihydrogen phosphate R* and 8.0 g of *sodium chloride R* in water. Dilute to 1000.0 mL with the same solvent. Adjust the pH if necessary.

Phosphate buffer solution pH 7.4. *4004800.*

Add 250.0 mL of *0.2 M potassium dihydrogen phosphate R* to 393.4 mL of *0.1 M sodium hydroxide*.

Tris(hydroxymethyl)aminomethane buffer solution pH 7.4. *4012100.*

Dissolve 30.3 g of *tris(hydroxymethyl)aminomethane R* in approximately 200 mL of *water R*. Add 183 mL of *1 M hydrochloric acid*. Dilute to 500.0 mL with *water R*. *Note: the pH is 7.7-7.8 at room temperature and 7.4 at 37 °C. This solution is stable for several months at 4 °C.*

Tris(hydroxymethyl)aminomethane sodium chloride buffer solution pH 7.4. *4004900.*

Dissolve 6.08 g of *tris(hydroxymethyl)aminomethane R*, 8.77 g of *sodium chloride R* in 500 mL of *distilled water R*. Add 10.0 g of *bovine albumin R*. Adjust the pH using *hydrochloric acid R*. Dilute to 1000.0 mL with *distilled water R*.

Tris(hydroxymethyl)aminomethane sodium chloride buffer solution pH 7.4 R1. *4012200.*

Dissolve 0.1 g of *bovine albumin R* in a mixture containing 2 mL of *tris(hydroxymethyl)aminomethane buffer solution pH 7.4 R* and 50 mL of a 5.84 mg/mL solution of *sodium chloride R*. Dilute to 100.0 mL with *water R*.

Tris-sodium acetate buffer solution pH 7.4. *4012900.*

Dissolve 6.3 g of *tris(hydroxymethyl)aminomethane R* and 4.9 g of *anhydrous sodium acetate R* in 900 mL of *water R*. Adjust to pH 7.4 with *sulfuric acid R* and dilute to 1000 mL with *water R*.

Tris-sodium acetate-sodium chloride buffer solution pH 7.4. *4013000.*

Dissolve 30.0 g of *tris(hydroxymethyl)aminomethane R*, 14.5 g of *anhydrous sodium acetate R* and 14.6 g of *sodium chloride R* in 900 mL of *water R*. Add 0.50 g of *bovine albumin R*. Adjust to pH 7.4 with *sulfuric acid R* and dilute to 1000 mL with *water R*.

Borate buffer solution pH 7.5. *4005200.*

Dissolve 2.5 g of *sodium chloride R*, 2.85 g of *disodium tetraborate R* and 10.5 g of *boric acid R* in *water R* and dilute to 1000.0 mL with the same solvent. Adjust the pH if necessary.

Storage: at 2 °C to 8 °C.

Buffer (HEPES) solution pH 7.5. *4009700.*

Dissolve 2.38 g of *HEPES R* in about 90 mL of *water R*. Adjust the pH to 7.5 with *sodium hydroxide solution R*. Dilute to 100 mL with *water R*.

0.05 M Phosphate buffer solution pH 7.5. *4014400.*

Dissolve 0.89 g of *disodium hydrogen phosphate dihydrate R* in about 80 mL of *water R*. Adjust to pH 7.5 with an 8.5 per cent *V/V* solution of *phosphoric acid R* and dilute to 100.0 mL with *water R*.

0.2 M Phosphate buffer solution pH 7.5. *4005400.*

Dissolve 27.22 g of *potassium dihydrogen phosphate R* in 930 mL of *water R*, adjust to pH 7.5 with a 300 g/L solution of *potassium hydroxide R* and dilute to 1000.0 mL with *water R*.

0.33 M Phosphate buffer solution pH 7.5. *4005300.*

Dissolve 119.31 g of *disodium hydrogen phosphate dodecahydrate R* in *water R* and dilute to 1000.0 mL with the same solvent (solution A). Dissolve 45.36 g of *potassium dihydrogen phosphate R* in *water R* and dilute to 1000.0 mL with the same solvent (solution B). Mix 85 mL of solution A and 15 mL of solution B. Adjust the pH if necessary.

0.25 M Sodium phosphate buffer solution pH 7.5. *4016100.*

Dissolve 3.90 g of *sodium dihydrogen phosphate R* in 70 mL of *water R*, adjust to pH 7.5 with a 300 g/L solution of *sodium hydroxide R* and dilute to 100 mL with *water R*.

0.05 M Tris-hydrochloride buffer solution pH 7.5. *4005600.*

Dissolve 6.057 g of *tris(hydroxymethyl)aminomethane R* in *water R* and adjust the pH with *hydrochloric acid R*. Dilute to 1000.0 mL with *water R*.

0.1 M Tris-hydrochloride buffer solution pH 7.5. *4016200.*

Dissolve 3.03 g of *tris(hydroxymethyl)aminomethane R* in 200 mL of *water R*, adjust to pH 7.5 with *hydrochloric acid R* and dilute to 250 mL with *water R*.

1 M Tris-hydrochloride buffer solution pH 7.5. *4014500.*

Dissolve 12.11 g of *tris(hydroxymethyl)aminomethane R* in 90 mL of *water R*, adjust to pH 7.5 with *hydrochloric acid R* and dilute to 100.0 mL with *water R*.

Tris(hydroxymethyl)aminomethane buffer solution pH 7.5. *4005500.*

Dissolve 7.27 g of *tris(hydroxymethyl)aminomethane R* and 5.27 g of *sodium chloride R* in *water R*, and adjust the pH if necessary. Dilute to 1000.0 mL with *water R*.

Tris(hydroxymethyl)aminomethane buffer solution pH 7.5 R1. *4016400.*

Dissolve 1.21 g of *tris(hydroxymethyl)aminomethane R* in 900 mL of *water R* and add 10 mL of *0.01 M calcium chloride solution R*. Adjust the pH if necessary with *sodium hydroxide solution R* or *hydrochloric acid R*, and dilute to 1000.0 mL with *water R*.

Sodium citrate buffer solution pH 7.8 (0.034 M sodium citrate, 0.101 M sodium chloride). *4009800.*

Dissolve 10.0 g of *sodium citrate R* and 5.90 g of *sodium chloride R* in 900 mL of *water R*. Adjust the pH by addition of *hydrochloric acid R* and dilute to 1000 mL with *water R*.

0.0015 M Borate buffer solution pH 8.0. *4006000.*

Dissolve 0.572 g of *disodium tetraborate R* and 2.94 g of *calcium chloride R* in 800 mL of *water R*. Adjust the pH with *1 M hydrochloric acid*. Dilute to 1000.0 mL with *water R*.

Buffer solution pH 8.0. *4005900.*

To 50.0 mL of *0.2 M potassium dihydrogen phosphate R* add 46.8 mL of *0.2 M sodium hydroxide*. Dilute to 200.0 mL with *water R*.

Buffer solution pH 8.0 R1. *4010400.*

Dissolve 20 g of *dipotassium hydrogen phosphate R* in 900 mL of *water R*. Adjust the pH with *phosphoric acid R*. Dilute to 1000 mL with *water R*.

0.02 M Phosphate buffer solution pH 8.0. *4006100.*

To 50.0 mL of *0.2 M potassium dihydrogen phosphate R* add 46.8 mL of *0.2 M sodium hydroxide*. Dilute to 500.0 mL with *water R*.

0.1 M Phosphate buffer solution pH 8.0. *4008400.*

Dissolve 0.523 g of *potassium dihydrogen phosphate R* and 16.73 g of *dipotassium hydrogen phosphate R* in *water R* and dilute to 1000.0 mL with the same solvent.

1 M Phosphate buffer solution pH 8.0. *4007800.*

Dissolve 136.1 g of *potassium dihydrogen phosphate R* in *water R*, adjust the pH with *1 M sodium hydroxide*. Dilute to 1000.0 mL with *water R*.

0.02 M Sodium phosphate buffer solution pH 8.0. *4013700.*

Dissolve 0.31 g of *sodium dihydrogen phosphate R* in 70 mL of *water R* and adjust to pH 8.0 with *1 M sodium hydroxide*, then dilute to 100 mL with *water R*.

1 M Tris-hydrochloride buffer solution pH 8.0. *4012700.*

Dissolve 121.1 g of *tris(hydroxymethyl)aminomethane R* and 1.47 g of *calcium chloride R* in 900 mL of *water R*. Adjust the pH with *hydrochloric acid R* and dilute to 1000.0 mL with *water R*.

Tris-hydrochloride buffer solution pH 8.0. *4012300.*

Dissolve 1.21 g of *tris(hydroxymethyl)aminomethane R* and 29.4 mg of *calcium chloride R* in *water R*. Adjust the pH with *1 M hydrochloric acid* and dilute to 100.0 mL with *water R*.

Tris-sodium acetate buffer solution pH 8.0. *4013100.*

Dissolve 6.3 g of *tris(hydroxymethyl)aminomethane R* and 4.9 g of *anhydrous sodium acetate R* in 900 mL of *water R*. Adjust to pH 8.0 with *sulfuric acid R* and dilute to 1000 mL with *water R*.

Tris-sodium acetate-sodium chloride buffer solution pH 8.0. *4013200.*

Dissolve 30.0 g of *tris(hydroxymethyl)aminomethane R*, 14.5 g of *anhydrous sodium acetate R* and 14.6 g of *sodium chloride R* in 900 mL of *water R*. Add 0.50 g of *bovine albumin R*. Adjust to pH 8.0 with *sulfuric acid R* and dilute to 1000 mL with *water R*.

Tris(hydroxymethyl)aminomethane buffer solution pH 8.1. *4006200.*

Dissolve 0.294 g of *calcium chloride R* in 40 mL of *tris(hydroxymethyl)aminomethane solution R* and adjust the pH with *1 M hydrochloric acid*. Dilute to 100.0 mL with *water R*.

Guanidine-tris(hydroxymethyl)aminomethane buffer solution pH 8.3. *4016300.*

Dissolve 1.21 g of *tris(hydroxymethyl)aminomethane R* in 87.5 mL of a 764 g/L solution of *guanidine hydrochloride R*. Adjust to pH 8.3 with *hydrochloric acid R* and dilute to 100 mL with *water R*.

Tris-glycine buffer solution pH 8.3. *4006300.*

Dissolve 6.0 g of *tris(hydroxymethyl)aminomethane R* and 28.8 g of *glycine R* in *water R* and dilute to 1000.0 mL with the same solvent. Dilute 1 volume to 10 volumes with *water R* immediately before use.

Tris-hydrochloride buffer solution pH 8.3. *4011800.*

Dissolve 9.0 g of *tris(hydroxymethyl)aminomethane R* in 2.9 L of *water R*. Adjust the pH with *1 M hydrochloric acid*. Adjust the volume to 3 L with *water R*.

Barbital buffer solution pH 8.4. *4006400.*

Dissolve 8.25 g of *barbital sodium R* in *water R* and dilute to 1000.0 mL with the same solvent.

Tris-EDTA BSA buffer solution pH 8.4. *4006500.*

Dissolve 6.1 g of *tris(hydroxymethyl)aminomethane R*, 2.8 g of *sodium edetate R*, 10.2 g of *sodium chloride R* and 10 g of *bovine albumin R* in *water R*, adjust to pH 8.4 using *1 M hydrochloric acid* and dilute to 1000.0 mL with *water R*.

Tris(hydroxymethyl)aminomethane-EDTA buffer solution pH 8.4. *4006600.*

Dissolve 5.12 g of *sodium chloride R*, 3.03 g of *tris(hydroxymethyl)aminomethane R* and 1.40 g of *sodium edetate R* in 250 mL of *distilled water R*. Adjust the pH to 8.4 using *hydrochloric acid R*. Dilute to 500.0 mL with *distilled water R*.

Tris(hydroxymethyl)aminomethane-EDTA buffer solution pH 8.4 R1. *4015100.*

Dissolve 10.20 g of *sodium chloride R*, 6.10 g of *tris(hydroxymethyl)aminomethane R*, 2.80 g of *sodium edetate R* and 1.00 g of *macrogol 6000 R* or 2.00 g of *bovine albumin R* or of *human albumin R* in 800 mL of *water R*. Adjust to pH 8.4 with *hydrochloric acid R* and dilute to 1.0 L with *water R*.

Guanidine-tris(hydroxymethyl)aminomethane-EDTA buffer solution pH 8.5. *4014600.*

Dissolve 1.0 g of *sodium edetate R*, 12.1 g of *tris(hydroxymethyl)aminomethane R* and 57.0 g of *guanidine hydrochloride R* in 35 mL of *water R*. Adjust to pH 8.5 with *hydrochloric acid R* and dilute to 100 mL with *water R*.

Phosphate buffer solution pH 8.5. *4013300.*

Dissolve 3.5 g of *dipotassium hydrogen phosphate R* and 4.5 g of *sodium chloride R* in 500 mL of *water R*. Adjust the pH with a mixture of equal volumes of *dilute phosphoric acid R* and *water R*.

Tris acetate buffer solution pH 8.5. *4006700.*

Dissolve 0.294 g of *calcium chloride R* and 12.11 g of *tris(hydroxymethyl)aminomethane R* in *water R*. Adjust the pH with *acetic acid R*. Dilute to 1000.0 mL with *water R*.

Barbital buffer solution pH 8.6 R1. *4006900.*

Dissolve in *water R* 1.38 g of *barbital R*, 8.76 g of *barbital sodium R* and 0.38 g of *calcium lactate pentahydrate R* and dilute to 1000.0 mL with the same solvent.

Guanidine-tris(hydroxymethyl)aminomethane-EDTA buffer solution pH 8.6. *4016500.*

Dissolve 0.018 g of *sodium edetate R*, 2.2 g of *tris(hydroxymethyl)aminomethane R* and 28.7 g of *guanidine hydrochloride R* in 20 mL of *water R*. Adjust to pH 8.6 with *acetic acid R* and dilute to 50 mL with *water R*.

1.5 M Tris-hydrochloride buffer solution pH 8.8. *4009900.*

Dissolve 90.8 g of *tris(hydroxymethyl)aminomethane R* in 400 mL of *water R*. Adjust the pH with *hydrochloric acid R* and dilute to 500.0 mL with *water R*.

3 M Tris-hydrochloride buffer solution pH 8.8. *4015000.*

Dissolve 363.3 g of *tris(hydroxymethyl)aminomethane R* in 500 mL of *water R*. Adjust the pH with *hydrochloric acid R* and dilute to 1 L with *water R*.

Buffer (phosphate) solution pH 9.0. *4008300.*

Dissolve 1.74 g of *potassium dihydrogen phosphate R* in 80 mL of *water R*, adjust the pH with a 1 M potassium hydroxide solution prepared from *potassium hydroxide R* and dilute to 100.0 mL with *water R*.

Buffer solution pH 9.0. *4007000.*

Dissolve 6.18 g of *boric acid R* in *0.1 M potassium chloride R* and dilute to 1000.0 mL with the same solvent. Mix 1000.0 mL of this solution and 420.0 mL of *0.1 M sodium hydroxide*.

Buffer solution pH 9.0 R1. *4007100.*

Dissolve 6.20 g of *boric acid R* in 500 mL of *water R* and adjust the pH with *1 M sodium hydroxide* (about 41.5 mL). Dilute to 1000.0 mL with *water R*.

0.05 M Tris-hydrochloride buffer solution pH 9.0. *4013500.*

Dissolve 0.605 g of *tris(hydroxymethyl)aminomethane R* in *water R*. Adjust the pH with *1 M hydrochloric acid* and dilute to 100.0 mL with *water R*.

Tris(hydroxymethyl)aminomethane buffer solution pH 9.0. *4015200.*

Dissolve 1.21 g of *tris(hydroxymethyl)aminomethane R* in 950 mL of *water for chromatography R*. Adjust to pH 9.0 with *acetic acid R* and dilute to 1000.0 mL with *water for chromatography R*.

Tris(hydroxymethyl)aminomethane buffer solution pH 9.0 R1. *4016600.*

Dissolve 12.1 g of *tris(hydroxymethyl)aminomethane R* in 950 mL of *water R*. Adjust to pH 9.0 with *acetic acid R* and dilute to 1000.0 mL with *water R*.

Ammonium chloride buffer solution pH 9.5. *4007200.*

Dissolve 33.5 g of *ammonium chloride R* in 150 mL of *water R*, add 42.0 mL of *concentrated ammonia R* and dilute to 250.0 mL with *water R*.

Storage: in a polyethylene container.

Ammonium chloride buffer solution pH 10.0. *4007300.*

Dissolve 5.4 g of *ammonium chloride R* in 20 mL of *water R*, add 35.0 mL of *ammonia R* and dilute to 100.0 mL with *water R*.

Borate buffer solution pH 10.0. *4016000.*

Introduce 12.4 g of *boric acid R* into a 500.0 mL volumetric flask. Add 300 mL of *water R* to suspend the boric acid. Add 100 mL of a 56 g/L solution of *potassium hydroxide R* and mix to dissolve the boric acid. Adjust to pH 10.0 by slowly adding a 56 g/L solution of *potassium hydroxide R* (about 60 mL is usually needed). Mix. Dilute almost to volume with *water R*. If necessary, adjust the pH with *boric acid R* or with a 56 g/L solution of *potassium hydroxide R*. Dilute to 500.0 mL with *water R*.

Diethanolamine buffer solution pH 10.0. *4007500.*

Dissolve 96.4 g of *diethanolamine R* in *water R* and dilute to 400 mL with the same solvent. Add 0.5 mL of an 186 g/L solution of *magnesium chloride R* and adjust the pH with *1 M hydrochloric acid*. Dilute to 500.0 mL with *water R*.

0.1 M Ammonium carbonate buffer solution pH 10.3. *4011900.*

Dissolve 7.91 g of *ammonium carbonate R* in 800 mL of *water R*. Adjust the pH with *dilute sodium hydroxide solution R*. Dilute to 1000.0 mL with *water R*.

Ammonium chloride buffer solution pH 10.4. *4011000.*

Dissolve 70 g of *ammonium chloride R* in 200 mL of *water R*, add 330 mL of *concentrated ammonia R* and dilute to 1000.0 mL with *water R*. If necessary, adjust to pH 10.4 with *ammonia R*.

Borate buffer solution pH 10.4. *4011100.*

Dissolve 24.64 g of *boric acid R* in 900 mL of *distilled water R*. Adjust the pH using a 400 g/L solution of *sodium hydroxide R*. Dilute to 1000 mL with *distilled water R*.

Ammonium chloride buffer solution pH 10.7. *4013400.*

Dissolve 67.5 g of *ammonium chloride R* in *water R*, add 570 mL of *concentrated ammonia R* and dilute to 1000.0 mL with *water R*.

Buffer solution pH 10.9. *4007600.*

Dissolve 6.75 g of *ammonium chloride R* in *ammonia R* and dilute to 100.0 mL with the same solvent.

Total-ionic-strength-adjustment buffer. *4007700.*

Dissolve 58.5 g of *sodium chloride R*, 57.0 mL of *glacial acetic acid R*, 61.5 g of *sodium acetate R* and 5.0 g of *cyclohexylenedinitrilotetra-acetic acid R* in *water R* and dilute to 500.0 mL with the same solvent. Adjust to pH 5.0 to 5.5 with a 335 g/L solution of *sodium hydroxide R* and dilute to 1000.0 mL with *distilled water R*.

Total-ionic-strength-adjustment buffer R1. *4008800.*

Dissolve 210 g of *citric acid monohydrate R* in 400 mL of *distilled water R*. Adjust to pH 7.0 with *concentrated ammonia R*. Dilute to 1000.0 mL with *distilled water R* (solution A). Dissolve 132 g of *ammonium phosphate R* in *distilled water R* and dilute to 1000.0 mL with the same solvent (solution B). To a suspension of 292 g of (ethylenedinitrilo)tetra-*acetic acid R* in about 500 mL of *distilled water R*, add about 200 mL of *concentrated ammonia R* to dissolve. Adjust the pH to 6 to 7 with *concentrated ammonia R*. Dilute to 1000.0 mL with *distilled water R* (solution C). Mix equal volumes of solution A, B, and C and adjust to pH 7.5 with *concentrated ammonia R*.

Buffer solution pH 11. *4014000.*

Dissolve 6.21 g of *boric acid R*, 4.00 g of *sodium hydroxide R* and 3.70 g of *potassium chloride R* in 500 mL of *water R* and dilute to 1000 mL with the same solvent.

0.1 M Phosphate buffer solution pH 11.3. *4015400.*

Dissolve 17.4 g of *dipotassium hydrogen phosphate R* in about 950 mL of *water R*, adjust to pH 11.3 using a 100 g/L solution of *potassium hydroxide R* and dilute to 1.0 L with *water R*. Filter through a membrane filter (nominal pore size 0.45 µm).

4.2. VOLUMETRIC ANALYSIS

07/2017:40201

4.2.1. PRIMARY STANDARDS FOR VOLUMETRIC SOLUTIONS

Primary standards for volumetric solutions are indicated by the suffix RV. Primary standards of suitable quality may be obtained from commercial sources or prepared by the following methods.

For primary standards from commercial sources, a pre-treatment step may be necessary. Follow the supplier's instructions.

A secondary standard may be used provided its traceability to a primary standard has been demonstrated.

Arsenious trioxide. As_2O_3. (M_r 197.8). *2000100.* [1327-53-3]. Arsenic(III) oxide.

Sublime *arsenious trioxide R* in a suitable apparatus.

Storage: over *anhydrous silica gel R*.

Benzoic acid. $C_7H_6O_2$. (M_r 122.1). *2000200.* [65-85-0].

Sublime *benzoic acid R* in a suitable apparatus.

Ferrous ethylenediammonium sulfate. $Fe(C_2H_{10}N_2)(SO_4)_2,4H_2O$. ($M_r$ 382.1). *2000900.* [113193-60-5]. Ethylenediammonium iron(II) disulfate tetrahydrate. Ethylenediammonium tetraaquabis(sulfato)iron(II).

Content: minimum 99.5 per cent.

Potassium bromate. $KBrO_3$. (M_r 167.0). *2000300.* [7758-01-2].

Crystallise *potassium bromate R* from boiling *water R*. Collect the crystals and dry to constant mass at 180 °C.

Potassium hydrogen phthalate. $C_8H_5KO_4$. (M_r 204.2). *2000400.* [877-24-7]. Potassium 2-carboxybenzoate.

Recrystallise *potassium hydrogen phthalate R* from boiling *water R*, collect the crystals at a temperature above 35 °C and dry to constant mass at 110 °C.

Sodium chloride. NaCl. (M_r 58.44). *2000600.* [7647-14-5].

To 1 volume of the *saturated sodium chloride solution R* add 2 volumes of *hydrochloric acid R*. Collect the crystals formed and wash with *hydrochloric acid R1*. Remove the hydrochloric acid by heating on a water-bath and dry the crystals to constant mass at 300 °C.

Sulfanilic acid. $C_6H_7NO_3S$. (M_r 173.2). *2000700.* [121-57-3]. 4-Aminobenzenesulfonic acid.

Recrystallise *sulfanilic acid R* from boiling *water R*. Filter and dry to constant mass at 100-105 °C.

Trometamol. $C_4H_{11}NO_3$. (M_r 121.1). *2001000.* [77-86-1]. 2-Amino-2-(hydroxymethyl)propane-1,3-diol. Tris(hydroxymethyl)aminomethane.

Content: minimum 99.5 per cent.

Zinc. Zn. (M_r 65.4). *2000800.* [7440-66-6].

Content: minimum 99.9 per cent.

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4.2.2. VOLUMETRIC SOLUTIONS

Volumetric solutions are prepared according to the usual chemical analytical methods. The accuracy of the apparatus used is verified to ensure that it is appropriate for the intended use.

The concentration of volumetric solutions is indicated in terms of molarity. Molarity expresses, as the number of moles, the amount of substance dissolved in 1 L of solution. A solution which contains *x* moles of substance per litre is said to be *x* M.

Volumetric solutions do not differ from the prescribed strength by more than 10 per cent. The molarity of the volumetric solutions is determined by an appropriate number of titrations. The repeatability does not exceed 0.2 per cent (relative standard deviation).

Volumetric solutions are standardised by the methods described below. When a volumetric solution is to be used in an assay in which the end-point is determined by an electrochemical process (for example, amperometry or potentiometry) the solution is standardised by the same method. The composition of the medium in which a volumetric solution is standardised should be the same as that in which it is to be used.

Solutions more dilute than those described below are either prepared by adapting the quantities stated or by dilution, with *carbon dioxide-free water R* (unless otherwise prescribed), of a more concentrated solution that has been previously standardised. In the first case, the correction factor is determined on the volumetric solution to be used in the monograph. In the latter case, the correction factor of the dilute solution is the same as that of the standardised solution from which it was prepared.

Commercially available volumetric solutions traceable to a primary standard may be used provided their titre is determined or verified prior to first use.

Titres of volumetric solutions are verified at appropriate intervals that are defined in the quality system procedures.

0.1 M Ammonium and cerium nitrate. *3000100.*

Shake for 2 min a solution containing 56 mL of *sulfuric acid R* and 54.82 g of *ammonium and cerium nitrate R*, then add 5 successive quantities, each of 100 mL, of *water R*, shaking after each addition. Dilute the clear solution to 1000.0 mL with *water R*. Standardise the solution after 10 days.

Standardisation. Dissolve 0.300 g of *ferrous ethylenediammonium sulfate RV* in 50 mL of a diluted solution of *sulfuric acid R* (49 g/L H_2SO_4). Titrate with the ammonium and cerium nitrate solution, determining the end-point potentiometrically (*2.2.20*) or using 0.1 mL of *ferroin R* as indicator.

1 mL of *0.1 M ammonium and cerium nitrate* is equivalent to 38.21 mg of $Fe(C_2H_{10}N_2)(SO_4)_2,4H_2O$.

Storage: protected from light.

0.1 M Ammonium and cerium sulfate. *3000300.*

Dissolve 65.0 g of *ammonium and cerium sulfate R* in a mixture of 500 mL of *water R* and 30 mL of *sulfuric acid R*. Allow to cool and dilute to 1000.0 mL with *water R*.

Standardisation. Dissolve 0.300 g of *ferrous ethylenediammonium sulfate RV* in 50 mL of a diluted solution of *sulfuric acid R* (49 g/L H_2SO_4). Titrate with the ammonium and cerium sulfate solution, determining the end-point potentiometrically (*2.2.20*) or using 0.1 mL of *ferroin R* as indicator.

1 mL of *0.1 M ammonium and cerium sulfate* is equivalent to 38.21 mg of $Fe(C_2H_{10}N_2)(SO_4)_2,4H_2O$.

Dilution. Use a diluted solution of *sulfuric acid R* (59 g/L H_2SO_4) while cooling the solution.

0.1 M Ammonium thiocyanate. *3000500.*

Dissolve 7.612 g of *ammonium thiocyanate R* in *water R* and dilute to 1000.0 mL with the same solvent.

Standardisation. To 20.0 mL of *0.1 M silver nitrate* add 25 mL of *water R*, 2 mL of *dilute nitric acid R* and 2 mL of *ferric ammonium sulfate solution R2*. Titrate with the ammonium thiocyanate solution until a reddish-yellow colour is obtained.

0.1 M Barium chloride. *3000600.*

Dissolve 24.4 g of *barium chloride R* in *water R* and dilute to 1000.0 mL with the same solvent.

Standardisation. To 10.0 mL of the barium chloride solution add 60 mL of *water R*, 3 mL of *concentrated ammonia R* and 0.5-1 mg of *phthalein purple R*. Titrate with *0.1 M sodium edetate*. When the solution begins to decolorise, add 50 mL of *ethanol (96 per cent) R* and continue the titration until the blue-violet colour disappears.

0.05 M Barium perchlorate. *3000700.*

Dissolve 15.8 g of *barium hydroxide R* in a mixture of 7.5 mL of *perchloric acid R* and 75 mL of *water R*, adjust the solution to pH 3 by adding *perchloric acid R* and filter if necessary. Add 150 mL of *ethanol (96 per cent) R* and dilute to 250 mL with *water R*. Dilute to 1000.0 mL with *buffer solution pH 3.7 R*.

Standardisation. To 5.0 mL of *0.05 M sulfuric acid* add 5 mL of *water R*, 50 mL of *buffer solution pH 3.7 R* and 0.5 mL of *alizarin S solution R*. Titrate with the barium perchlorate solution until an orange-red colour appears. Standardise immediately before use.

Dilution. Use *buffer solution pH 3.7 R*.

0.005 M Barium perchlorate. *3010200.*

Dilute 10.0 mL of *0.05 M barium perchlorate* to 100.0 mL with a buffer solution prepared as follows: to 15.0 mL of *acetic acid R* add 60.0 mL of *2-propanol R*. Adjust to pH 3.7 with *ammonia R* and dilute to 100.0 mL with *water R*.

0.004 M Benzethonium chloride. *3000900.*

Dissolve in *water R* 1.792 g of *benzethonium chloride R*, previously dried to constant mass at 100-105 °C, and dilute to 1000.0 mL with the same solvent.

Standardisation. 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 *0.1 M perchloric acid*, using 0.05 mL of *crystal violet solution R* as indicator. Carry out a blank titration.

1 mL of *0.1 M perchloric acid* is equivalent to 44.81 mg of $C_{27}H_{42}ClNO_2$.

0.01 M Bismuth nitrate. *3010000.*

Dissolve 4.86 g of *bismuth nitrate pentahydrate R* in 60 mL of *dilute nitric acid R* and dilute to 1000.0 mL with *water R*.

Standardisation. To 25.0 mL of the bismuth nitrate solution, add 50 mL of *water R* and titrate with *0.01 M sodium edetate* using 0.05 mL of a 1 g/L solution of *xylenol orange R* as indicator.

0.0167 M Bromide-bromate. *3001000.*

Dissolve 2.7835 g of *potassium bromate RV* and 13 g of *potassium bromide R* in *water R* and dilute to 1000.0 mL with the same solvent.

0.1 M Cerium sulfate. *3001100.*

Dissolve 40.4 g of *cerium sulfate R* in a mixture of 500 mL of *water R* and 50 mL of *sulfuric acid R*. Allow to cool and dilute to 1000.0 mL with *water R*.

Standardisation. Dissolve 0.300 g of *ferrous ethylenediammonium sulfate RV* in 50 mL of a diluted solution of *sulfuric acid R* (49 g/L H_2SO_4). Titrate with the cerium sulfate solution, determining the end-point potentiometrically (*2.2.20*) or using 0.1 mL of *ferroin R* as indicator.

1 mL of *0.1 M cerium sulfate* is equivalent to 38.21 mg of $Fe(C_2H_{10}N_2)(SO_4)_2,4H_2O$.

0.02 M Copper sulfate. *3001200.*

Dissolve 5.0 g of *copper sulfate pentahydrate R* in *water R* and dilute to 1000.0 mL with the same solvent.

Standardisation. To 20.0 mL of the copper sulfate solution add 2 g of *sodium acetate R* and 0.1 mL of *pyridylazonaphthol solution R*. Titrate with *0.02 M sodium edetate* until the colour changes from violet-blue to bright green. Titrate slowly towards the end of the titration.

0.1 M Ferric ammonium sulfate. *3001300.*

Dissolve 50.0 g of *ferric ammonium sulfate R* in a mixture of 6 mL of *sulfuric acid R* and 300 mL of *water R* and dilute to 1000.0 mL with *water R*.

Standardisation. To 10.0 mL of the ferric ammonium sulfate solution add 35 mL of *water R*, 3 mL of *hydrochloric acid R* and 1 g of *potassium iodide R*. Allow to stand for 10 min. Titrate with *0.1 M sodium thiosulfate*, determining the end-point potentiometrically (*2.2.20*) or using 1 mL of *starch solution R* as indicator.

1 mL of *0.1 M sodium thiosulfate* is equivalent to 48.22 mg of $FeNH_4(SO_4)_2,12H_2O$.

0.1 M Ferrous sulfate. *3001400.*

Dissolve 27.80 g of *ferrous sulfate R* in 500 mL of *dilute sulfuric acid R* and dilute to 1000.0 mL with *water R*.

Standardisation. To 25.0 mL of the ferrous sulfate solution add 3 mL of *phosphoric acid R* and titrate immediately with *0.02 M potassium permanganate*. Standardise immediately before use.

1 M Hydrochloric acid. *3001800.*

Dilute 103.0 g of *hydrochloric acid R* to 1000.0 mL with *water R*.

Standardisation. Dissolve 0.950 g of *trometamol RV* in 50 mL of *water R*. Titrate with the hydrochloric acid solution, determining the end-point potentiometrically (*2.2.20*) or using 0.1 mL of *methyl orange solution R* as indicator until a yellowish-red colour is obtained.

1 mL of *1 M hydrochloric acid* is equivalent to 121.1 mg of $C_4H_{11}NO_3$.

0.1 M Hydrochloric acid. *3002100.*

Dilute 100.0 mL of *1 M hydrochloric acid* to 1000.0 mL with *carbon dioxide-free water R*.

Standardisation. Carry out the titration described for *1 M hydrochloric acid* using 95 mg of *trometamol RV* dissolved in 50 mL of *water R*.

1 mL of *0.1 M hydrochloric acid* is equivalent to 12.11 mg of $C_4H_{11}NO_3$.

0.5 M Iodine. *3009400.*

Dissolve 127 g of *iodine R* and 200 g of *potassium iodide R* in *water R* and dilute to 1000.0 mL with the same solvent.

Standardisation. To 2.0 mL of the iodine solution add 1 mL of *dilute acetic acid R* and 50 mL of *water R*. Titrate with *0.1 M sodium thiosulfate*, using *starch solution R* as indicator.

Storage: protected from light.

0.05 M Iodine. *3002700.*

Dissolve 12.7 g of *iodine R* and 20 g of *potassium iodide R* in *water R* and dilute to 1000.0 mL with the same solvent.

Standardisation. To 10.0 mL of the iodine solution add 1 mL of *dilute acetic acid R* and 40 mL of *water R*. Titrate with *0.1 M sodium thiosulfate*, determining the end-point potentiometrically (*2.2.20*) or using *starch solution R* as indicator.

Storage: protected from light.

0.01 M Iodine. *3002900.*

Add 0.3 g of *potassium iodide R* to 20.0 mL of *0.05 M iodine* and dilute to 100.0 mL with *water R*.

0.1 M Lanthanum nitrate. *3010100.*

Dissolve 43.30 g of *lanthanum nitrate R* in *water R* and dilute to 1000.0 mL with the same solvent.

Standardisation. To 20.0 mL of the lanthanum nitrate solution, add 15 mL of *water R* and 25 mL of *0.1 M sodium edetate*. Add about 50 mg of *xylenol orange triturate R* and about 2 g of *hexamethylenetetramine R*. Titrate with *0.1 M zinc sulfate* until the colour changes from yellow to violet-pink.

1 mL of *0.1 M sodium edetate* is equivalent to 43.30 mg of $La(NO_3)_3,6H_2O$.

0.1 M Lead nitrate. *3003100.*

Dissolve 33 g of *lead nitrate R* in *water R* and dilute to 1000.0 mL with the same solvent.

Standardisation. Take 20.0 mL of the lead nitrate solution and carry out the determination of lead by complexometry (*2.5.11*).

0.1 M Lithium methoxide. *3003300.*

Dissolve 0.694 g of *lithium R* in 150 mL of *anhydrous methanol R* and dilute to 1000.0 mL with *toluene R*.

Standardisation. To 10 mL of *dimethylformamide R* add 0.05 mL of a 3 g/L solution of *thymol blue R* in *methanol R* and titrate with the lithium methoxide solution until a pure blue colour is obtained. Immediately add 0.100 g of *benzoic acid RV*. Stir to effect solution and titrate with the lithium methoxide solution until the pure blue colour is again obtained. Protect the solution from atmospheric carbon dioxide throughout the titration. From the volume of titrant used in the second titration ascertain the exact strength of the lithium methoxide solution. Standardise immediately before use.

1 mL of *0.1 M lithium methoxide* is equivalent to 12.21 mg of $C_7H_6O_2$.

0.1 M Magnesium chloride. *3003400.*

Dissolve 20.33 g of *magnesium chloride R* in *water R* and dilute to 1000.0 mL with the same solvent.

Standardisation. Carry out the determination of magnesium by complexometry (*2.5.11*).

1 M Nitric acid. *3003600.*

Dilute 96.6 g of *nitric acid R* to 1000.0 mL with *water R*.

Standardisation. Dissolve 0.950 g of *trometamol RV* in 50 mL of *water R*. Titrate with the nitric acid solution, determining the end-point potentiometrically (*2.2.20*) or using 0.1 mL of *methyl orange solution R* as indicator until a reddish-yellow colour is obtained.

1 mL of *1 M nitric acid* is equivalent to 121.1 mg of $C_4H_{11}NO_3$.

0.1 M Perchloric acid. *3003900.*

Place 8.5 mL of *perchloric acid R* in a volumetric flask containing about 900 mL of *glacial acetic acid R* and mix. Add 30 mL of *acetic anhydride R*, dilute to 1000.0 mL with *glacial acetic acid R*, mix and allow to stand for 24 h. Determine the water content (*2.5.12*) without addition of methanol and, if necessary, adjust the water content to 0.1-0.2 per cent by adding either *acetic anhydride R* or *water R*. Allow to stand for 24 h.

Standardisation. Dissolve 0.170 g of *potassium hydrogen phthalate RV* in 50 mL of *anhydrous acetic acid R*, warming gently if necessary. Allow to cool protected from air, and titrate with the perchloric acid solution, determining the end-point potentiometrically (*2.2.20*) or using 0.05 mL of *crystal violet solution R* as indicator. Note the temperature of the perchloric acid solution at the time of the titration. If the temperature at which an assay is carried out is different from that at which the *0.1 M perchloric acid* has been standardised, the volume used in the assay becomes:

$$V_c = V[1 + (t_1 - t_2)0.0011]$$

t_1 = temperature during standardisation,

t_2 = temperature during the assay,

V_c = corrected volume,

V = observed volume.

1 mL of *0.1 M perchloric acid* is equivalent to 20.42 mg of $C_8H_5KO_4$.

Dilution. Use *anhydrous acetic acid R*.

0.033 M Potassium bromate. *3004200.*

Dissolve 5.5670 g of *potassium bromate RV* in *water R* and dilute to 1000.0 mL with the same solvent.

0.1 M Potassium hydrogen phthalate. *3004700.*

In a conical flask containing about 800 mL of *anhydrous acetic acid R*, dissolve 20.42 g of *potassium hydrogen phthalate RV*. Heat on a water-bath until completely dissolved, protected from humidity. Cool to 20 °C and dilute to 1000.0 mL with *anhydrous acetic acid R*.

0.1 M Potassium hydroxide. *3004800.*

Dissolve 6 g of *potassium hydroxide R* in *carbon dioxide-free water R* and dilute to 1000.0 mL with the same solvent.

Standardisation. Dissolve 0.150 g of *potassium hydrogen phthalate RV* in 50 mL of *water R*. Titrate with the potassium hydroxide solution, determining the end-point potentiometrically (*2.2.20*) or using 0.1 mL of *phenolphthalein solution R* as indicator.

1 mL of *0.1 M potassium hydroxide* is equivalent to 20.42 mg of $C_8H_5KO_4$.

0.5 M Potassium hydroxide in alcohol (60 per cent *V/V*). *3004900.*

Dissolve 3 g of *potassium hydroxide R* in *aldehyde-free alcohol R* (60 per cent *V/V*) and dilute to 100.0 mL with the same solvent.

Standardisation. Dissolve 0.500 g of *benzoic acid RV* in 10 mL of *water R* and 40 mL of *ethanol (96 per cent) R*. Titrate with the potassium hydroxide solution, determining the end-point potentiometrically (*2.2.20*) or using 0.1 mL of *phenolphthalein solution R* as indicator.

1 mL of *0.5 M potassium hydroxide in alcohol (60 per cent V/V)* is equivalent to 61.06 mg of $C_7H_6O_2$.

0.5 M Potassium hydroxide, alcoholic. *3005000.*

Dissolve 3 g of *potassium hydroxide R* in 5 mL of *water R* and dilute to 100.0 mL with *aldehyde-free alcohol R*.

Standardisation. Dissolve 0.500 g of *benzoic acid RV* in 10 mL of *water R* and 40 mL of *ethanol (96 per cent) R*. Titrate with the potassium hydroxide solution, determining the end-point potentiometrically (*2.2.20*) or using 0.1 mL of *phenolphthalein solution R* as indicator.

1 mL of *0.5 M alcoholic potassium hydroxide* is equivalent to 61.06 mg of $C_7H_6O_2$.

Dilution. Use *aldehyde-free alcohol R*.

0.05 M Potassium iodate. *3005200.*

Dissolve 10.70 g of *potassium iodate R* in *water R* and dilute to 1000.0 mL with the same solvent.

Standardisation. To 3.0 mL of the potassium iodate solution add 40.0 mL of *water R*, 1 g of *potassium iodide R* and 5 mL of *dilute sulfuric acid R*. Titrate with *0.1 M sodium thiosulfate*, determining the end-point potentiometrically (*2.2.20*) or using 1 mL of *starch solution R*, added towards the end of the titration, as indicator.

1 mL of *0.1 M sodium thiosulfate* is equivalent to 3.567 mg of KIO_3.

0.001 M Potassium iodide. *3009200.*

Dilute 10.0 mL of *potassium iodide solution R* to 100.0 mL with *water R*. Dilute 5.0 mL of this solution to 500.0 mL with *water R*.

0.02 M Potassium permanganate. *3005300.*

Dissolve 3.2 g of *potassium permanganate R* in *water R* and dilute to 1000.0 mL with the same solvent. Heat the solution for 1 h on a water-bath, allow to cool and filter through a sintered-glass filter (*2.1.2*).

Standardisation. Dissolve 0.300 g of *ferrous ethylenediammonium sulfate RV* in 50 mL of a diluted solution of *sulfuric acid R* (49 g/L H_2SO_4). Titrate with the potassium permanganate solution, determining the end-point potentiometrically (*2.2.20*) or by the colour of the solution changing to pink. Standardise immediately before use.

1 mL of *0.02 M potassium permanganate* is equivalent to 38.21 mg of $Fe(C_2H_{10}N_2)(SO_4)_2,4H_2O$.

Storage: protected from light.

0.1 M Silver nitrate. *3005600.*

Dissolve 17.0 g of *silver nitrate R* in *water R* and dilute to 1000.0 mL with the same solvent.

Standardisation. Dissolve 50 mg of *sodium chloride RV* in *water R*, add 5 mL of *dilute nitric acid R* and dilute to 50 mL with *water R*. Titrate with the silver nitrate solution, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M silver nitrate* is equivalent to 5.844 mg of NaCl.

Storage: protected from light.

0.1 M Sodium arsenite. *3005800.*

Dissolve *arsenious trioxide RV* equivalent to 4.946 g of As_2O_3 in a mixture of 20 mL of *strong sodium hydroxide solution R* and 20 mL of *water R*, dilute to 400 mL with *water R* and add *dilute hydrochloric acid R* until the solution is neutral to *blue litmus paper R*. Dissolve 2 g of *sodium hydrogen carbonate R* in the solution and dilute to 500.0 mL with *water R*.

0.1 M Sodium edetate. *3005900.*

Dissolve 37.5 g of *sodium edetate R* in 500 mL of *water R*, add 100 mL of *1 M sodium hydroxide* and dilute to 1000.0 mL with *water R*.

Standardisation. Dissolve 0.120 g of *zinc RV* in 4 mL of *hydrochloric acid R1*. Add *dilute sodium hydroxide solution R* until the solution is weakly acid and carry out the assay of zinc by complexometry (*2.5.11*).

1 mL of *0.1 M sodium edetate* is equivalent to 6.538 mg of Zn.

Storage: in a polyethylene container.

1 M Sodium hydroxide. *3006300.*

Dissolve 42 g of *sodium hydroxide R* in *carbon dioxide-free water R* and dilute to 1000.0 mL with the same solvent.

Standardisation. Dissolve 1.50 g of *potassium hydrogen phthalate RV* in 50 mL of *water R*. Titrate with the sodium hydroxide solution, determining the end-point potentiometrically (*2.2.20*) or using 0.1 mL of *phenolphthalein solution R* as indicator.

1 mL of *1 M sodium hydroxide* is equivalent to 204.2 mg of $C_8H_5KO_4$.

If sodium hydroxide free from carbonate is prescribed, prepare it as follows. Dissolve *sodium hydroxide R* in *water R* to give a concentration of 400-600 g/L and allow to stand. Decant the clear supernatant, taking precautions to avoid the introduction

of carbon dioxide, and dilute with *carbon dioxide-free water R* to the required molarity. The solution complies with the following test. Titrate 20.0 mL of hydrochloric acid of the same molarity with the solution of sodium hydroxide, using 0.1 mL of *phenolphthalein solution R* as indicator. At the end-point add just sufficient of the acid to discharge the pink colour and concentrate the solution to 20 mL by boiling. During boiling add just sufficient acid to discharge the pink colour, which should not reappear after prolonged boiling. The volume of acid used does not exceed 0.1 mL.

0.1 M Sodium hydroxide. *3006600.*

Dilute 100.0 mL of *1 M sodium hydroxide* to 1000.0 mL with *carbon dioxide-free water R*.

Standardisation. Carry out the titration described for *1 M sodium hydroxide* using 0.150 g of *potassium hydrogen phthalate RV* in 50 mL of *water R*.

1 mL of *0.1 M sodium hydroxide* is equivalent to 20.42 mg of $C_8H_5KO_4$.

Standardisation (for use in the assay of halide salts of organic bases). Dissolve 0.100 g of *benzoic acid RV* in a mixture of 5 mL of *0.01 M hydrochloric acid* and 50 mL of *ethanol (96 per cent) R*. Carry out the titration (*2.2.20*), using the sodium hydroxide solution. Note the volume added between the 2 points of inflexion.

1 mL of *0.1 M sodium hydroxide* is equivalent to 12.21 mg of $C_7H_6O_2$.

0.1 M Sodium hydroxide, ethanolic. *3007000.*

To 250 mL of *anhydrous ethanol R* add 3.3 g of *strong sodium hydroxide solution R*.

Standardisation. Dissolve 0.100 g of *benzoic acid RV* in 10 mL of *water R* and 40 mL of *ethanol (96 per cent) R*. Titrate with the ethanolic sodium hydroxide solution, determining the end-point potentiometrically (*2.2.20*) or using 0.2 mL of *thymolphthalein solution R* as indicator. Standardise immediately before use.

1 mL of *0.1 M ethanolic sodium hydroxide* is equivalent to 12.21 mg of $C_7H_6O_2$.

0.1 M Sodium methoxide. *3007100.*

Cool 175 mL of *anhydrous methanol R* in iced *water R* and add, in small portions, about 2.5 g of freshly cut *sodium R*. When the metal has dissolved, dilute to 1000.0 mL with *toluene R*.

Standardisation. To 10 mL of *dimethylformamide R* add 0.05 mL of a 3 g/L solution of *thymol blue R* in *methanol R*, and titrate with the sodium methoxide solution until a pure blue colour is obtained. Immediately add 0.100 g of *benzoic acid RV*. Stir until dissolution and titrate with the sodium methoxide solution until the pure blue colour is again obtained. Protect the solution from atmospheric carbon dioxide throughout the titration. From the volume of titrant used in the second titration ascertain the exact strength of the sodium methoxide solution. Standardise immediately before use.

1 mL of *0.1 M sodium methoxide* is equivalent to 12.21 mg of $C_7H_6O_2$.

0.1 M Sodium nitrite. *3007200.*

Dissolve 7.5 g of *sodium nitrite R* in *water R* and dilute to 1000.0 mL with the same solvent.

Standardisation. Dissolve 0.150 g of *sulfanilic acid RV* in 50 mL of *dilute hydrochloric acid R* and carry out the determination of primary aromatic amino-nitrogen (*2.5.8*), using the sodium nitrite solution and determining the end-point electrometrically. Standardise immediately before use.

1 mL of *0.1 M sodium nitrite* is equivalent to 17.32 mg of $C_6H_7NO_3S$.

0.1 M Sodium periodate. *3009500.*

Dissolve 21.4 g of *sodium periodate R* in about 500 mL of *water R* and dilute to 1000.0 mL with the same solvent.

Standardisation. In a stoppered flask, introduce 5.0 mL of the sodium periodate solution and add 100 mL of *water R*. Add 10 mL of *potassium iodide solution R* and 5 mL of *hydrochloric acid R1*, close, shake and allow to stand for 2 min. Titrate with *0.1 M sodium thiosulfate* until the yellow colour almost disappears. Determine the end-point potentiometrically (*2.2.20*) or add 2 mL of *starch solution R* and titrate slowly until the colour is completely discharged.

1 mL of *0.1 M sodium thiosulfate* is equivalent to 2.674 mg of $NaIO_4$ or 0.125 mL of *0.1 M sodium periodate*.

0.1 M Sodium thiosulfate. *3007300.*

Dissolve 25 g of *sodium thiosulfate R* and 0.2 g of *sodium carbonate R* in *carbon dioxide-free water R* and dilute to 1000.0 mL with the same solvent.

Standardisation. To 10.0 mL of *0.033 M potassium bromate*, add 40 mL of *water R*, 10 mL of *potassium iodide solution R* and 5 mL of *hydrochloric acid R1*. Titrate with the sodium thiosulfate solution, using 1 mL of *starch solution R*, added towards the end of the titration, as indicator.

1 mL of *0.1 M sodium thiosulfate* is equivalent to 2.783 mg of $KBrO_3$ or 0.5 mL of *0.033 M potassium bromate*.

0.5 M Sulfuric acid. *3007800.*

Dissolve 28 mL of *sulfuric acid R* in *water R* and dilute to 1000.0 mL with the same solvent.

Standardisation. Dissolve 0.950 g of *trometamol RV* in 50 mL of *water R*. Titrate with the sulfuric acid solution, determining the end-point potentiometrically (*2.2.20*) or using 0.1 mL of *methyl orange solution R* as indicator until the solution turns reddish-yellow.

1 mL of *0.5 M sulfuric acid* is equivalent to 121.1 mg of $C_4H_{11}NO_3$.

0.1 M Tetrabutylammonium hydroxide. *3008300.*

Dissolve 40 g of *tetrabutylammonium iodide R* in 90 mL of *anhydrous methanol R*, add 20 g of finely powdered *silver oxide R* and shake vigorously for 1 h. Centrifuge a few millilitres of the mixture and test the supernatant for iodides. If a positive reaction is obtained, add an additional 2 g of *silver oxide R* and shake for a further 30 min. Repeat this procedure until the liquid is free from iodides, filter the mixture through a fine sintered-glass filter (*2.1.2*) and rinse the reaction vessel and filter with three quantities, each of 50 mL, of *toluene R*. Add the washings to the filtrate and dilute to 1000.0 mL with *toluene R*. Pass dry carbon dioxide-free nitrogen through the solution for 5 min.

Standardisation. To 10 mL of *dimethylformamide R* add 0.05 mL of a 3 g/L solution of *thymol blue R* in *methanol R* and titrate with the tetrabutylammonium hydroxide solution until a pure blue colour is obtained. Immediately add 0.100 g of *benzoic acid RV*. Stir to effect solution, and titrate with the tetrabutylammonium hydroxide solution until the pure blue colour is again obtained. Protect the solution from atmospheric carbon dioxide throughout the titration. From the volume of titrant used in the second titration ascertain the exact strength of the tetrabutylammonium hydroxide solution. Standardise immediately before use.

1 mL of *0.1 M tetrabutylammonium hydroxide* is equivalent to 12.21 mg of $C_7H_6O_2$.

0.1 M Tetrabutylammonium hydroxide in 2-propanol. *3008400.*

Prepare as described for *0.1 M tetrabutylammonium hydroxide* using *2-propanol R* instead of *toluene R* and standardise as described.

0.05 M Zinc chloride. *3008500.*

Dissolve 6.82 g of *zinc chloride R*, weighed with appropriate precautions, in *water R*. If necessary, add dropwise *dilute hydrochloric acid R* until the opalescence disappears. Dilute to 1000.0 mL with *water R*.

Standardisation. To 20.0 mL of the zinc chloride solution add 5 mL of *dilute acetic acid R* and carry out the determination of zinc by complexometry (*2.5.11*).

0.1 M Zinc sulfate. *3008600.*

Dissolve 29 g of *zinc sulfate R* in *water R* and dilute to 1000.0 mL with the same solvent.

Standardisation. To 20.0 mL of the zinc sulfate solution add 5 mL of *dilute acetic acid R* and carry out the determination of zinc by complexometry (*2.5.11*).

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5.1.4. MICROBIOLOGICAL QUALITY OF NON-STERILE PHARMACEUTICAL PREPARATIONS AND SUBSTANCES FOR PHARMACEUTICAL USE(1)

◊*This chapter does not apply to products containing viable micro-organisms as active ingredients.*◊

The presence of certain micro-organisms 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 therefore have to ensure a low bioburden of finished dosage forms by implementing current guidelines on Good Manufacturing Practice during the manufacture, storage and distribution of pharmaceutical preparations.

Microbial examination of non-sterile products is performed according to the methods given in general chapters *2.6.12* and *2.6.13*. 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 Tables 5.1.4.-1 and 5.1.4.-2. Acceptance criteria are based on individual results or on the average of replicate counts when replicate counts are performed (e.g. direct plating methods).

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 5.1.4.-1 includes a list of specified micro-organisms 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 micro-organisms depending on the nature of the starting materials and the manufacturing process.

If it has been shown that none of the prescribed tests will allow valid enumeration of micro-organisms 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 micro-organisms listed in Table 5.1.4.-1, the significance of other micro-organisms recovered is evaluated in terms of:

- use of the product: hazard varies according to the route of administration (eye, nose, respiratory tract);
- nature of the product: its ability to support growth, the presence of adequate antimicrobial preservation;
- method of application;
- 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 specialised 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.

Table 5.1.4.-1. – *Acceptance criteria for microbiological quality of non-sterile dosage forms*

Route of administration	TAMC (CFU/g or CFU/mL)	TYMC (CFU/g or CFU/mL)	Specified micro-organisms
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 nebulisation)	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 (1 g or 1 mL)
◆Special Ph. Eur. provision for oral dosage forms containing raw materials of natural (animal, vegetal or mineral) origin for which antimicrobial pretreatment is not feasible and for which the competent 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)◆
◆Special Ph. Eur. provision for premixes for medicated feeding stuffs for veterinary use using excipients of plant origin for which antimicrobial treatment is not feasible.	10^5	10^4	Not more than 10^4 CFU of bile-tolerant gram-negative bacteria (1 g or 1 mL) Absence of *Escherichia coli* (1 g or 1 mL) Absence of *Salmonella* (25 g or 25 mL)◆

(1) This chapter has undergone pharmacopoeial harmonisation. See chapter *5.8. Pharmacopoeial harmonisation*.

Table 5.1.4.-2. – *Acceptance criteria for microbiological quality of non-sterile substances for pharmaceutical use*

	TAMC (CFU/g or CFU/mL)	**TYMC (CFU/g or CFU/mL)**
Substances for pharmaceutical use	10^3	10^2

◆*Recommended acceptance criteria for microbiological quality of herbal medicinal products for oral use and extracts used in their preparation are given in general chapter 5.1.8.*◆

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5.4. RESIDUAL SOLVENTS

LIMITING RESIDUAL SOLVENT LEVELS IN ACTIVE SUBSTANCES, EXCIPIENTS AND MEDICINAL PRODUCTS

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) has adopted Impurities Guidelines for Residual Solvents which prescribes limits for the content of solvents which may remain in active substances, excipients and medicinal products after processing. This guideline, the text of which is reproduced below, excludes existing marketed products. The European Pharmacopoeia is, however, applying the same principles enshrined in the guideline to existing active substances, excipients and medicinal products whether or not they are the subject of a monograph of the Pharmacopoeia. All substances and products are to be tested for the content of solvents likely to be present in a substance or product.

Where the limits to be applied comply with those given below, tests for residual solvents are not generally mentioned in specific monographs since the solvents employed may vary from one manufacturer to another and the requirements of this general chapter are applied via the general monograph on *Substances for Pharmaceutical Use (2034)*. The competent authority is to be informed of the solvents employed during the production process. This information is also given in the dossier submitted for a certificate of suitability of the monographs of the European Pharmacopoeia and is mentioned on the certificate.

Where only Class 3 solvents are used, a test for loss on drying may be applied or a specific determination of the solvent may be made. If for a Class 3 solvent a justified and authorised limit higher than 0.5 per cent is applied, a specific determination of the solvent is required.

When Class 1 residual solvents or Class 2 residual solvents (or Class 3 residual solvents which exceed the 0.5 per cent) are used, the methodology described in the general method (*2.4.24*) is to be applied wherever possible. Otherwise an appropriate validated method is to be employed.

When a quantitative determination of a residual solvent is carried out, the result is taken into account for the calculation of the content of the substance except where a test for drying is carried out.

IMPURITIES: GUIDELINES FOR RESIDUAL SOLVENTS (CHMP/ICH/82260/2006)

1. INTRODUCTION

The objective of this guideline is to recommend acceptable amounts of residual solvents in pharmaceuticals for the safety of the patient. The guideline recommends the use of less toxic solvents and describes levels considered to be toxicologically acceptable for some residual solvents.

Residual solvents in pharmaceuticals are defined here as organic volatile chemicals that are used or produced in the manufacture of active substances or excipients, or in the preparation of medicinal products. The solvents are not completely removed by practical manufacturing techniques. Appropriate selection of the solvent for the synthesis of active substance may enhance the yield, or determine characteristics such as crystal form, purity, and solubility. Therefore, the solvent may sometimes be a critical parameter in the synthetic process. This guideline does not address solvents deliberately used as excipients nor does it address solvates. However, the content of solvents in such products should be evaluated and justified.

Since there is no therapeutic benefit from residual solvents, all residual solvents should be removed to the extent possible to meet product specifications, good manufacturing practices, or other quality-based requirements. Medicinal products should contain no higher levels of residual solvents than can be supported by safety data. Some solvents that are known to cause unacceptable toxicities (Class 1, Table 1) should be avoided in the production of active substances, excipients, or medicinal products unless their use can be strongly justified in a risk-benefit assessment. Some solvents associated with less severe toxicity (Class 2, Table 2) should be limited in order to protect patients from potential adverse effects. Ideally, less toxic solvents (Class 3, Table 3) should be used where practical. The complete list of solvents included in this guideline is given in Appendix 1.

The lists are not exhaustive and other solvents can be used and later added to the lists. Recommended limits of Class 1 and 2 solvents or classification of solvents may change as new safety data becomes available. Supporting safety data in a marketing application for a new medicinal product containing a new solvent may be based on concepts in this guideline or the concept of qualification of impurities as expressed in the guideline for active substances (*Q3A*, Impurities in New Active Substances) or medicinal products (*Q3B*, Impurities in New Medicinal Products), or all three guidelines.

2. SCOPE OF THE GUIDELINE

Residual solvents in active substances, excipients, and in medicinal products are within the scope of this guideline. Therefore, testing should be performed for residual solvents when production or purification processes are known to result in the presence of such solvents. It is only necessary to test for solvents that are used or produced in the manufacture or purification of active substances, excipients, or medicinal product. Although manufacturers may choose to test the medicinal product, a cumulative method may be used to calculate the residual solvent levels in the medicinal product from the levels in the ingredients used to produce the medicinal product. If the calculation results in a level equal to or below that recommended in this guideline, no testing of the medicinal product for residual solvents need be considered. If however, the calculated level is above the recommended level, the medicinal product should be tested to ascertain whether the formulation process has reduced the relevant solvent level to within the acceptable amount. Medicinal product should also be tested if a solvent is used during its manufacture.

This guideline does not apply to potential new active substances, excipients, or medicinal products used during the clinical research stages of development, nor does it apply to existing marketed medicinal products.

The guideline applies to all dosage forms and routes of administration. Higher levels of residual solvents may be acceptable in certain cases such as short term (30 days or less) or topical application. Justification for these levels should be made on a case by case basis.

See Appendix 2 for additional background information related to residual solvents.

3. GENERAL PRINCIPLES

3.1. CLASSIFICATION OF RESIDUAL SOLVENTS BY RISK ASSESSMENT

The term "tolerable daily intake" (TDI) is used by the International Program on Chemical Safety (IPCS) to describe exposure limits of toxic chemicals and "acceptable daily intake" (ADI) is used by the World Health Organization (WHO) and other national and international health authorities and institutes. The new term "permitted daily exposure" (PDE) is defined in the present guideline as a pharmaceutically acceptable intake of residual solvents to avoid confusion of differing values for ADI's of the same substance.

Residual solvents assessed in this guideline are listed in Appendix 1 by common names and structures. They were evaluated for their possible risk to human health and placed into one of three classes as follows:

Class 1 solvents: solvents to be avoided

Known human carcinogens, strongly suspected human carcinogens, and environmental hazards.

Class 2 solvents: solvents to be limited

Non-genotoxic animal carcinogens or possible causative agents of other irreversible toxicity such as neurotoxicity or teratogenicity.

Solvents suspected of other significant but reversible toxicities.

Class 3 solvents: solvents with low toxic potential

Solvents with low toxic potential to man; no health-based exposure limit is needed. Class 3 solvents have PDEs of 50 mg or more per day.

3.2. METHODS FOR ESTABLISHING EXPOSURE LIMITS

The method used to establish permitted daily exposures for residual solvents is presented in Appendix 3. Summaries of the toxicity data that were used to establish limits are published in *Pharmeuropa*, Vol. 9, No. 1, Supplement April 1997.

3.3. OPTIONS FOR DESCRIBING LIMITS OF CLASS 2 SOLVENTS

Two options are available when setting limits for Class 2 solvents.

Option 1: the concentration limits in parts per million stated in Table 2 can be used. They were calculated using equation (1) below by assuming a product mass of 10 g administered daily.

$$\text{Concentration (ppm)} = \frac{1000 \times \text{PDE}}{\text{dose}} \qquad (1)$$

Here, PDE is given in terms of mg/day and dose is given in g/day.

These limits are considered acceptable for all substances, excipients, or products. Therefore this option may be applied if the daily dose is not known or fixed. If all excipients and active substances in a formulation meet the limits given in Option 1, then these components may be used in any proportion. No further calculation is necessary provided the daily dose does not exceed 10 g. Products that are administered in doses greater than 10 g per day should be considered under Option 2.

Option 2: it is not considered necessary for each component of the medicinal product to comply with the limits given in Option 1. The PDE in terms of mg/day as stated in Table 2 can be used with the known maximum daily dose and equation (1) above to determine the concentration of residual solvent allowed in a medicinal product. Such limits are considered acceptable provided that is has been demonstrated that the residual solvent has been reduced to the practical minimum. The limits should be realistic in relation to analytical precision, manufacturing capability, reasonable variation in the manufacturing process, and the limits should reflect contemporary manufacturing standards.

Option 2 may be applied by adding the amounts of a residual solvent present in each of the components of the medicinal product. The sum of the amounts of solvent per day should be less than that given by the PDE.

Consider an example of the use of Option 1 and Option 2 applied to acetonitrile in a medicinal product. The permitted daily exposure to acetonitrile is 4.1 mg per day; thus, the Option 1 limit is 410 ppm. The maximum administered daily mass of a medicinal product is 5.0 g, and the medicinal product contains two excipients. The composition of the medicinal product and the calculated maximum content of residual acetonitrile are given in the following table.

Component	Amount in formulation	Acetonitrile content	Daily exposure
Active substance	0.3 g	800 ppm	0.24 mg
Excipient 1	0.9 g	400 ppm	0.36 mg
Excipient 2	3.8 g	800 ppm	3.04 mg
Medicinal product	5.0 g	728 ppm	3.64 mg

Excipient 1 meets the Option 1 limit, but the active substance, excipient 2, and medicinal product do not meet the Option 1 limit. Nevertheless, the product meets the Option 2 limit of 4.1 mg per day and thus conforms to the recommendations in this guideline.

Consider another example using acetonitrile as residual solvent. The maximum administered daily mass of a medicinal product is 5.0 g, and the medicinal product contains two excipients. The composition of the medicinal product and the calculated maximum content of residual acetonitrile is given in the following table.

Component	Amount in formulation	Acetonitrile content	Daily exposure
Active substance	0.3 g	800 ppm	0.24 mg
Excipient 1	0.9 g	2000 ppm	1.80 mg
Excipient 2	3.8 g	800 ppm	3.04 mg
Medicinal product	5.0 g	1016 ppm	5.08 mg

In this example, the product meets neither the Option 1 nor the Option 2 limit according to this summation. The manufacturer could test the medicinal product to determine if the formulation process reduced the level of acetonitrile. If the level of acetonitrile was not reduced during formulation to the allowed limit, then the manufacturer of the medicinal product should take other steps to reduce the amount of acetonitrile in the medicinal product. If all of these steps fail to reduce the level of residual solvent, in exceptional cases the manufacturer could provide a summary of efforts made to reduce the solvent level to meet the guideline value, and provide a risk-benefit analysis to support allowing the product to be utilised containing residual solvent at a higher level.

3.4. ANALYTICAL PROCEDURES

Residual solvents are typically determined using chromatographic techniques such as gas chromatography. Any harmonised procedures for determining levels of residual solvents as described in the pharmacopoeias should be used, if feasible. Otherwise, manufacturers would be free to select the most appropriate validated analytical procedure for a particular application. If only Class 3 solvents are present, a non-specific method such as loss on drying may be used.

Validation of methods for residual solvents should conform to ICH guidelines "Text on Validation of Analytical Procedures" and "Extension of the ICH Text on Validation of Analytical Procedures".

3.5. REPORTING LEVELS OF RESIDUAL SOLVENTS

Manufacturers of pharmaceutical products need certain information about the content of residual solvents in excipients or active substances in order to meet the criteria of this guideline. The following statements are given as acceptable examples of the information that could be provided from a supplier of excipients or active substances to a pharmaceutical manufacturer. The supplier might choose one of the following as appropriate:

– only Class 3 solvents are likely to be present. Loss on drying is less than 0.5 per cent;

– only Class 2 solvents X, Y, ... are likely to be present. All are below the Option 1 limit;

(Here the supplier would name the Class 2 solvents represented by X, Y, ...)

– only Class 2 solvents X, Y, ... and Class 3 solvents are likely to be present. Residual Class 2 solvents are below the Option 1 limit and residual Class 3 solvents are below 0.5 per cent.

If Class 1 solvents are likely to be present, they should be identified and quantified. "Likely to be present" refers to the solvent used in the final manufacturing step and to solvents that are used in earlier manufacturing steps and not removed consistently by a validated process.

If solvents of Class 2 or Class 3 are present at greater than their Option 1 limits or 0.5 per cent, respectively, they should be identified and quantified.

4. LIMITS OF RESIDUAL SOLVENTS

4.1. SOLVENTS TO BE AVOIDED

Solvents in Class 1 should not be employed in the manufacture of active substances, excipients, and medicinal products because of their unacceptable toxicity or their deleterious environmental effect. However, if their use is unavoidable in order to produce a medicinal product with a significant therapeutic advance, then their levels should be restricted as shown in Table 1, unless otherwise justified. 1,1,1-Trichloroethane is included in Table 1 because it is an environmental hazard. The stated limit of 1500 ppm is based on a review of the safety data.

Table 1. – *Class 1 solvents in pharmaceutical products (solvents that should be avoided)*

Solvent	Concentration limit (ppm)	Concern
Benzene	2	Carcinogen
Carbon tetrachloride	4	Toxic and environmental hazard
1,2-Dichloroethane	5	Toxic
1,1-Dichloroethene	8	Toxic
1,1,1-Trichloroethane	1500	Environmental hazard

4.2. SOLVENTS TO BE LIMITED

Solvents in Table 2 should be limited in pharmaceutical products because of their inherent toxicity. PDEs are given to the nearest 0.1 mg/day, and concentrations are given to the nearest 10 ppm. The stated values do not reflect the necessary analytical precision of determination. Precision should be determined as part of the validation of the method.

Table 2. – *Class 2 solvents in pharmaceutical products*

Solvent	PDE (mg/day)	Concentration limit (ppm)
Acetonitrile	4.1	410
Chlorobenzene	3.6	360
Chloroform	0.6	60
Cumene	0.7	70
Cyclohexane	38.8	3880
1,2-Dichloroethene	18.7	1870
Dichloromethane	6.0	600
1,2-Dimethoxyethane	1.0	100
N,N-Dimethylacetamide	10.9	1090
N,N-Dimethylformamide	8.8	880
1,4-Dioxane	3.8	380
2-Ethoxyethanol	1.6	160
Ethyleneglycol	6.2	620
Formamide	2.2	220
Hexane	2.9	290
Methanol	30.0	3000
2-Methoxyethanol	0.5	50
Methylbutylketone	0.5	50
Methylcyclohexane	11.8	1180
Methylisobutylketone	45.0	4500
N-Methylpyrrolidone	5.3	530
Nitromethane	0.5	50
Pyridine	2.0	200
Sulfolane	1.6	160

Solvent	PDE (mg/day)	Concentration limit (ppm)
Tetrahydrofuran	7.2	720
Tetralin	1.0	100
Toluene	8.9	890
1,1,2-Trichloroethene	0.8	80
Xylene*	21.7	2170

*usually 60 per cent *m*-xylene, 14 per cent *p*-xylene, 9 per cent *o*-xylene with 17 per cent ethyl benzene.

4.3. SOLVENTS WITH LOW TOXIC POTENTIAL

Solvents in Class 3 (shown in Table 3) may be regarded as less toxic and of lower risk to human health. Class 3 includes no solvent known as a human health hazard at levels normally accepted in pharmaceuticals. However, there are no long-term toxicity or carcinogenicity studies for many of the solvents in Class 3. Available data indicate that they are less toxic in acute or short-term studies and negative in genotoxicity studies. It is considered that amounts of these residual solvents of 50 mg per day or less (corresponding to 5000 ppm or 0.5 per cent under Option l) would be acceptable without justification. Higher amounts may also be acceptable provided they are realistic in relation to manufacturing capability and good manufacturing practice.

Table 3. – *Class 3 solvents which should be limited by GMP or other quality-based requirements*

Acetic acid	Heptane
Acetone	Isobutyl acetate
Anisole	Isopropyl acetate
1-Butanol	Methyl acetate
2-Butanol	3-Methyl-1-butanol
Butyl acetate	Methylethylketone
tert-Butylmethyl ether	2-Methyl-1-propanol
Dimethyl sulfoxide	Pentane
Ethanol	1-Pentanol
Ethyl acetate	1-Propanol
Ethyl ether	2-Propanol
Ethyl formate	Propyl acetate
Formic acid	Triethylamine

4.4. SOLVENTS FOR WHICH NO ADEQUATE TOXICOLOGICAL DATA WAS FOUND

The following solvents (Table 4) may also be of interest to manufacturers of excipients, active substances, or medicinal products. However, no adequate toxicological data on which to base a PDE was found. Manufacturers should supply justification for residual levels of these solvents in pharmaceutical products.

Table 4. – *Solvents for which no adequate toxicological data was found*

1,1-Diethoxypropane	Methylisopropylketone
1,1-Dimethoxymethane	Methyltetrahydrofuran
2,2-Dimethoxypropane	Petroleum ether
Isooctane	Trichloroacetic acid
Isopropyl ether	Trifluoroacetic acid

GLOSSARY

Genotoxic carcinogens: carcinogens which produce cancer by affecting genes or chromosomes.

LOEL: abbreviation for *lowest-observed effect level.*

Lowest-observed effect level: the lowest dose of substance in a study or group of studies that produces biologically significant increases in frequency or severity of any effects in the exposed humans or animals.

Modifying factor: a factor determined by professional judgement of a toxicologist and applied to bioassay data to relate that data safely to humans.

Neurotoxicity: the ability of a substance to cause adverse effects on the nervous system.

NOEL: abbreviation for *no-observed-effect level.*

No-observed-effect level: the highest dose of substance at which there are no biologically significant increases in frequency or severity of any effects in the exposed humans or animals.

PDE: abbreviation for *permitted daily exposure.*

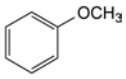
Permitted daily exposure: the maximum acceptable intake per day of residual solvent in pharmaceutical products.

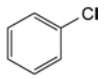
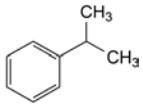
Reversible toxicity: the occurrence of harmful effects that are caused by a substance and which disappear after exposure to the substance ends.

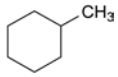
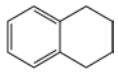
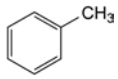
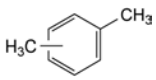
Strongly suspected human carcinogen: a substance for which there is no epidemiological evidence of carcinogenesis but there are positive genotoxicity data and clear evidence of carcinogenesis in rodents.

Teratogenicity: the occurrence of structural malformations in a developing foetus when a substance is administered during pregnancy.

APPENDIX 1. LIST OF SOLVENTS INCLUDED IN THE GUIDELINE

Solvent	Other Names	Structure	Class
Acetic acid	Ethanoic acid	CH_3COOH	Class 3
Acetone	2-Propanone Propan-2-one	CH_3COCH_3	Class 3
Acetonitrile		CH_3CN	Class 2
Anisole	Methoxybenzene		Class 3
Benzene	Benzol		Class 1
1-Butanol	*n*-Butyl alcohol Butan-1-ol	$CH_3[CH_2]_3OH$	Class 3
2-Butanol	*sec*-Butyl alcohol Butan-2-ol	$CH_3CH_2CH(OH)CH_3$	Class 3
Butyl acetate	Acetic acid butyl ester	$CH_3COO[CH_2]_3CH_3$	Class 3

Solvent	Other Names	Structure	Class
tert-Butylmethyl ether	2-Methoxy-2-methylpropane	$(CH_3)_3COCH_3$	Class 3
Carbon tetrachloride	Tetrachloromethane	CCl_4	Class 1
Chlorobenzene			Class 2
Chloroform	Trichloromethane	$CHCl_3$	Class 2
Cumene	Isopropylbenzene (1-Methylethyl)benzene		Class 2
Cyclohexane	Hexamethylene		Class 2
1,2-Dichloroethane	*sym*-Dichloroethane Ethylene dichloride Ethylene chloride	CH_2ClCH_2Cl	Class 1
1,1-Dichloroethene	1,1-Dichloroethylene Vinylidene chloride	$H_2C=CCl_2$	Class 1
1,2-Dichloroethene	1,2-Dichloroethylene Acetylene dichloride	$ClHC=CHCl$	Class 2
Dichloromethane	Methylene chloride	CH_2Cl_2	Class 2
1,2-Dimethoxyethane	Ethyleneglycol dimethyl ether Monoglyme Dimethyl cellosolve	$H_3COCH_2CH_2OCH_3$	Class 2
N,*N*-Dimethylacetamide	DMA	$CH_3CON(CH_3)_2$	Class 2
N,*N*-Dimethylformamide	DMF	$HCON(CH_3)_2$	Class 2
Dimethyl sulfoxide	Methylsulfinylmethane Methyl sulfoxide DMSO	$(CH_3)_2SO$	Class 3
1,4-Dioxane	*p*-Dioxane [1,4]Dioxane		Class 2
Ethanol	Ethyl alcohol	CH_3CH_2OH	Class 3
2-Ethoxyethanol	Cellosolve	$CH_3CH_2OCH_2CH_2OH$	Class 2
Ethyl acetate	Acetic acid ethyl ester	$CH_3COOCH_2CH_3$	Class 3
Ethyleneglycol	1,2-Dihydroxyethane 1,2-Ethanediol	$HOCH_2CH_2OH$	Class 2
Ethyl ether	Diethyl ether Ethoxyethane 1,1′-Oxybisethane	$CH_3CH_2OCH_2CH_3$	Class 3
Ethyl formate	Formic acid ethyl ester	$HCOOCH_2CH_3$	Class 3
Formamide	Methanamide	$HCONH_2$	Class 2
Formic acid		$HCOOH$	Class 3
Heptane	*n*-Heptane	$CH_3[CH_2]_5CH_3$	Class 3
Hexane	*n*-Hexane	$CH_3[CH_2]_4CH_3$	Class 2
Isobutyl acetate	Acetic acid isobutyl ester	$CH_3COOCH_2CH(CH_3)_2$	Class 3
Isopropyl acetate	Acetic acid isopropyl ester	$CH_3COOCH(CH_3)_2$	Class 3
Methanol	Methyl alcohol	CH_3OH	Class 2
2-Methoxyethanol	Methyl cellosolve	$CH_3OCH_2CH_2OH$	Class 2
Methyl acetate	Acetic acid methyl ester	CH_3COOCH_3	Class 3
3-Methyl-1-butanol	Isoamyl alcohol Isopentyl alcohol 3-Methylbutan-1-ol	$(CH_3)_2CHCH_2CH_2OH$	Class 3
Methylbutylketone	2-Hexanone Hexan-2-one	$CH_3[CH_2]_3COCH_3$	Class 2

Solvent	Other Names	Structure	Class
Methylcyclohexane	Cyclohexylmethane		Class 2
Methylethylketone	2-Butanone MEK Butan-2-one	$CH_3CH_2COCH_3$	Class 3
Methylisobutylketone	4-Methylpentan-2-one 4-Methyl-2-pentanone MIBK	$CH_3COCH_2CH(CH_3)_2$	Class 2
2-Methyl-1-propanol	Isobutyl alcohol 2-Methylpropan-1-ol	$(CH_3)_2CHCH_2OH$	Class 3
N-Methylpyrrolidone	1-Methylpyrrolidin-2-one 1-Methyl-2-pyrrolidinone		Class 2
Nitromethane		CH_3NO_2	Class 2
Pentane	*n*-Pentane	$CH_3[CH_2]_3CH_3$	Class 3
1-Pentanol	Amyl alcohol Pentan-1-ol Pentyl alcohol	$CH_3[CH_2]_3CH_2OH$	Class 3
1-Propanol	Propan-1-ol Propyl alcohol	$CH_3CH_2CH_2OH$	Class 3
2-Propanol	Propan-2-ol Isopropyl alcohol	$(CH_3)_2CHOH$	Class 3
Propyl acetate	Acetic acid propyl ester	$CH_3COOCH_2CH_2CH_3$	Class 3
Pyridine			Class 2
Sulfonane	Tetrahydrothiophene 1,1-dioxide		Class 2
Tetrahydrofuran	Tetramethylene oxide Oxacyclopentane		Class 2
Tetralin	1,2,3,4-Tetrahydronaphthalene		Class 2
Toluene	Methylbenzene		Class 2
1,1,1-Trichloroethane	Methylchloroform	CH_3CCl_3	Class 1
1,1,2-Trichloroethene	Trichloroethene	$HClC{=}CCl_2$	Class 2
Triethylamine	*N*,*N*-Diethylethanamine	$N(CH_2CH_3)_3$	Class 3
Xylene*	Dimethybenzene Xylol		Class 2

*usually 60 per cent *m*-xylene, 14 per cent *p*-xylene, 9 per cent *o*-xylene with 17 per cent ethyl benzene.

APPENDIX 2. ADDITIONAL BACKGROUND

A2.1. ENVIRONMENTAL REGULATION OF ORGANIC VOLATILE SOLVENTS

Several of the residual solvents frequently used in the production of pharmaceuticals are listed as toxic chemicals in Environmental Health Criteria (EHC) monographs and the Integrated Risk Information System (IRIS). The objectives of such groups as the International Programme on Chemical Safety (IPCS), the United States Environmental Protection Agency (USEPA) and the United States Food and Drug Administration (USFDA) include the determination of acceptable exposure levels. The goal is protection of human health and maintenance of environmental integrity against the possible deleterious effects of chemicals resulting from long-term environmental exposure. The methods involved in the estimation of maximum safe exposure limits are usually based on long-term studies. When long-term study data are unavailable, shorter term study data can be used with modification of the approach such as use of larger safety factors. The approach described therein relates primarily to long-term or life-time exposure of the general population in the ambient environment, i.e. ambient air, food, drinking water and other media.

A2.2. RESIDUAL SOLVENTS IN PHARMACEUTICALS

Exposure limits in this guideline are established by referring to methodologies and toxicity data described in EHC and IRIS monographs. However, some specific assumptions about residual solvents to be used in the synthesis and formulation of pharmaceutical products should be taken into account in establishing exposure limits. They are:

1) Patients (not the general population) use pharmaceuticals to treat their diseases or for prophylaxis to prevent infection or disease.

2) The assumption of life-time patient exposure is not necessary for most pharmaceutical products but may be appropriate as a working hypothesis to reduce risk to human health.

3) Residual solvents are unavoidable components in pharmaceutical production and will often be a part of medicinal products.

4) Residual solvents should not exceed recommended levels except in exceptional circumstances.

5) Data from toxicological studies that are used to determine acceptable levels for residual solvents should have been generated using appropriate protocols such as those described for example, by OECD, EPA, and the FDA Red Book.

APPENDIX 3. METHODS FOR ESTABLISHING EXPOSURE LIMITS

The Gaylor-Kodell method of risk assessment (Gaylor, D. W. and Kodell, R. L. Linear Interpolation algorithm for low dose assessment of toxic substance. J. Environ. Pathology, 4, 305, 1980) is appropriate for Class 1 carcinogenic solvents. Only in cases where reliable carcinogenicity data are available should extrapolation by the use of mathematical models be applied to setting exposure limits. Exposure limits for Class 1 solvents could be determined with the use of a large safety factor (i.e., 10 000 to 100 000) with respect to the no-observed-effect level (NOEL). Detection and quantification of these solvents should be by state-of-the-art analytical techniques.

Acceptable exposure levels in this guideline for Class 2 solvents were established by calculation of PDE values according to the procedures for setting exposure limits in pharmaceuticals (*Pharmacopeial Forum*, Nov-Dec 1989), and the method adopted by IPCS for Assessing Human Health Risk of Chemicals (*Environmental Health Criteria 170*, WHO, 1994). These methods are similar to those used by the USEPA (IRIS) and the USFDA (*Red Book*) and others. The method is outlined here to give a better understanding of the origin of the PDE values. It is not necessary to perform these calculations in order to use the PDE values tabulated in Section 4 of this document.

PDE is derived from the no-observed-effect level (NOEL), or the lowest-observed effect level (LOEL), in the most relevant animal study as follows:

$$\text{PDE} = \frac{\text{NOEL} \times \text{Weight Adjustment}}{\text{F1} \times \text{F2} \times \text{F3} \times \text{F4} \times \text{F5}}$$

The PDE is derived preferably from a NOEL. If no NOEL is obtained, the LOEL may be used. Modifying factors proposed here, for relating the data to humans, are the same kind of "uncertainty factors" used in Environmental Health Criteria (*Environmental Health Criteria* 170, World Health Organization, Geneva, 1994), and "modifying factors" or "safety factors" in *Pharmacopoeial Forum*. The assumption of 100 per cent systemic exposure is used in all calculations regardless of route of administration.

The modifying factors are as follows:

F1 = a factor to account for extrapolation between species:

- F1 = 2 for extrapolation from dogs to humans;
- F1 = 2.5 for extrapolation from rabbits to humans;
- F1 = 3 for extrapolation from monkeys to humans;
- F1 = 5 for extrapolation from rats to humans;
- F1 = 10 for extrapolation from other animals to humans;
- F1 = 12 for extrapolation from mice to humans.

F1 takes into account the comparative surface area: body weight ratios for the species concerned and for man. Surface area (S) is calculated as:

$$S = km^{0.67}$$

in which m = body mass, and the constant k has been taken to be 10. The body weight used in the equation are those shown below in Table A3.-1.

Table A3.-1. – *Values used in the calculations in this document*

Rat body weight	425 g
Pregnant rat body weight	330 g
Mouse body weight	28 g
Pregnant mouse body weight	30 g
Guinea-pig body weight	500 g
Rhesus monkey body weight	2.5 kg
Rabbit body weight (pregnant or not)	4 kg
Beagle dog body weight	11.5 kg
Rat respiratory volume	290 L/day
Mouse respiratory volume	43 L/day
Rabbit respiratory volume	1440 L/day
Guinea-pig respiratory volume	430 L/day
Human respiratory volume	28800 L/day
Dog respiratory volume	9000 L/day
Monkey respiratory volume	1150 L/day
Mouse water consumption	5 mL/day
Rat water consumption	30 mL/day
Rat food consumption	30 g/day

F2 = a factor of 10 to account for variability between individuals.

A factor of 10 is generally given for all organic solvents, and 10 is used consistently in this guideline.

F3 = a variable factor to account for toxicity studies of short-term exposure:

F3 = 1 for studies that last at least one half-lifetime (1 year for rodents or rabbits; 7 years for cats, dogs and monkeys);

F3 = 1 for reproductive studies in which the whole period of organogenesis is covered;

F3 = 2 for a 6 month study in rodents, or a 3.5 year study in non-rodents;

F3 = 5 for a 3 month study in rodents, or a 2 year study in non-rodents;

F3 = 10 for studies of a shorter duration.

In all cases, the higher factor has been used for study durations between the time points, e.g. a factor of 2 for a 9 month rodent study.

F4 = a factor that may be applied in cases of severe toxicity, e.g. non-genotoxic carcinogenicity, neurotoxicity or teratogenicity.

In studies of reproductive toxicity, the following factors are used:

F4 = 1 for foetal toxicity associated with maternal toxicity;

F4 = 5 for foetal toxicity without maternal toxicity;

F4 = 5 for a teratogenic effect with maternal toxicity;

F4 = 10 for a teratogenic effect without maternal toxicity.

F5 = a variable factor that may be applied if the no-effect level was not established.

When only a LOEL is available, a factor of up to 10 can be used depending on the severity of the toxicity.

The weight adjustment assumes an arbitrary adult human body weight for either sex of 50 kg. This relatively low weight provides an additional safety factor against the standard weights of 60 kg or 70 kg that are often used in this type of calculation. It is recognised that some adult patients weigh less than 50 kg; these patients are considered to be accommodated by the built-in safety factors used to determine a PDE. If the solvent was present in a formulation specifically intended for paediatric use, an adjustment for a lower body weight would be appropriate.

As an example of the application of this equation, consider the toxicity study of acetonitrile in mice that is summarised in *Pharmeuropa*, Vol. 9. No. 1, Supplement, April 1997, page S24. The NOEL is calculated to be 50.7 mg kg^{-1} day^{-1}. The PDE for acetonitrile in this study is calculated as follows:

$$\text{PDE} = \frac{50.7\text{mg kg}^{-1}\,\text{day}^{-1} \times 50\text{ kg}}{12 \times 10 \times 5 \times 1 \times 1} = 4.22\text{ mg day}^{-1}$$

In this example,

F1 = 12 to account for the extrapolation from mice to humans;

F2 = 10 to account for differences between individual humans;

F3 = 5 because the duration of the study was only 13 weeks;

F4 = 1 because no severe toxicity was encountered;

F5 = 1 because the no-effect level was determined.

The equation for an ideal gas, $PV = nRT$, is used to convert concentrations of gases used in inhalation studies from units of ppm to units of mg/L or mg/m^3. Consider as an example the rat reproductive toxicity study by inhalation of carbon tetrachloride (molecular weight 153.84) summarised in *Pharmeuropa*, Vol. 9, No. 1, Supplement, April 1997, page S9.

$$\frac{n}{V} = \frac{P}{RT} = \frac{300 \times 10^{-6}\text{ atm} \times 153\,840\text{ mg mol}^{-1}}{0.082\text{ L atm K}^{-1}\text{mol}^{-1} \times 298\text{ K}}$$

$$= \frac{46.15\text{ mg}}{24.45\text{ L}} = 1.89\text{ mg / L}$$

The relationship 1000 L = 1 m^3 is used to convert to mg/m^3.

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5.9. POLYMORPHISM

Polymorphism (or crystal polymorphism) is a phenomenon related to the solid state; it is the ability of a compound in the solid state to exist in different crystalline forms having the same chemical composition. Substances that exist in a non-crystalline solid state are said to be amorphous.

When this phenomenon is observed for a chemical element (for example, sulfur), the term allotropy is used instead of polymorphism.

The term pseudopolymorphism is used to describe solvates (including hydrates), where a solvent is present in the crystal matrix in stoichiometric proportions; the term may also be extended to include compounds where the solvent is trapped in the matrix in variable proportions. 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".

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

The identity of chemical composition implies that all crystalline and amorphous forms of a given species have the same chemical behaviour in solution or as a melt; in contrast, their physico-chemical and physical characteristics (solubility, hardness, compressibility, density, melting point, etc.), and therefore their reactivity and bioavailability may be different at the solid state.

When a compound shows polymorphism, the form for which the free enthalpy is lowest at a given temperature and pressure is the most thermodynamically stable. The other forms are said to be in a metastable state. At normal temperature and pressure, a metastable form may remain unchanged or may change to a thermodynamically more stable form.

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 the more 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 characterised by a transition temperature. However, if only one of the forms is stable over the entire temperature range, the change is irreversible or monotropic.

Different crystalline forms or solvates may be produced by varying the crystallisation conditions (temperature, pressure, solvent, concentration, rate of crystallisation, seeding of the crystallisation medium, presence and concentration of impurities, etc.).

The following techniques may be used to study polymorphism:

- X-ray diffraction of powders (*2.9.33*),
- X-ray diffraction of single crystals,
- thermal analysis (*2.2.34*) (differential scanning calorimetry, thermogravimetry, thermomicroscopy),
- microcalorimetry,
- moisture absorption analysis,
- optical and electronic microscopy,
- solid-state nuclear magnetic resonance,
- infrared absorption spectrophotometry (*2.2.24*),
- Raman spectroscopy (*2.2.48*),
- measurement of solubility and intrinsic dissolution rate,
- density measurement.

These techniques are often complementary and it is indispensable to use several of them.

Pressure/temperature and energy/temperature diagrams based on analytical data are valuable tools for fully understanding the energetic relationship (enantiotropism, monotropism) and the thermodynamic stability of the individual modifications of a polymorphic compound.

For solvates, differential scanning calorimetry and thermogravimetry are preferable, combined with measurements of solubility, intrinsic dissolution rate and X-ray diffraction.

For hydrates, water sorption/desorption isotherms are determined to demonstrate the zones of relative stability.

In general, hydrates are less soluble in water than anhydrous forms, and likewise solvates are less soluble in their solvent than unsolvated forms.

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5.10. CONTROL OF IMPURITIES IN SUBSTANCES FOR PHARMACEUTICAL USE

Preamble

The monographs of the European Pharmacopoeia on substances for pharmaceutical use are designed to ensure acceptable quality for users. The role of the Pharmacopoeia in public health protection requires that adequate control of impurities be provided by monographs. The quality required is based on scientific, technical and regulatory considerations.

Requirements concerning impurities are given in specific monographs and in the general monograph *Substances for pharmaceutical use (2034)*. Specific monographs and the general monograph are complementary: specific monographs prescribe acceptance criteria for impurities whereas the general monograph deals with the need for qualification, identification and reporting of any organic impurities that occur in *active substances*.

The thresholds for reporting, identification and qualification contained in the general monograph *Substances for pharmaceutical use (2034)* apply to all related substances. However, if a monograph does not contain a related substances test based on a quantitative method, any new impurities occurring above a threshold may be overlooked since the test is not capable to detect those impurities.

The provisions of the Related substances section of the general monograph *Substances for pharmaceutical use (2034)*, notably those concerning thresholds, do not apply to excipients; also excluded from the provisions of this section are: biological and biotechnological products; oligonucleotides; radiopharmaceuticals; fermentation products and semi-synthetic products derived therefrom; herbal products and crude products of animal and plant origin. Although the thresholds stated in the general monograph do not apply, the general concepts of reporting, identification (wherever possible) and qualification of impurities are equally valid for these classes.

Basis for the elaboration of monographs of the European Pharmacopoeia

European Pharmacopoeia monographs are elaborated on substances that are present in medicinal products that have been authorised by the competent authorities of Parties to the *European Pharmacopoeia Convention*. Consequently, these monographs do not necessarily cover all sources of substances for pharmaceutical use on the world market.

Organic and inorganic impurities present in those substances that have been evaluated by the competent authorities are qualified with respect to safety at the maximum authorised content (at the maximum daily dose) unless new safety data that become available following evaluation justify lower limits.

European Pharmacopoeia monographs on substances for pharmaceutical use are elaborated by groups of experts and working parties collaborating with national pharmacopoeia authorities, the competent authorities for marketing authorisation, national control laboratories and the European Pharmacopoeia laboratory; they are also assisted by the producers of the substances and/or the pharmaceutical manufacturers that use these substances.

Control of impurities in substances for pharmaceutical use

The quality with respect to impurities is controlled by a set of tests within a monograph. These tests are intended to cover organic and inorganic impurities that are relevant in view of the sources of active substances in authorised medicinal products.

Control of residual solvents is provided by the general monograph *Substances for pharmaceutical use (2034)* and general chapter *5.4. Residual solvents*. The certificate of suitability of a monograph of the European Pharmacopoeia for a given source of a substance indicates the residual solvents that are controlled together with the specified acceptance criteria and the validated control method where this differs from those described in general chapter *2.4.24. Identification and control of residual solvents*.

Monographs on organic chemicals usually have a test entitled "Related substances" that covers relevant organic impurities. This test may be supplemented by specific tests where the general test does not control a given impurity or where there are particular reasons (for example, safety reasons) for requiring special control.

Where a monograph has no Related substances (or equivalent) test but only specific tests, the user of a substance must nevertheless ensure that there is suitable control of organic impurities; those occurring above the identification threshold are to be identified (wherever possible) and, unless justified, those occurring above the qualification threshold are to be qualified (see also under Recommendations to users of monographs of active substances).

Where the monograph covers substances with different impurity profiles, it may have a single related substances test to cover all impurities mentioned in the Impurities section or several tests may be necessary to give control of all known profiles. Compliance may be established by carrying out only the tests relevant to the known impurity profile for the source of the substance.

Instructions for control of impurities may be included in the Production 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 too technically complex for general use or cannot be applied to the final drug substance and/or where validation of the production process (including the purification step) will give sufficient control.

Impurities section in monographs on active substances

The Impurities section in a monograph includes impurities (chemical structure and name wherever possible), which are usually organic, that are known to be detected by the tests prescribed in the monograph. It is based on information available at the time of elaboration or revision of the monograph and is not necessarily exhaustive. The section includes specified impurities and, where so indicated, other detectable impurities.

Specified impurities have an acceptance criterion not greater than that authorised by the competent authorities.

Other detectable impurities are potential impurities with a defined structure but not known to be normally present above the identification threshold in substances used in medicinal products that have been authorised by the competent authorities of Parties to the Convention. They are given in the Impurities section for information.

Where an impurity other than a specified impurity is found in an active substance it is the responsibility of the user of the substance to check whether it has to be identified/qualified, depending on its content, nature, maximum daily dose and relevant identification/qualification threshold, in accordance with the general monograph on *Substances for pharmaceutical use (2034)*, Related substances section.

It should be noted that specific thresholds are applied to substances exclusively for veterinary use.

Interpretation of the test for related substances in the monographs on active substances

A specific monograph on a substance for pharmaceutical use is to be read and interpreted in conjunction with the general monograph on *Substances for pharmaceutical use (2034)*.

Where a general acceptance criterion for impurities ("any other impurity", "other impurities", "any impurity") equivalent to a nominal content greater than the applicable identification threshold (see the general monograph on *Substances for pharmaceutical use (2034)*) is prescribed, this is valid only for specified impurities mentioned in the Impurities section. The need for identification (wherever possible), reporting, specification and qualification of other impurities that occur must be considered according to the requirements of the general monograph. It is the responsibility of the user of the substance to determine the validity of the acceptance criteria for impurities not mentioned in the Impurities section and for those indicated as other detectable impurities.

Acceptance criteria for the related substances test are presented in different ways in existing monographs; the decision tree (Figure 5.10.-1) may be used as an aid in the interpretation of general acceptance criteria and their relation with the Impurities section of the monograph.

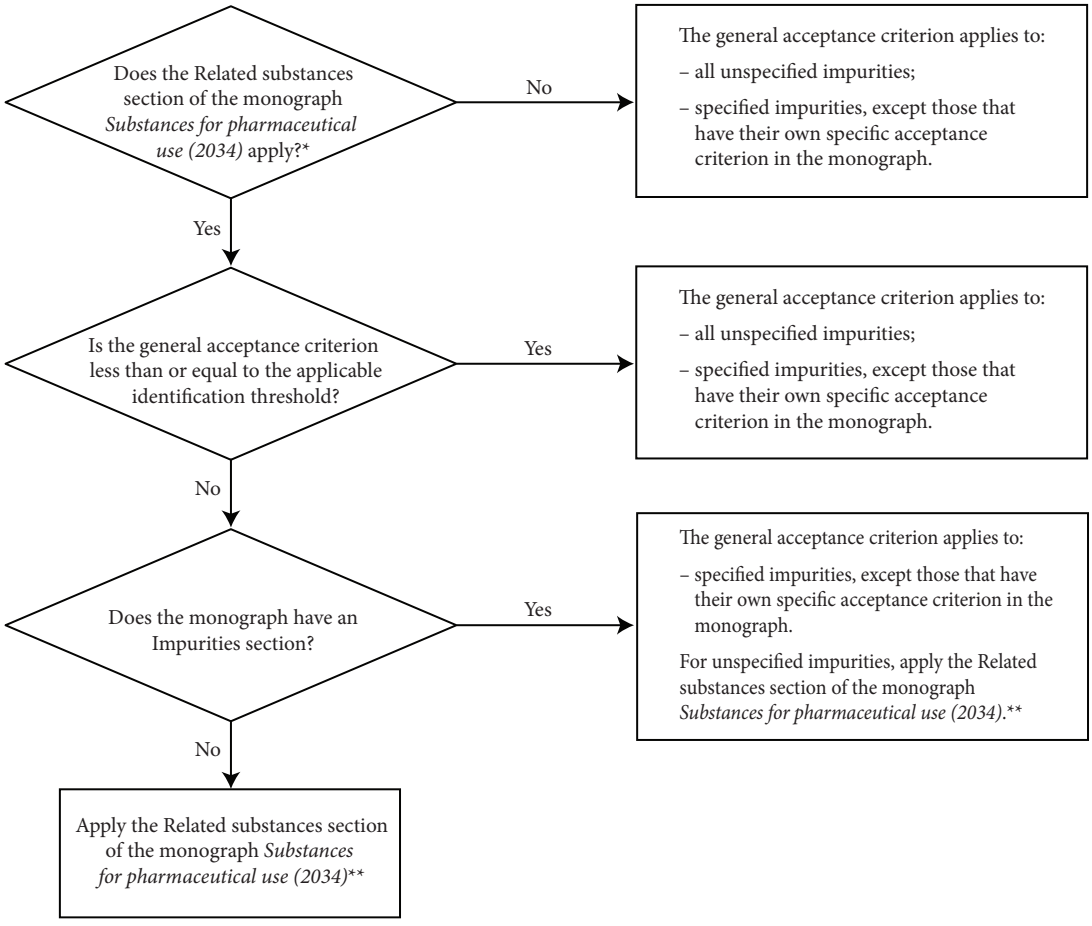
General acceptance criteria for "other" impurities are expressed in various ways in the monographs: "any other impurity", "other impurities", "any impurity", "any spot", "any band", etc. The general acceptance criteria may apply to certain specified impurities only or to unspecified impurities and certain specified impurities, depending on the nature of the active substance and the applicable identification threshold. Pending editorial adaptation of already published monographs using unequivocal terminology, the decision tree (Figure 5.10.-1) may be used to determine the acceptance criterion to be applied.

Recommendations to users of monographs of active substances

Monographs give a specification for suitable quality of substances with impurity profiles corresponding to those taken into account during elaboration and/or revision of the monograph. It is the responsibility of the user of the substance to check that the monograph provides adequate control of impurities for a substance for pharmaceutical use from a given source, notably by using the procedure for certification of suitability of the monographs of the European Pharmacopoeia.

A monograph with a related substances test based on a quantitative method (such as liquid chromatography, gas chromatography and capillary electrophoresis) provides adequate control of impurities for a substance from a given source if impurities present in amounts above the applicable identification threshold are specified impurities mentioned in the Impurities section.

If the substance contains impurities other than those mentioned in the Impurities section, it has to be verified that these impurities are detectable by the method described



* The requirements of this section apply to active substances with the exception of: biological and biotechnological products; oligonucleotides; radiopharmaceuticals; products of fermentation and semi-synthetic products derived therefrom; crude products of animal or plant origin; herbal products.

** To apply the Related substances section of the monograph *Substances for pharmaceutical use (2034)*:
– an individual acceptance criterion must be defined for any impurity that may be present above the identification threshold;
– any impurity with an acceptance criterion above the identification threshold must wherever possible be identified;
– any impurity with an acceptance criterion above the qualification threshold must be qualified.

Figure 5.10.-1. – *Decision tree for interpretation of general acceptance criteria for 'other' impurities in monographs*

in the monograph, otherwise a new method must be developed and revision of the monograph must be requested. Depending on the contents found and the limits proposed, the identification and/or the qualification of these impurities must be considered.

Where a single related substances test covers different impurity profiles, only impurities for the known profile from a single source need to be reported in the certificate of analysis unless the marketing authorisation holder uses active substances with different impurity profiles.

Identification of impurities (peak assignment)

Where a monograph has an individual limit for an impurity, it is often necessary to define means of identification, for example using a reference substance, a representative chromatogram or relative retention. The user of the substance may find it necessary to identify impurities other than those for which the monograph provides a means of identification, for example to check the suitability of the specification for a given impurity profile by comparison with the Impurities section. The European Pharmacopoeia does not provide reference substances, representative chromatograms or information on relative retentions for this purpose, unless prescribed in the monograph. Users will therefore have to apply the available scientific techniques for identification.

New impurities/Specified impurities above the specified limit

Where a new manufacturing process or change in an established process leads to the occurrence of a new impurity, it is necessary to apply the provisions of the general monograph on *Substances for pharmaceutical use (2034)* regarding identification and qualification and to verify the suitability of the monograph for control of the impurity. A certificate of suitability is a means for confirming for a substance from a given source that the new impurity is adequately controlled or the certificate contains a method for control with a defined acceptance criterion. In the latter case revision of the monograph will be initiated.

Where a new manufacturing process or change in an established process leads to the occurrence of a specified impurity above the specified limit, it is necessary to apply the provisions of the general monograph on *Substances for pharmaceutical use (2034)* regarding qualification.

Expression of acceptance criteria

The acceptance criteria for related substances are expressed in monographs either in terms of comparison of peak areas (comparative tests) or as numerical values.

Chromatographic methods

General chapter *2.2.46. Chromatographic separation techniques* deals with various aspects of impurities control.

Information is available via the EDQM website on commercial names for columns and other reagents and equipment found suitable during monograph development, where this is considered useful.

GLOSSARY

Disregard limit: in chromatographic tests, the nominal content at or below which peaks/signals are not taken into account for calculating a sum of impurities. The numerical values for the disregard limit and the reporting threshold are usually the same.

Identification threshold: a limit above which an impurity is to be identified.

Identified impurity: an impurity for which structural characterisation has been achieved.

Impurity: any component of a substance for pharmaceutical use that is not the chemical entity defined as the substance.

Nominal concentration: concentration calculated on the basis of the concentration of the prescribed reference and taking account of the prescribed correction factor.

Other detectable impurities: potential impurities with a defined structure that are known to be detected by the tests in a monograph but not known to be normally present above the identification threshold in substances used in medicinal products that have been authorised by the competent authorities of Parties to the Convention. They are unspecified impurities and are thus limited by a general acceptance criterion.

Potential impurity: an impurity that theoretically can arise during manufacture or storage. It may or may not actually appear in the substance. Where a potential impurity is known to be detected by the tests in a monograph but not known to be normally present in substances used in medicinal products that have been authorised by the competent authorities of Parties to the Convention, it will be included in the Impurities section under *Other detectable impurities* for information.

Qualification: the process of acquiring and evaluating data that establishes the biological safety of an individual impurity or a given impurity profile at the level(s) specified.

Qualification threshold: a limit above which an impurity is to be qualified.

Related substances: title used in monographs for general tests for organic impurities.

Reporting threshold: a limit above which an impurity is to be reported. Synonym: reporting level.

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

Unidentified impurity: an impurity for which a structural characterisation has not been achieved and that is defined solely by qualitative analytical properties (for example, relative retention).

Unspecified impurity: an impurity that is limited by a general acceptance criterion and not individually listed with its own specific acceptance criterion.

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SUBSTANCES FOR PHARMACEUTICAL USE

Corpora ad usum pharmaceuticum

DEFINITION

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

This general monograph does not apply to herbal drugs, herbal drugs for homoeopathic preparations, herbal drug preparations, herbal drug extracts, or mother tinctures for homoeopathic preparations, which are the subject of separate general monographs (*Herbal drugs (1433)*, *Herbal drugs for homoeopathic preparations (2045)*, *Herbal drug preparations (1434)*, *Herbal drug extracts (0765)*, *Mother tinctures for homoeopathic preparations (2029)*). It does not apply to raw materials for homoeopathic preparations, except where there is an individual monograph for the substance in the non-homoeopathic part of the Pharmacopoeia.

This monograph does not apply to chemical precursors for radiopharmaceutical preparations which are the subject of a separate monograph (*Chemical precursors for radiopharmaceutical preparations (2902)*).

Where a substance for pharmaceutical use not described in an individual monograph of the Pharmacopoeia is used in a medicinal product prepared for the special needs of individual patients, the need for compliance with the present general monograph is decided in the light of a risk assessment that takes account of the available quality of the substance and its intended use.

Where medicinal products are manufactured using substances for pharmaceutical use of human or animal origin, the requirements of chapter *5.1.7. Viral safety* apply.

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

Substance for pharmaceutical use of special grade. Unless otherwise indicated or restricted in the individual monographs, a substance for pharmaceutical use is intended for human and veterinary use, and is of appropriate quality for the manufacture of all dosage forms in which it can be used.

Polymorphism. Individual monographs do not usually specify crystalline or amorphous forms, unless bioavailability is affected. All forms of a substance for pharmaceutical use comply with the requirements of the monograph, unless otherwise indicated.

PRODUCTION

Substances for pharmaceutical use are manufactured by procedures that are designed to ensure a consistent quality and comply with the requirements of the individual monograph or approved specification.

The manufacture of active substances must take place under conditions of good manufacturing practice.

The provisions of general chapter *5.10* apply to the control of impurities in substances for pharmaceutical use.

Whether or not it is specifically stated in the individual monograph that the substance for pharmaceutical use:

- is a recombinant protein or another substance obtained as a direct gene product based on genetic modification, where applicable, the substance also complies with the requirements of the general monograph *Products of recombinant DNA technology (0784)*;
- is obtained from animals susceptible to transmissible spongiform encephalopathies other than by experimental challenge, where applicable, the substance also complies with the requirements of the general monograph *Products with risk of transmitting agents of animal spongiform encephalopathies (1483)*;
- is a substance derived from a fermentation process, whether or not the micro-organisms involved are modified by traditional procedures or recombinant DNA (rDNA) technology, where applicable, the substance also complies with the requirements of the general monograph *Products of fermentation (1468)*.

If solvents are used during production, they are of suitable quality. In addition, their toxicity and their residual level are taken into consideration (*5.4*). If water is used during production, it is of suitable quality.

The identity of elemental impurities derived from intentionally added catalysts and reagents is known, and strategies for controlling them should be established using the principles of risk management.

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

Powdered substances may be processed to obtain a certain degree of fineness (*2.9.35*).

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

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

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

If substances are processed with excipients, these excipients comply with the requirements of the relevant monograph or, where no such monograph exists, the approved specification.

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

CHARACTERS

The statements under the heading Characters (e.g. statements about the solubility or a decomposition point) are not to be interpreted in a strict sense and are not requirements. They are given for information.

Where a substance may show polymorphism, this may be stated under Characters in order to draw this to the attention of the user who may have to take this characteristic into consideration during formulation of a preparation.

IDENTIFICATION

Where under Identification an individual monograph contains subdivisions entitled 'First identification' and 'Second identification', the test or tests that constitute the 'First identification' may be used in all circumstances. The test or tests that constitute the 'Second identification' may be used in pharmacies provided it can be demonstrated that the substance or preparation is fully traceable to a batch certified to comply with all the other requirements of the monograph.

Certain monographs give two or more sets of tests for the purpose of the first identification, which are equivalent and may be used independently. One or more of these sets usually contain a cross-reference to a test prescribed in the Tests section of the monograph. It may be used to simplify the work of the analyst carrying out the identification and the prescribed tests. For example, one identification set cross-refers to a test for enantiomeric purity while the other set gives a test for specific optical rotation : the intended purpose of the two is the same, that is, verification that the correct enantiomer is present.

TESTS

Polymorphism (*5.9*). If the nature of a crystalline or amorphous form imposes restrictions on its use in preparations, the nature of the specific crystalline or amorphous form is identified, its morphology is adequately controlled and its identity is stated on the label.

Related substances. Unless otherwise prescribed or justified and authorised, organic impurities in active substances are to be reported, identified wherever possible, and qualified as indicated in Table 2034.-1 or in Table 2034.-2 for peptides obtained by chemical synthesis.

Table 2034.-1. – *Reporting, identification and qualification of organic impurities in active substances*

Use	Maximum daily dose	Reporting threshold	Identification threshold	Qualification threshold
Human use or human and veterinary use	≤ 2 g/day	> 0.05 per cent	> 0.10 per cent or a daily intake of > 1.0 mg (whichever is the lower)	> 0.15 per cent or a daily intake of > 1.0 mg (whichever is the lower)
Human use or human and veterinary use	> 2 g/day	> 0.03 per cent	> 0.05 per cent	> 0.05 per cent
Veterinary use only	Not applicable	> 0.10 per cent	> 0.20 per cent	> 0.50 per cent

Table 2034.-2. – *Reporting, identification and qualification of organic impurities in peptides obtained by chemical synthesis*

Reporting threshold	Identification threshold	Qualification threshold
> 0.1 per cent	> 0.5 per cent	> 1.0 per cent

Specific thresholds may be applied for impurities known to be unusually potent or to produce toxic or unexpected pharmacological effects.

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

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

The requirements above do not apply to biological and biotechnological products, oligonucleotides, products of fermentation and semi-synthetic products derived therefrom, to crude products of animal or plant origin or herbal products.

Elemental impurities. Permitted daily exposures for elemental impurities (e.g. as included in the ICH Q3D guideline, the principles of which are reproduced in general chapter *5.20. Elemental impurities*) apply to the medicinal product. Individual monographs on substances for pharmaceutical use therefore do not contain specifications for elemental impurities unless otherwise prescribed.

Residual solvents are limited according to the principles defined in chapter *5.4*, using general method *2.4.24* or another suitable method. Where a quantitative determination of a residual solvent is carried out and a test for loss on drying is not carried out, the content of residual solvent is taken into account for calculation of the assay content of the substance, the specific optical rotation and the specific absorbance.

Microbiological quality. Individual monographs give acceptance criteria for microbiological quality wherever such control is necessary. Table 5.1.4.-2. – *Acceptance criteria for microbiological quality of non-sterile substances for pharmaceutical use* in chapter *5.1.4. Microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use* gives recommendations on microbiological quality that are of general relevance for substances subject to microbial contamination. Depending on the nature of the substance and its intended use, different acceptance criteria may be justified.

Sterility (*2.6.1*). If intended for use in the manufacture of sterile dosage forms without a further appropriate sterilisation procedure, or if offered as sterile grade, the substance for pharmaceutical use complies with the test for sterility.

Bacterial endotoxins (*2.6.14*). The substance for pharmaceutical use complies with the test for bacterial endotoxins if it is labelled as a bacterial endotoxin-free grade or if it is intended for use in the manufacture of parenteral preparations or preparations for irrigation without a further appropriate procedure for the removal of bacterial endotoxins. The limit, when not indicated in the individual monograph, is determined in accordance with the recommendations of general chapter *5.1.10. Guidelines for using the test for bacterial endotoxins*.

Pyrogens (*2.6.8*). If the test for pyrogens is justified rather than the test for bacterial endotoxins and if a pyrogen-free grade is offered, the substance for pharmaceutical use complies with the test for pyrogens. The limit and test method are stated in the individual monograph or approved by the competent authority. Based on appropriate test validation for bacterial endotoxins and pyrogens, the test for bacterial endotoxins may replace the test for pyrogens.

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

ASSAY

Unless justified and authorised, contents of substances for pharmaceutical use are determined. Suitable methods are used.

LABELLING

In general, labelling is subject to supranational and national regulation and to international agreements. The statements under the heading Labelling therefore are not comprehensive and, moreover, for the purposes of the Pharmacopoeia only those statements that are necessary to demonstrate compliance

or non-compliance with the monograph are mandatory. Any other labelling statements are included as recommendations. When the term 'label' is used in the Pharmacopoeia, the labelling statements may appear on the container, the package, a leaflet accompanying the package or a certificate of analysis accompanying the article, as decided by the competent authority.

Where appropriate, the label states that the substance is:

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

Where applicable, the label states:

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

04/2018:1468

PRODUCTS OF FERMENTATION

Producta ab fermentatione

This monograph applies to indirect gene products obtained by fermentation. It is not applicable to:

- *monographs in the Pharmacopoeia concerning vaccines for human or veterinary use*;
- *products derived from continuous cell lines of human or animal origin*;
- *direct gene products that result from the transcription and translation from nucleic acid to protein, whether or not subject to post-translational modification*;
- *products obtained by semi-synthesis from a product of fermentation and those obtained by biocatalytic transformation*;
- *whole broth concentrates or raw fermentation products.*

This monograph provides general requirements for the development and manufacture of products of fermentation. These requirements are not necessarily comprehensive in a given case and requirements complementary or additional to those prescribed in this monograph may be imposed in an individual monograph or by the competent authority.

DEFINITION

For the purposes of this monograph, products of fermentation are active or inactive pharmaceutical substances produced by controlled fermentation as indirect gene products. They are primary or secondary metabolites of micro-organisms such as bacteria, yeasts, fungi and micro-algae, whether or not modified by traditional procedures or recombinant DNA (rDNA) technology. Such metabolites include vitamins, amino acids, antibiotics, alkaloids and polysaccharides.

They may be obtained by batch or continuous fermentation processes followed by procedures such as extraction, concentration, purification and isolation.

PRODUCTION

Production is based on a process that has been validated and shown to be suitable. The extent of validation depends on the critical nature of the respective process step.

CHARACTERISATION OF THE PRODUCER MICRO-ORGANISM

The history of the micro-organism used for production is documented. The micro-organism is adequately characterised. This may include determination of the phenotype of the micro-organism, macroscopic and microscopic methods and biochemical tests and, if appropriate, determination of the genotype of the micro-organism and molecular genetic tests.

PROCESSES USING A SEED-LOT SYSTEM

The *master cell bank* is a homogeneous suspension or lyophilisate of the original cells distributed into individual containers for storage. The viability and productivity of the cells under the selected storage conditions and their suitability for initiating a satisfactory production process after storage must be demonstrated.

Propagation of the master cell bank may take place through a seed-lot system that uses a working cell bank.

The *working cell bank* is a homogeneous suspension or lyophilisate of the cell material derived from the master cell bank, distributed in equal volumes into individual containers for storage (for example, in liquid nitrogen).

Production may take place by batch or continuous culture and may be terminated under defined conditions.

All containers in a cell bank are stored under identical conditions. Once removed from storage, the individual ampoules, vials or culture straws are not returned to the cell bank.

PROCESSES USING STAGED GROWTH IN CULTURES

The contents of a container of the working cell bank are used, if necessary after resuspension, to prepare an inoculum in a suitable medium. After a suitable period of growth, the cultures are used to initiate the fermentation process, if necessary following preculture in a prefermentor. The conditions to be used at each stage of the process are defined and must be met with each production run.

CHANGE CONTROL

If the production process is altered in a way that causes a significant change in the impurity profile of the product, the critical steps associated with this change in impurity profile are revalidated.

If a significant change has taken place in the micro-organism used for production that causes a significant change in the impurity profile of the product, the critical steps of the production process associated with this change, particularly the procedure for purification and isolation, are revalidated.

Revalidation includes demonstration that new impurities present in the product as a result of the change are adequately controlled by the test procedures. If necessary, additional or alternative tests must be introduced with appropriate limits. If the change in the process or in the micro-organism results in an increase in the level of an impurity already present, the acceptability of such an increase is addressed.

When a master cell bank is replaced, the critical steps of the production process must be revalidated to the extent necessary to demonstrate that no adverse change has occurred in the quality and safety of the product. Particular attention must be given to possible changes in the impurity profile of the product if a modified or new micro-organism is introduced into the process.

RAW MATERIALS

The raw materials employed in the fermentation and/or down-stream processing are of suitable quality for the intended purpose. They are tested to ensure that they comply with written specifications. Special attention must be paid to the levels of free histidine in fish peptones as the presence of free histidine may lead to histamine formation in certain conditions.

Levels of bioburden in media or in the inlet air for aeration are reduced to an adequately low level to ensure that if microbial contamination occurs, it does not adversely affect the quality, purity and safety of the product. Addition of components such as nutrients, precursors, and substrates during fermentation takes place aseptically.

IN-PROCESS CONTROLS

In-process controls are in place to ensure the consistency of the conditions during fermentation and down-stream processing and of the quality of the isolated product. Particular attention must be paid to ensure that any microbial contamination that adversely affects the quality, purity and safety of the product is detected by the controls applied.

Production conditions may be monitored, as appropriate, by suitable procedures for example to control and check:

- temperature,
- pH,
- rate of aeration,
- rate of agitation,
- pressure,

and to monitor the concentration of the required product.

DOWN-STREAM PROCESSING

At the end of fermentation, the producer micro-organism is inactivated or removed. Further processing is designed to reduce residues originating from the culture medium to an acceptable level and to ensure that the desired product is recovered with consistent quality.

Various purification processes may be used, for example, charcoal treatment, ultrafiltration and solvent extraction. It must be demonstrated that the process or processes chosen reduce to a minimum or remove:

- residues from the producer micro-organism, culture media, substrates and precursors,
- unwanted transformation products of substrates and precursors.

If necessary, suitable tests are performed either as in-process controls or on the isolated product of fermentation.

IDENTIFICATION, TESTS AND ASSAY

The requirements with which the product must comply throughout its period of validity, as well as specific test methods, are stated in the individual monographs.

04/2019:2619

PHARMACEUTICAL PREPARATIONS

Pharmaceutica

INTRODUCTION

This monograph is intended to be a reference source of standards in the European Pharmacopoeia on active substances, excipients and dosage forms, which are to be applied in the manufacture/preparation of pharmaceuticals, but not a guide on how to manufacture as there is specific guidance available covering methods of manufacture and associated controls.

It does not cover investigational medicinal products, but competent authorities may refer to pharmacopoeial standards when authorising clinical trials using investigational medicinal products.

DEFINITION

Pharmaceutical preparations are medicinal products generally consisting of active substances that may be combined with excipients, formulated into a dosage form suitable for the intended use, where necessary after reconstitution, presented in a suitable and appropriately labelled container.

Pharmaceutical preparations may be licensed by the competent authority, or unlicensed and made to the specific needs of patients according to legislation. There are 2 categories of unlicensed pharmaceutical preparations:

- extemporaneous preparations, i.e. pharmaceutical preparations individually prepared for a specific patient or patient group, supplied after preparation;
- stock preparations, i.e. pharmaceutical preparations prepared in advance and stored until a request for a supply is received.

In addition to this monograph, pharmaceutical preparations also comply with the General Notices and with the relevant general chapters of the Pharmacopoeia. General chapters are normally given for information and become mandatory when referred to in a general or specific monograph, unless such reference is made in a way that indicates that it is not the intention to make the text referred to mandatory but rather to cite it for information.

Where relevant, pharmaceutical preparations also comply with the dosage form monographs (e.g. *Capsules (0016)*, *Tablets (0478)*) and general monographs relating to pharmaceutical preparations (e.g. *Allergen products (1063)*, *Herbal teas (1435)*, *Homoeopathic preparations (1038)*, *Homoeopathic pillules, coated (2786)*, *Homoeopathic pillules, impregnated (2079)*, *Immunosera for human use, animal (0084)*, *Immunosera for veterinary use (0030)*, *Live biotherapeutic products for human use (3053)*, *Monoclonal antibodies for human use (2031)*, *Radiopharmaceutical preparations (0125)*, *Vaccines for human use (0153)*, *Vaccines for veterinary use (0062)*).

ETHICAL CONSIDERATIONS AND GUIDANCE IN THE PREPARATION OF UNLICENSED PHARMACEUTICAL PREPARATIONS

The underlying principle of legislation for pharmaceutical preparations is that, subject to specific exemptions, no pharmaceutical preparation may be placed on the market without an appropriate marketing authorisation. The exemptions from the formal licensing requirement allow the supply of unlicensed products to meet the special needs of individual patients. However, when deciding to use an unlicensed preparation all health professionals involved (e.g. the prescribing practitioners and/or the preparing pharmacists) have, within their area of responsibilities, a duty of care to the patient receiving the pharmaceutical preparation.

In considering the preparation of an unlicensed pharmaceutical preparation, a suitable level of risk assessment is undertaken.

The risk assessment identifies:

- the criticality of different parameters (e.g. quality of active substances, excipients and containers; design of the preparation process; extent and significance of testing; stability of the preparation) to the quality of the preparation; and
- the risk that the preparation may present to a particular patient group.

Based on the risk assessment, the person responsible for the preparation must ensure, with a suitable level of assurance, that the pharmaceutical preparation is, throughout its shelf-life, of an appropriate quality and suitable and fit for its purpose. For stock preparations, storage conditions and shelf-life have to be justified on the basis of, for example, analytical data or professional judgement, which may be based on literature references.

PRODUCTION

Manufacture/preparation must take place within the framework of a suitable quality system and be compliant with the standards relevant to the type of product being made. Licensed products must comply with the requirements of their licence. For unlicensed products a risk assessment as outlined in the section 'Ethical considerations and guidance in the preparation of unlicensed pharmaceutical preparations' is of special importance, as these products are not previously assessed by the competent authority.

Where pharmaceutical preparations are manufactured/prepared using materials of human or animal origin, the general requirements of general chapters *5.1.7. Viral safety* and *5.2.6. Evaluation of safety of veterinary vaccines and immunosera* and of the general monograph *Products with risk of transmitting agents of animal spongiform encephalopathies (1483)* apply, where appropriate.

Formulation. During pharmaceutical development or prior to manufacture/preparation, suitable ingredients, processes, tests and specifications are identified and justified in order to ensure the suitability of the product for the intended purpose. This includes consideration of the properties required in order to identify whether specific ingredient properties or process steps are critical to the required quality of the pharmaceutical preparation.

Active substances and excipients. Active substances and excipients used in the formulation of pharmaceutical preparations comply with the requirements of the relevant general monographs, e.g. *Substances for pharmaceutical use (2034)*, *Essential oils (2098)*, *Herbal drug extracts (0765)*, *Herbal drugs (1433)*, *Herbal drug preparations (1434)*, *Herbal drugs for homoeopathic preparations (2045)*, *Mother tinctures for homoeopathic preparations (2029)*, *Methods of preparation of homoeopathic stocks and potentisation (2371)*, *Products of fermentation (1468)*, *Products of recombinant DNA technology (0784)*, *Vegetable fatty oils (1579)*.

In addition, where specific monographs exist, the quality of the active substances and excipients used complies with the corresponding monographs.

Where no specific monographs exist, the required quality must be defined, taking into account the intended use and the involved risk.

When physicochemical characteristics of active substances and functionality-related characteristics (FRCs) of excipients (e.g. particle-size distribution, viscosity, polymorphism) are critical in relation to their role in the manufacturing process and quality attributes of the pharmaceutical preparation, they must be identified and controlled.

Detailed information on FRCs is given in general chapter *5.15. Functionality-related characteristics of excipients*.

Microbiological quality. The formulation of the pharmaceutical preparation and its container must ensure that the microbiological quality is suitable for the intended use.

During development, it shall be demonstrated that the antimicrobial activity of the preparation as such or, if necessary, with the addition of a suitable preservative or preservatives, or by the selection of an appropriate container, provides adequate protection from adverse effects that may arise from microbial contamination or proliferation during the storage and use of the preparation. A suitable test method together with criteria for evaluating the preservative properties of the formulation are provided in general chapter *5.1.3. Efficacy of antimicrobial preservation*.

If preparations do not have adequate antimicrobial efficacy and do not contain antimicrobial preservatives they are supplied in single-dose containers, or in multidose containers that prevent microbial contamination of the contents after opening.

In the manufacture/preparation of non-sterile pharmaceutical preparations, suitable measures are taken to ensure their microbial quality; recommendations on this aspect are provided in general chapters *5.1.4. Microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use* and *5.1.8. Microbiological quality of herbal medicinal products for oral use and extracts used in their preparation*.

Sterile preparations are manufactured/prepared using materials and methods designed to ensure sterility and to avoid the introduction of contaminants and the growth of micro-organisms; recommendations on this aspect are provided in general chapter *5.1.1. Methods of preparation of sterile products*.

Containers. A suitable container is selected. Consideration is given to the intended use of the preparation, the properties of the container, the required shelf-life, and product/container incompatibilities. Where applicable, containers for pharmaceutical preparations comply with the requirements for containers (*3.2* and subsections) and materials used for the manufacture of containers (*3.1* and subsections).

Stability. Stability requirements of pharmaceutical preparations are dependent on their intended use and on the desired storage time.

Where applicable, the probability and criticality of possible degradation products of the active substance(s) and/or reaction products of the active substance(s) with an excipient and/or the immediate container must be assessed. Depending on the result of this assessment, limits of degradation and/or reaction products are set and monitored in the pharmaceutical preparation. Licensed products require a stability exercise.

Methods used for the purpose of stability testing for all relevant characteristics of the preparation are validated as stability indicating, i.e. the methods allow the quantification of the relevant degradation products and physical characteristic changes.

TESTS

Relevant tests to apply in order to ensure the appropriate quality of a particular dosage form are described in the specific dosage form monographs.

Where it is not practical, for unlicensed pharmaceutical preparations, to carry out the tests (e.g. batch size, time restraints), other suitable methods are implemented to ensure that the appropriate quality is achieved in accordance with the risk assessment carried out and any local guidance or legal requirements.

Stock preparations are normally tested to a greater extent than extemporaneous preparations.

The following tests are applicable to many preparations and are therefore listed here.

Appearance. The appearance (e.g. size, shape and colour) of the pharmaceutical preparation is controlled.

Identity and purity tests. Where applicable, the following tests are carried out on the pharmaceutical preparation:

- identification of the active substance(s);
- identification of specific excipient(s), such as preservatives;
- purity tests (e.g. investigation of degradation products, residual solvents (*2.4.24*) or other related impurities, sterility (*2.6.1*));
- safety tests (e.g. safety tests for biological products).

Elemental impurities. General chapter *5.20. Elemental impurities* applies to pharmaceutical preparations except products for veterinary use, unlicensed preparations and other products that are excluded from the scope of this chapter.

For pharmaceutical preparations outside the scope of general chapter *5.20*, manufacturers of these products remain responsible for controlling the levels of elemental impurities using the principles of risk management.

If appropriate, testing is performed using suitable analytical procedures according to general chapter *2.4.20. Determination of elemental impurities*.

Uniformity (*2.9.40* or *2.9.5*/*2.9.6*). Pharmaceutical preparations presented in single-dose units comply with the test(s) as prescribed in the relevant specific dosage form monograph. If justified and authorised, general chapter *2.9.40* can be applicable only at the time of release.

Special uniformity requirements apply in the following cases:

- for herbal drugs and herbal drug preparations, compliance with general chapter *2.9.40* is not required;
- for homoeopathic preparations, the provisions of general chapters *2.9.6* and *2.9.40* are normally not appropriate, however in certain circumstances compliance with these chapters may be required by the competent authority;
- for single- and multivitamin and trace-element preparations, compliance with general chapters *2.9.6* and *2.9.40* (*content uniformity only*) is not required;
- in justified and authorised circumstances, for other preparations, compliance with general chapters *2.9.6* and *2.9.40* may not be required by the competent authority.

Reference standards. Reference standards may be needed at various stages for quality control of pharmaceutical preparations. They are established and monitored taking due account of general chapter *5.12. Reference standards*.

ASSAY

Unless otherwise justified and authorised, contents of active substances and specific excipients such as preservatives are determined in pharmaceutical preparations. Limits must be defined and justified.

Suitable and validated methods are used. If assay methods prescribed in the respective active substance monographs are used, it must be demonstrated that they are not affected by the presence of the excipients and/or by the formulation.

Reference standards. See Tests.

LABELLING AND STORAGE

The relevant labelling requirements given in the general dosage form monographs apply. In addition, relevant European Union or other applicable regulations apply.

GLOSSARY

Formulation: the designing of an appropriate formula (including materials, processes, etc.) that will ensure that the patient receives the suitable pharmaceutical preparation in an appropriate form that has the required quality and that will be stable and effective for the required length of time.

Licensed pharmaceutical preparation: a medicinal product that has been granted a marketing authorisation by a competent authority. Synonym: authorised pharmaceutical preparation.

Manufacture: all operations of purchase of materials and products, Production, Quality Control, release, storage, distribution of medicinal products and the related controls.

Preparation (of an unlicensed pharmaceutical preparation): the 'manufacture' of unlicensed pharmaceutical preparations by or at the request of pharmacies or other healthcare establishments (the term 'preparation' is used instead of 'manufacture' in order clearly to distinguish it from the industrial manufacture of licensed pharmaceutical preparations).

Reconstitution: manipulation to enable the use or application of a medicinal product with a marketing authorisation in accordance with the instructions given in the summary of product characteristics or the patient information leaflet.

Risk assessment: the identification of hazards and the analysis and evaluation of risks associated with exposure to those hazards.

Unlicensed pharmaceutical preparation: a medicinal product that is exempt from the need of having a marketing authorisation issued by a competent authority but is made for specific patients' needs according to legislation.

04/2018:0016

CAPSULES

Capsulae

The requirements of this monograph do not necessarily apply to preparations that are presented as capsules intended for use other than by oral administration. Requirements for such preparations may be found, where appropriate, in other general monographs, for example Rectal preparations (1145) and Vaginal preparations (1164).

DEFINITION

Capsules are solid preparations with hard or soft shells of various shapes and capacities, containing a single dose of active substance(s). They are usually intended for oral administration.

The capsule shells are made of gelatin or other substances, the consistency of which may be adjusted by the addition of substances such as glycerol or sorbitol. Excipients such as surface-active agents, opaque fillers, antimicrobial preservatives, sweeteners, colouring matter authorised by the competent authority and flavouring substances may be added. The capsules may bear surface markings.

The contents of capsules may be solid, semi-solid or liquid. They consist of one or more active substances with or without excipients such as solvents, diluents, lubricants and disintegrating agents. The contents do not cause deterioration of the shell. The shell, however, is attacked by the digestive fluids and the contents are released.

Where applicable, containers for capsules comply with the requirements of *Materials used for the manufacture of containers* (*3.1* and subsections) and *Containers* (*3.2* and subsections).

Several categories of capsules may be distinguished:

- hard capsules;
- soft capsules;
- gastro-resistant capsules;
- modified-release capsules;
- cachets.

PRODUCTION

In the manufacture, packaging, storage and distribution of capsules, suitable measures are taken to ensure their microbial quality; recommendations on this aspect are provided in general chapter *5.1.4. Microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use*.

TESTS

Uniformity of dosage units. Capsules comply with the test for uniformity of dosage units (*2.9.40*) or, where justified and authorised, with the tests for uniformity of content and/or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content (*2.9.6*). Unless otherwise prescribed or justified and authorised, capsules with a content of active substance less than 2 mg or less than 2 per cent of the fill mass comply with test B for uniformity of content of single-dose preparations. If the preparation has more than one active substance, the requirement applies only to those ingredients that correspond to the above conditions.

Uniformity of mass (*2.9.5*). Capsules comply with the test for uniformity of mass of single-dose preparations. If the test for uniformity of content is prescribed for all the active substances, the test for uniformity of mass is not required.

Dissolution. Unless otherwise justified and authorised, a suitable test is carried out, for example one of the tests described in general chapter *2.9.3. Dissolution test for solid dosage forms*.

Where a dissolution test is prescribed, a disintegration test may not be required.

STORAGE

Store at a temperature not exceeding 30 °C.

LABELLING

The label states the name of any added preservative.

Hard capsules

DEFINITION

Hard capsules are solid single-dose preparations. They consist of a hard shell, the capacity of which can be varied, containing a solid, semi-solid or liquid preparation. The shell is made of gelatin or other substances, and consists of 2 prefabricated cylindrical sections that are open at one end and rounded and closed at the other. The contents are filled into one of the sections and the capsule is closed by slipping the other section over it.

PRODUCTION

The active substance(s), usually in solid form (powder or granules), are filled into one of the sections that is then closed by slipping the other section over it. The security of the closure may be strengthened by suitable means.

TESTS

Disintegration (*2.9.1*). Hard capsules comply with the test. Use *water R* as the liquid medium. When justified and authorised, *0.1 M hydrochloric acid* or *artificial gastric juice R* may be used as the liquid medium. If the capsules float on the surface of the water, a disc may be added. Operate the apparatus for 30 min, unless otherwise justified and authorised.

Soft capsules

DEFINITION

Soft capsules are solid single-dose preparations. They consist of a soft shell, the capacity and shape of which can be varied, containing a semi-solid or liquid preparation. The shell is made of gelatin or other substances and may have one or more solid active substances incorporated into it. The shell is usually thicker than that of a hard capsule and consists of one part, as soft capsules are generally formed, filled and sealed in a single operation.

PRODUCTION

Soft capsules are usually formed, filled and sealed in one operation, but for extemporaneous use the shell may be prefabricated. The shell material may contain an active substance.

Liquids may be enclosed directly; solids are usually dissolved or dispersed in a suitable vehicle to give a solution or dispersion of a paste-like consistency.

There may be partial migration of the constituents from the capsule contents into the shell and vice versa because of the nature of the materials and the surfaces in contact.

TESTS

Disintegration (*2.9.1*). Soft capsules comply with the test. Use *water R* as the liquid medium. When justified and authorised, *0.1 M hydrochloric acid* or *artificial gastric juice R* may be used

as the liquid medium. Add a disc to each tube. Liquid active substances dispensed in soft capsules may attack the disc; in such circumstances and where authorised, the disc may be omitted. Operate the apparatus for 30 min, unless otherwise justified and authorised. If the capsules fail to comply because of adherence to the discs, the results are invalid. Repeat the test on a further 6 capsules omitting the discs.

Modified-release capsules

DEFINITION

Modified-release capsules are hard or soft capsules in which the contents or the shell or both contain special excipients or are prepared by a special process designed to modify the rate, the place or the time at which the active substance(s) are released.

Modified-release capsules include prolonged-release capsules, delayed-release capsules and pulsatile-release capsules.

Gastro-resistant capsules

DEFINITION

Gastro-resistant capsules are delayed-release capsules that are intended to resist the gastric fluid and to release their active substance or substances in the intestinal fluid. Usually they are prepared by filling capsule shells with granules or with particles covered with a gastro-resistant coating. In other cases, the shell of the capsule is covered with a gastro-resistant coating or the shell itself has gastro-resistant properties.

TESTS

Disintegration (*2.9.1*). Capsules with a gastro-resistant shell comply with the test with the following modifications. Use *0.1 M hydrochloric acid* as the liquid medium and operate the apparatus for 2 h, or other such time as may be authorised, without the discs. Examine the state of the capsules. The time of resistance to the acid medium varies according to the formulation of the capsules to be examined. It is typically 2 h to 3 h but even with authorised deviations it must not be less than 1 h. No capsule shows signs of disintegration or rupture permitting the escape of the contents. Replace the acid by *phosphate buffer solution pH 6.8 R*. When justified and authorised, a buffer solution of pH 6.8 with added pancreas powder (for example, 0.35 g of *pancreas powder R* per 100 mL of buffer solution) may be used. Add a disc to each tube. Operate the apparatus for 60 min. If the capsules fail to comply because of adherence to the discs, the results are invalid. Repeat the test on a further 6 capsules omitting the discs.

Cachets

DEFINITION

Cachets are solid preparations consisting of a hard shell containing a single dose of one or more active substances. The cachet shell is made of unleavened bread usually from rice flour and consists of 2 prefabricated flat cylindrical sections. Before administration, the cachets are immersed in water for a few seconds, placed on the tongue and swallowed with a draught of water.

01/2008:1163
corrected 10.0

EYE PREPARATIONS

Ophthalmica

DEFINITION

Eye preparations are sterile liquid, semi-solid or solid preparations intended for administration upon the eyeball and/or to the conjunctiva, or for insertion in the conjunctival sac.

Where applicable, containers for eye preparations comply with the requirements of materials used for the manufacture of containers (*3.1* and subsections) and containers (*3.2* and subsections).

Several categories of eye preparations may be distinguished:

- eye drops;
- eye lotions;
- powders for eye drops and powders for eye lotions;
- semi-solid eye preparations;
- ophthalmic inserts.

PRODUCTION

During the development of an eye preparation whose formulation contains an antimicrobial preservative, the necessity for and the efficacy of the chosen preservative shall be demonstrated to the satisfaction of the competent authority. A suitable test method together with criteria for judging the preservative properties of the formulation are provided in chapter *5.1.3. Efficacy of antimicrobial preservation*.

Eye preparations are prepared using materials and methods designed to ensure sterility and to avoid the introduction of contaminants and the growth of micro-organisms; recommendations on this aspect are provided in chapter *5.1.1. Methods of preparation of sterile products*.

In the manufacture of eye preparations containing dispersed particles, measures are taken to ensure a suitable and controlled particle size with regard to the intended use.

During development, it must be demonstrated that the nominal contents can be withdrawn from the container of liquid and semi-solid eye preparations supplied in single-dose containers.

TESTS

Sterility (*2.6.1*). Eye preparations comply with the test. Applicators supplied separately also comply with the test. Remove the applicator with aseptic precautions from its package and transfer it to a tube of culture medium so that it is completely immersed. Incubate and interpret the results as described in the test.

STORAGE

Unless otherwise justified and authorised, store in a sterile, tamper-evident container.

LABELLING

The label states the name of any added antimicrobial preservative.

Eye drops

DEFINITION

Eye drops are sterile aqueous or oily solutions, emulsions or suspensions of one or more active substances intended for instillation into the eye.

Eye drops may contain excipients, for example, to adjust the tonicity or the viscosity of the preparation, to adjust or stabilise the pH, to increase the solubility of the active substance, or to stabilise the preparation. These substances do not adversely affect the intended medicinal action or, at the concentrations used, cause undue local irritation.

Aqueous preparations supplied in multidose containers contain a suitable antimicrobial preservative in appropriate concentration except when the preparation itself has adequate antimicrobial properties. The antimicrobial preservative chosen must be compatible with the other ingredients of the preparation and must remain effective throughout the period of time during which eye drops are in use.

If eye drops do not contain antimicrobial preservatives they are supplied in single-dose containers or in multidose containers preventing microbial contamination of the contents after opening.

Eye drops intended for use in surgical procedures do not contain antimicrobial preservatives.

Eye drops that are solutions, examined under suitable conditions of visibility, are practically clear and practically free from particles.

Eye drops that are suspensions may show a sediment that is readily redispersed on shaking to give a suspension which remains sufficiently stable to enable the correct dose to be delivered.

Multidose preparations are supplied in containers that allow successive drops of the preparation to be administered. The containers contain at most 10 mL of the preparation, unless otherwise justified and authorised.

TESTS

Particle size. Unless otherwise justified and authorised, eye drops in the form of a suspension comply with the following test: introduce a suitable quantity of the suspension into a counting cell or with a micropipette onto a slide, as appropriate, and scan under a microscope an area corresponding to 10 µg of the solid phase. For practical reasons, it is recommended that the whole sample is first scanned at low magnification (e.g. × 50) and particles greater than 25 µm are identified. These larger particles can then be measured at a larger magnification (e.g. × 200 to × 500). For each 10 µg of solid active substance, not more than 20 particles have a maximum dimension greater than 25 µm, and not more than 2 of these particles have a maximum dimension greater than 50 µm. None of the particles has a maximum dimension greater than 90 µm.

LABELLING

The label states, for multidose containers, the period after opening the container after which the contents must not be used. This period does not exceed 4 weeks, unless otherwise justified and authorised.

Eye lotions

DEFINITION

Eye lotions are sterile aqueous solutions intended for use in rinsing or bathing the eye or for impregnating eye dressings.

Eye lotions may contain excipients, for example to adjust the tonicity or the viscosity of the preparation or to adjust or stabilise the pH. These substances do not adversely affect the intended action or, at the concentrations used, cause undue local irritation.

Eye lotions supplied in multidose containers contain a suitable antimicrobial preservative in appropriate concentration except when the preparation itself has adequate antimicrobial properties. The antimicrobial preservative chosen is compatible with the other ingredients of the preparation and remains effective throughout the period of time during which the eye lotions are in use.

If eye lotions do not contain antimicrobial preservatives, they are supplied in single-dose containers. Eye lotions intended for use in surgical procedures or in first-aid treatment do not contain an antimicrobial preservative and are supplied in single-dose containers.

Eye lotions, examined under suitable conditions of visibility, are practically clear and practically free from particles.

The containers for multidose preparations do not contain more than 200 mL of eye lotion, unless otherwise justified and authorised.

LABELLING

The label states:

- where applicable, that the contents are to be used on one occasion only;
- for multidose containers, the period after opening the container after which the contents must not be used; this period does not exceed 4 weeks, unless otherwise justified and authorised.

Powders for eye drops and powders for eye lotions

DEFINITION

Powders for the preparation of eye drops and eye lotions are supplied in a dry, sterile form to be dissolved or suspended in an appropriate liquid vehicle at the time of administration. They may contain excipients to facilitate dissolution or dispersion, to prevent caking, to adjust the tonicity, to adjust or stabilise the pH or to stabilise the preparation.

After dissolution or suspension in the prescribed liquid, they comply with the requirements for eye drops or eye lotions, as appropriate.

TESTS

Uniformity of dosage units (*2.9.40*). Single-dose powders for eye drops and eye lotions comply with the test or, where justified and authorised, with the tests for uniformity of content and/or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content (*2.9.6*). Unless otherwise prescribed or justified and authorised, single-dose powders for eye drops and eye lotions with a content of active substance less then 2 mg or less than 2 per cent of the total mass comply with test B. If the preparation has more than one active substance, the requirement applies only to those substances that correspond to the above condition.

Uniformity of mass (*2.9.5*). Single-dose powders for eye drops and eye lotions comply with the test. If the test for uniformity of content is prescribed for all the active substances, the test for uniformity of mass is not required.

Semi-solid eye preparations

DEFINITION

Semi-solid eye preparations are sterile ointments, creams or gels intended for application to the conjunctiva or to the eyelids. They contain one or more active substances dissolved or dispersed in a suitable basis. They have a homogeneous appearance.

Semi-solid eye preparations comply with the requirements of the monograph *Semi-solid preparations for cutaneous application (0132)*. The basis is non-irritant to the conjunctiva.

Semi-solid eye preparations are packed in small, sterilised collapsible tubes fitted or provided with a sterilised cannula. The containers contain at most 10 g of the preparation, unless otherwise justified and authorised. The tubes must be well-closed to prevent microbial contamination. Semi-solid eye preparations may also be packed in suitably designed single-dose containers. The containers, or the nozzles of tubes, are of such a shape as to facilitate administration without contamination.

TESTS

Particle size. Semi-solid eye preparations containing dispersed solid particles comply with the following test: spread gently a quantity of the preparation corresponding to at least 10 µg of solid active substance as a thin layer. Scan under a microscope the whole area of the sample. For practical reasons, it is recommended that the whole sample is first scanned at a small magnification (e.g. × 50) and particles greater than 25 µm are identified. These larger particles can then be measured at a larger magnification (e.g. × 200 to × 500). For each 10 µg of solid active substance, not more than 20 particles have a maximum dimension greater than 25 µm, and not more than 2 of these particles have a maximum dimension greater than 50 µm. None of the particles has a maximum dimension greater than 90 µm.

LABELLING

The label states, for multidose containers, the period after opening the container after which the contents must not be used. This period does not exceed 4 weeks, unless otherwise justified and authorised.

Ophthalmic inserts

DEFINITION

Ophthalmic inserts are sterile, solid or semi-solid preparations of suitable size and shape, designed to be inserted in the conjunctival sac, to produce an ocular effect. They generally consist of a reservoir of active substance embedded in a matrix or bounded by a rate-controlling membrane. The active substance, which is more or less soluble in lacrymal liquid, is released over a determined period of time.

Ophthalmic inserts are individually distributed into sterile containers.

PRODUCTION

In the manufacture of ophthalmic inserts, measures are taken to ensure a suitable dissolution behaviour.

TESTS

Uniformity of dosage units (*2.9.40*). Ophthalmic inserts comply with the test or, where justified and authorised, with the test for uniformity of content shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content (*2.9.6*). Ophthalmic inserts comply, where applicable, with test A.

LABELLING

The label states:

- where applicable, the total quantity of active substance per insert;
- where applicable, the dose released per unit time.

01/2018:0672

LIQUID PREPARATIONS FOR ORAL USE

Praeparationes liquidae peroraliae

Where justified and authorised, the requirements of this monograph do not apply to liquid preparations for oral use intended for veterinary use.

DEFINITION

Liquid preparations for oral use are usually solutions, emulsions or suspensions containing one or more active substances in a suitable vehicle; they may, however, consist of liquid active substances used as such (oral liquids).

Some preparations for oral use are prepared by dilution of concentrated liquid preparations, or from powders or granules for the preparation of oral solutions or suspensions, for oral drops or for syrups, using a suitable vehicle.

The vehicle for any preparation for oral use is chosen having regard to the nature of the active substance(s) and to provide organoleptic characteristics appropriate to the intended use of the preparation.

Liquid preparations for oral use may contain suitable antimicrobial preservatives, antioxidants and other excipients such as dispersing, suspending, thickening, emulsifying, buffering, wetting, solubilising, stabilising, flavouring and sweetening agents and colouring matter, authorised by the competent authority.

Emulsions may show evidence of phase separation but are readily redispersed on shaking. Suspensions may show a sediment, which is readily dispersed on shaking to give a suspension that remains sufficiently stable to enable the correct dose to be delivered.

Where applicable, containers for liquid preparations for oral use comply with the requirements of *Materials used for the manufacture of containers* (*3.1* and subsections) and *Containers* (*3.2* and subsections).

Several categories of preparations may be distinguished;

- oral solutions, emulsions and suspensions;
- powders and granules for oral solutions and suspensions;
- oral drops;
- powders for oral drops;
- syrups;
- powders and granules for syrups.

PRODUCTION

During development of a preparation for oral use whose formulation contains an antimicrobial preservative, the need for and the efficacy of the chosen preservative shall be demonstrated to the satisfaction of the competent authority. A suitable test method together with criteria for judging the preservative properties of the formulation are provided in general chapter *5.1.3. Efficacy of antimicrobial preservation*.

During development, it must be demonstrated that the nominal content can be withdrawn from the container, for liquid preparations for oral use presented in single-dose containers.

In the manufacturing, packaging, storage and distribution of liquid preparations for oral use, suitable measures are taken to ensure their microbial quality; recommendations on this aspect are provided in general chapter *5.1.4. Microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use*.

In the manufacture of liquid preparations for oral use containing dispersed particles, measures are taken to ensure a suitable and controlled particle size with regard to the intended use.

TESTS

Uniformity of dosage units. Solutions, suspensions and emulsions in single-dose containers comply with the test for uniformity of dosage units (*2.9.40*) or, where justified and authorised, with the test for uniformity of content or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content (*2.9.6*). Unless otherwise prescribed or justified and authorised, single-dose preparations that are suspensions or emulsions comply with the following test. After shaking, empty each container as completely as possible and carry out the test on the individual contents. They comply with test B for uniformity of content of single-dose preparations.

Uniformity of mass. Single-dose preparations that are solutions comply with the following test: weigh individually the contents of 20 containers, emptied as completely as possible, and determine the average mass. Not more than 2 of the individual masses deviate by more than 10 per cent from the average mass and none deviate by more than 20 per cent.

Dose and uniformity of dose of oral drops. Into a suitable graduated cylinder, introduce by means of the dropping device the number of drops usually prescribed for one dose, or introduce by means of the measuring device the usually prescribed quantity. The dropping speed does not exceed 2 drops per second. Weigh the liquid, repeat the addition, weigh again and carry on repeating the addition and weighing until a total of 10 masses are obtained. No single mass deviates by more than 10 per cent from the average mass.

If the labelling defines a mass, the total of 10 masses does not differ by more than 15 per cent from the nominal mass of 10 doses defined on the labelling. If the labelling defines a volume, measure the total volume of 10 doses. The volume does not differ by more than 15 per cent from the nominal volume of 10 doses defined on the labelling.

Uniformity of mass of delivered doses from multidose containers (*2.9.27*). Liquid preparations for oral use supplied in multidose containers comply with the test. Oral drops are not subject to the provisions of this test.

LABELLING

The label states the name of any added preservative.

Oral solutions, emulsions and suspensions

DEFINITION

Oral solutions, emulsions and suspensions are supplied in single-dose or multidose containers. Each dose from a multidose container is administered by means of a device suitable for measuring the prescribed volume. The device is usually a spoon or a cup for volumes of 5 mL or multiples thereof or an oral syringe for other volumes.

Powders and granules for oral solutions and suspensions

DEFINITION

Powders and granules for the preparation of oral solutions or suspensions are intended to be reconstituted with the prescribed liquid to produce a liquid preparation for oral use. They may contain excipients, in particular to facilitate dispersion or dissolution and to prevent caking.

After dissolution or suspension, they comply with the requirements for oral solutions or oral suspensions, as appropriate.

TESTS

Uniformity of dosage units. Single-dose powders and single-dose granules comply with the test for uniformity of dosage units (*2.9.40*) or, where justified and authorised, with the tests for uniformity of content and/or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content (*2.9.6*). Unless otherwise prescribed or justified and authorised, single-dose powders and single-dose granules with a content of active substance less than 2 mg or less than 2 per cent of the total mass comply with test B for uniformity of content of single-dose preparations. If the preparation has more than one active substance, the requirement applies only to those substances that correspond to the above conditions.

Uniformity of mass (*2.9.5*). Single-dose powders and single-dose granules comply with the test for uniformity of mass of single-dose preparations. If the test for uniformity of content is prescribed for all the active substances, the test for uniformity of mass is not required.

LABELLING

The label states:

- the method of preparation of the solution or suspension;
- the conditions and the duration of storage after reconstitution.

Oral drops

DEFINITION

Oral drops are solutions, emulsions or suspensions that are administered in small volumes such as drops by means of a suitable device.

LABELLING

The label states the number of drops per millilitre of preparation or per gram of preparation if the dose is measured in drops.

Powders for oral drops

DEFINITION

Powders for the preparation of oral drops are intended to be reconstituted with the prescribed liquid to produce a liquid preparation for oral use. They may contain excipients to facilitate dissolution or suspension in the prescribed liquid or to prevent caking.

After dissolution or suspension, they comply with the requirements for oral drops.

TESTS

Uniformity of dosage units. Single-dose powders for oral drops comply with the test for uniformity of dosage units (*2.9.40*) or, where justified and authorised, with the tests for uniformity of content and/or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content (*2.9.6*). Unless otherwise prescribed or justified and authorised, single-dose powders for oral drops with a content of active substance less than 2 mg or less than 2 per cent of the total mass comply with test B for uniformity of content of single-dose preparations. If the preparation has more than one active substance, the requirement applies only to those substances that correspond to the above conditions.

Uniformity of mass (*2.9.5*). Single-dose powders for oral drops comply with the test for uniformity of mass of single-dose preparations. If the test for uniformity of content is prescribed for all the active substances, the test for uniformity of mass is not required.

Syrups

DEFINITION

Syrups are aqueous preparations characterised by a sweet taste and a viscous consistency. They may contain sucrose at a concentration of at least 45 per cent *m/m*. The sweet taste can also be obtained by using other polyols or sweetening agents. Syrups usually contain aromatic or other flavouring agents. Each dose from a multidose container is administered by means of a device suitable for measuring the prescribed volume. The device is usually a spoon or a cup for volumes of 5 mL or multiples thereof.

LABELLING

The label states the name and concentration of the polyol or sweetening agent.

Powders and granules for syrups

DEFINITION

Powders and granules for syrups are intended to be reconstituted with the prescribed liquid to produce a liquid preparation for oral use. They may contain excipients to facilitate dissolution.

After dissolution, they comply with the requirements for syrups.

TESTS

Uniformity of dosage units. Single-dose powders and granules for syrups comply with the test for uniformity of dosage units (*2.9.40*) or, where justified and authorised, with the tests for uniformity of content and/or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content (*2.9.6*). Unless otherwise prescribed or justified and authorised, single-dose powders and granules for syrups with a content of active substance less than 2 mg or less than 2 per cent of the total mass comply with test B for uniformity of content of single-dose preparations. If the preparation has more than one active substance, the requirement applies only to those substances that correspond to the above conditions.

Uniformity of mass (*2.9.5*). Single-dose powders and granules for syrups comply with the test for uniformity of mass of single-dose preparations. If the test for uniformity of content is prescribed for all the active substances, the test for uniformity of mass is not required.

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PARENTERAL PREPARATIONS

Parenteralia

The requirements of this monograph do not necessarily apply to products derived from human blood, to immunological preparations, or radiopharmaceutical preparations. Special requirements may apply to preparations for veterinary use depending on the species of animal for which the preparation is intended.

DEFINITION

Parenteral preparations are sterile preparations intended for administration by injection, infusion or implantation into the human or animal body.

Parenteral preparations may require the use of excipients, for example to make the preparation isotonic with respect to blood, to adjust the pH, to increase solubility, to prevent deterioration of the active substances or to provide adequate antimicrobial properties, but not to adversely affect the intended medicinal action of the preparation or, at the concentrations used, to cause toxicity or undue local irritation.

Containers for parenteral preparations are made as far as possible from materials that are sufficiently transparent to permit the visual inspection of the contents, except for implants and in other justified and authorised cases.

Where applicable, the containers for parenteral preparations comply with the requirements for *Materials used for the manufacture of containers* (*3.1* and subsections) and *Containers* (*3.2* and subsections).

Parenteral preparations intended for chronic use or total parenteral nutrition should have appropriate limits for specific components or elements, taking long-term toxicity into account.

Parenteral preparations are supplied in glass containers (*3.2.1*) or in other containers such as plastic containers (*3.2.2*, *3.2.2.1* and *3.2.9*) and prefilled syringes. The tightness of the container is ensured by suitable means. Closures ensure a good seal, prevent the access of micro-organisms and other contaminants and usually permit the withdrawal of a part or the whole of the contents without removal of the closure. The plastic materials or elastomers (*3.2.9*) used to manufacture the closures are sufficiently firm and elastic to allow the passage of a needle with the least possible shedding of particles. Closures for multidose containers are sufficiently elastic to ensure that the puncture is resealed when the needle is withdrawn.

Several categories of parenteral preparations may be distinguished:

- injections;
- infusions;
- concentrates for injections or infusions;
- powders for injections or infusions;
- gels for injections;
- implants.

PRODUCTION

During the development of a parenteral preparation, the formulation for which contains an antimicrobial preservative, the effectiveness of the chosen preservative shall be demonstrated to the satisfaction of the competent authority. A suitable test method together with criteria for judging the preservative properties of the formulation are provided under *Efficacy of antimicrobial preservation* (*5.1.3*).

Parenteral preparations are prepared using materials and methods designed to ensure sterility and to avoid the introduction of contaminants and the growth of micro-organisms. Recommendations on this aspect are provided in the text *Methods of preparation of sterile products* (*5.1.1*).

Water used in the manufacture of parenteral preparations complies with the requirements of water for injections in bulk stated in the monograph *Water for injections (0169)*.

TESTS

Particulate contamination: sub-visible particles (*2.9.19*). For preparations for human use, solutions for infusion or solutions for injection comply with the test.

In the case of preparations for subcutaneous or intramuscular injection, higher limits may be appropriate. Radiopharmaceutical preparations are exempt from these requirements. Preparations for which the label states that the product is to be used with a final filter are exempt from these requirements, providing it has been demonstrated that the filter delivers a solution that complies with the test.

For preparations for veterinary use, when supplied in containers with a nominal content of more than 100 mL and when the content is equivalent to a dose of more than 1.4 mL per kilogram of body mass, solutions for infusion or solutions for injection comply with the test for particulate contamination: sub-visible particles.

Sterility (*2.6.1*). Parenteral preparations comply with the test for sterility.

STORAGE

In a sterile, airtight, tamper-evident container.

LABELLING

The label states:

- the name and concentration of any added antimicrobial preservative;
- where applicable, that the solution is to be used in conjunction with a final filter;
- where applicable, that the preparation is free from bacterial endotoxins or that it is apyrogenic.

Injections

DEFINITION

Injections are sterile solutions, emulsions or suspensions. They are prepared by dissolving, emulsifying or suspending the active substance(s) and any added excipients in water, in a suitable non-aqueous liquid, that may be non-sterile where justified, or in a mixture of these vehicles.

Solutions for injection, examined under suitable conditions of visibility, are clear and practically free from particles.

Emulsions for injection do not show any evidence of phase separation. Suspensions for injection may show a sediment which is readily dispersed on shaking to give a suspension which remains sufficiently stable to enable the correct dose to be withdrawn.

Multidose preparations. Multidose aqueous injections contain a suitable antimicrobial preservative at an appropriate concentration except when the preparation itself has adequate antimicrobial properties. When a preparation for parenteral administration is presented in a multidose container, the precautions to be taken for its administration and more particularly for its storage between successive withdrawals are given.

Antimicrobial preservatives. Aqueous preparations which are prepared using aseptic precautions and which cannot be terminally sterilised may contain a suitable antimicrobial preservative in an appropriate concentration.

No antimicrobial preservative is added when:

- the volume to be injected in a single dose exceeds 15 mL, unless otherwise justified;
- the preparation is intended for administration by routes where, for medical reasons, an antimicrobial preservative is not acceptable, such as intracisternally, epidurally, intrathecally or by any route giving access to the cerebrospinal fluid, or intra- or retro-ocularly.

Such preparations are presented in single-dose containers.

PRODUCTION

In the manufacture of injections containing dispersed particles, measures are taken to ensure a suitable and controlled particle size with regard to the intended use.

Single-dose preparations. The volume of the injection in a single-dose container is sufficient to permit the withdrawal and administration of the nominal dose using a normal technique (*2.9.17*).

TESTS

Uniformity of dosage units. Single-dose suspensions for injection comply with the test for uniformity of dosage units (*2.9.40*) or, where justified and authorised, with the test for uniformity of content shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content (*2.9.6*). Unless otherwise prescribed or justified and authorised, single-dose suspensions for injection with a content of active substance less than 2 mg or less than 2 per cent of the total mass comply with test A for uniformity of content of single-dose preparations. If the preparation contains more than one active substance, the requirement applies only to those substances that correspond to the above conditions.

Bacterial endotoxins - pyrogens. A test for bacterial endotoxins (*2.6.14*) is carried out or, where justified and authorised, the test for pyrogens (*2.6.8*). Recommendations on the limits for bacterial endotoxins are given in general chapter *5.1.10*.

Preparations for human use. The preparation complies with a test for bacterial endotoxins (*2.6.14*) or with the test for pyrogens (*2.6.8*).

Preparations for veterinary use. When the volume to be injected in a single dose is 15 mL or more and is equivalent to a dose of 0.2 mL or more per kilogram of body mass, the preparation complies with a test for bacterial endotoxins (*2.6.14*) or with the test for pyrogens (*2.6.8*).

Any preparation. Where the label states that the preparation is free from bacterial endotoxins or apyrogenic, respectively, the preparation complies with a test for bacterial endotoxins (*2.6.14*) or with the test for pyrogens (*2.6.8*), respectively.

Infusions

DEFINITION

Infusions are sterile, aqueous solutions or emulsions with water as the continuous phase. They are usually made isotonic with respect to blood. They are principally intended for administration in large volume. Infusions do not contain any added antimicrobial preservative.

Solutions for infusion, examined under suitable conditions of visibility, are clear and practically free from particles.

Emulsions for infusion do not show any evidence of phase separation.

PRODUCTION

In the manufacture of infusions containing dispersed particles, measures are taken to ensure a suitable and controlled particle size with regard to the intended use.

The volume of the infusion in the container is sufficient to permit the withdrawal and administration of the nominal dose using a normal technique (*2.9.17*).

TESTS

Bacterial endotoxins - pyrogens. They comply with a test for bacterial endotoxins (*2.6.14*) or, where justified and authorised, with the test for pyrogens (*2.6.8*). For the latter test inject 10 mL per kilogram of body mass into each rabbit, unless otherwise justified and authorised.

Concentrates for injections or infusions

DEFINITION

Concentrates for injections or infusions are sterile solutions intended for injection or infusion after dilution. They are diluted to a prescribed volume with a prescribed liquid before administration. After dilution, they comply with the requirements for injections or for infusions.

TESTS

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

Powders for injections or infusions

DEFINITION

Powders for injections or infusions are solid, sterile substances distributed in their final containers and which, when shaken with the prescribed volume of a prescribed sterile liquid rapidly form either clear and practically particle-free solutions or uniform suspensions. After dissolution or suspension, they comply with the requirements for injections or for infusions.

Freeze-dried products for parenteral administration are considered as powders for injections or infusions.

PRODUCTION

The uniformity of content and uniformity of mass of freeze-dried products for parenteral administration are ensured by the in-process control of the amount of the solution prior to freeze-drying.

TESTS

Uniformity of dosage units. Powders for injections or infusions comply with the test for uniformity of dosage units (*2.9.40*) or, where justified and authorised, with the tests for uniformity of content and/or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content (*2.9.6*). Unless otherwise prescribed or justified and authorised, powders for injections or infusions with a content of active substance less than 2 mg or less than 2 per cent of the total mass, or with a unit mass equal to or less than 40 mg comply with test A for uniformity of content of single-dose preparations. If the preparation contains more than one active substance, the requirement applies only to those substances that correspond to the above conditions.

Uniformity of mass (*2.9.5*). Powders for injections or infusions comply with the test for uniformity of mass of single-dose preparations. If the test for uniformity of content is prescribed for all the active substances, the test for uniformity of mass is not required.

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

LABELLING

The label states the instructions for the preparation of injections and infusions.

Gels for injections

DEFINITION

Gels for injections are sterile gels with a viscosity suitable to guarantee a modified release of the active substance(s) at the site of injection.

Implants

DEFINITION

Implants are sterile, solid preparations of a size and shape suitable for parenteral implantation and release of the active substance(s) over an extended period of time. Each dose is provided in a sterile container.

TESTS

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

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TABLETS

Compressi

The requirements of this monograph do not necessarily apply to preparations that are presented as tablets intended for use other than by oral administration. Requirements for such preparations may be found, where appropriate, in other general monographs; for example Rectal preparations (1145), Vaginal preparations (1164) and Oromucosal preparations (1807). This monograph does not apply to lozenges, oral pastes and oral gums. Where justified and authorised, the requirements of this monograph do not apply to tablets for veterinary use. Tablets for use in the mouth comply with the requirements of the monograph Oromucosal preparations (1807).

DEFINITION

Tablets are solid preparations each containing a single dose of one or more active substances. They are obtained by compressing uniform volumes of particles or by another suitable manufacturing technique, such as extrusion, moulding or freeze-drying (lyophilisation). Tablets are intended for oral administration. Some are swallowed whole, some after being chewed, some are dissolved or dispersed in water before being administered and some are retained in the mouth where the active substance is liberated.

The particles consist of one or more active substances with or without excipients such as diluents, binders, disintegrating agents, glidants, lubricants, substances capable of modifying the behaviour of the preparation in the digestive tract, colouring matter authorised by the competent authority and flavouring substances.

Tablets are usually straight, solid cylinders, the end surfaces of which are flat or convex and the edges of which may be bevelled. They may have break-marks and may bear a symbol or other markings. Tablets may be coated.

Where applicable, containers for tablets comply with the requirements for materials used for the manufacture of containers (*3.1* and subsections) and containers (*3.2* and subsections).

Several categories of tablets for oral use may be distinguished:

- uncoated tablets;
- coated tablets;
- gastro-resistant tablets;
- modified-release tablets;
- effervescent tablets;
- soluble tablets;
- dispersible tablets;
- orodispersible tablets;
- chewable tablets;
- oral lyophilisates.

PRODUCTION

Tablets are usually prepared by compressing uniform volumes of particles or particle aggregates produced by granulation methods. In the manufacture of tablets, measures are taken to ensure that they possess a suitable mechanical strength to avoid crumbling or breaking on handling or subsequent processing. This may be demonstrated using the tests described in general chapters *2.9.7. Friability of uncoated tablets* and *2.9.8. Resistance to crushing of tablets*.

In the manufacture, packaging, storage and distribution of tablets, suitable measures are taken to ensure their microbiological quality; recommendations on this aspect are provided in general chapter *5.1.4. Microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use*.

Subdivision of tablets. Tablets may bear a break-mark or break-marks for the purpose of being subdivided into parts, either to ease the intake of the medicinal product or to deliver fractional doses. In cases where fractions of tablets are necessary to deliver the intended dose stated on the label, the efficacy of the breakmark is assessed during product development or for validation purposes by determining the uniformity of mass of the sub-divided parts using the following test.

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

TESTS

Uniformity of dosage units (*2.9.40*). Tablets comply with the test or, where justified and authorised, with the tests for uniformity of content and/or uniformity of mass shown below. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

Uniformity of content (*2.9.6*). Unless otherwise prescribed or justified and authorised, tablets with a content of active substance less than 2 mg or less than 2 per cent of the total mass comply with test A. If the preparation has more than 1 active substance, the requirement applies only to those substances that correspond to the above conditions.

Unless otherwise justified and authorised, coated tablets other than film-coated tablets comply with test A irrespective of their content of active substance(s).

Uniformity of mass (*2.9.5*). Uncoated tablets and, unless otherwise justified and authorised, film-coated tablets comply with the test. If the test for uniformity of content is prescribed or justified and authorised for all the active substances, the test for uniformity of mass is not required.

Dissolution. Unless otherwise justified and authorised, a suitable test is carried out, for example one of the tests described in general chapter *2.9.3. Dissolution test for solid dosage forms*.

Where a dissolution test is prescribed, a disintegration test may not be required.

Uncoated tablets

DEFINITION

Uncoated tablets include single-layer tablets resulting from a single compression of particles and multi-layer tablets consisting of concentric or parallel layers obtained by successive compression of particles of different composition. The excipients used are not specifically intended to modify the release of the active substance in the digestive fluids.

Uncoated tablets conform to the general definition of tablets. A broken section, when examined under a lens, shows either a relatively uniform texture (single-layer tablets) or a stratified texture (multi-layer tablets) but no signs of coating.

TESTS

Disintegration (*2.9.1*). Uncoated tablets comply with the test using *water R* as the liquid medium. Add a disc to each tube. Operate the apparatus for 15 min, unless otherwise justified and authorised, and examine the state of the tablets. If the tablets fail to comply because of adherence to the discs, the results are invalid. Repeat the test on a further 6 tablets, omitting the discs.

Coated tablets

DEFINITION

Coated tablets are tablets covered with one or more layers of mixtures of various substances such as natural or synthetic resins, gums, gelatin, inactive and insoluble fillers, sugars, plasticisers, polyols, waxes, colouring matter authorised by the competent authority and sometimes flavouring substances and active substances. The substances used as coatings are usually applied as a solution or suspension in conditions in which evaporation of the vehicle occurs. When the coating is a very thin polymeric coating, the tablets are known as film-coated tablets.

Coated tablets have a smooth surface, which is often coloured and may be polished; a broken section, when examined under a lens, shows a core surrounded by one or more continuous layers with a different texture.

PRODUCTION

Where justified, uniformity of mass or uniformity of content of coated tablets other than film-coated tablets may be ensured by control of the cores.

TESTS

Disintegration (*2.9.1*). Coated tablets other than film-coated tablets comply with the test using *water R* as the liquid medium. Add a disc to each tube. Operate the apparatus for 60 min, unless otherwise justified and authorised, and examine the state of the tablets. If any of the tablets has not disintegrated, repeat the test on a further 6 tablets, replacing *water R* with *0.1 M hydrochloric acid*. If 1 or 2 tablets fail to disintegrate, repeat the test on 12 additional tablets.

The requirements of the test are met if not fewer than 16 of the 18 tablets tested have disintegrated.

Film-coated tablets comply with the disintegration test prescribed above except that the apparatus is operated for 30 min, unless otherwise justified and authorised.

If coated tablets or film-coated tablets fail to comply because of adherence to the discs, the results are invalid. Repeat the test on a further 6 tablets, omitting the discs.

Gastro-resistant tablets

DEFINITION

Gastro-resistant tablets are delayed-release tablets that are intended to resist the gastric fluid and to release their active substance(s) in the intestinal fluid. Usually they are prepared by covering tablets with a gastro-resistant coating or from granules or particles already covered with a gastro-resistant coating.

Tablets covered with a gastro-resistant coating conform to the definition of coated tablets.

TESTS

Disintegration (*2.9.1*). Tablets covered with a gastro-resistant coating comply with the test with the following modifications. Use *0.1 M hydrochloric acid* as the liquid medium. Operate the apparatus for 2 h, or another such time as may be justified and authorised, without the discs, and examine the state of the tablets. The time of resistance to the acid medium varies according to the formulation of the tablets to be examined. It is typically 2 h to 3 h but even with authorised deviations is not less than 1 h. No tablet shows signs of either disintegration (apart from fragments of coating) or cracks that would allow the escape of the contents. Replace the acid by *phosphate buffer solution pH 6.8 R* and add a disc to each tube. Operate the apparatus for 60 min and examine the state of the tablets. If the tablets fail to comply because of adherence to the discs, the results are invalid. Repeat the test on a further 6 tablets, omitting the discs.

Modified-release tablets

DEFINITION

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

Modified-release tablets include prolonged-release tablets, delayed-release tablets and pulsatile-release tablets.

Effervescent tablets

DEFINITION

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

TESTS

Disintegration. Place 1 tablet in a beaker containing 200 mL of *water R* at 15-25 °C; numerous bubbles of gas are evolved. When the evolution of gas around the tablet or its fragments ceases the tablet has disintegrated, being either dissolved or dispersed in the water so that no agglomerates of particles remain. Repeat the operation on 5 other tablets. The tablets comply with the test if each of the 6 tablets used disintegrates in the manner prescribed within 5 min, unless otherwise justified and authorised.

Soluble tablets

DEFINITION

Soluble tablets are uncoated or film-coated tablets. They are intended to be dissolved in water before administration. The solution produced may be slightly opalescent due to the added excipients used in the manufacture of the tablets.

TESTS

Disintegration (*2.9.1*). Soluble tablets disintegrate within 3 min, using *water R* at 15-25 °C as the liquid medium.

Dispersible tablets

DEFINITION

Dispersible tablets are uncoated or film-coated tablets intended to be dispersed in water before administration, giving a homogeneous dispersion.

TESTS

Disintegration (*2.9.1*). Dispersible tablets disintegrate within 3 min, using *water R* at 15-25 °C as the liquid medium.

Fineness of dispersion. Place 2 tablets in 100 mL of *water R* and stir until completely dispersed. A smooth dispersion is produced, which passes through a sieve screen with a nominal mesh aperture of 710 µm.

Orodispersible tablets

DEFINITION

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

TESTS

Disintegration (*2.9.1*). Orodispersible tablets disintegrate within 3 min, using *water R* as the liquid medium.

Chewable tablets

DEFINITION

Chewable tablets are intended to be chewed before being swallowed.

PRODUCTION

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

Oral lyophilisates

DEFINITION

Oral lyophilisates are solid single-dose preparations made by freeze-drying of a liquid or semi-solid preparation. These fast-releasing preparations are intended to be placed in the mouth where their contents are released in saliva and swallowed or, alternatively, are intended to be dissolved or dispersed in water before oral administration.

PRODUCTION

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

TESTS

Disintegration. Place 1 oral lyophilisate in a beaker containing 200 mL of *water R* at 15-25 °C. It disintegrates within 3 min. Repeat the test on 5 other oral lyophilisates. They comply with the test if all 6 have disintegrated.

Water (*2.5.12*). Oral lyophilisates comply with the test; the limits are approved by the competent authority.

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SEMI-SOLID PREPARATIONS FOR CUTANEOUS APPLICATION

Praeparationes molles ad usum dermicum

The requirements of this monograph apply to all semi-solid preparations for cutaneous application. Where appropriate, additional requirements specific to semi-solid preparations intended to be applied to particular surfaces or mucous membranes may be found in other general monographs, for example Ear preparations (0652), Nasal preparations (0676), Rectal preparations (1145), Eye preparations (1163) and Vaginal preparations (1164).

DEFINITION

Semi-solid preparations for cutaneous application are intended for local or transdermal delivery of active substances, or for their emollient or protective action. They are of homogeneous appearance.

Semi-solid preparations for cutaneous application consist of a simple or compound basis in which, usually, 1 or more active substances are dissolved or dispersed. According to its composition, the basis may influence the activity of the preparation.

The basis may consist of natural or synthetic substances and may be single phase or multiphase. According to the nature of the basis, the preparation may have hydrophilic or hydrophobic properties; it may contain suitable excipients such as antimicrobial preservatives, antioxidants, stabilisers, emulsifiers, thickeners and penetration enhancers.

Semi-solid preparations for cutaneous application intended for use on severely injured skin are sterile.

Where applicable, containers for semi-solid preparations for cutaneous application comply with the requirements of *Materials used for the manufacture of containers* (*3.1* and subsections) and *Containers* (*3.2* and subsections).

Several categories of semi-solid preparations for cutaneous application may be distinguished:

- ointments;
- creams;
- gels;
- pastes;
- poultices;
- medicated plasters;
- cutaneous patches.

According to their structure, ointments, creams and gels generally show viscoelastic behaviour and are non-Newtonian in character, e.g. plastic, pseudoplastic or thixotropic type flow at high shear rates. Pastes frequently exhibit dilatancy.

PRODUCTION

During development of semi-solid preparations for cutaneous application whose formulation contains an antimicrobial preservative, the need for and the efficacy of the chosen preservative shall be demonstrated to the satisfaction of the competent authority. A suitable test method together with criteria for judging the preservative properties of the formulation are provided in *Efficacy of antimicrobial preservation* (*5.1.3*). In the manufacture, packaging, storage and distribution of semi-solid preparations for cutaneous application, suitable measures are taken to ensure their microbiological quality; recommendations on this are provided in *5.1.4. Microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use*. Sterile semi-solid preparations for cutaneous application are prepared using materials and methods designed to ensure sterility and to avoid the introduction of contaminants and the growth of micro-organisms; recommendations on this are provided in *Methods of preparation of sterile products* (*5.1.1*).

During development, it must be demonstrated that the nominal content can be withdrawn from the container of semi-solid preparations for cutaneous application presented in single-dose containers.

In the manufacture of semi-solid preparations for cutaneous application, suitable measures are taken to ensure that the defined rheological properties are fulfilled. Where appropriate, the following non-mandatory tests may be carried out: measurement of consistency by penetrometry (*2.9.9*), viscosity (apparent viscosity) (*2.2.10*) and a suitable test to demonstrate the appropriate release of the active substance(s).

In the manufacture of semi-solid preparations for cutaneous application containing 1 or more active substances that are not dissolved in the basis (e.g. emulsions or suspensions), measures are taken to ensure appropriate homogeneity of the preparation to be delivered.

In the manufacture of semi-solid preparations for cutaneous application containing dispersed particles, measures are taken to ensure a suitable and controlled particle size with regard to the intended use.

TESTS

Uniformity of dosage units. Semi-solid preparations that are supplied either in single-dose containers that represent 1 dose of medicinal product or in metered-dose containers, and that are intended for transdermal delivery of the active substance(s) in view of a systemic effect, comply with the test for uniformity of dosage units (*2.9.40*). Semi-solid preparations in which the active substance(s) are dissolved comply with the test for mass variation; semi-solid preparations in which the active substance(s) are suspended comply with the test for content uniformity. Follow the procedure described for liquid dosage forms. Herbal drugs and herbal drug preparations present in the dosage form are not subject to the provisions of this paragraph.

For semi-solid preparations presented in metered-dose containers and in which the active substance(s) are dissolved, proceed as follows. Discharge once to waste. Wait for a minimum of 5 s, shake for 5 s if necessary, and discharge again to waste. Repeat this procedure for a further 3 actuations. Weigh the container, discharge once to waste and weigh the container again. Calculate the difference between the 2 masses. Repeat the procedure for a further 9 containers. Determine the mass variation (*2.9.40*).

For semi-solid preparations supplied in metered-dose containers and in which the active substance(s) are suspended, proceed as follows. Use an apparatus capable of quantitatively retaining the dose leaving the metered-dose container. Shake 1 container for 5 s and discharge once to waste. Wait for a minimum of 5 s, shake for 5 s and discharge again to waste. Repeat this procedure for a further 3 actuations. After 2 s, fire 1 dose from the metered-dose container into the collecting vessel. Collect the contents of the collecting vessel by successive rinses. Determine the content of active substance in the combined rinses. Repeat the procedure for a further 9 containers. Determine the content uniformity (*2.9.40*).

Sterility (*2.6.1*). Where the label indicates that the preparation is sterile, it complies with the test for sterility.

STORAGE

If the preparation contains water or other volatile ingredients, store in an airtight container. If the preparation is sterile, store in a sterile, airtight, tamper-evident container.

LABELLING

The label states:

– the name of any excipient;

– where applicable, that the preparation is sterile.

Ointments

DEFINITION

An ointment consists of a single-phase basis in which solids or liquids may be dispersed.

Hydrophobic ointments

Hydrophobic ointments can absorb only small amounts of water. Typical bases used for their formulation are hard, liquid and light liquid paraffins, vegetable oils, animal fats, synthetic glycerides, waxes and liquid polyalkylsiloxanes.

Water-emulsifying ointments

Water-emulsifying ointments can absorb larger amounts of water and thereby produce water-in-oil or oil-in-water emulsions after homogenisation, depending on the nature of the emulsifiers: water-in-oil emulsifying agents such as wool alcohols, sorbitan esters, monoglycerides and fatty alcohols, or oil-in-water emulsifying agents such as sulfated fatty alcohols, polysorbates, macrogol cetostearyl ether or esters of fatty acids with macrogols may be used for this purpose. Their bases are those of the hydrophobic ointments.

Hydrophilic ointments

Hydrophilic ointments are preparations having bases that are miscible with water. The bases usually consist of mixtures of liquid and solid macrogols (polyethylene glycols). They may contain appropriate amounts of water.

Creams

DEFINITION

Creams are multiphase preparations consisting of a lipophilic phase and an aqueous phase.

Lipophilic creams

Lipophilic creams have as the continuous phase the lipophilic phase. They usually contain water-in-oil emulsifying agents such as wool alcohols, sorbitan esters and monoglycerides.

Hydrophilic creams

Hydrophilic creams have as the continuous phase the aqueous phase. They contain oil-in-water emulsifying agents such as sodium or trolamine soaps, sulfated fatty alcohols, polysorbates and polyoxyl fatty acid and fatty alcohol esters combined, if necessary, with water-in-oil emulsifying agents.

Gels

DEFINITION

Gels consist of liquids gelled by means of suitable gelling agents.

Lipophilic gels

Lipophilic gels (oleogels) are preparations whose bases usually consist of liquid paraffin with polyethylene or fatty oils gelled with colloidal silica or aluminium or zinc soaps.

Hydrophilic gels

Hydrophilic gels (hydrogels) are preparations whose bases usually consist of water, glycerol or propylene glycol gelled with suitable gelling agents such as poloxamers, starch, cellulose derivatives, carbomers and magnesium-aluminium silicates.

Pastes

DEFINITION

Pastes are semi-solid preparations for cutaneous application containing large proportions of solids finely dispersed in the basis.

Poultices

DEFINITION

Poultices consist of a hydrophilic heat-retentive basis in which solid or liquid active substances are dispersed. They are usually spread thickly on a suitable dressing and heated before application to the skin.

Medicated plasters

DEFINITION

Medicated plasters are flexible preparations containing 1 or more active substances. They are intended to be applied to the skin. They are designed to maintain the active substance(s) in close contact with the skin such that these may be absorbed slowly, or act as protective or keratolytic agents.

Medicated plasters consist of an adhesive basis, which may be coloured, containing 1 or more active substances, spread as a uniform layer on an appropriate support made of natural or synthetic material. They are not irritant or sensitising to the skin. The adhesive layer is covered by a suitable protective liner, which is removed before applying the plaster to the skin. When removed, the protective liner does not detach the preparation from the outer, supporting layer.

Medicated plasters are presented in a range of sizes directly adapted to their intended use or as larger sheets to be cut before use. Medicated plasters adhere firmly to the skin when gentle pressure is applied and can be peeled off without causing appreciable injury to the skin or detachment of the preparation from the outer, supporting layer.

TESTS

Dissolution. A suitable test may be required to demonstrate the appropriate release of the active substance(s), for example one of the tests described in *Dissolution test for transdermal patches* (*2.9.4*).

Cutaneous patches

DEFINITION

Cutaneous patches are flexible preparations containing 1 or more active substances. They are intended to be applied to the skin. They are designed to maintain the active substance(s) in close contact with the skin such that these may act locally.

Cutaneous patches consist of an adhesive basis, which may be coloured, containing 1 or more active substances, spread as a uniform layer on an appropriate support made of natural or synthetic material. The adhesive basis is not irritant or sensitising to the skin. The adhesive layer is covered by a suitable protective liner, which is removed before applying the patch to the skin. When removed, the protective liner does not detach the preparation from the outer, supporting layer.

Cutaneous patches are presented in a range of sizes adapted to their intended use. They adhere firmly to the skin when gentle pressure is applied and can be peeled off without causing appreciable injury to the skin or detachment of the preparation from the outer, supporting layer.

TESTS

Dissolution. A suitable test may be required to demonstrate the appropriate release of the active substance(s), for example one of the tests described in *Dissolution test for transdermal patches* (*2.9.4*).

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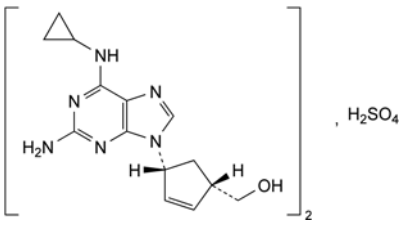
ABACAVIR SULFATE

Abacaviri sulfas



$C_{28}H_{38}N_{12}O_6S$ M_r 671
[188062-50-2]

DEFINITION

Bis[[(1*S*,4*R*)-4-[2-amino-6-(cyclopropylamino)-9*H*-purin-9-yl]cyclopent-2-enyl]methanol] sulfate.

Content: 99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white powder.

Solubility: soluble in water, practically insoluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *abacavir sulfate CRS*.

B. Enantiomeric purity (see Tests).

C. Solution S (see Tests) gives reaction (a) of sulfates (*2.3.1*).

TESTS

Solution S. Dissolve 0.250 g in *water R* and dilute to 25.0 mL with the same solvent.

Enantiomeric purity. Liquid chromatography (*2.2.29*).

Solution A. Mix 0.5 mL of *trifluoroacetic acid R* and 100 mL of *methanol R*.

Solution B. Mix 30 volumes of *methanol R*, 30 volumes of *2-propanol R* and 40 volumes of *heptane R*.

Test solution. Dissolve 40 mg of the substance to be examined in 30 mL of solution A. Sonicate until dissolution is complete. Add 30 mL of *2-propanol R* and dilute to 100.0 mL with *heptane R*.

Reference solution (a). Dissolve 2 mg of *abacavir for system suitability CRS* (containing impurities A and D) in 1.5 mL of solution A. Sonicate until dissolution is complete. Add 1.5 mL of *2-propanol R* and dilute to 5.0 mL with *heptane R*.

Reference solution (b). Dilute 1.0 mL of the test solution to 100.0 mL with solution B. Dilute 1.0 mL of this solution to 10.0 mL with solution B.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm;
– *stationary phase*: *amylose derivative of silica gel for chiral separation R* (10 µm);
– *temperature*: 30 °C.

Mobile phase:

– *mobile phase A*: *diethylamine R*, *2-propanol R*, *heptane R* (0.1:15:85 *V/V/V*);
– *mobile phase B*: *heptane R*, *2-propanol R* (50:50 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 25	100	0
25 - 27	100 → 0	0 → 100
27 - 37	0	100

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 286 nm.

Injection: 20 µL.

Identification of impurities: use the chromatogram supplied with *abacavir for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A and D.

Relative retention with reference to abacavir (retention time = about 17 min): impurity D = about 0.8; impurity A = about 0.9.

System suitability: reference solution (a):

– *resolution*: minimum 1.5 between the peaks due to impurities D and A; minimum 1.5 between the peaks due to impurity A and abacavir.

Limit:

– *impurity A*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent).

Related substances. Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use and transfer them to low-adsorption, inert glass vials.*

Test solution. Dissolve 25 mg of the substance to be examined in *water R* and dilute to 100.0 mL with the same solvent. Sonicate until dissolution is complete.

Reference solution (a). Dissolve 2.5 mg of *abacavir for peak identification CRS* (containing impurities B and D) in 10.0 mL of *water R*.

Reference solution (b). Dilute 1.0 mL of the test solution to 100.0 mL with *water R*. Dilute 1.0 mL of this solution to 10.0 mL with *water R*.

Column:

– *size*: *l* = 0.15 m, Ø = 3.9 mm;
– *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
– *temperature*: 30 °C.

Mobile phase:

– *mobile phase A*: dilute 0.5 mL of *trifluoroacetic acid R* in 1000 mL of *water R*;
– *mobile phase B*: *water R*, *methanol R* (15:85 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	95	5
5 - 25	95 → 70	5 → 30
25 - 40	70 → 10	30 → 90

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 254 nm.

Injection: 20 µL.

Identification of impurities: use the chromatogram supplied with *abacavir for peak identification CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities B and D.

Relative retention with reference to abacavir (retention time = about 22 min): impurity D = about 1.04; impurity B = about 1.3.

System suitability: reference solution (a):

– *peak-to-valley ratio*: minimum 3.0, where H_p = height above the baseline of the peak due to impurity D and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to abacavir.

Limits:

– *impurity B*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

– *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

– *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

– *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water (*2.5.32*): maximum 0.5 per cent, determined on 60.0 mg.

Sulfated ash (*2.4.14*): maximum 0.2 per cent, determined on 1.0 g.

ASSAY

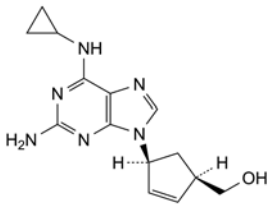
Dissolve 0.300 g in 50 mL of *water R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M sodium hydroxide* is equivalent to 33.54 mg of $C_{28}H_{38}N_{12}O_6S$.

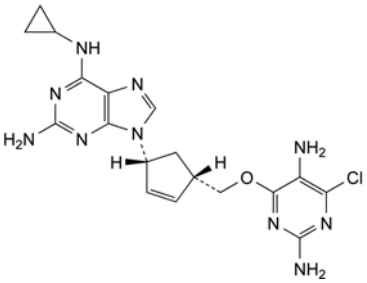
IMPURITIES

Specified impurities: A, B.

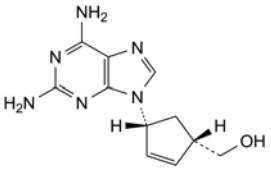
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *C, D, E, F.*



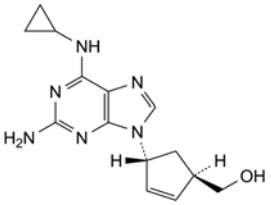
A. [(1*R*,4*S*)-4-[2-amino-6-(cyclopropylamino)-9*H*-purin-9-yl]cyclopent-2-enyl]methanol,



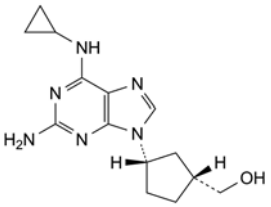
B. 6-(cyclopropylamino)-9-[(1*R*,4*S*)-4-[[(2,5-diamino-6-chloropyrimidin-4-yl)oxy]methyl]cyclopent-2-enyl]-9*H*-purine-2-amine,



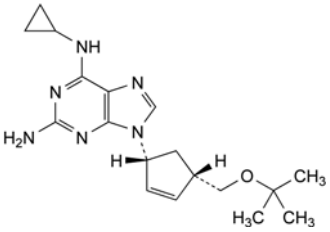
C. [(1*S*,4*R*)-4-(2,6-diamino-9*H*-purin-9-yl)cyclopent-2-enyl]methanol,



D. [(1*R*,4*R*)-4-[2-amino-6-(cyclopropylamino)-9*H*-purin-9-yl]cyclopent-2-enyl]methanol,



E. [(1*R*,3*S*)-3-[2-amino-6-(cyclopropylamino)-9*H*-purin-9-yl]cyclopentyl]methanol,



F. 6-(cyclopropylamino)-9-[(1*R*,4*S*)-4-[[(1,1-dimethylethyl)oxy]methyl]cyclopent-2-enyl]-9*H*-purine-2-amine.

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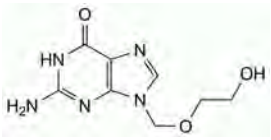
ACICLOVIR

Aciclovirum



$C_8H_{11}N_5O_3$ M_r 225.2
[59277-89-3]

DEFINITION

2-Amino-9-[(2-hydroxyethoxy)methyl]-1,9-dihydro-6*H*-purin-6-one.

Content: 98.5 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: slightly soluble in water, very slightly soluble in ethanol (96 per cent), practically insoluble in heptane. It dissolves in dilute solutions of mineral acids and alkali hydroxides.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *aciclovir CRS*.

TESTS

Appearance of solution. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method II*).

Dissolve 0.25 g in a 4 g/L solution of *sodium hydroxide R* and dilute to 25 mL with the same solvent.

Related substances. Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Solvent mixture: *dimethyl sulfoxide R*, *water R* (20:80 *V/V*).

Phosphate buffer solution pH 2.5. 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 solution pH 3.1. 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*.

Test solution. Dissolve 25 mg of the substance to be examined in 5.0 mL of *dimethyl sulfoxide R* and dilute to 25.0 mL with *water R*.

Reference solution (a). Dissolve 5 mg of *aciclovir for system suitability CRS* (containing impurities A, B, J, K, N, O and P) in 1 mL of *dimethyl sulfoxide R* and dilute to 5.0 mL with *water R*.

Reference solution (b). Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c). Dissolve the contents of a vial of *aciclovir for peak identification 1 CRS* (containing impurities C and I) in 200 µL of *dimethyl sulfoxide R* and dilute to 1.0 mL with *water R*.

Reference solution (d). Dissolve the contents of a vial of *aciclovir for peak identification 2 CRS* (containing impurities F and G) in 1.0 mL of reference solution (a).

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

- *mobile phase A*: *acetonitrile R*, phosphate buffer solution pH 3.1 (1:99 *V/V*);
- *mobile phase B*: *acetonitrile R*, phosphate buffer solution pH 2.5 (50:50 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	100	0
5 - 27	100 → 80	0 → 20
27 - 40	80	20

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 254 nm.

Injection: 10 µL of the test solution and reference solutions (b), (c) and (d).

Identification of impurities: use the chromatogram supplied with *aciclovir for peak identification 1 CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities C and I; use the chromatogram supplied with *aciclovir for peak identification 2 CRS* and the chromatogram obtained with reference solution (d) to identify the peaks due to impurities A, B, F, G, J, K, N, O and P.

Relative retention with reference to aciclovir (retention time = about 13 min): impurity B = about 0.4; impurity P = about 0.7; impurity C = about 0.9; impurity N = about 1.37; impurities O and Q = about 1.42; impurity I = about 1.57; impurity J = about 1.62; impurity F = about 1.7; impurity A = about 1.8; impurities K and R = about 2.5; impurity G = about 2.6.

System suitability:

- *resolution*: minimum 1.5 between the peaks due to impurity C and aciclovir in the chromatogram obtained with reference solution (c); minimum 1.5 between the peaks due to impurities F and A and minimum 1.5 between the peaks due to impurities K and G in the chromatogram obtained with reference solution (d).

Limits:

- *correction factor*: for the calculation of content, multiply the peak area of impurity I by 1.5;
- *impurity B*: not more than 7 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.7 per cent);
- *sum of impurities O and Q*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
- *sum of impurities K and R*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
- *impurities A, G, J, N, P*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
- *impurities C, F, I*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
- *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent);
- *total*: not more than 15 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent);
- *disregard limit*: 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.03 per cent).

Water (*2.5.12*): maximum 6.0 per cent, determined on 0.500 g.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

Bacterial endotoxins (*2.6.14, Method D*): less than 0.50 IU/mg, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

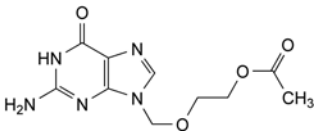
Dissolve 0.150 g in 60 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

1 mL of *0.1 M perchloric acid* is equivalent to 22.52 mg of $C_8H_{11}N_5O_3$.

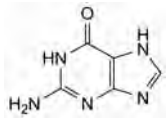
IMPURITIES

Specified impurities: A, B, C, F, G, I, J, K, N, O, P, Q, R.

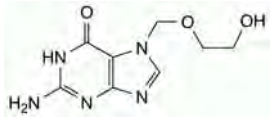
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *L, M*.



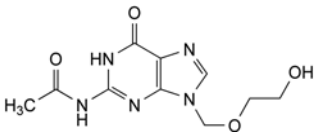
A. 2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl acetate,



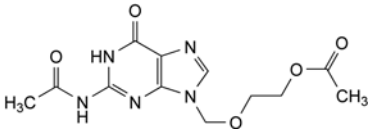
B. 2-amino-1,7-dihydro-6*H*-purin-6-one (guanine),



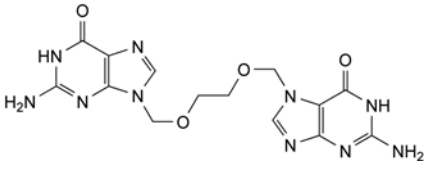
C. 2-amino-7-[(2-hydroxyethoxy)methyl]-1,7-dihydro-6*H*-purin-6-one,



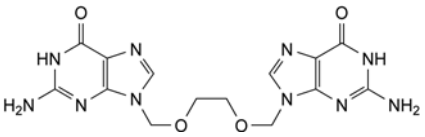
F. *N*-[9-[(2-hydroxyethoxy)methyl]-6-oxo-6,9-dihydro-1*H*-purin-2-yl]acetamide,



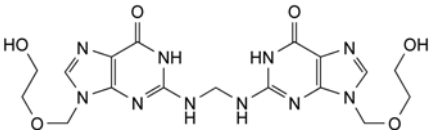
G. 2-[[2-(acetylamino)-6-oxo-1,6-dihydro-9*H*-purin-9-yl]methoxy]ethyl acetate,



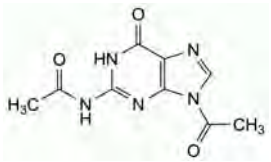
I. 2-amino-7-[[2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethoxy]methyl]-1,7-dihydro-6*H*-purin-6-one,



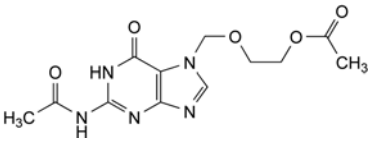
J. 9,9′-[ethylenebis(oxymethylene)]bis(2-amino-1,9-dihydro-6*H*-purin-6-one),



K. 2,2′-(methylenediimino)bis[9-[(2-hydroxyethoxy)methyl]-1,9-dihydro-6*H*-purin-6-one],



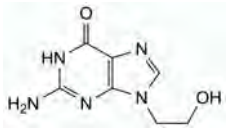
L. *N*-(9-acetyl-6-oxo-6,9-dihydro-1*H*-purin-2-yl)acetamide (*N*^{2},9-diacetylguanine),



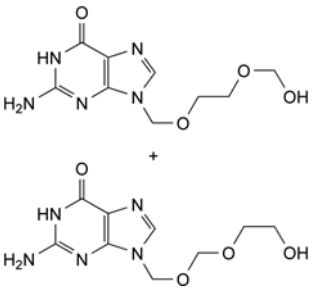
M. 2-[[2-(acetylamino)-6-oxo-1,6-dihydro-7*H*-purin-7-yl]methoxy]ethyl acetate,

N. unknown structure,

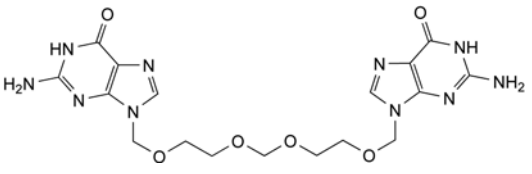
O. unknown structure,



P. 2-amino-9-(2-hydroxyethyl)-1,9-dihydro-6*H*-purin-6-one,



Q. mixture of 2-amino-9-[[2-(hydroxymethoxy)ethoxy]methyl]-1,9-dihydro-6*H*-purin-6-one and 2-amino-9-[[2-(hydroxyethoxy)methoxy]methyl]-1,9-dihydro-6*H*-purin-6-one,

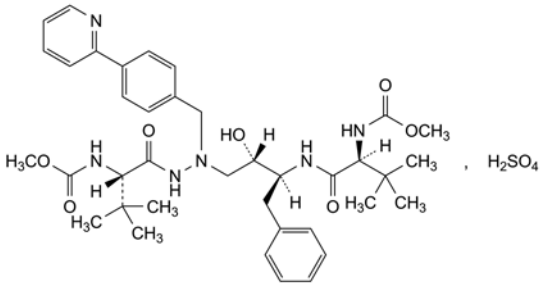


R. 9,9′-[methylenebis(oxyethyleneoxymethylene)]bis(2-amino-1,9-dihydro-6*H*-purin-6-one).

04/2019:2898
corrected 10.1

ATAZANAVIR SULFATE

Atazanaviri sulfas



$C_{38}H_{54}N_6O_{11}S$ M_r 803
[229975-97-7]

DEFINITION

Methyl [(5*S*,10*S*,11*S*,14*S*)-11-benzyl-5-*tert*-butyl-10-hydroxy-15,15-dimethyl-3,6,13-trioxo-8-[[4-(pyridin-2-yl)phenyl]methyl]-2-oxa-4,7,8,12-tetraazahexadecan-14-yl]carbamate sulfate.

Content: 98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or pale yellow, slightly hygroscopic, crystalline powder that may contain agglomerates.

Solubility: slightly soluble in water, freely soluble in ethanol (96 per cent), practically insoluble in heptane.

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *atazanavir sulfate CRS*.

C. It gives reaction (a) of sulfates (*2.3.1*).

TESTS

Specific optical rotation (*2.2.7*): – 44 to – 40 (anhydrous substance), measured at 25 °C.

Dissolve 0.100 g in 8 mL of *methanol R*, using sonication if necessary, and dilute to 10.0 mL with the same solvent.

Related substances. Liquid chromatography (*2.2.29*).

Solvent mixture. Mix equal volumes of *acetonitrile R1* and a freshly prepared 2.73 g/L solution of *potassium dihydrogen phosphate R* in *water for chromatography R* previously adjusted to pH 3.5 with *dilute phosphoric acid R*.

Test solution (a). Dissolve 20.0 mg of the substance to be examined in 40 mL of the solvent mixture, sonicate for 3 min and dilute to 50.0 mL with the solvent mixture.

Test solution (b). Dissolve 50.0 mg of the substance to be examined in 40 mL of the solvent mixture, sonicate for 3 min and dilute to 50.0 mL with the solvent mixture.

Reference solution (a). Dissolve 20.0 mg of *atazanavir sulfate CRS* in 40 mL of the solvent mixture, sonicate for 3 min and dilute to 50.0 mL with the solvent mixture.

Reference solution (b). Dilute 1.0 mL of test solution (a) to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c). Dissolve 4 mg of *atazanavir for system suitability CRS* (containing impurity F) in 8 mL of the solvent mixture, sonicate for 3 min and dilute to 10 mL with the solvent mixture.

Reference solution (d). Dissolve 2.0 mg of *atazanavir impurity K CRS* in 9 mL of the solvent mixture, sonicate for 3 min and dilute to 10.0 mL with the solvent mixture. Dilute 5.0 mL of the solution to 100.0 mL with the solvent mixture. Dilute 3.0 mL of this solution to 20.0 mL with the solvent mixture.

Column:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3.0 µm);
- *temperature*: 25 °C.

Mobile phase:
- *mobile phase A*: mix 25 volumes of *acetonitrile R1* and 75 volumes of a freshly prepared 2.73 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 3.5 with *dilute phosphoric acid R*;
- *mobile phase B*: mix 25 volumes of a freshly prepared 2.73 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 3.5 with *dilute phosphoric acid R*, and 75 volumes of *acetonitrile R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	100	0
5 - 45	100 → 0	0 → 100

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 215 nm.

Injection: 10 µL of test solution (a) and reference solutions (b) and (c).

Identification of impurities: use the chromatogram supplied with *atazanavir for system suitability CRS* and the chromatogram obtained with reference solution (c) to identify the peak due to impurity F.

Relative retention with reference to atazanavir (retention time = about 30 min): impurity F = about 0.99.

System suitability: reference solution (c):
- *resolution*: minimum 1.5 between the peaks due to impurity F and atazanavir.

Calculation of percentage contents:
- for each impurity, use the concentration of atazanavir sulfate in reference solution (b).

Limits:
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 0.5 per cent;
- *reporting threshold*: 0.05 per cent; disregard any peak with a relative retention with reference to atazanavir of less than 0.2.

Impurity K. Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Mobile phase:

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	95	5
5 - 8	95 → 0	5 → 100
8 - 14	0	100

Injection: 20 µL of test solution (b) and reference solution (d).

Identification of impurities: use the chromatogram obtained with reference solution (d) to identify the peak due to impurity K.

Relative retention with reference to atazanavir (retention time = about 10 min): impurity K = about 0.4.

Calculation of percentage content:
- for impurity K, use the concentration of impurity K in reference solution (d).

Limit:

– *impurity K*: maximum 0.15 per cent.

Water (*2.5.32*): maximum 2.5 per cent, determined on 0.100 g by direct sample introduction.

Sulfated ash (*2.4.14*): maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Mobile phase: solvent mixture.

Injection: test solution (a) and reference solutions (a) and (c).

Run time: 1.6 times the retention time of atazanavir.

Relative retention with reference to atazanavir (retention time = about 9.5 min): impurity F = about 0.94.

System suitability: reference solution (c):

– *resolution*: minimum 1.5 between the peaks due to impurity F and atazanavir.

Calculate the percentage content of $C_{38}H_{54}N_6O_{11}S$ using the chromatogram obtained with reference solution (a) and taking into account the assigned content of *atazanavir sulfate CRS*.

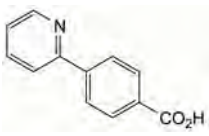
STORAGE

In an airtight container.

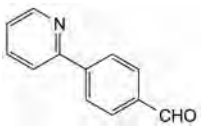
IMPURITIES

Specified impurities: K.

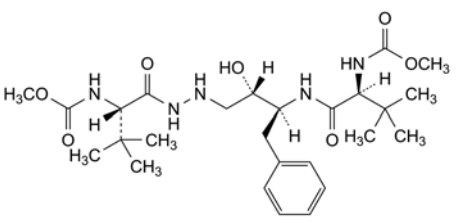
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, B, C, D, E, F, G, H, I, J.*



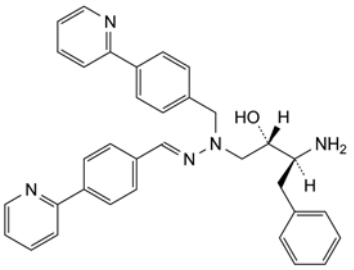
A. 4-(pyridin-2-yl)benzoic acid,



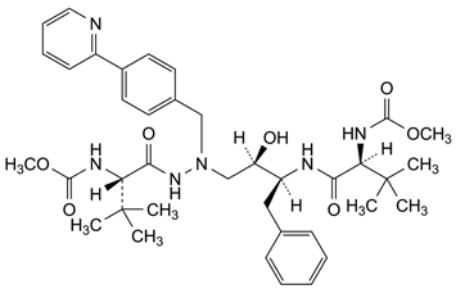
B. 4-(pyridin-2-yl)benzaldehyde,



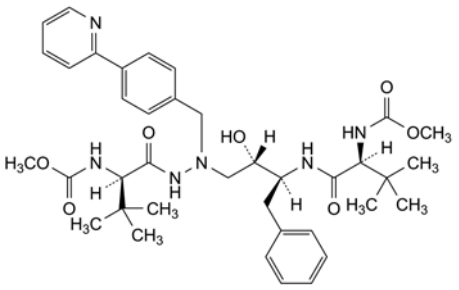
C. methyl [(5*S*,10*S*,11*S*,14*S*)-11-benzyl-5-*tert*-butyl-10-hydroxy-15,15-dimethyl-3,6,13-trioxo-2-oxa-4,7,8,12-tetraazahexadecan-14-yl]carbamate,



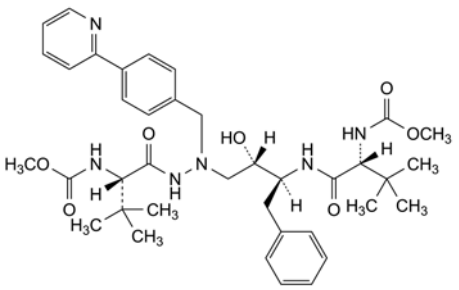
D. (2*S*,3*S*)-3-amino-4-phenyl-1-[(*E*)-1-[[4-(pyridin-2-yl)phenyl]methyl]-2-[[4-(pyridin-2-yl)phenyl]methylidene]hydrazin-1-yl]butan-2-ol,



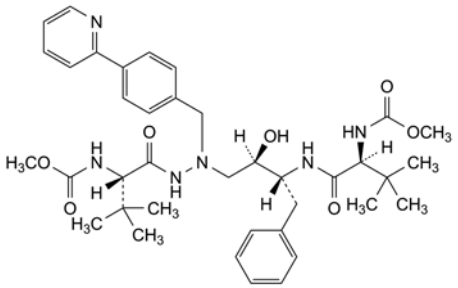
E. methyl [(5*S*,10*R*,11*S*,14*S*)-11-benzyl-5-*tert*-butyl-10-hydroxy-15,15-dimethyl-3,6,13-trioxo-8-[[4-(pyridin-2-yl)phenyl]methyl]-2-oxa-4,7,8,12-tetraazahexadecan-14-yl]carbamate,



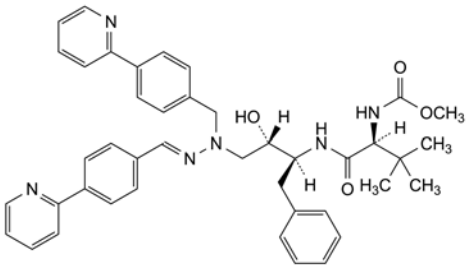
F. methyl [(5*R*,10*S*,11*S*,14*S*)-11-benzyl-5-*tert*-butyl-10-hydroxy-15,15-dimethyl-3,6,13-trioxo-8-[[4-(pyridin-2-yl)phenyl]methyl]-2-oxa-4,7,8,12-tetraazahexadecan-14-yl]carbamate,



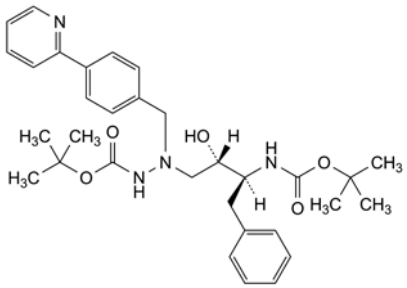
G. methyl [(5*S*,10*S*,11*S*,14*R*)-11-benzyl-5-*tert*-butyl-10-hydroxy-15,15-dimethyl-3,6,13-trioxo-8-[[4-(pyridin-2-yl)phenyl]methyl]-2-oxa-4,7,8,12-tetraazahexadecan-14-yl]carbamate,



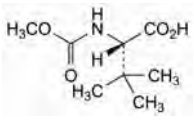
H. methyl [(5*S*,10*R*,11*R*,14*S*)-11-benzyl-5-*tert*-butyl-10-hydroxy-15,15-dimethyl-3,6,13-trioxo-8-[[4-(pyridin-2-yl)phenyl]methyl]-2-oxa-4,7,8,12-tetraazahexadecan-14-yl]carbamate,



I. methyl [(2*S*)-1-[[(2*S*,3*S*)-3-hydroxy-1-phenyl-4-[(*E*)-1-[[4-(pyridin-2-yl)phenyl]methyl]-2-[[4-(pyridin-2-yl)phenyl]methylidene]hydrazin-1-yl]butan-2-yl]amino]-3,3-dimethyl-1-oxobutan-2-yl]carbamate,



J. *tert*-butyl 2-[(2*S*,3*S*)-3-(*tert*-butoxyformamido)-2-hydroxy-4-phenylbutyl]-2-[[4-(pyridin-2-yl)phenyl]methyl]hydrazine-1-carboxylate,

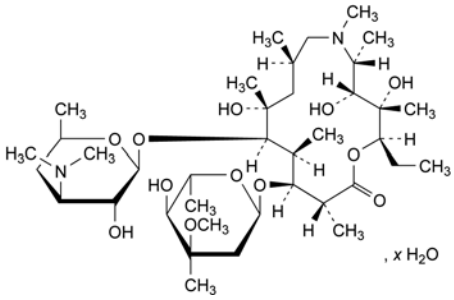


K. (2*S*)-2-(methoxyformamido)-3,3-dimethylbutanoic acid.

01/2018:1649
corrected 9.6

AZITHROMYCIN

Azithromycinum



$C_{38}H_{72}N_2O_{12},xH_2O$ M_r 749 (anhydrous substance)
with x = 1 or 2

Azithromycin monohydrate: [121470-24-4]
Azithromycin dihydrate: [117772-70-0]

DEFINITION

(2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one. The degree of hydration is 1 or 2.

Semi-synthetic product derived from a fermentation product.

Content: 96.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white powder.

Solubility: practically insoluble in water, freely soluble in anhydrous ethanol and in methylene chloride.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *azithromycin CRS*.

If the spectra obtained in the solid state show differences, prepare further spectra using 90 g/L solutions in *methylene chloride R*.

TESTS

Solution S. Dissolve 0.500 g in *anhydrous ethanol R* and dilute to 50.0 mL with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*): 9.0 to 11.0.

Dissolve 0.100 g in 25.0 mL of *methanol R* and dilute to 50.0 mL with *carbon dioxide-free water R*.

Specific optical rotation (*2.2.7*): − 49 to − 45 (anhydrous substance), determined on solution S.

Related substances. Liquid chromatography (*2.2.29*).

Solvent mixture. Prepare a 1.73 g/L solution of *ammonium dihydrogen phosphate R* adjusted to pH 10.0 with *ammonia R*. Transfer 350 mL of this solution to a suitable container. Add 300 mL of *acetonitrile R* and 350 mL of *methanol R*. Mix well.

Test solution. Dissolve 0.200 g of the substance to be examined in the solvent mixture and dilute to 25.0 mL with the solvent mixture.

Reference solution (a). Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture.

Reference solution (b). Dissolve the contents of a vial of *azithromycin for system suitability CRS* (containing impurities F, H and J) in 1.0 mL of the solvent mixture and sonicate for 5 min.

Reference solution (c). Dissolve 8.0 mg of *azithromycin for peak identification CRS* (containing impurities A, B, C, E, F, G, I, J, L, M, N, O and P) in 1.0 mL of the solvent mixture.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl amorphous organosilica polymer for chromatography R* (5 µm);
- *temperature*: 60 °C.

Mobile phase:

- *mobile phase A*: 1.80 g/L solution of *anhydrous disodium hydrogen phosphate R* adjusted to pH 8.9 with *dilute phosphoric acid R* or with *dilute sodium hydroxide solution R*;
- *mobile phase B*: *methanol R2, acetonitrile R1* (25:75 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 25	50 → 45	50 → 55
25 - 30	45 → 40	55 → 60
30 - 80	40 → 25	60 → 75
80 - 81	25 → 50	75 → 50
81 - 93	50	50

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 210 nm.

Injection: 50 µL.

Identification of impurities: use the chromatogram supplied with *azithromycin for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B, C, E, F, G, I, J, L, M, N, O and P; use the chromatogram supplied with *azithromycin for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peak due to impurity H.

Relative retention with reference to azithromycin (retention time = 45-50 min): impurity L = about 0.29; impurity M = about 0.37; impurity E = about 0.43; impurity F = about 0.51; impurity D = about 0.54; impurity J = about 0.54; impurity Q = about 0.54; impurity I = about 0.61; impurity C = about 0.73; impurity N = about 0.76; impurity H = about 0.79; impurity A = about 0.83; impurity P = about 0.92; impurity O = about 1.23; impurity G = about 1.26; impurity B = about 1.31.

System suitability: reference solution (b):

- *peak-to-valley ratio*: minimum 1.4, where H_p = height above the baseline of the peak due to impurity J and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity F.

Limits:

- *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity F = 0.3; impurity G = 0.2; impurity H = 0.1; impurity L = 2.3; impurity M = 0.6; impurity N = 0.7;
- *impurity B*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent);
- *impurities A, C, E, F, H, I, L, M, N, O, P*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *sum of impurities D, J and Q*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

- *impurity G*: not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *any other impurity*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (3.0 per cent);
- *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent); disregard the peaks eluting before impurity L and after impurity B.

Water (*2.5.12*): 1.8 per cent to 6.5 per cent, determined on 0.200 g.

Sulfated ash (*2.4.14*): maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*).

Solution A. Mix 60 volumes of *acetonitrile R* and 40 volumes of a 6.7 g/L solution of *dipotassium hydrogen phosphate R* adjusted to pH 8.0 with *phosphoric acid R*.

Test solution. Dissolve 53.0 mg of the substance to be examined in 2 mL of *acetonitrile R* and dilute to 100.0 mL with solution A.

Reference solution (a). Dissolve 53.0 mg of *azithromycin CRS* in 2 mL of *acetonitrile R* and dilute to 100.0 mL with solution A.

Reference solution (b). Dissolve 5 mg of the substance to be examined and 5 mg of *azithromycin impurity A CRS* in 0.5 mL of *acetonitrile R* and dilute to 10 mL with solution A.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl vinyl polymer for chromatography R* (5 µm);
- *temperature*: 40 °C.

Mobile phase: mix 60 volumes of *acetonitrile R1* and 40 volumes of a 6.7 g/L solution of *dipotassium hydrogen phosphate R* adjusted to pH 11.0 with a 560 g/L solution of *potassium hydroxide R*.

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 210 nm.

Injection: 10 µL.

Run time: 1.5 times the retention time of azithromycin.

Retention time: azithromycin = about 10 min.

System suitability: reference solution (b):

- *resolution*: minimum 3.0 between the peaks due to impurity A and azithromycin.

Calculate the percentage content of $C_{38}H_{72}N_2O_{12}$ taking into account the assigned content of *azithromycin CRS*.

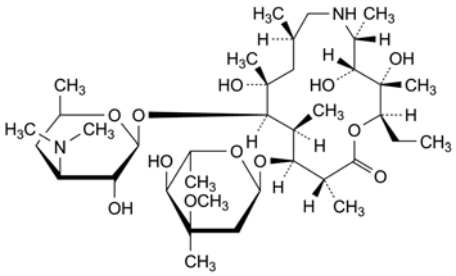
STORAGE

In an airtight container.

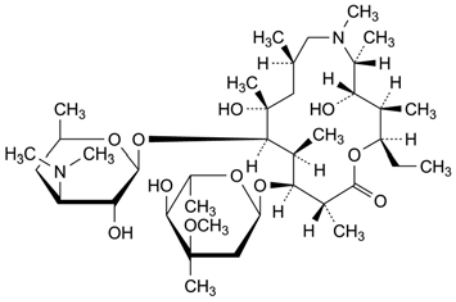
IMPURITIES

Specified impurities: *A, B, C, D, E, F, G, H, I, J, L, M, N, O, P, Q*.

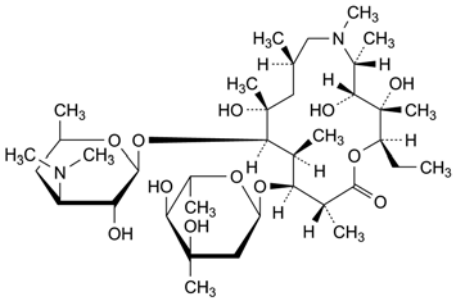
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *K*.



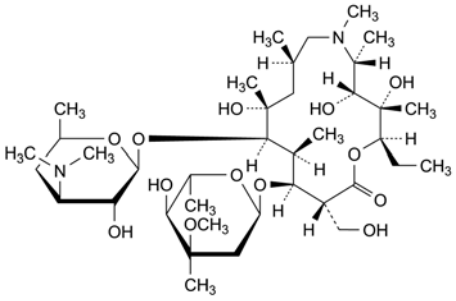
A. 6-demethylazithromycin,



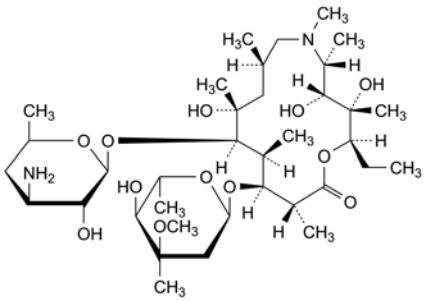
B. 3-deoxyazithromycin (azithromycin B),



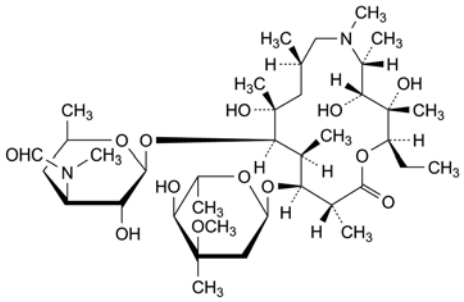
C. 3″-*O*-demethylazithromycin (azithromycin C),



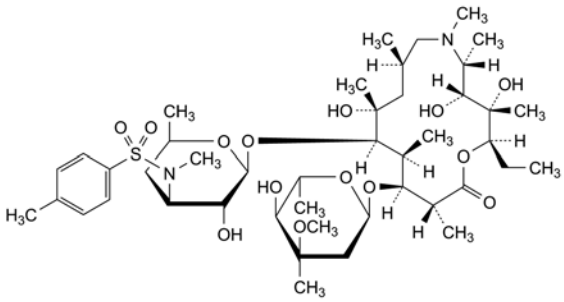
D. 14-demethyl-14-(hydroxymethyl)azithromycin (azithromycin F),



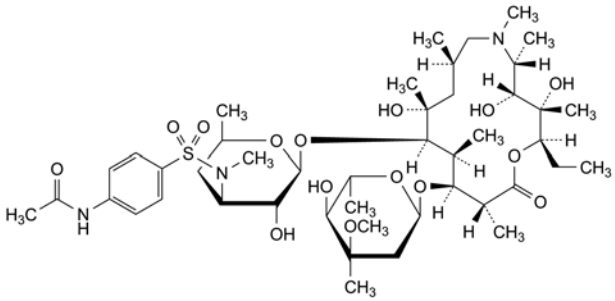
E. 3′-(*N*,*N*-didemethyl)azithromycin (aminoazithromycin),



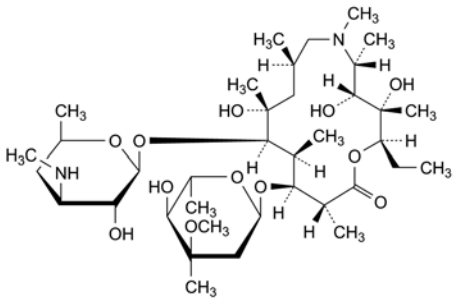
F. 3′-*N*-demethyl-3′-*N*-formylazithromycin,



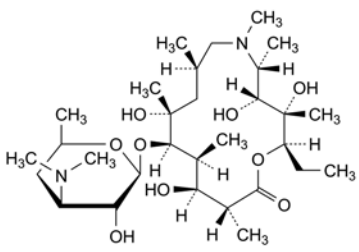
G. 3′-*N*-demethyl-3′-*N*-[(4-methylphenyl)sulfonyl]-azithromycin,



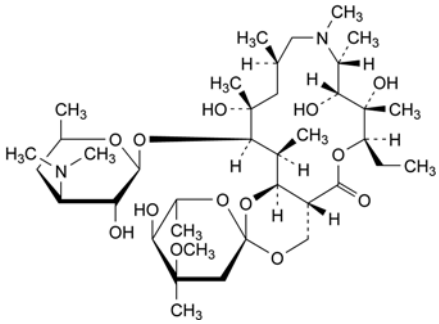
H. 3′-*N*-[[4-(acetylamino)phenyl]sulfonyl]-3′-*N*-demethylazithromycin,



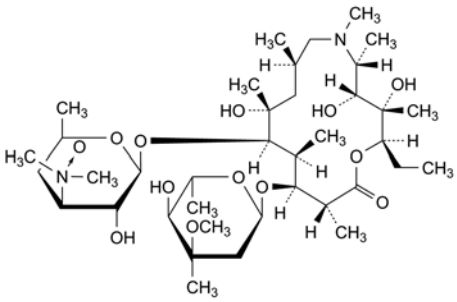
I. 3′-*N*-demethylazithromycin,



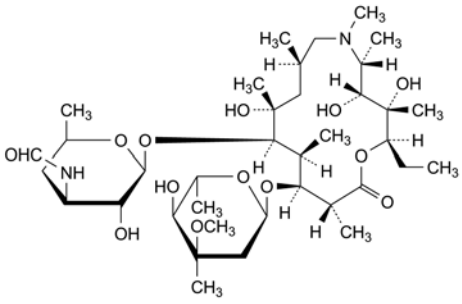
J. 13-*O*-decladinosylazithromycin,



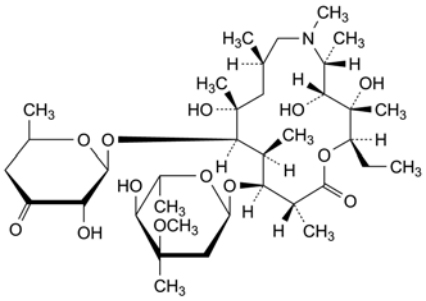
K. C^{14},1″-epoxyazithromycin (azithromycin E),



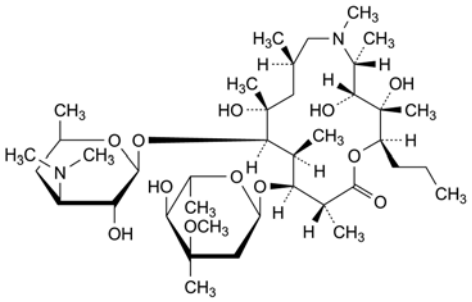
L. azithromycin 3′-*N*-oxide,



M. 3′-(*N*,*N*-didemethyl)-3′-*N*-formylazithromycin,

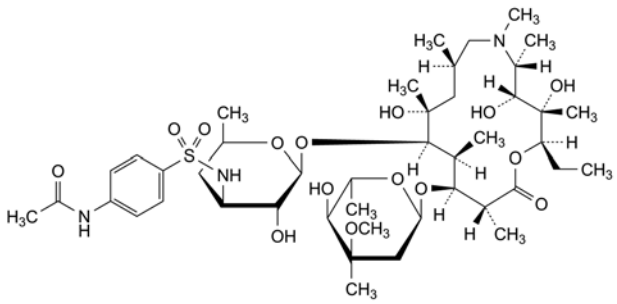


N. 3′-de(dimethylamino)-3′-oxoazithromycin,



O. 2-desethyl-2-propylazithromycin,

P. unknown structure,

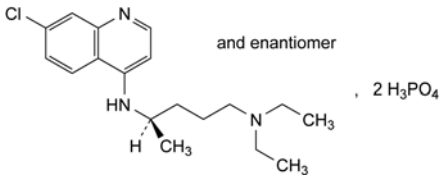


Q. 3′-*N*-[[4-(acetylamino)phenyl]sulfonyl]-3′-(*N*,*N*-didemethyl)azithromycin.

01/2017:0544

CHLOROQUINE PHOSPHATE

Chloroquini phosphas



$C_{18}H_{32}ClN_3O_8P_2$ M_r 515.9
[50-63-5]

DEFINITION

Chloroquine phosphate contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of N^4-(7-chloroquinolin-4-yl)-N^1,N^1-diethylpentane-1,4-diamine bis(dihydrogen phosphate), calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, hygroscopic, freely soluble in water, very slightly soluble in alcohol and in methanol.

It exists in 2 forms, one of which melts at about 195 °C and the other at about 218 °C.

IDENTIFICATION

First identification: B, D.

Second identification: A, C, D.

A. Dissolve 0.100 g in *water R* and dilute to 100.0 mL with the same solvent. Dilute 1.0 mL of this solution to 100.0 mL with *water R*. Examined between 210 nm and 370 nm (*2.2.25*), the solution shows absorption maxima at 220 nm, 235 nm, 256 nm, 329 nm and 342 nm. The specific absorbances at the maxima are respectively 600 to 660, 350 to 390, 300 to 330, 325 to 355 and 360 to 390.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with the base isolated from *chloroquine sulfate CRS*. Record the spectra using solutions prepared as follows: dissolve separately 0.1 g of the substance to be examined and 80 mg of the reference substance in 10 mL of *water R*, add 2 mL of *dilute sodium hydroxide solution R* and shake with 2 quantities, each of 20 mL, of *methylene chloride R*; combine the organic layers, wash with *water R*, dry over *anhydrous sodium sulfate R*, evaporate to dryness and dissolve the residues separately, each in 2 mL of *methylene chloride R*.

C. Dissolve 25 mg in 20 mL of *water R* and add 8 mL of *picric acid solution R1*. The precipitate, washed with *water R*, with *alcohol R* and finally with *methylene chloride R*, melts (*2.2.14*) at 206-209 °C.

D. Dissolve 0.1 g in 10 mL of *water R*, add 2 mL of *dilute sodium hydroxide solution R* and shake with 2 quantities, each of 20 mL, of *methylene chloride R*. The aqueous layer, acidified by the addition of *nitric acid R*, gives reaction (b) of phosphates (*2.3.1*).

TESTS

Solution S. Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 25 mL with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_5 or GY_5 (*2.2.2, Method II*).

pH (*2.2.3*). The pH of solution S is 3.8 to 4.3.

Related substances. Examine by thin-layer chromatography (*2.2.27*), using *silica gel* GF_{254} *R* as the coating substance.

Test solution. Dissolve 0.50 g of the substance to be examined in *water R* and dilute to 10 mL with the same solvent.

Reference solution (a). Dilute 1 mL of the test solution to 100 mL with *water R*.

Reference solution (b). Dilute 5 mL of reference solution (a) to 10 mL with *water R*.

Apply to the plate 2 µL of each solution. Develop over a path of 12 cm using a mixture of 10 volumes of *diethylamine R*, 40 volumes of *cyclohexane R* and 50 volumes of *chloroform R*. Allow the plate to dry in air. Examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (1.0 per cent) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Loss on drying (*2.2.32*): maximum 2.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Dissolve 0.200 g in 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 25.79 mg of $C_{18}H_{32}ClN_3O_8P_2$.

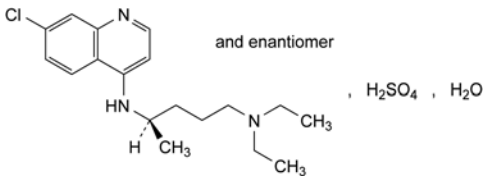
STORAGE

In an airtight container, protected from light.

01/2017:0545

CHLOROQUINE SULFATE

Chloroquini sulfas



$C_{18}H_{28}ClN_3O_4S,H_2O$ M_r 436.0

DEFINITION

Chloroquine sulfate contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of N^4-(7-chloroquinolin-4-yl)-N^1,N^1-diethylpentane-1,4-diamine sulfate, calculated with reference to the anhydrous substance.

CHARACTERS

A white or almost white, crystalline powder, freely soluble in water and in methanol, very slightly soluble in ethanol (96 per cent).

It melts at about 208 °C (instantaneous method).

IDENTIFICATION

First identification: B, D.

Second identification: A, C, D.

A. Dissolve 0.100 g in *water R* and dilute to 100.0 mL with the same solvent. Dilute 1.0 mL of this solution to 100.0 mL with *water R*. Examined between 210 nm and 370 nm (*2.2.25*), the solution shows absorption maxima at 220 nm, 235 nm, 256 nm, 329 nm and 342 nm. The specific absorbances at the maxima are respectively 730 to 810, 430 to 470, 370 to 410, 400 to 440 and 430 to 470.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with the base isolated from *chloroquine sulfate CRS*. Record the spectra using solutions prepared as follows: dissolve separately 0.1 g of the substance to be examined and of the reference substance in 10 mL of *water R*, add 2 mL of *dilute sodium hydroxide solution R* and shake with 2 quantities, each of 20 mL, of *methylene chloride R*; combine the organic layers, wash with *water R*, dry over *anhydrous sodium sulfate R*, evaporate to dryness and dissolve the residues separately each in 2 mL of *methylene chloride R*.

C. Dissolve 25 mg in 20 mL of *water R* and add 8 mL of *picric acid solution R1*. The precipitate, washed with *water R*, with *ethanol (96 per cent) R* and finally with *ether R*, melts (*2.2.14*) at 206 °C to 209 °C.

D. It gives reaction (a) of sulfates (*2.3.1*).

TESTS

Solution S. Dissolve 2.0 g in *carbon dioxide-free water R* and dilute to 25 mL with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_5 or GY_5 (*2.2.2, Method II*).

pH (*2.2.3*). The pH of solution S is 4.0 to 5.0.

Related substances. Examine by thin-layer chromatography (*2.2.27*), using *silica gel* GF_{254} *R* as the coating substance.

Test solution. Dissolve 0.50 g of the substance to be examined in *water R* and dilute to 10 mL with the same solvent.

Reference solution (a). Dilute 1 mL of the test solution to 100 mL with *water R*.

Reference solution (b). Dilute 5 mL of reference solution (a) to 10 mL with *water R*.

Apply separately to the plate 2 µL of each solution. Develop over a path of 12 cm using a mixture of 10 volumes of *diethylamine R*, 40 volumes of *cyclohexane R* and 50 volumes of *methylene chloride R*. Allow the plate to dry in air. Examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (1.0 per cent) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Water (*2.5.12*): 3.0 per cent to 5.0 per cent, determined on 0.500 g.

Sulfated ash (*2.4.14*). Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.400 g in 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 41.8 mg of $C_{18}H_{28}ClN_3O_4S$.

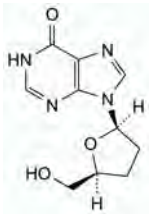
STORAGE

Store in an airtight container, protected from light.

01/2017:2200

DIDANOSINE

Didanosinum



$C_{10}H_{12}N_4O_3$ M_r 236.2
[69655-05-6]

DEFINITION

9-(2,3-Dideoxy-β-D-*glycero*-pentofuranosyl)-1,9-dihydro-6*H*-purin-6-one (2′,3′-dideoxyinosine).

Content: 98.5 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: sparingly soluble in water, freely soluble in dimethyl sulfoxide, slightly soluble in methanol and in ethanol (96 per cent).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *didanosine CRS*.

B. Specific optical rotation (*2.2.7*): – 28.2 to – 24.2 (anhydrous substance).
Dissolve 0.100 g in *water R* and dilute to 10.0 mL with the same solvent.

TESTS

Related substances. Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Solvent mixture. Mix 8 volumes of mobile phase B and 92 volumes of mobile phase A.

Test solution. Dissolve 25.0 mg of the substance to be examined in 50.0 mL of the solvent mixture.

Reference solution (a). Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b). Dissolve 5.0 mg of *didanosine impurity A CRS* in the solvent mixture and dilute to 100.0 mL with the solvent mixture. Dilute 1.0 mL to 20.0 mL with the solvent mixture.

Reference solution (c). Dissolve 5 mg of *didanosine for system suitability CRS* (containing impurities A to F) in the solvent mixture and dilute to 10 mL with the solvent mixture.

Reference solution (d). Dissolve 5 mg of *didanosine impurity G CRS* in the solvent mixture and dilute to 100 mL with the solvent mixture. Dilute 1 mL to 20 mL with the solvent mixture.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
- *mobile phase A*: mix 8 volumes of *methanol R* and 92 volumes of a 3.86 g/L solution of *ammonium acetate R* adjusted to pH 8.0 with *concentrated ammonia R*;
- *mobile phase B*: mix 30 volumes of *methanol R* and 70 volumes of a 3.86 g/L solution of *ammonium acetate R* adjusted to pH 8.0 with *concentrated ammonia R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 18	100	0
18 - 25	100 → 0	0 → 100
25 - 45	0	100
45 - 50	0 → 100	100 → 0
50 - 60	100	0

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 254 nm.

Injection: 20 µL.

Identification of impurities: use the chromatogram supplied with *didanosine for system suitability CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A to F and use the chromatogram obtained with reference solution (d) to identify the peak due to impurity G.

Relative retention with reference to didanosine (retention time = about 13-15 min): impurity A = about 0.3; impurity B = about 0.4; impurity C = about 0.44; impurity D = about 0.48; impurity E = about 0.5; impurity F = about 0.8; impurity G = about 1.6.

System suitability: reference solution (c):
- *resolution*: minimum 2.5 between the peaks due to impurity C and impurity D.

Limits:
- *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *impurities B, C, D, E, F, G*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (*2.5.12*): maximum 2.0 per cent, determined on 0.500 g.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

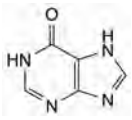
Dissolve 0.200 g in 50 mL of *glacial acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 23.62 mg of $C_{10}H_{12}N_4O_3$.

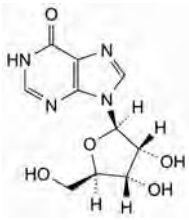
IMPURITIES

Specified impurities: A, B, C, D, E, F, G.

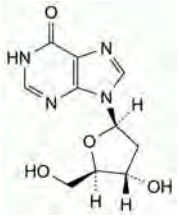
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *H, I.*



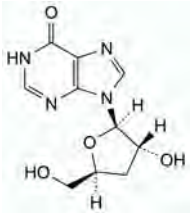
A. 1,7-dihydro-6*H*-purin-6-one (hypoxanthine),



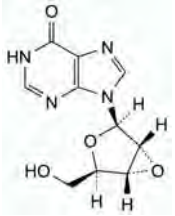
B. 9-β-D-ribofuranosyl-1,9-dihydro-6*H*-purin-6-one (inosine),



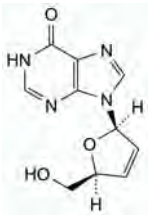
C. 9-(2-deoxy-β-D-*erythro*-pentofuranosyl)-1,9-dihydro-6*H*-purin-6-one (2′-deoxyinosine),



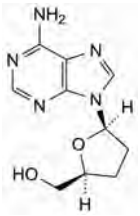
D. 9-(3-deoxy-β-D-*erythro*-pentofuranosyl)-1,9-dihydro-6*H*-purin-6-one (3′-deoxyinosine),



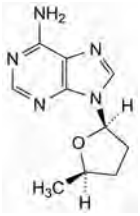
E. 9-(2,3-anhydro-β-D-ribofuranosyl)-1,9-dihydro-6*H*-purin-6-one (2′,3′-anhydroinosine),



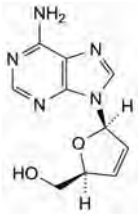
F. 9-(2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)-1,9-dihydro-6*H*-purin-6-one (2′,3′-dideoxy-2′,3′-didehydroinosine),



G. 9-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)-9*H*-purin-6-amine (2′,3′-dideoxyadenosine),



H. 9-(2,3,5-trideoxy-β-D-*glycero*-pentofuranosyl)-9*H*-purin-6-amine (2′,3′,5′-trideoxyadenosine),

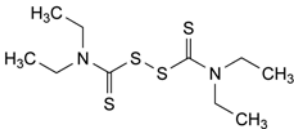


I. 9-(2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)-9*H*-purin-6-amine (2′,3′-dideoxy-2′,3′-didehydroadenosine).

01/2017:0603

DISULFIRAM

Disulfiramum



$C_{10}H_{20}N_2S_4$ M_r 296.5
[97-77-8]

DEFINITION

Disulfiram contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of tetraethyldisulfanedicarbothioamide, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, practically insoluble in water, freely soluble in methylene chloride, sparingly soluble in alcohol.

IDENTIFICATION

First identification: A, B.

Second identification: A, C, D.

A. Melting point (*2.2.14*): 70 °C to 73 °C.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *disulfiram CRS*. Examine the substances prepared as discs.

C. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dissolve about 10 mg in 10 mL of *methanol R*. Add 2 mL of a 0.5 g/L solution of *cupric chloride R* in *methanol R*. A yellow colour develops which becomes greenish-yellow.

TESTS

Related substances. Examine by thin-layer chromatography (*2.2.27*), using as the coating substance a suitable silica gel with a fluorescent indicator having an optimal intensity at 254 nm.

Test solution (a). Dissolve 0.20 g of the substance to be examined in *ethyl acetate R* and dilute to 10 mL with the same solvent.

Test solution (b). Dilute 1 mL of test solution (a) to 10 mL with *ethyl acetate R*.

Reference solution (a). Dissolve 10 mg of *disulfiram CRS* in *ethyl acetate R* and dilute to 5 mL with the same solvent.

Reference solution (b). Dilute 1 mL of test solution (b) to 20 mL with *ethyl acetate R*.

Apply to the plate 10 µL of each solution. Develop over a path of 15 cm using a mixture of 30 volumes of *butyl acetate R* and 70 volumes of *hexane R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Diethyldithiocarbamate. Dissolve 0.20 g in 10 mL of *peroxide-free ether R*, add 5 mL of *buffer solution pH 8.0 R* and shake vigorously. Discard the upper layer and wash the lower layer with 10 mL of *peroxide-free ether R*. Add to the lower layer 0.2 mL of a 4 g/L solution of *copper sulfate pentahydrate R* and 5 mL of *cyclohexane R*. Shake. Any yellow colour in the upper layer is not more intense than that of a standard prepared at the same time using 0.2 mL of a freshly prepared 0.15 g/L solution of *sodium diethyldithiocarbamate R* (150 ppm).

Loss on drying (*2.2.32*). Not more than 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 50 °C.

Sulfated ash (*2.4.14*). Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

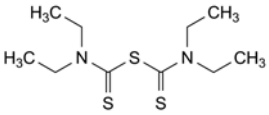
Dissolve 0.450 g in 80 mL of *acetone R* and add 20 mL of a 20 g/L solution of *potassium nitrate R*. Titrate with *0.1 M silver nitrate*. Determine the end-point potentiometrically (*2.2.20*), using a silver electrode and a silver-silver chloride double-junction electrode saturated with potassium nitrate.

1 mL of *0.1 M silver nitrate* is equivalent to 59.30 mg of $C_{10}H_{20}N_2S_4$.

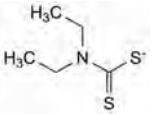
STORAGE

Store protected from light.

IMPURITIES



A. diethylthiocarbamic thioanhydride (sulfiram),

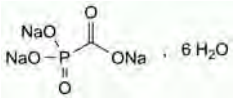


B. diethyldithiocarbamate.

01/2017:1520
corrected 10.0

FOSCARNET SODIUM HEXAHYDRATE

Foscarnetum natricum hexahydricum



$CNa_3O_5P,6H_2O$ M_r 300.0
[34156-56-4]

DEFINITION

Trisodium phosphonatoformate hexahydrate.

Content: 98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *foscarnet sodium hexahydrate CRS*.

B. It gives reaction (a) of sodium (*2.3.1*).

TESTS

Solution S. Dissolve 0.5 g in *carbon dioxide-free water R* and dilute to 25 mL with the same solvent.

Appearance of solution. Solution S is not more opalescent than reference suspension I (*2.2.1*) and is colourless (*2.2.2, Method II*).

pH (*2.2.3*): 9.0 to 11.0 for solution S.

Impurity D. Gas chromatography (*2.2.28*).

Test solution. Dissolve 0.250 g of the substance to be examined in 9.0 mL of a 6 g/L solution of *glacial acetic acid R* using a magnetic stirrer. Add 1.0 mL of *anhydrous ethanol R* and mix.

Reference solution. Dissolve 25.0 mg of *foscarnet impurity D CRS* in *anhydrous ethanol R* and dilute to 100.0 mL with the same solvent. Dilute 1.0 mL of this solution to 10.0 mL with *anhydrous ethanol R*.

Column:
- *material*: fused silica;
- *size*: *l* = 25 m, Ø = 0.31 mm;
- *stationary phase*: *phenyl(5)methyl(95)polysiloxane R* (film thickness 0.5 µm).

Carrier gas: *helium for chromatography R*.

Split ratio: 1:20.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 8	100 → 180
Injection port		200
Detector		250

Detection: flame ionisation.

Injection: 3 µL

Limit:
- *impurity D*: not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent).

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 25 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (a). Dilute 1.0 mL of the test solution to 50.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b). Dissolve 5 mg of *foscarnet impurity B CRS* in the mobile phase, add 2 mL of the test solution and dilute to 50 mL with the mobile phase.

Reference solution (c). Dissolve the contents of a vial of *foscarnet impurity mixture CRS* (impurities A and C) in 1 mL of mobile phase.

Column:
- *size*: *l* = 0.10 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase: dissolve 3.22 g of *sodium sulfate decahydrate R* in *water for chromatography R*, add 3 mL of *glacial acetic acid R* and 6 mL of a 44.61 g/L solution of *sodium pyrophosphate R* and dilute to 1000 mL with *water for chromatography R* (solution A); dissolve 3.22 g of *sodium sulfate decahydrate R* in *water for chromatography R*, add 6.8 g of *sodium acetate R* and 6 mL of a 44.61 g/L solution of *sodium pyrophosphate R* and dilute to 1000 mL with *water for chromatography R* (solution B). Mix about 700 mL of solution A and about 300 mL of solution B to obtain a solution of pH 4.4. To 1000 mL of this solution, add 0.25 g of *tetrahexylammonium hydrogen sulfate R* and 100 mL of *methanol R1*.

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 230 nm.

Injection: 40 µL.

Run time: 2.5 times the retention time of foscarnet.

Identification of impurities: use the chromatogram supplied with *foscarnet impurity mixture CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A and C; use the chromatogram obtained with reference solution (b) to identify the peak due to impurity B.

Relative retention with reference to foscarnet (retention time = about 5 min): impurity A = about 0.7; impurity B = about 1.5; impurity C = about 2.0.

System suitability: reference solution (b):
- *resolution*: minimum 7.0 between the peaks due to foscarnet and impurity B.

Limits:
- *impurities A, B, C*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *unspecified impurities*: for each impurity, not more than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent);
- *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent);
- *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.04 per cent); disregard any peak with a relative retention less than 0.6.

Phosphate and phosphite. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 60.0 mg of the substance to be examined in *water R* and dilute to 25.0 mL with the same solvent.

Reference solution (a). Dissolve 28 mg of *sodium dihydrogen phosphate monohydrate R* in *water R* and dilute to 100 mL with the same solvent.

Reference solution (b). Dissolve 43 mg of *sodium phosphite pentahydrate R* in *water R* and dilute to 100 mL with the same solvent.

Reference solution (c). Dilute 1.0 mL of reference solution (a) and 1.0 mL of reference solution (b) to 25 mL with *water R*.

Reference solution (d). Dilute 3 mL of reference solution (a) and 3 mL of reference solution (b) to 25 mL with *water R*.

Column:

– *size*: *l* = 0.05 m, Ø = 4.6 mm;

– *stationary phase*: *anion-exchange resin R*.

Mobile phase: dissolve 0.102 g of *potassium hydrogen phthalate R* in *water for chromatography R*, add 2.5 mL of *1 M nitric acid* and dilute to 1000 mL with *water for chromatography R*.

Flow rate: 1.4 mL/min.

Detection: spectrophotometer at 290 nm (indirect detection).

Injection: 20 µL of the test solution and reference solutions (c) and (d).

System suitability: reference solution (d):

– *resolution*: minimum 2.0 between the peaks due to phosphate (1st peak) and phosphite;

– *signal-to-noise ratio*: minimum 10 for the principal peak.

Limits:

– *phosphate*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.3 per cent);

– *phosphite*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.3 per cent).

Loss on drying (*2.2.32*): 35.0 per cent to 37.0 per cent, determined on 1.000 g by drying in an oven at 150 °C.

Bacterial endotoxins (*2.6.14*): less than 83.3 IU/g, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Dissolve 0.200 g in 50 mL of *water R*. Titrate with *0.05 M sulfuric acid*, determining the end-point potentiometrically (*2.2.20*) at the 1st point of inflexion.

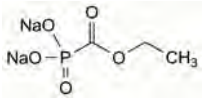
1 mL of *0.05 M sulfuric acid* is equivalent to 19.20 mg of CNa_3O_5P.

STORAGE

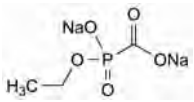
Protected from light.

IMPURITIES

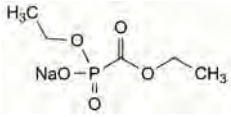
Specified impurities: A, B, C, D.



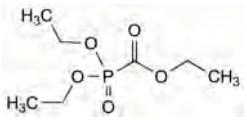
A. disodium (ethoxycarbonyl)phosphonate,



B. disodium (ethoxyoxydophosphanyl)formate,



C. ethyl sodium (ethoxycarbonyl)phosphonate,

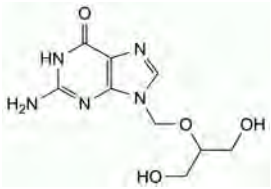


D. ethyl (diethoxyphosphoryl)formate.

01/2017:1752

GANCICLOVIR

Ganciclovirum



$C_9H_{13}N_5O_4$ M_r 255.2
[82410-32-0]

DEFINITION

2-Amino-9-[[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl]-1,9-dihydro-6*H*-purin-6-one.

Content: 99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white, hygroscopic, crystalline powder.

Solubility: slightly soluble in water, very slightly soluble in ethanol (96 per cent). It dissolves in dilute solutions of mineral acids and alkali hydroxides.

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *ganciclovir CRS*.

If the spectra obtained in the solid state show differences, dissolve 0.10 g of the substance to be examined and the reference substance separately in about 3.6 mL of *water R* at 80 °C. Allow to cool in an ice-bath and filter the precipitate. Dry in an oven at 105 °C for 3 h and record new spectra using the residues.

TESTS

Appearance of solution. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_5 (*2.2.2, Method II*).

Dissolve 1.25 g in a 40 g/L solution of *sodium hydroxide R* and dilute to 25 mL with the same solution.

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 30 mg of the substance to be examined in the mobile phase with the aid of ultrasound and dilute to 50.0 mL with the mobile phase.

Reference solution (a). Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b). Dissolve 3 mg of *ganciclovir CRS* in the mobile phase with the aid of ultrasound and dilute to 5.0 mL with the mobile phase.

Reference solution (c). Dissolve the contents of a vial of *ganciclovir impurity mixture CRS* (impurities A, B, C, D, E and F) in 1.0 mL of reference solution (b).

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *strong cation-exchange silica gel for chromatography R* (10 µm);
- *temperature*: 40 °C.

Mobile phase: mix equal volumes of *acetonitrile R* and a 0.05 per cent *V*/*V* solution of *trifluoroacetic acid R*.

Flow rate: 1.5 mL/min.

Detection: spectophotometer at 254 nm.

Injection: 20 µL.

Run time: 2.5 times the retention time of ganciclovir.

Identification of impurities: use the chromatogram supplied with *ganciclovir impurity mixture CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B, C, D, E and F.

Relative retention with reference to ganciclovir (retention time = about 14 min): impurity A = about 0.6; impurity B = about 0.67; impurity C = about 0.71; impurity D = about 0.8; impurity E = about 0.9; impurity F = about 2.0.

System suitability: reference solution (c):

- *peak-to-valley ratio*: minimum 5, where H_p = height above the baseline of the peak due to impurity E and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to ganciclovir.

Limits:

- *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 1.3; impurity F = 0.7;
- *impurity F*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent);
- *impurity B*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *impurities A, C, D, E*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent);
- *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent);
- *total*: not more than 6 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.6 per cent);
- *disregard limit*: 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.03 per cent).

Water (*2.5.12*): maximum 4.0 per cent, determined on 0.300 g.

Use *methanol R* as solvent. The substance to be examined has limited solubility in methanol. The sample will appear as a slurry. Replace the solvent after each titration.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

Bacterial endotoxins (*2.6.14*): less than 0.84 IU/mg, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Dissolve 0.200 g in 10 mL of *anhydrous formic acid R* and dilute to 60 mL with *glacial acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 25.52 mg of $C_9H_{13}N_5O_4$.

STORAGE

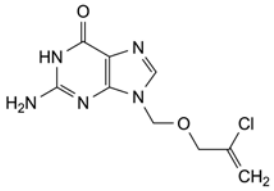
In an airtight container.

IMPURITIES

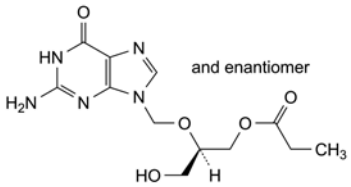
Specified impurities: *A, B, C, D, E, F.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use*

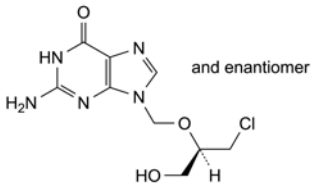
(2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *H, I, J.*



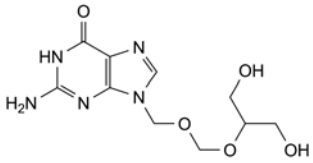
A. 2-amino-9-[[(2-chloroprop-2-en-1-yl)oxy]methyl]-1,9-dihydro-6*H*-purin-6-one,



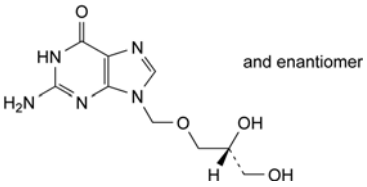
B. (2*RS*)-2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]-3-hydroxypropyl propionate,



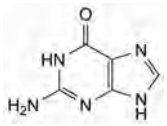
C. 2-amino-9-[[(1*RS*)-2-chloro-1-(hydroxymethyl)ethoxy]-methyl]-1,9-dihydro-6*H*-purin-6-one,



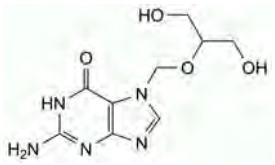
D. 2-amino-9-[[[2-hydroxy-1-(hydroxymethyl)ethoxy]-methoxy]methyl]-1,9-dihydro-6*H*-purin-6-one,



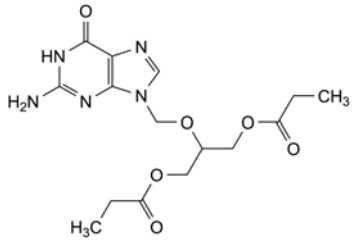
E. 2-amino-9-[[(2*RS*)-2,3-dihydroxypropoxy]methyl]-1,9-dihydro-6*H*-purin-6-one,



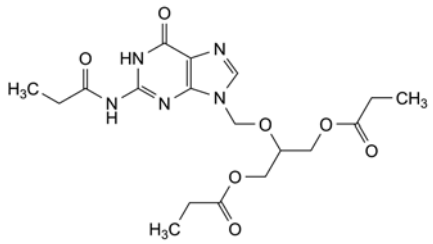
F. 2-amino-1,9-dihydro-6*H*-purin-6-one (guanine),



H. 2-amino-7-[[2-hydroxy-1-(hydroxymethyl)ethoxy]-methyl]-1,7-dihydro-6*H*-purin-6-one,



I. 2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]propane-1,3-diyl dipropanoate,

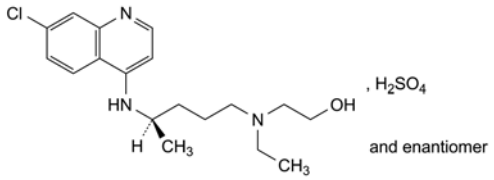


J. 2-[2-(propanoylamino)-6-oxo-1,6-dihydro-9*H*-purin-9-yl]methoxy]propane-1,3-diyl dipropanoate.

01/2017:2849
corrected 10.0

HYDROXYCHLOROQUINE SULFATE

Hydroxychloroquini sulfas



$C_{18}H_{28}ClN_3O_5S$ M_r 434.0
[747-36-4]

DEFINITION

2-[[(4*RS*)-4-[(7-Chloroquinolin-4-yl)amino]pentyl]-(ethyl)amino]ethan-1-ol sulfate.

Content: 98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance: white or slightly yellowish, crystalline powder.

Solubility: freely soluble in water, practically insoluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *hydroxychloroquine sulfate CRS*.

B. It gives reaction (a) of sulfates (*2.3.1*).

TESTS

Appearance of solution. The solution is clear and not more intensely coloured than reference solution Y_7 (*2.2.2, Method I*).

Dissolve 1.0 g in *water R* and dilute to 10.0 mL with the same solvent.

Related substances. Liquid chromatography (*2.2.29*).

Solvent mixture. Mix 500 mL of *water R*, 500 mL of *methanol R* and 4 mL of a 10 per cent *V/V* solution of *sulfuric acid R*.

Buffer solution. Dissolve 1.36 g of *potassium dihydrogen phosphate R* in 900 mL of *water for chromatography R*. Add 0.15 g of *sodium heptanesulfonate R*, adjust to pH 7.0 with *triethylamine R* and dilute to 1000 mL with *water for chromatography R*.

Test solution (a). Dissolve 25.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Test solution (b). Dilute 1.0 mL of test solution (a) to 10.0 mL with the solvent mixture.

Reference solution (a). Dissolve 5 mg of *hydroxychloroquine for system suitability CRS* (containing impurities B and C) in the solvent mixture and dilute to 10 mL with the solvent mixture.

Reference solution (b). Dilute 1.0 mL of test solution (a) to 10.0 mL with the solvent mixture. Dilute 2.0 mL of this solution to 100.0 mL with the solvent mixture.

Reference solution (c). Dissolve 25.0 mg of *hydroxychloroquine sulfate CRS* in the solvent mixture and dilute to 50.0 mL with the solvent mixture. Dilute 1.0 mL of the solution to 10.0 mL with the solvent mixture.

Column:

- *size*: *l* = 0.05 m, Ø = 2.1 mm;
- *stationary phase*: *end-capped ethylene-bridged octadecylsilyl silica gel for chromatography (hybrid material) R* (1.7 µm);
- *temperature*: 40 °C.

Mobile phase:

- *mobile phase A*: *methanol R*, buffer solution (10:90 *V/V*);
- *mobile phase B*: buffer solution, *methanol R* (15:85 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 1	100	0
1 - 11	100 → 0	0 → 100

Flow rate: 0.7 mL/min.

Detection: spectrophotometer at 254 nm.

Injection: 4 µL of test solution (a) and reference solutions (a) and (b).

Identification of impurities: use the chromatogram supplied with *hydroxychloroquine for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities B and C.

Relative retention with reference to hydroxychloroquine (retention time = about 6 min): impurity B = about 0.8; impurity C = about 0.9.

System suitability: reference solution (a):

- *resolution*: minimum 3.0 between the peaks due to impurity C and hydroxychloroquine; minimum 3.0 between the peaks due to impurities B and C.

Calculation of percentage contents:

- *correction factor*: multiply the peak area of impurity B by 1.6;
- for each impurity, use the concentration of hydroxychloroquine sulfate in reference solution (b).

Limits:

- *impurity C*: maximum 0.4 per cent;
- *impurity B*: maximum 0.15 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 0.6 per cent;
- *reporting threshold*: 0.05 per cent.

Loss on drying (*2.2.32*): maximum 2.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

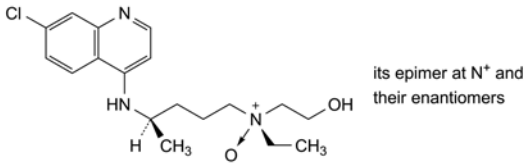
Injection: 4 µL of test solution (b) and reference solution (c).

Calculate the percentage content of $C_{18}H_{28}ClN_3O_5S$ taking into account the assigned content of *hydroxychloroquine sulfate CRS*.

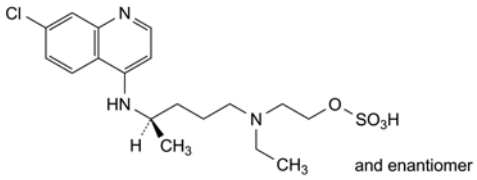
IMPURITIES

Specified impurities: B, C.

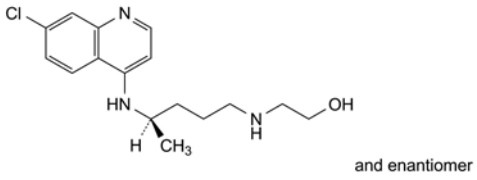
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, D, E, F, G.*



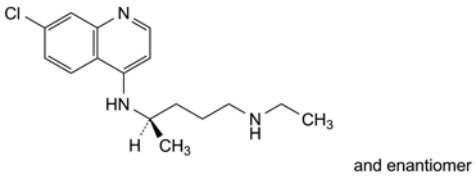
A. mixture of diastereoisomers of 4-[(7-chloroquinolin-4-yl)amino]-*N*-ethyl-*N*-(hydroxyethyl)pentan-1-amine *N*-oxide,



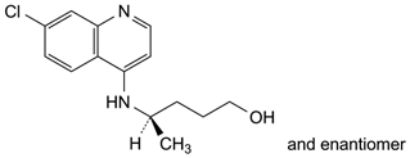
B. 2-[[(4*RS*)-4-[(7-chloroquinolin-4-yl)amino]pentyl]-(ethyl)amino]ethyl hydrogen sulfate,



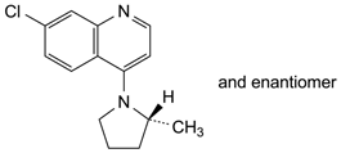
C. 2-[[(4*RS*)-4-[(7-chloroquinolin-4-yl)amino]pentyl]-amino]ethan-1-ol,



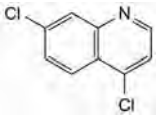
D. (4*RS*)-N^4-(7-chloroquinolin-4-yl)-N^1-ethylpentane-1,4-diamine,



E. (4*RS*)-4-[(7-chloroquinolin-4-yl)amino]pentan-1-ol,



F. 7-chloro-4-[(2*RS*)-2-methylpyrrolidin-1-yl]quinoline,

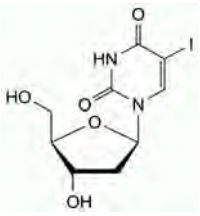


G. 4,7-dichloroquinoline.

01/2008:0669

IDOXURIDINE

Idoxuridinum



$C_9H_{11}IN_2O_5$ M_r 354.1
[54-42-2]

DEFINITION

Idoxuridine contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of 5-iodo-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrimidine-2,4(1*H*,3*H*)-dione, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, slightly soluble in water and in ethanol (96 per cent). It dissolves in dilute solutions of alkali hydroxides.

It melts at about 180 °C, with decomposition.

IDENTIFICATION

First identification: A.

Second identification: B, C, D.

A. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *idoxuridine CRS*. Examine the substances as discs prepared using 1 mg of the substance to be examined and of the reference substance each in 0.3 g of *potassium bromide R*.

B. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (c).

C. Heat about 5 mg in a test-tube over a naked flame. Violet vapour is evolved.

D. Disperse about 2 mg in 1 mL of *water R* and add 2 mL of *diphenylamine solution R2*. Heat in a water-bath for 10 min. A persistent light-blue colour develops.

TESTS

Solution S. Dissolve 0.500 g in *1 M sodium hydroxide* and dilute to 50.0 mL with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*). Dissolve 0.10 g in *carbon dioxide-free water R* and dilute to 100 mL with the same solvent. The pH of the solution is 5.5 to 6.5.

Specific optical rotation (*2.2.7*): + 28 to + 32, determined on solution S and calculated with reference to the dried substance.

Related substances. Examine by thin-layer chromatography (*2.2.27*), using as coating substance a suitable silica gel with a fluorescent indicator having an optimal intensity at 254 nm.

Test solution (a). Dissolve 0.20 g of the substance to be examined in a mixture of 1 volume of *concentrated ammonia R* and 5 volumes of *methanol R* and dilute to 5 mL with the same mixture of solvents.

Test solution (b). Dilute 1 mL of test solution (a) to 10 mL with a mixture of 1 volume of *concentrated ammonia R* and 5 volumes of *methanol R*.

Reference solution (a). Dissolve 20 mg of *5-iodouracil R*, 20 mg of *2′-deoxyuridine R* and 20 mg of *5-bromo-2′-deoxyuridine R* in a mixture of 1 volume of *concentrated ammonia R* and 5 volumes of *methanol R* and dilute to 100 mL with the same mixture of solvents.

Reference solution (b). Dissolve 0.20 g of the substance to be examined in 5 mL of reference solution (a).

Reference solution (c). Dissolve 20 mg of *idoxuridine CRS* in a mixture of 1 volume of *concentrated ammonia R* and 5 volumes of *methanol R* and dilute to 5 mL with the same mixture of solvents.

Reference solution (d). Dilute 1 mL of test solution (b) to 20 mL with a mixture of 1 volume of *concentrated ammonia R* and 5 volumes of *methanol R*.

Apply separately to the plate 5 µL of each solution. Develop twice over a path of 15 cm using a mixture of 10 volumes of *concentrated ammonia R*, 40 volumes of *chloroform R* and 50 volumes of *2-propanol R*, drying the plate in a current of cold air after each development. Examine in ultraviolet light at 254 nm. In the chromatogram obtained with test solution (a): any spots corresponding to 5-iodouracil, 2′-deoxyuridine and 5-bromo-2′-deoxyuridine are not more intense than the corresponding spots in the chromatogram obtained with reference solution (a) (0.5 per cent); any spot, apart from the principal spot and the spots corresponding to 5-iodouracil, 2′-deoxyuridine and 5-bromo-2′-deoxyuridine, is not more intense than the spot in the chromatogram obtained with reference solution (d) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (b) shows four clearly separated spots.

Iodide. Dissolve 0.25 g in 25 mL of *0.1 M sodium hydroxide*, add 5 mL of *dilute hydrochloric acid R* and dilute to 50 mL with *water R*. Allow to stand for 10 min and filter. To 25 mL of the filtrate add 5 mL of *dilute hydrogen peroxide solution R* and 10 mL of *chloroform R* and shake. Any pink colour in the organic layer is not more intense than that in a standard prepared at the same time in the same manner using 1 mL of a 0.33 g/L solution of *potassium iodide R* instead of the substance to be examined (0.1 per cent).

Loss on drying (*2.2.32*). Not more than 1.0 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C.

Sulfated ash (*2.4.14*). Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.3000 g in 20 mL of *dimethylformamide R*. Titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M tetrabutylammonium hydroxide* is equivalent to 35.41 mg of $C_9H_{11}IN_2O_5$.

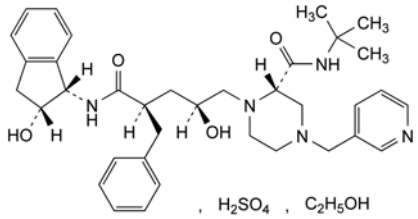
STORAGE

Store protected from light.

01/2017:2214

INDINAVIR SULFATE

Indinaviri sulfas



$C_{36}H_{49}N_5O_8S,C_2H_6O$ M_r 758
[157810-81-6]

DEFINITION

(2*S*)-1-[(2*S*,4*R*)-4-Benzyl-2-hydroxy-5-[[(1*S*,2*R*)-2-hydroxy-2,3-dihydro-1*H*-inden-1-yl]amino]-5-oxopentyl]-*N*-(1,1-dimethylethyl)-4-(pyridin-3-ylmethyl)piperazine-2-carboxamide sulfate ethanolate.

Content: 98.0 per cent to 102.0 per cent (anhydrous and ethanol-free substance).

PRODUCTION

A test for enantiomeric purity is carried out unless it has been demonstrated that the manufacturing process is enantioselective for the substance.

CHARACTERS

Appearance: white or almost white, hygroscopic powder.

Solubility: freely soluble in water, soluble in methanol, practically insoluble in heptane.

IDENTIFICATION

A. Specific optical rotation (*2.2.7*): + 122 to + 129 (anhydrous and ethanol-free substance), determined at 365 nm and at 25 °C.

Dissolve 0.500 g in *water R* and dilute to 50.0 mL with the same solvent.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *Ph. Eur. reference spectrum of indinavir sulfate*.

C. It gives reaction (a) of sulfates (*2.3.1*).

D. Ethanol (see Tests).

TESTS

Related substances. Liquid chromatography (*2.2.29*).

Solution A. Thoroughly mix equal volumes of mobile phase A and *acetonitrile R1*.

Test solution. Dissolve 50.0 mg of the substance to be examined in solution A and dilute to 100.0 mL with the same solution.

Reference solution (a). Dissolve 4 mg of *indinavir for system suitability CRS* (containing impurities B, C and E) in solution A and dilute to 10 mL with the same solution.

Reference solution (b). Dilute 1.0 mL of the test solution to 100.0 mL with solution A. Dilute 1.0 mL of this solution to 10.0 mL with solution A.

Reference solution (c). Dissolve 5.0 mg of *cis-aminoindanol R* (impurity A) in solution A and dilute to 10.0 mL with the same solution. Dilute 1.0 mL of the solution to 100.0 mL with solution A. Dilute 1.0 mL of this solution to 10.0 mL with solution A.

Reference solution (d). To 30 mg of the substance to be examined add 0.25 mL of *2 M hydrochloric acid R* and allow to stand at room temperature for 1 h. Dilute to 100 mL with a mixture of 2 volumes of *acetonitrile R1* and 3 volumes of mobile phase A and mix (*in situ* degradation to obtain impurity D).

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

- *mobile phase A*: solution containing 0.27 g/L of *potassium dihydrogen phosphate R* and 1.40 g/L of *dipotassium hydrogen phosphate R*; filter and degas;
- *mobile phase B*: *acetonitrile R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	80	20
5 - 40	80 → 30	20 → 70
40 - 45	30	70
45 - 47	30 → 80	70 → 20
47 - 52	80	20

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 220 nm.

Injection: 20 µL.

Identification of impurities: use the chromatogram supplied with *indinavir for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities B, C and E; use the chromatogram obtained with reference solution (d) to identify the peak due to impurity D.

Relative retention with reference to indinavir (retention time = about 25 min): impurity A = about 0.2; impurity B = about 0.8; impurity C = about 0.98; impurity D = about 1.1; impurity E = about 1.3.

System suitability: reference solution (a):

- *resolution*: minimum 1.8 between the peaks due to impurity C and indinavir.

Limits:

- *correction factor*: for the calculation of content, multiply the peak area of impurity D by 1.8;
- *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent);
- *impurity D*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
- *impurities B, C, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
- *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent);
- *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *disregard limit*: 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.03 per cent).

Ethanol. Gas chromatography (*2.2.28*).

Internal standard solution. Dilute 1.0 mL of *propanol R* to 200.0 mL with *water R*.

Test solution. Dissolve 0.400 g of the substance to be examined in 50.0 mL of *water R*, add 8.0 mL of the internal standard solution and dilute to 100.0 mL with *water R*.

Reference solution. Dilute 1.0 mL of *anhydrous ethanol R* to 200.0 mL. Dilute 2.0 mL of this solution and 2.0 mL of the internal standard solution to 25.0 mL with *water R*.

Column:

– *material*: fused silica;

– *size*: *l* = 30 m, Ø = 0.53 mm;

– *stationary phase*: *macrogol 20 000 R* (film thickness 1.0 µm).

Carrier gas: *helium for chromatography R*.

Flow rate: 10 mL/min.

Split ratio: 1:10.

Temperature:

– *column*: 35 °C;

– *injection port*: 140 °C;

– *detector*: 220 °C.

Detection: flame ionisation.

Injection: 1.0 µL.

System suitability: reference solution:

– *retention time*: ethanol = 2 min to 4 min;

– *resolution*: minimum 5.0 between the peaks due to ethanol and propanol.

Calculate the percentage content of ethanol taking the density (*2.2.5*) to be 0.790 g/mL.

Limit:

– *ethanol*: 5.0 per cent to 8.0 per cent *m/m*.

Water (*2.5.12*): maximum 1.5 per cent, determined on 0.500 g.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*).

Solution B. Add 20 mL of *dibutylammonium phosphate for ion-pairing R* to 1000 mL of *water R*. Adjust to pH 6.5 with *1 M sodium hydroxide*.

Test solution. Dissolve 60.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution. Dissolve 50.0 mg of *indinavir CRS* in the mobile phase and dilute to 100.0 mL with the mobile phase.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm;

– *stationary phase*: *base-deactivated octylsilyl silica gel for chromatography R* (5 µm);

– *temperature*: 40 °C.

Mobile phase: *acetonitrile R*, solution B (45:55 *V/V*).

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 260 nm.

Injection: 10 µL.

Run time: twice the retention time of indinavir.

Retention time: indinavir = about 10 min.

Calculate the percentage content of $C_{36}H_{49}N_5O_8S$ using the declared content of *indinavir CRS* and multiplying by a correction factor of 1.1598.

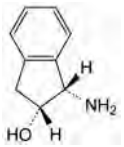
STORAGE

In an airtight container, protected from light.

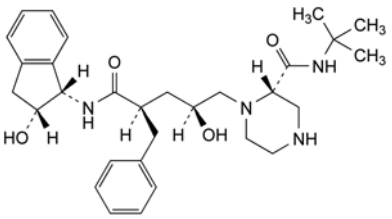
IMPURITIES

Specified impurities: A, B, C, D, E.

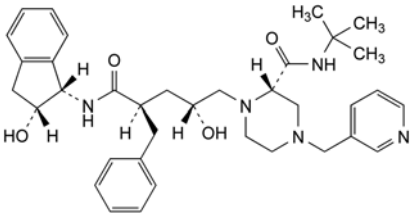
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *F.*



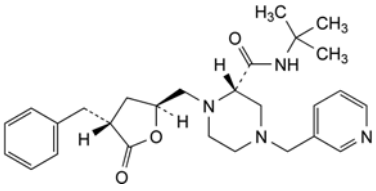
A. (1*S*,2*R*)-1-amino-2,3-dihydro-1*H*-inden-2-ol (*cis*-aminoindanol),



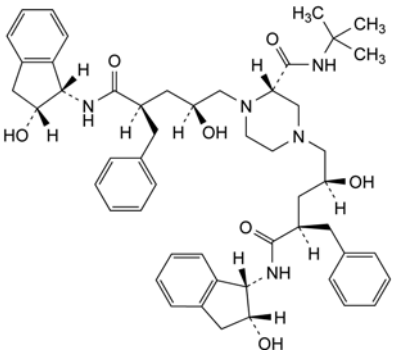
B. (2*S*)-1-[(2*S*,4*R*)-4-benzyl-2-hydroxy-5-[[(1*S*,2*R*)-2-hydroxy-2,3-dihydro-1*H*-inden-1-yl]amino]-5-oxopentyl]-*N*-(1,1-dimethylethyl)piperazine-2-carboxamide,



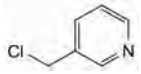
C. (2*S*)-1-[(2*R*,4*R*)-4-benzyl-2-hydroxy-5-[[(1*S*,2*R*)-2-hydroxy-2,3-dihydro-1*H*-inden-1-yl]amino]-5-oxopentyl]-*N*-(1,1-dimethylethyl)-4-(pyridin-3-ylmethyl)piperazine-2-carboxamide,



D. (3*R*,5*S*)-3-benzyl-5-[[(2*S*)-2-[(1,1-dimethylethyl)-carbamoyl]-4-(pyridin-3-ylmethyl)piperazin-1-yl]methyl]-4,5-dihydrofuran-2(3*H*)-one,



E. (2*S*)-1,4-bis[(2*S*,4*R*)-4-benzyl-2-hydroxy-5-[[(1*S*,2*R*)-2-hydroxy-2,3-dihydro-1*H*-inden-1-yl]amino]-5-oxopentyl]-*N*-(1,1-dimethylethyl)piperazine-2-carboxamide,

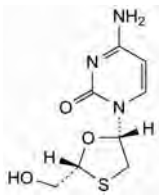


F. 3-(chloromethyl)pyridine (nicotinyl chloride).

01/2017:2217

LAMIVUDINE

Lamivudinum



$C_8H_{11}N_3O_3S$ M_r 229.3
[134678-17-4]

DEFINITION

4-Amino-1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2(1*H*)-one.

Content: 97.5 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white powder.

Solubility: soluble in water, sparingly soluble in methanol, slightly soluble in ethanol (96 per cent).

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification: *B, C.*

Second identification: *A, B.*

A. Specific optical rotation (*2.2.7*): – 99 to – 97 (dried substance).

Dissolve 0.250 g in *water R* and dilute to 50.0 mL with the same solvent.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *lamivudine CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

C. Enantiomeric purity (see Tests).

TESTS

Absorbance (*2.2.25*): maximum 0.3 at 440 nm, using a path length of 4 cm.

Dissolve 1.00 g in *water R*, using sonication if necessary, and dilute to 20.0 mL with the same solvent.

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (a). Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b). Dissolve 5 mg of *salicylic acid R* in the mobile phase and dilute to 100.0 mL with the mobile phase. Dilute 1.0 mL of the solution to 100.0 mL with the mobile phase.

Reference solution (c). Dissolve 50.0 mg of *lamivudine CRS* in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (d). Dissolve 5 mg of *cytosine R* and 5 mg of *uracil R* in the mobile phase and dilute to 100.0 mL with the mobile phase. Dilute 2.0 mL of the solution to 10.0 mL with the mobile phase.

Reference solution (e). Dissolve 5 mg of *lamivudine for system suitability 1 CRS* (containing impurities A and B) in 2 mL of the mobile phase. Add 1.0 mL of reference solution (d) and dilute to 10.0 mL with the mobile phase.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 35 °C.

Mobile phase: mix 5 volumes of *methanol R* and 95 volumes of a 1.9 g/L solution of *ammonium acetate R*, previously adjusted to pH 3.8 with *glacial acetic acid R*.

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 277 nm.

Injection: 10 µL.

Run time: 3 times the retention time of lamivudine.

Identification of impurities: use the chromatograms obtained with reference solutions (b) and (e) to identify the peaks due to impurities A, B, E, F and C.

Relative retention with reference to lamivudine (retention time = about 9 min): impurity E = about 0.28; impurity F = about 0.32; impurity A = about 0.36; impurity B = about 0.91; impurity J = about 1.45; impurity C = about 2.32.

System suitability: reference solution (e):
- *resolution*: minimum 1.5 between the peaks due to impurities F and A; minimum 1.5 between the peaks due to impurity B and lamivudine.

Limits:
- *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity E = 0.6; impurity F = 2.2; impurity J = 2.2;
- *impurity A*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
- *impurity B*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *impurity C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
- *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- *total*: not more than 6 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.6 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Enantiomeric purity. Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution. Dissolve 25.0 mg of the substance to be examined in *water R* and dilute to 100.0 mL with the same solvent.

Reference solution. Dissolve the contents of a vial of *lamivudine for system suitability 2 CRS* (containing impurity D) in 1.0 mL of *water R*.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *silica gel BC for chiral chromatography R* (5 µm);

– *temperature*: maintain at constant temperature between 15 °C and 30 °C; the temperature may be adjusted to optimise the resolution between lamivudine and impurity D; a lower temperature favours improved resolution.

Mobile phase: mix 5 volumes of *methanol R* and 95 volumes of a 7.7 g/L solution of *ammonium acetate R*.

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 270 nm.

Injection: 10 µL.

Run time: twice the retention time of lamivudine.

Relative retention with reference to lamivudine (retention time = about 8 min): impurity D = about 1.2; impurity B and enantiomer = about 1.3 and 1.5.

System suitability: reference solution:

– *peak-to-valley-ratio*: minimum 15, where H_p = height above the baseline of the peak due to impurity D and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to lamivudine.

Calculate the sum of the percentage contents of all impurity peaks with a relative retention from 1.2 to 1.5. Subtract the percentage content of impurity B as obtained in the test for related substances.

Limit:

– *impurity D*: maximum 0.3 per cent.

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection: test solution and reference solution (c).

Calculate the percentage content of $C_8H_{11}N_3O_3S$ using the chromatograms obtained with the test solution and reference solution (c) and the declared content of $C_8H_{11}N_3O_3S$ in *lamivudine CRS*.

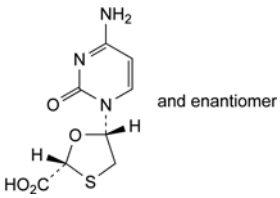
STORAGE

Protected from light.

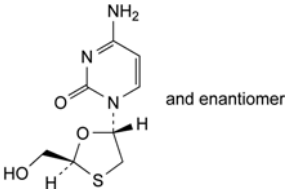
IMPURITIES

Specified impurities: *A, B, C, D.*

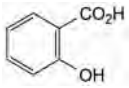
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *E, F, G, H, I, J.*



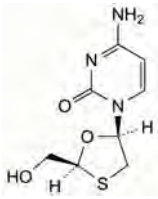
A. (2*RS*,5*SR*)-5-(4-amino-2-oxopyrimidin-1(2*H*)-yl)-1,3-oxathiolane-2-carboxylic acid,



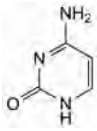
B. 4-amino-1-[(2*RS*,5*RS*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2(1*H*)-one ((±)-*trans*-lamivudine),



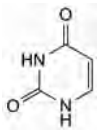
C. 2-hydroxybenzenecarboxylic acid (salicylic acid),



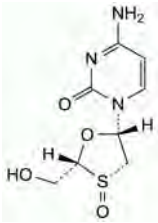
D. 4-amino-1-[(2*S*,5*R*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2(1*H*)-one,



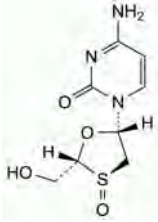
E. 4-aminopyrimidin-2(1*H*)-one (cytosine),



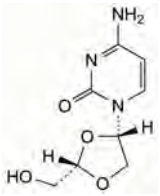
F. pyrimidine-2,4(1*H*,3*H*)-dione (uracil),



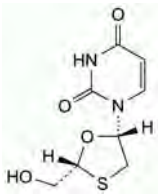
G. 4-amino-1-[(2*R*,3*S*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2(1*H*)-one *S*-oxide,



H. 4-amino-1-[(2*R*,3*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2(1*H*)-one *S*-oxide,



I. 4-amino-1-[(2*S*,4*S*)-2-(hydroxymethyl)-1,3-dioxolan-4-yl]pyrimidin-2(1*H*)-one,

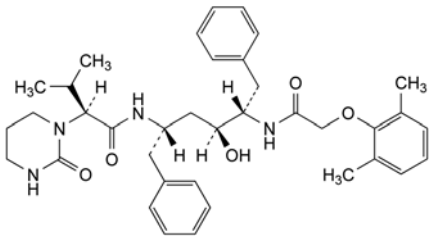


J. 1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidine-2,4(1*H*,3*H*)-dione.

01/2017:2615

LOPINAVIR

Lopinavirum



$C_{37}H_{48}N_4O_5$ M_r 629
[192725-17-0]

DEFINITION

(2*S*)-*N*-[(1*S*,3*S*,4*S*)-1-Benzyl-4-[[2-(2,6-dimethylphenoxy)-acetyl]amino]-3-hydroxy-5-phenylpentyl]-3-methyl-2-[2-oxo-tetrahydropyrimidin-1(2*H*)-yl]butanamide.

Content: 98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or yellowish-white, slightly hygroscopic powder.

Solubility: practically insoluble in water, very soluble in methanol and in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *lopinavir CRS*.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

TESTS

Specific optical rotation (*2.2.7*): − 27.0 to − 22.0 (anhydrous substance).

Dissolve 0.200 g in *methanol R* and dilute to 25.0 mL with the same solvent.

Related substances

A. Liquid chromatography (*2.2.29*).

Solvent mixture: *acetonitrile R1*, *water R* (50:50 *V/V*).

Phosphate buffer solution. Dissolve 0.9 g of *dipotassium hydrogen phosphate R* and 2.7 g of *potassium dihydrogen phosphate R* in 900 mL of *water R* and mix well. Adjust to pH 6.0 with *phosphoric acid R*, dilute to 1000 mL with *water R* and filter.

Test solution (a). Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 100.0 mL with the solvent mixture.

Test solution (b). Dilute 5.0 mL of test solution (a) to 100.0 mL with the solvent mixture.

Reference solution (a). Dissolve 50.0 mg of *lopinavir CRS* in the solvent mixture and dilute to 100.0 mL with the solvent mixture. Dilute 5.0 mL of the solution to 100.0 mL with the solvent mixture.

Reference solution (b). Dilute 5.0 mL of test solution (b) to 250.0 mL with the solvent mixture.

Reference solution (c). Dissolve 2.5 mg of *lopinavir for system suitability CRS* (containing impurities A, B, C, F, G, I, N, Q, R, S and T) in the solvent mixture and dilute to 5.0 mL with the solvent mixture.

Reference solution (d). Dissolve 2.5 mg of *lopinavir for peak identification CRS* (containing impurities D and O) in the solvent mixture and dilute to 5.0 mL with the solvent mixture.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (4 µm);
- *temperature*: 50 °C.

Mobile phase:

- *mobile phase A*: *acetonitrile R1*, phosphate buffer solution (45:55 *V/V*);
- *mobile phase B*: phosphate buffer solution, *acetonitrile R1* (25:75 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 60	100	0
60 - 61	100 → 0	0 → 100
61 - 81	0	100

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 215 nm.

Injection: 20 µL of test solution (a) and reference solutions (b), (c) and (d).

Identification of impurities: use the chromatogram supplied with *lopinavir for system suitability CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B, C, F, G, I and N; use the chromatogram supplied with *lopinavir for peak identification CRS* and the chromatogram obtained with reference solution (d) to identify the peak due to impurity D.

Relative retention r (not r_G) with reference to lopinavir (retention time = about 37 min): impurity A = about 0.03; impurity B = about 0.07; impurity C = about 0.10; impurity D = about 0.13; impurity F = about 0.59; impurity G = about 0.62; impurity I = about 1.1; impurity N = about 1.4.

System suitability: reference solution (c):

- *resolution*: minimum 1.5 between the peaks due to impurities F and G.

Calculation of percentage contents:

- for impurity A, multiply the peak area by the correction factor 1.6;
- for impurity B, multiply the peak area by the correction factor 1.3;
- for impurity C, multiply the peak area by the correction factor 1.5;
- for impurity D, multiply the peak area by the correction factor 1.3;
- for each impurity, use the concentration of lopinavir in reference solution (b).

Limits:

- *impurities B, I*: for each impurity, maximum 0.2 per cent;
- *impurities A, C, D, F, G*: for each impurity, maximum 0.15 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *reporting threshold*: 0.05 per cent; disregard any peak eluting after impurity N.

B. Liquid chromatography (*2.2.29*) as described in test A for related substances with the following modifications.

Mobile phase: mobile phase A, mobile phase B (30:70 *V/V*).

Run time: 8.3 times the retention time of lopinavir.

Identification of impurities: use the chromatogram supplied with *lopinavir for system suitability CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities Q, R, S and T; use the chromatogram supplied with *lopinavir for peak identification CRS* and the chromatogram obtained with reference solution (d) to identify the peak due to impurity O.

Relative retention r (not r_G) with reference to lopinavir (retention time = about 6 min): impurity N = about 1.4; impurity O = about 1.5; impurity Q = about 4.4; impurity R = about 6.0; impurity S = about 7.1; impurity T = about 8.5.

System suitability: reference solution (c):

– *resolution*: minimum 3.0 between the peaks due to impurities S and T.

Calculation of percentage contents:

– for impurity O, multiply the peak area by the correction factor 1.3;

– for impurity Q, multiply the peak area by the correction factor 0.7;

– for each impurity, use the concentration of lopinavir in reference solution (b).

Limits:

– *impurities O, Q, R, T*: for each impurity, maximum 0.15 per cent;

– *unspecified impurities*: for each impurity, maximum 0.10 per cent;

– *reporting threshold*: 0.05 per cent; disregard any peak eluting before and including impurity N;

– *total of all impurities eluting before and including impurity N in test A and after impurity N in test B*: maximum 0.7 per cent.

Water (*2.5.12*): maximum 4.4 per cent, determined on 0.250 g.

Sulfated ash (*2.4.14*): maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in test A for related substances with the following modifications.

Mobile phase: mobile phase A.

Injection: test solution (b) and reference solution (a).

Run time: 1.6 times the retention time of lopinavir.

Calculate the percentage content of $C_{37}H_{48}N_4O_5$ taking into account the assigned content of *lopinavir CRS*.

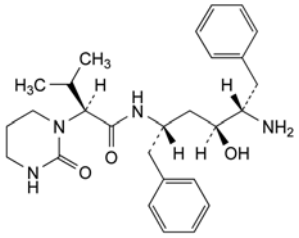
STORAGE

In an airtight container.

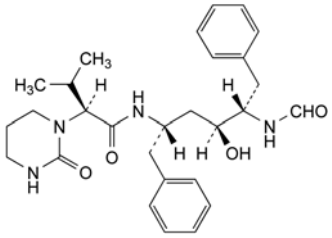
IMPURITIES

Specified impurities: A, B, C, D, F, G, I, O, Q, R, T.

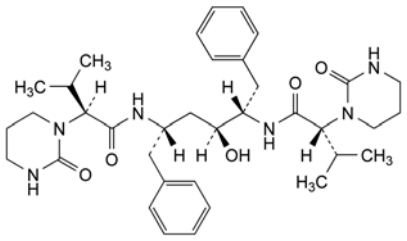
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *E, H, J, K, L, M, N, P, S.*



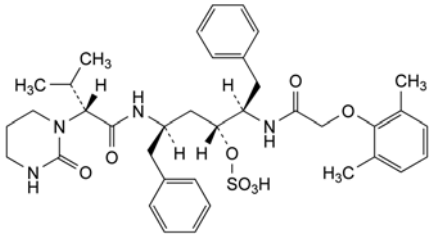
A. (2*S*)-*N*-[(1*S*,3*S*,4*S*)-1-benzyl-4-amino-3-hydroxy-5-phenylpentyl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide,



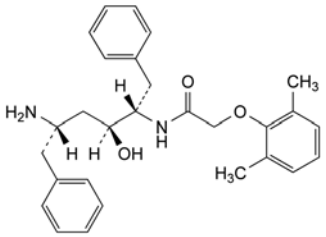
B. (2*S*)-*N*-[(1*S*,3*S*,4*S*)-1-benzyl-4-(formylamino)-3-hydroxy-5-phenylpentyl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide,



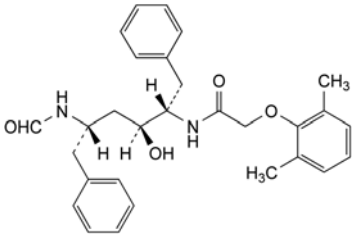
C. (2*R*)-*N*-[(1*S*,2*S*,4*S*)-1-benzyl-2-hydroxy-4-[[(2*S*)-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanoyl]amino]-5-phenylpentyl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide,



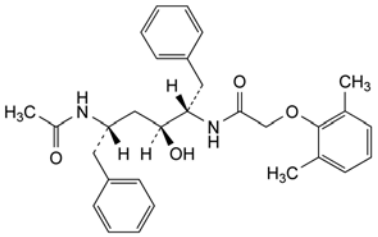
D. (1*R*,3*R*)-1-[(1*R*)-1-[[2-(2,6-dimethylphenoxy)acetyl]-amino]-2-phenylethyl]-3-[[(2*R*)-3-methyl-2-[2-oxo-tetrahydropyrimidin-1(2*H*)-yl]butanoyl]amino]-4-phenylbutyl hydrogen sulfate,



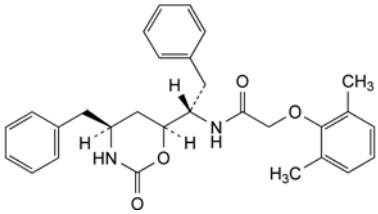
E. *N*-[(1*S*,2*S*,4*S*)-4-amino-1-benzyl-2-hydroxy-5-phenylpentyl]-2-(2,6-dimethylphenoxy)acetamide,



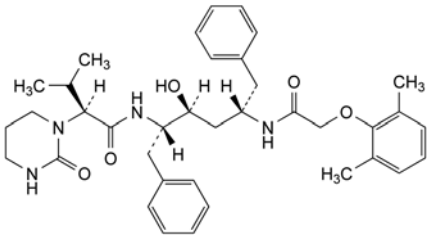
F. *N*-[(1*S*,2*S*,4*S*)-1-benzyl-4-(formylamino)-2-hydroxy-5-phenylpentyl]-2-(2,6-dimethylphenoxy)acetamide,



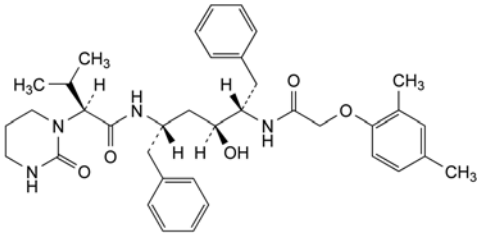
G. *N*-[(1*S*,2*S*,4*S*)-(4-acetylamino)-1-benzyl-2-hydroxy-5-phenylpentyl]-2-(2,6-dimethylphenoxy)acetamide,



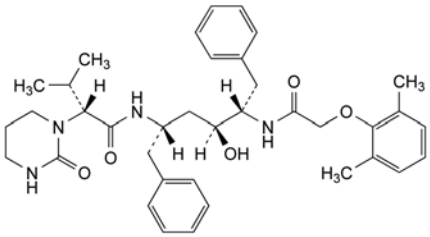
H. *N*-[(1*S*)-1-[(4*S*,6*S*)-4-benzyl-2-oxo-1,3-oxazinan-6-yl]-2-phenylethyl]-2-(2,6-dimethylphenoxy)acetamide,



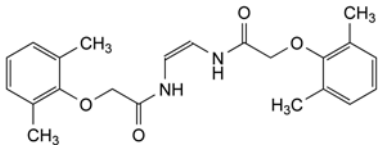
I. (2*S*)-*N*-[(1*S*,2*S*,4*S*)-1-benzyl-4-[[2-(2,6-dimethylphenoxy)-acetyl]amino]-2-hydroxy-5-phenylpentyl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide,



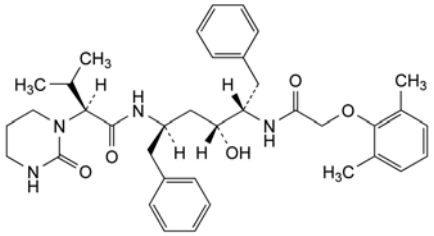
J. (2*S*)-*N*-[(1*S*,3*S*,4*S*)-1-benzyl-4-[[2-(2,4-dimethylphenoxy)-acetyl]amino]-3-hydroxy-5-phenylpentyl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide,



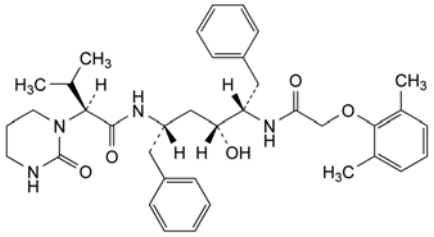
K. (2*R*)-*N*-[(1*S*,3*S*,4*S*)-1-benzyl-4-[[2-(2,6-dimethylphenoxy)-acetyl]amino]-3-hydroxy-5-phenylpentyl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide,



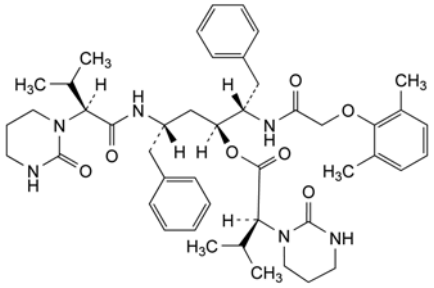
L. *N*,*N*′-(*Z*)-ethene-1,2-diylbis[2-(2,6-dimethylphenoxy)-acetamide],



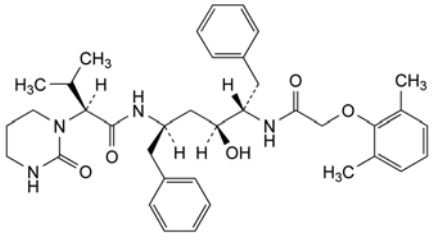
M. (2*S*)-*N*-[(1*R*,3*R*,4*S*)-1-benzyl-4-[[2-(2,6-dimethyl-phenoxy)acetyl]amino]-3-hydroxy-5-phenylpentyl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]-butanamide,



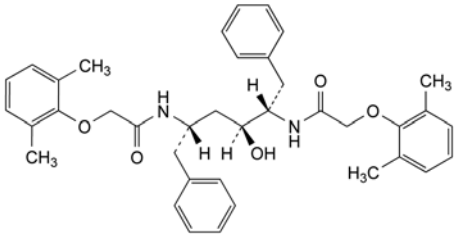
N. (2*S*)-*N*-[(1*S*,3*R*,4*S*)-1-benzyl-4-[[2-(2,6-dimethylphenoxy)-acetyl]amino]-3-hydroxy-5-phenylpentyl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide,



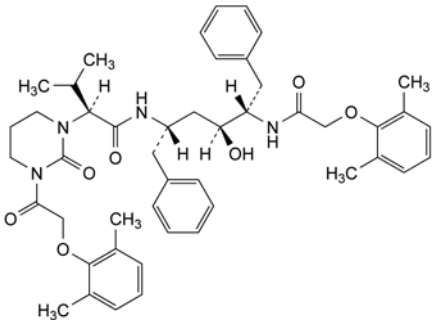
O. (1*S*,3*S*)-1-[(1*S*)-1-[[2-(2,6-dimethylphenoxy)acetyl]-amino]-2-phenylethyl]-3-[[(2*S*)-3-methyl-2-[2-oxo-tetrahydropyrimidin-1(2*H*)-yl]butanoyl]amino]-4-phenylbutyl (2*S*)-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanoate,



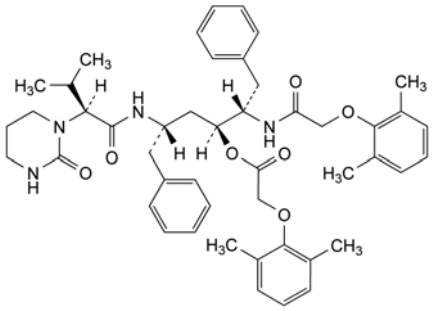
P. (2*S*)-*N*-[(1*R*,3*S*,4*S*)-1-benzyl-4-[[2-(2,6-dimethylphenoxy)-acetyl]amino]-3-hydroxy-5-phenylpentyl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide,



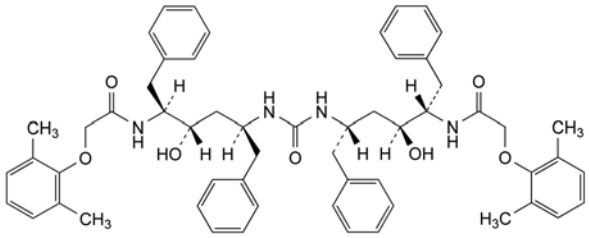
Q. *N*-[(1*S*,2*S*,4*S*)-1-benzyl-4-[[2-(2,6-dimethylphenoxy)-acetyl]amino]-2-hydroxy-5-phenylpentyl]-2-(2,6-dimethylphenoxy)acetamide,



R. (2*S*)-*N*-[(1*S*,3*S*,4*S*)-1-benzyl-4-[[2-(2,6-dimethylphenoxy)-acetyl]amino]-3-hydroxy-5-phenylpentyl]-2-[3-[2-(2,6-dimethylphenoxy)acetyl]-2-oxotetrahydropyrimidin-1(2*H*)-yl]-3-methylbutanamide,



S. (1*S*,3*S*)-1-[(1*S*)-1-[[2-(2,6-dimethylphenoxy)acetyl]-amino]-2-phenylethyl]-3-[[(2*S*)-3-methyl-2-[2-oxo-tetrahydropyrimidin-1(2*H*)-yl]butanoyl]amino]-4-phenylbutyl 2-(2,6-dimethylphenoxy)acetate,

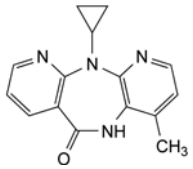


T. *N*,*N*′-bis[(1*S*,3*S*,4*S*)-1-benzyl-4-[[2-(2,6-dimethyl-phenoxy)acetyl]amino]-3-hydroxy-5-phenylpentyl]urea.

01/2017:2255
corrected 9.3

NEVIRAPINE

Nevirapinum



$C_{15}H_{14}N_4O$ M_r 266.3
[129618-40-2]

DEFINITION

11-Cyclopropyl-4-methyl-5,11-dihydro-6*H*-dipyrido [3,2-*b*:2′,3′-*e*][1,4]diazepin-6-one.

Content: 97.5 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white powder.

Solubility: practically insoluble in water, sparingly soluble or slightly soluble in methylene chloride, slightly soluble in methanol.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *anhydrous nevirapine CRS*.

B. Loss on drying (see Tests).

TESTS

Related substances. Liquid chromatography (*2.2.29*).

Test solution (a). Dissolve 24.0 mg of the substance to be examined in a mixture of 4 mL of *acetonitrile R* and 80 mL of the mobile phase and sonicate until dissolution is complete. Dilute to 100.0 mL with the mobile phase.

Test solution (b). Dilute 3.0 mL of test solution (a) to 25.0 mL with the mobile phase.

Reference solution (a). Dilute 1.0 mL of test solution (a) to 100.0 mL with the mobile phase. Dilute 5.0 mL of this solution to 50.0 mL with the mobile phase.

Reference solution (b). Add 2.0 mL of the mobile phase to a vial of *nevirapine for peak identification CRS* (containing impurities A, B and C), mix and sonicate for 1 min.

Reference solution (c). Dissolve 24.0 mg of *anhydrous nevirapine CRS* in a mixture of 4 mL of *acetonitrile R* and 80 mL of the mobile phase and sonicate until complete dissolution. Dilute to 100.0 mL with the mobile phase. Dilute 3.0 mL of this solution to 25.0 mL with the mobile phase.

Column:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped amidohexadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 35 °C.

Mobile phase: mix 20 volumes of *acetonitrile R* and 80 volumes of a 2.88 g/L solution of *ammonium dihydrogen phosphate R*, previously adjusted to pH 5.0 using *dilute sodium hydroxide solution R*.

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 220 nm.

Injection: 50 µL of test solution (a) and reference solutions (a) and (b).

Run time: 10 times the retention time of nevirapine.

Identification of impurities: use the chromatogram supplied with *nevirapine for peak identification CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B and C.

Relative retention with reference to nevirapine (retention time = about 8 min): impurity B = 0.7; impurity A = 1.5; impurity C = 2.8.

System suitability: reference solution (b):
- *resolution*: minimum 5 between the peaks due to impurity B and nevirapine.

Limits:
- *impurities A, B, C*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- *total*: not more than 6 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.6 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

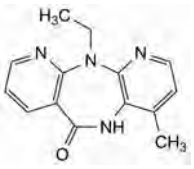
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection: 25 µL of test solution (b) and reference solution (c).

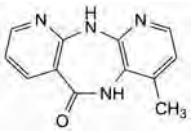
Calculate the percentage content of $C_{15}H_{14}N_4O$ from the declared content of *anhydrous nevirapine CRS*.

IMPURITIES

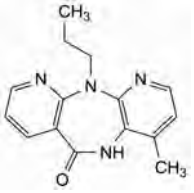
Specified impurities: A, B, C.



A. 11-ethyl-4-methyl-5,11-dihydro-6*H*-dipyrido-[3,2-b:2′,3′-*e*][1,4]diazepin-6-one,



B. 4-methyl-5,11-dihydro-6*H*-dipyrido[3,2-b:2′,3′-*e*]-[1,4]diazepin-6-one,

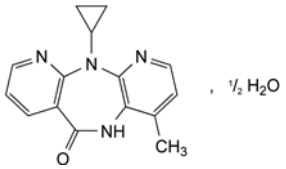


C. 4-methyl-11-propyl-5,11-dihydro-6*H*-dipyrido[3,2-b:2′,3′-*e*][1,4]diazepin-6-one.

01/2017:2479
corrected 10.0

NEVIRAPINE HEMIHYDRATE

Nevirapinum hemihydricum



$C_{15}H_{14}N_4O,½H_2O$ M_r 275.3
[220988-26-1]

DEFINITION

11-Cyclopropyl-4-methyl-5,11-dihydro-6*H*-dipyrido[3,2-*b*:2′,3′-*e*][1,4]diazepin-6-one hemihydrate.

Content: 97.5 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white powder.

Solubility: practically insoluble in water, slightly soluble in methanol and in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *nevirapine hemihydrate CRS*.

B. Water (see Tests).

TESTS

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 20.0 mg of the substance to be examined in *methanol R* and sonicate until dissolution is complete. Dilute to 50.0 mL with *methanol R*.

Reference solution (a). Dilute 1.0 mL of the test solution to 100.0 mL with *methanol R*. Dilute 1.0 mL of this solution to 10.0 mL with *methanol R*.

Reference solution (b). Add 1 mL of *methanol R* to a vial of *nevirapine for peak identification CRS* (containing impurities A, B and C), mix and sonicate for 1 min.

Reference solution (c). Dissolve 20.0 mg of *anhydrous nevirapine CRS* in *methanol R* and sonicate until dissolution is complete. Dilute to 50.0 mL with *methanol R*.

Column:
- *size*: *l* = 50 mm, Ø = 2.1 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (1.8 µm);
- *temperature*: 40 °C.

Mobile phase:
- *mobile phase A*: dissolve 0.77 g of *ammonium acetate R* in 900 mL of *water for chromatography R*, adjust to pH 5.6 with *acetic acid R* and dilute to 1000 mL with *water for chromatography R*;
- *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 – 1.35	90	10
1.35 – 3.85	90 → 67	10 → 33
3.85 – 6.70	67 → 60	33 → 40
6.70 – 7.65	60	40

Flow rate: 0.7 mL/min.

Detection: spectrophotometer at 282 nm.

Injection: 2.0 µL of the test solution and reference solutions (a) and (b).

Identification of impurities: use the chromatogram supplied with *nevirapine for peak identification CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B and C.

Relative retention with reference to nevirapine (retention time = about 3 min): impurity B = about 0.9; impurity A = about 1.2; impurity C = about 1.3.

System suitability:
- *resolution*: minimum 5.0 between the peaks due to impurity B and nevirapine and minimum 5.0 between the peaks due to nevirapine and impurity A in the chromatogram obtained with reference solution (b);
- *symmetry factor*: maximum 1.7 for the peak due to nevirapine in the chromatogram obtained with reference solution (a).

Calculation of percentage contents:
- for each impurity, use the concentration of nevirapine hemihydrate in reference solution (a).

Limits:
- *impurities A, B, C*: for each impurity, maximum 0.2 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 0.6 per cent;
- *reporting threshold*: 0.05 per cent.

Water (*2.5.12*): 3.1 per cent to 3.9 per cent, determined on 0.300 g.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

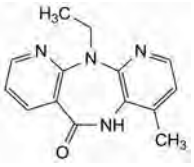
Injection: 2.0 µL of the test solution and reference solution (c).

Calculate the percentage content of $C_{15}H_{14}N_4O$ taking into account the assigned content of *anhydrous nevirapine CRS*.

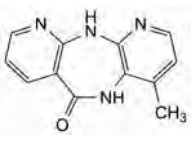
IMPURITIES

Specified impurities: A, B, C.

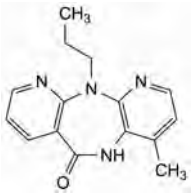
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *D.*



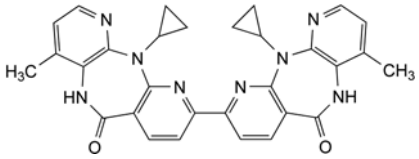
A. 11-ethyl-4-methyl-5,11-dihydro-6*H*-dipyrido[3,2-*b*:2′,3′-*e*][1,4]diazepin-6-one,



B. 4-methyl-5,11-dihydro-6*H*-dipyrido[3,2-*b*:2′,3′-*e*][1,4]diazepin-6-one,



C. 4-methyl-11-propyl-5,11-dihydro-6*H*-dipyrido[3,2-*b*:2′,3′-*e*][1,4]diazepin-6-one,

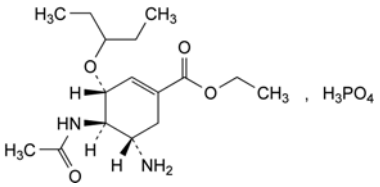


D. 11,11′-dicyclopropyl-4,4′-dimethyl-5,5′,11,11′-tetrahydro-6*H*,6′*H*-9,9′-bidipyrido[3,2-*b*:2′,3′-*e*][1,4]diazepine-6,6′-dione.

04/2011:2422
corrected 10.0

OSELTAMIVIR PHOSPHATE

Oseltamiviri phosphas



$C_{16}H_{31}N_2O_8P$ M_r 410.4
[204255-11-8]

DEFINITION

Ethyl (3*R*,4*R*,5*S*)-4-acetamido-5-amino-3-(1-ethylpropoxy)-cyclohex-1-ene-1-carboxylate phosphate.

Content: 98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white powder.

Solubility: freely soluble in water and in methanol, practically insoluble in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *oseltamivir phosphate (impurity B-free) CRS*.
If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

C. Dissolve 200 mg in 10 mL of *water R*. It gives reaction (b) of phosphates (*2.3.1*).

TESTS

Specific optical rotation (*2.2.7*): – 30.7 to – 32.6 (anhydrous substance), measured at 25 °C.

Dissolve 0.50 g in *water R* and dilute to 50.0 mL with the same solvent.

Impurity B. Liquid chromatography (*2.2.29*) coupled with mass spectrometry (*2.2.43*).

Test solution. Dissolve 0.100 g of the substance to be examined in *water for chromatography R* and dilute to 10.0 mL with the same solvent.

Reference solution (a). Dissolve 2.5 mg of *oseltamivir impurity B CRS* in 5.0 mL of *anhydrous ethanol R* and dilute to 50.0 mL with *water for chromatography R*. Dilute 2.0 mL of the solution to 100.0 mL with *water for chromatography R*.

Reference solution (b). Dissolve 50.0 mg of *oseltamivir phosphate (impurity B-free) CRS* in reference solution (a) and dilute to 5.0 mL with the same solution.

Column:
- *size*: *l* = 0.05 m, Ø = 3.0 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 40 °C.

Mobile phase: mix 10 volumes of a 1.54 g/L solution of *ammonium acetate R* in *water for chromatography R*, 30 volumes of *acetonitrile R1* and 60 volumes of *water for chromatography R*.

Flow rate: 1.5 mL/min.

Post-column split ratio: use a split ratio suitable for the mass detector (e.g. 1:3).

Detection:
- mass detector: the following settings have been found to be suitable and are given as examples; if the detector has different setting parameters, adjust the detector settings so as to comply with the system suitability criterion:
  - ionisation: ESI-positive;
  - detection *m/z*: 356.2;
  - dwell: 580 ms;
  - gain EMV: 1;
  - fragmentator voltage: 120 V;
  - gas temperature: 350 °C;
  - drying gas flow: 13 L/min,
  - nebuliser pressure: 345 kPa;
  - capillary voltage (Vcap): 3 kV.

Injection: 1 µL of the test solution and reference solution (b).

Run time: 3 min.

System suitability: reference solution (b):
- *repeatability*: maximum relative standard deviation of 15 per cent determined on 6 injections.

Limit:
- *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (100 ppm).

Impurity H. Gas chromatography (*2.2.28*).

Silylation reagent. Mix 1.0 mL of *chlorotrimethylsilane R*, 2.0 mL of *hexamethyldisilazane R* and 10.0 mL of *anhydrous pyridine R*.

Test solution. Introduce 15.0 mg of the substance to be examined into a 2 mL vial and add 1.0 mL of the silylation reagent. Close the vial, shake and heat at 60 °C for 20 min. Centrifuge and discard the precipitate.

Reference solution. Introduce 15.0 mg of *oseltamivir impurity H CRS* into a 2 mL vial and add 1.0 mL of *anhydrous pyridine R*. Close the vial and shake (solution A). (Note: impurity H is hygroscopic.) Introduce 15.0 mg of the substance to be examined into another 2 mL vial and add 1.0 mL of the silylation reagent. Close the vial, shake and heat at 60 °C for 20 min. Centrifuge and discard the precipitate (solution B). Introduce 10.0 µL of solution A and 10.0 µL of solution B into a volumetric flask and dilute to 10.0 mL with *anhydrous pyridine R*.

Column:
- *material*: fused silica;
- *size*: *l* = 30 m, Ø = 0.32 mm;
- *stationary phase*: *methylpolysiloxane R* (film thickness 0.25 µm).

Carrier gas: *helium for chromatography R*.

Flow rate: 1.2 mL/min.

Split ratio: 1:50.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 2	180
	2 - 11	180 → 250
	11 - 21	250
Injection port		260
Detector		260

Detection: flame ionisation.

Injection: 1 µL.

Relative retention with reference to oseltamivir phosphate (retention time = about 10 min): impurity H = about 0.5.

System suitability: reference solution:

– *repeatability*: maximum relative standard deviation of 5 per cent for the peak due to impurity H after 6 injections.

Limit:

– *impurity H*: not more than 1.5 times the area of the corresponding peak in the chromatogram obtained with the reference solution (0.15 per cent).

Related substances. Liquid chromatography (*2.2.29*).

Solvent mixture: *acetonitrile R1*, *methanol R2*, *water for chromatography R* (135:245:620 *V/V/V*).

Test solution. Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Reference solution (a). Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b). Dissolve 5 mg of *oseltamivir impurity A CRS* and 5.0 mg of *oseltamivir impurity C CRS* in the solvent mixture and dilute to 50.0 mL with the solvent mixture. Dilute 1.0 mL of the solution to 10.0 mL with the solvent mixture.

Reference solution (c). Dissolve 50.0 mg of *oseltamivir phosphate (impurity B-free) CRS* in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm;

– *stationary phase*: *end-capped octylsilyl silica gel for chromatography R* (5 µm);

– *temperature*: 50 °C.

Mobile phase: mix 135 volumes of *acetonitrile R1*, 245 volumes of *methanol R2* and 620 volumes of a 6.8 g/L solution of *potassium dihydrogen phosphate R* in *water for chromatography R*, adjusted to pH 6.0 with a 1 M potassium hydroxide solution prepared from *potassium hydroxide R*.

Flow rate: 1.2 mL/min.

Detection: spectrophotometer at 207 nm.

Injection: 15 µL of the test solution and reference solutions (a) and (b).

Run time: twice the retention time of oseltamivir phosphate.

Relative retention with reference to oseltamivir phosphate (retention time = about 17 min): impurity A = about 0.16; impurity C = about 0.17.

System suitability: reference solution (b):

– *resolution*: minimum 1.5 between the peaks due to impurities A and C.

Limits:

– *impurity C*: not more than 0.3 times the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

– *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

– *total*: not more than 7 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.7 per cent);

– *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (*2.5.12*): maximum 0.5 per cent, determined on 0.500 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection: test solution and reference solution (c).

Calculate the percentage content of $C_{16}H_{31}N_2O_8P$ from the declared content of *oseltamivir phosphate (impurity B-free) CRS*.

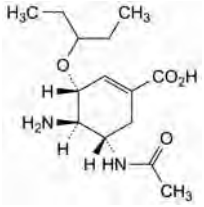
STORAGE

Protected from light.

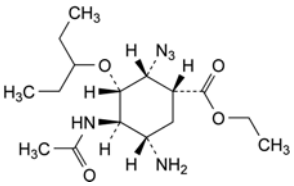
IMPURITIES

Specified impurities: B, C, H.

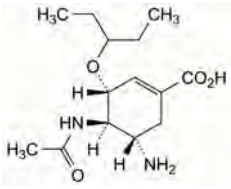
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, D, E, F, G.*



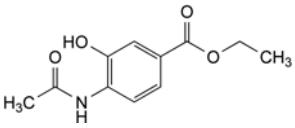
A. (3*R*,4*R*,5*S*)-5-acetamido-4-amino-3-(1-ethylpropoxy)-cyclohex-1-ene-1-carboxylic acid,



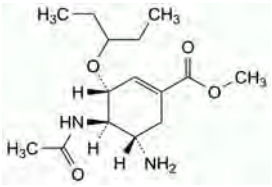
B. ethyl (1*R*,2*R*,3*S*,4*R*,5*S*)-4-acetamido-5-amino-2-azido-3-(1-ethylpropoxy)cyclohexanecarboxylate,



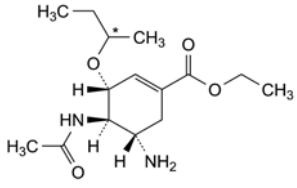
C. (3*R*,4*R*,5*S*)-4-acetamido-5-amino-3-(1-ethylpropoxy)-cyclohex-1-ene-1-carboxylic acid,



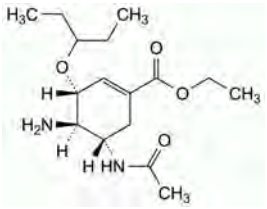
D. ethyl 4-acetamido-3-hydroxybenzoate,



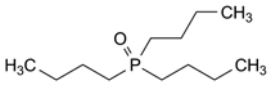
E. methyl (3*R*,4*R*,5*S*)-4-acetamido-5-amino-3-(1-ethyl-propoxy)cyclohex-1-ene-1-carboxylate,



F. ethyl (3*R*,4*R*,5*S*)-4-acetamido-5-amino-3-(1-methyl-propoxy)cyclohex-1-ene-1-carboxylate,



G. ethyl (3*R*,4*R*,5*S*)-5-acetamido-4-amino-3-(1-ethylpropoxy)cyclohex-1-ene-1-carboxylate,



H. tributylphosphane oxide.

07/2018:2939

RALTEGRAVIR CHEWABLE TABLETS

Raltegraviri compressi masticabiles

DEFINITION

Raltegravir chewable tablets contain *Raltegravir potassium (2887)*.

The tablets comply with the monograph Tablets (0478) and with the following additional requirements.

Content: 95.0 per cent to 105.0 per cent of the content of raltegravir ($C_{20}H_{21}FN_6O_5$) stated on the label.

IDENTIFICATION

Carry out either tests A, B or tests B, C.

A. Record the UV spectrum of the principal peak in the chromatograms obtained with the solutions used in the assay with a diode array detector in the range of 190-400 nm.

Results: the UV spectrum of the principal peak in the chromatogram obtained with the test solution is similar to the UV spectrum of the principal peak in the chromatogram obtained with reference solution (a).

B. Examine the chromatograms obtained in the assay.

Results: the principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

C. Infrared absorption spectrophotometry (*2.2.24*).

Preparation: crush a tablet to a powder and homogenise.

Comparison: *raltegravir potassium CRS*.

Results: the spectrum obtained shows absorption maxima at about 1633 cm^{-1}, 1515 cm^{-1}, 1188 cm^{-1}, 810 cm^{-1} and 728 cm^{-1}, similar to the spectrum obtained with *raltegravir potassium CRS*.

Other absorption maxima may be present in the spectra.

TESTS

Related substances. Liquid chromatography (*2.2.29*).

Solvent mixture: *acetonitrile R*, *water R* (30:70 *V/V*).

Test solution. Place 10 tablets in an appropriate volumetric flask to obtain a concentration of 1 mg/mL of raltegravir and make up to volume with the solvent mixture. Stir vigorously for 1 h. Centrifuge a portion of the solution and dilute 20.0 mL of the clear supernatant to 200.0 mL with the solvent mixture.

Reference solution (a). Dissolve 22.0 mg of *raltegravir potassium CRS* in the solvent mixture and dilute to 200.0 mL with the solvent mixture.

Reference solution (b). Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 2.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c). Dissolve 2 mg of *raltegravir impurity E CRS* in the solvent mixture and dilute to 100.0 mL with the solvent mixture. Dilute 1.0 mL of the solution to 100.0 mL with the test solution.

Reference solution (d). In order to prepare impurities C and D *in situ*, dissolve 20 mg of *raltegravir potassium R* in a 40 g/L solution of *sodium hydroxide R* and dilute to 10 mL with the same solvent. Stir the solution for 2 h at room temperature. To 5 mL of the solution add 5 mL of a 103 g/L solution of *hydrochloric acid R* and dilute to 50 mL with the solvent mixture.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 40 °C.

Mobile phase:

- *mobile phase A*: mix 20 volumes of *acetonitrile for chromatography R* and 80 volumes of a 1.36 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 3.0 with *phosphoric acid R*;
- *mobile phase B*: *acetonitrile for chromatography R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	100	0
2 - 27	100 → 50	0 → 50

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 220 nm.

Injection: 15 µL of the test solution and reference solutions (b), (c) and (d).

Identification of impurities: use the chromatogram obtained with reference solution (d) to identify the peaks due to impurities C and D; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity E.

Relative retention with reference to raltegravir (retention time = about 22 min): impurity D = about 0.7; impurity C = about 0.8; impurity E = about 0.96.

System suitability: reference solution (c):

- *resolution*: minimum 1.5 between the peaks due to impurity E and raltegravir.

Calculation of percentage contents:

- *correction factors*: multiply the peak areas of the following impurities by the corresponding correction factor: impurity C = 1.6; impurity D = 1.4;
- for each impurity, use the concentration of raltegravir in reference solution (b).

Limits:

- *impurity C*: maximum 0.3 per cent;
- *impurity D*: maximum 0.2 per cent;
- *unspecified impurities*: for each impurity, maximum 0.2 per cent;
- *total*: maximum 0.8 per cent;
- *reporting threshold*: 0.1 per cent.

Dissolution (*2.9.3*, *Apparatus 2*). The tablets comply with the test, unless otherwise justified and authorised.

Dissolution medium: *water R*, 900 mL.

Rotation speed: 50 r/min.

Time: 15 min.

Analysis. Liquid chromatography (*2.2.29*).

Test solutions. Solutions from the dissolution test.

Reference solution. Using sonication if necessary, dissolve a suitable quantity of *raltegravir potassium CRS* in a suitable quantity of a mixture of 30 volumes of *acetonitrile R* and 70 volumes of *water R* to obtain a concentration of raltegravir corresponding to the theoretical concentration of raltegravir in the test solution, based on the labelled content of the tablets.

Column:

- *size*: *l* = 0.1 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped monolithic octadecylsilyl silica gel for chromatography R*;
- *temperature*: 40 °C.

Mobile phase: mix 38 volumes of *acetonitrile R* and 62 volumes of a 1.36 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 3.0 with *phosphoric acid R*.

Flow rate: 5.0 mL/min.

Detection: spectrophotometer at 303 nm.

Injection: 30 µL.

Run time: 1 min.

System suitability: reference solution:

– *repeatability*: maximum relative standard deviation of 1.5 per cent determined on 6 injections.

Calculate the amount of dissolved raltegravir, expressed as a percentage of the content of raltegravir ($C_{20}H_{21}FN_6O_5$) stated on the label, taking into account the assigned content of *raltegravir potassium CRS* and a conversion factor of 0.9210.

Acceptance criterion:

– Q = 85 per cent after 15 min.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection: test solution and reference solution (a).

System suitability: reference solution (a):

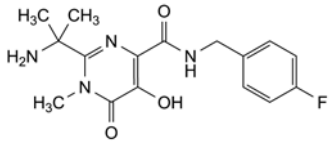
– *repeatability*: maximum relative standard deviation of 1.5 per cent determined on 6 injections.

Calculate the percentage content of raltegravir ($C_{20}H_{21}FN_6O_5$) taking into account the assigned content of *raltegravir potassium CRS* and a conversion factor of 0.9210.

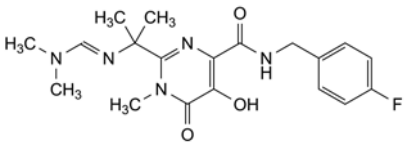
IMPURITIES

Specified impurities: C, D.

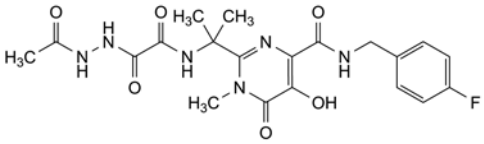
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph)*: A, B, E.*



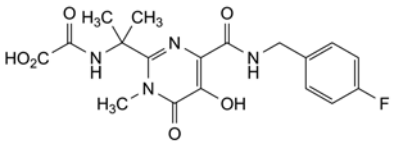
A. 2-(2-aminopropan-2-yl)-*N*-[(4-fluorophenyl)methyl]-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxamide,



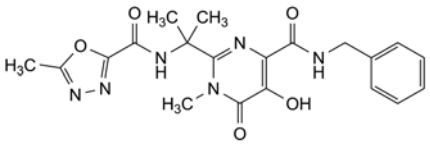
B. 2-[2-[(*E*)-[(dimethylamino)methylidene]amino]propan-2-yl]-*N*-[(4-fluorophenyl)methyl]-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxamide,



C. 2-[2-[2-(2-acetylhydrazin-1-yl)-2-oxoacetamido]propan-2-yl]-*N*-[(4-fluorophenyl)methyl]-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxamide,



D. *N*-[2-[4-[[(4-fluorophenyl)methyl]carbamoyl]-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl]propan-2-yl]oxamic acid,

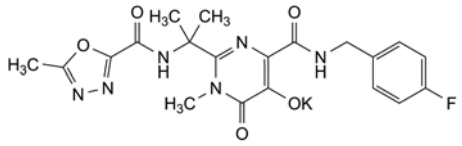


E. *N*-benzyl-5-hydroxy-1-methyl-2-[2-[(5-methyl-1,3,4-oxadiazol-2-yl)formamido]propan-2-yl]-6-oxo-1,6-dihydropyrimidine-4-carboxamide.

04/2018:2887
corrected 10.0

RALTEGRAVIR POTASSIUM

Raltegravirum kalicum



$C_{20}H_{20}FKN_6O_5$ M_r 482.5
[871038-72-1]

DEFINITION

Potassium 4-[[(4-fluorophenyl)methyl]carbamoyl]-1-methyl-2-[2-[(5-methyl-1,3,4-oxadiazol-2-yl)formamido]propan-2-yl]-6-oxo-1,6-dihydropyrimidin-5-olate.

Content: 98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white powder.

Solubility: soluble in water, very slightly soluble in ethanol (96 per cent), practically insoluble in heptane.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *raltegravir potassium CRS*.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

B. It gives reaction (b) of potassium (*2.3.1*).

TESTS

Related substances. Liquid chromatography (*2.2.29*).

Solvent mixture: *acetonitrile R*, *water R* (25:75 *V/V*).

Test solution. Dissolve 25.0 mg of the substance to be examined in 100 mL of the solvent mixture using sonication for 5 min. Add about 140 mL of the solvent mixture then dilute to 250.0 mL with the solvent mixture.

Reference solution (a). Dissolve 25.0 mg of *raltegravir potassium CRS* in 100 mL of the solvent mixture using sonication for 5 min. Add about 140 mL of the solvent mixture then dilute to 250.0 mL with the solvent mixture.

Reference solution (b). Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c). Dissolve 2 mg of *raltegravir impurity E CRS* in the solvent mixture and dilute to 20.0 mL with the solvent mixture. Dilute 1.0 mL of the solution to 50.0 mL with reference solution (a).

Reference solution (d). In order to prepare impurity C *in situ*, dissolve 20 mg of the substance to be examined in a 40 g/L solution of *sodium hydroxide R* and dilute to 10 mL with the same solvent. Stir the solution for 30 min. To 5 mL of the solution add 5 mL of a 103 g/L solution of *hydrochloric acid R* and dilute to 50 mL with the solvent mixture.

Reference solution (e). Dissolve 5 mg of *raltegravir for peak identification CRS* (containing impurities F and G) in 20 mL of the solvent mixture using sonication for 5 min. Add about 25 mL of the solvent mixture then dilute to 50 mL with the solvent mixture.

Column:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *phenylsilyl silica gel for chromatography R* (3.5 µm);
- *temperature*: 15 °C.

Mobile phase:
- *mobile phase A*: 0.1 per cent *V/V* solution of *phosphoric acid R*;
- *mobile phase B*: *acetonitrile for chromatography R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	75	25
2 - 5	75 → 60	25 → 40
5 - 10	60 → 55	40 → 45
10 - 19	55 → 10	45 → 90
19 - 22	10 → 5	90 → 95

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 220 nm.

Injection: 10 µL of the test solution and reference solutions (b), (c), (d) and (e).

Identification of impurities: use the chromatogram obtained with reference solution (d) to identify the peak due to impurity C; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity E; use the chromatogram supplied with *raltegravir for peak identification CRS* and the chromatogram obtained with reference solution (e) to identify the peaks due to impurities F and G.

Relative retention with reference to raltegravir (retention time = about 10 min): impurity C = about 0.7; impurity E = about 0.95; impurity G = about 1.1; impurity F = about 1.15.

System suitability: reference solution (c):
- *resolution*: minimum 1.5 between the peaks due to impurity E and raltegravir.

Calculation of percentage contents:
- *correction factor*: multiply the peak area of impurity C by 1.6;
- for each impurity, use the concentration of raltegravir potassium in reference solution (b).

Limits:
- *impurity C*: maximum 0.3 per cent;
- *impurities E, F, G*: for each impurity, maximum 0.15 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 0.5 per cent;
- *reporting threshold*: 0.05 per cent.

Water (*2.5.12*): maximum 0.6 per cent, determined on 0.500 g. Use as the solvent a mixture of equal volumes of *methanol R* and *formamide R*.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection: test solution and reference solution (a).

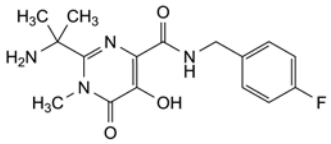
Calculate the percentage content of $C_{20}H_{20}FKN_6O_5$ taking into account the assigned content of *raltegravir potassium CRS*.

IMPURITIES

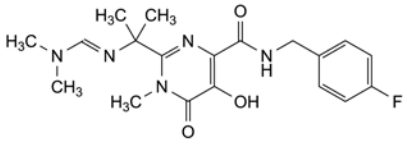
Specified impurities: C, E, F, G.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general

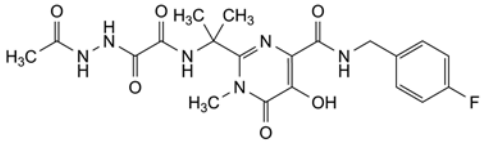
acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, B, D, H.*



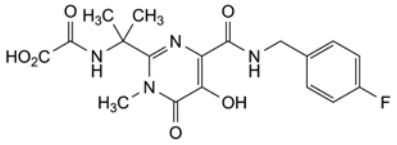
A. 2-(2-aminopropan-2-yl)-*N*-[(4-fluorophenyl)methyl]-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxamide,



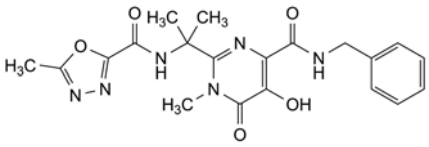
B. 2-[2-[(*E*)-[(dimethylamino)methylidene]amino]propan-2-yl]-*N*-[(4-fluorophenyl)methyl]-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxamide,



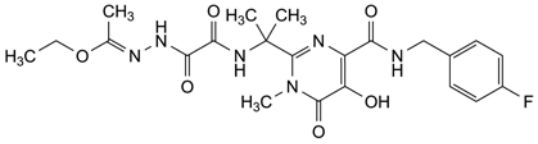
C. 2-[2-[2-(2-acetylhydrazin-1-yl)-2-oxoacetamido]propan-2-yl]-*N*-[(4-fluorophenyl)methyl]-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxamide,



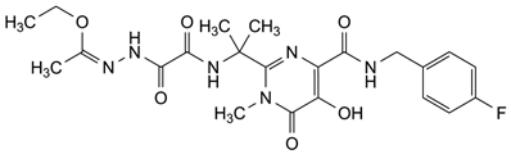
D. *N*-[2-[4-[[(4-fluorophenyl)methyl]carbamoyl]-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl]propan-2-yl]oxamic acid,



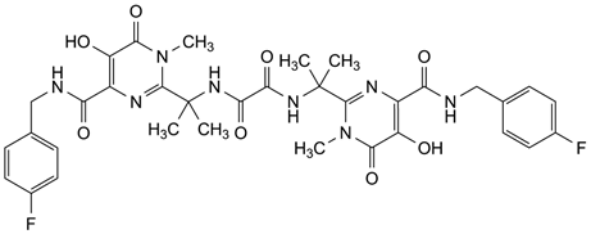
E. *N*-benzyl-5-hydroxy-1-methyl-2-[2-[(5-methyl-1,3,4-oxadiazol-2-yl)formamido]propan-2-yl]-6-oxo-1,6-dihydropyrimidine-4-carboxamide,



F. ethyl (1*E*)-*N*-[[2-[4-[[(4-fluorophenyl)methyl]carbamoyl]-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl]propan-2-yl]oxamoyl]ethanehydrazonate,



G. ethyl (1*Z*)-*N*-[[2-[4-[[(4-fluorophenyl)methyl]carbamoyl]-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl]propan-2-yl]oxamoyl]ethanehydrazonate,



H. *N*,*N*′-bis[2-[4-[[(4-fluorophenyl)methyl]carbamoyl]-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl]propan-2-yl]oxamide.

07/2018:2938

RALTEGRAVIR TABLETS

Raltegraviri compressi

DEFINITION

Raltegravir tablets contain *Raltegravir potassium (2887)*.

The tablets comply with the monograph Tablets (0478) and with the following additional requirements.

Content: 95.0 per cent to 105.0 per cent of the content of raltegravir ($C_{20}H_{21}FN_6O_5$) stated on the label.

IDENTIFICATION

Carry out either tests A, B or tests B, C.

A. Record the UV spectrum of the principal peak in the chromatograms obtained with the solutions used in the assay with a diode array detector in the range of 190-400 nm.

Results: the UV spectrum of the principal peak in the chromatogram obtained with the test solution is similar to the UV spectrum of the principal peak in the chromatogram obtained with reference solution (a).

B. Examine the chromatograms obtained in the assay.

Results: the principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

C. Infrared absorption spectrophotometry (*2.2.24*).

Preparation: crush a tablet to a powder and homogenise.

Comparison: *raltegravir potassium CRS*.

Results: the spectrum obtained shows absorption maxima at about 1633 cm^{-1}, 1515 cm^{-1}, 1188 cm^{-1}, 810 cm^{-1} and 728 cm^{-1}, similar to the spectrum obtained with *raltegravir potassium CRS*.

Other absorption maxima may be present in the spectra.

TESTS

Related substances. Liquid chromatography (*2.2.29*).

Solvent mixture: *acetonitrile R*, *water R* (30:70 *V/V*).

Test solution. Place 10 tablets in an appropriate volumetric flask to obtain a concentration of 8 mg/mL of raltegravir and make up to volume with the solvent mixture. Stir vigorously for 1 h. Centrifuge a portion of the solution and dilute 5.0 mL of the clear supernatant to 100.0 mL with the solvent mixture. Dilute 50.0 mL of this solution to 200.0 mL with the solvent mixture.

Reference solution (a). Dissolve 22.0 mg of *raltegravir potassium CRS* in the solvent mixture and dilute to 200.0 mL with the solvent mixture.

Reference solution (b). Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 2.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c). Dissolve 2 mg of *raltegravir impurity E CRS* in the solvent mixture and dilute to 100.0 mL with the solvent mixture. Dilute 1.0 mL of the solution to 100.0 mL with the test solution.

Reference solution (d). In order to prepare impurities C and D *in situ,* dissolve 20 mg of *raltegravir potassium R* in a 40 g/L solution of *sodium hydroxide R* and dilute to 10 mL with the same solvent. Stir the solution for 2 h at room temperature. To 5 mL of the solution add 5 mL of a 103 g/L solution of *hydrochloric acid R* and dilute to 50 mL with the solvent mixture.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm;
– *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
– *temperature*: 40 °C.

Mobile phase:

– *mobile phase A*: mix 20 volumes of *acetonitrile for chromatography R* and 80 volumes of a 1.36 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 3.0 with *phosphoric acid R*;
– *mobile phase B*: *acetonitrile for chromatography R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	100	0
2 - 27	100 → 50	0 → 50

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 220 nm.

Injection: 15 µL of the test solution and reference solutions (b), (c) and (d).

Identification of impurities: use the chromatogram obtained with reference solution (d) to identify the peaks due to impurities C and D; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity E.

Relative retention with reference to raltegravir (retention time = about 22 min): impurity D = about 0.7; impurity C = about 0.8; impurity E = about 0.96.

System suitability: reference solution (c):

– *resolution*: minimum 1.5 between the peaks due to impurity E and raltegravir.

Calculation of percentage contents:

– *correction factors*: multiply the peak areas of the following impurities by the corresponding correction factor: impurity C = 1.6; impurity D = 1.4;
– for each impurity, use the concentration of raltegravir in reference solution (b).

Limits:

– *impurity C*: maximum 0.5 per cent;
– *impurity D*: maximum 0.3 per cent;
– *unspecified impurities*: for each impurity, maximum 0.2 per cent;
– *total*: maximum 0.8 per cent;
– *reporting threshold*: 0.1 per cent.

Dissolution (*2.9.3, Apparatus 2*). The tablets comply with the test, unless otherwise justified and authorised. Use sinker devices.

Dissolution medium: *water R*, 900 mL.

Rotation speed: 100 r/min.

Time: 15 min and 60 min.

Analysis. Liquid chromatography (*2.2.29*).

Test solutions. Solutions from the dissolution test.

Reference solution. Using sonication, dissolve a suitable quantity of *raltegravir potassium CRS* in a suitable quantity of a mixture of 30 volumes of *acetonitrile R* and 70 volumes of *water R* to obtain a concentration of raltegravir corresponding to the theoretical concentration of raltegravir in the test solution, based on the labelled content of the tablets.

Column:

– *size*: *l* = 0.1 m, Ø = 4.6 mm;
– *stationary phase*: *end-capped monolithic octadecylsilyl silica gel for chromatography R*;
– *temperature*: 40 °C.

Mobile phase: mix 38 volumes of *acetonitrile R* and 62 volumes of a 1.36 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 3.0 with *phosphoric acid R*.

Flow rate: 5.0 mL/min.

Detection: spectrophotometer at 303 nm.

Injection: 10 µL.

Run time: 1 min.

System suitability: reference solution:

– *repeatability*: maximum relative standard deviation of 1.5 per cent determined on 6 injections.

Calculate the amount of dissolved raltegravir, expressed as a percentage of the content of raltegravir ($C_{20}H_{21}FN_6O_5$) stated on the label, taking into account the assigned content of *raltegravir potassium CRS* and a conversion factor of 0.9210.

Acceptance criteria:

– 15-45 per cent after 15 min;
– *Q* = 70 per cent after 60 min.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection: test solution and reference solution (a).

System suitability: reference solution (a):

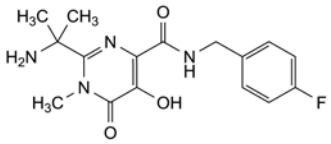
– *repeatability*: maximum relative standard deviation of 1.5 per cent determined on 6 injections.

Calculate the percentage content of raltegravir ($C_{20}H_{21}FN_6O_5$) taking into account the assigned content of *raltegravir potassium CRS* and a conversion factor of 0.9210.

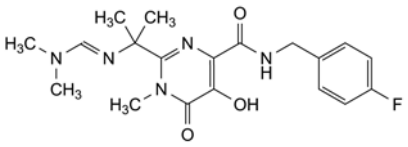
IMPURITIES

Specified impurities: C, D.

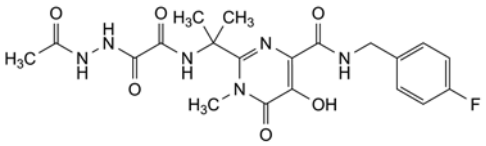
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph)*: A, B, E.*



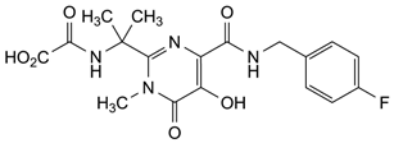
A. 2-(2-aminopropan-2-yl)-*N*-[(4-fluorophenyl)methyl]-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxamide,



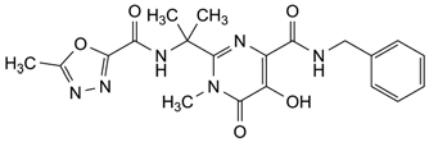
B. 2-[2-[(*E*)-[(dimethylamino)methylidene]amino]propan-2-yl]-*N*-[(4-fluorophenyl)methyl]-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxamide,



C. 2-[2-[2-(2-acetylhydrazin-1-yl)-2-oxoacetamido]propan-2-yl]-*N*-[(4-fluorophenyl)methyl]-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxamide,



D. *N*-[2-[4-[[(4-fluorophenyl)methyl]carbamoyl]-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl]propan-2-yl]oxamic acid,

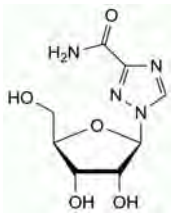


E. *N*-benzyl-5-hydroxy-1-methyl-2-[2-[(5-methyl-1,3,4-oxadiazol-2-yl)formamido]propan-2-yl]-6-oxo-1,6-dihydropyrimidine-4-carboxamide.

01/2017:2109

RIBAVIRIN

Ribavirinum



$C_8H_{12}N_4O_5$ M_r 244.2
[36791-04-5]

DEFINITION

1-β-D-Ribofuranosyl-1*H*-1,2,4-triazole-3-carboxamide.

Content: 98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: freely soluble in water, slightly soluble in ethanol (96 per cent), slightly soluble or very slightly soluble in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *ribavirin CRS*.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methylene chloride R*, evaporate to dryness and record new spectra using the residues.

TESTS

pH (*2.2.3*): 4.0 to 6.5.

Dissolve 0.200 g in *carbon dioxide-free water R* and dilute to 10.0 mL with the same solvent.

Specific optical rotation (*2.2.7*): – 33 to – 37 (dried substance).

Dissolve 0.250 g in *water R* and dilute to 25.0 mL with the same solvent. Determine the specific optical rotation within 10 min of preparing the solution.

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 50.0 mg of the substance to be examined in *water for chromatography R* and dilute to 100.0 mL with the same solvent.

Reference solution (a). In order to produce impurity A *in situ*, mix 5.0 mL of the test solution and 5.0 mL of a 42 g/L solution of *sodium hydroxide R* and allow to stand for 90 min. Neutralise with 5.0 mL of a 103 g/L solution of *hydrochloric acid R* and mix well.

Reference solution (b). Dilute 1.0 mL of the test solution to 100.0 mL with *water for chromatography R*. Dilute 1.0 mL of this solution to 10.0 mL with *water for chromatography R*.

Reference solution (c). Dissolve 50.0 mg of *ribavirin CRS* in *water for chromatography R* and dilute to 100.0 mL with the same solvent.

Column:

– *size*: *l* = 0.15 m, Ø = 4.6 mm;
– *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (3 µm) suitable for use with highly aqueous mobile phases ;
– *temperature*: 25 °C.

Mobile phase:

– *mobile phase A*: dissolve 1.0 g of *anhydrous sodium sulfate R* in 950 mL of *water for chromatography R*, add 2.0 mL of a 5 per cent *V/V* solution of *phosphoric acid R*, adjust to pH 2.8 with a 5 per cent *V/V* solution of *phosphoric acid R* and dilute to 1000 mL with *water for chromatography R*;
– *mobile phase B*: *acetonitrile R1*, mobile phase A (5:95 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	100	0
15 - 25	100 → 0	0 → 100
25 - 35	0	100

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 220 nm.

Injection: 5 µL of the test solution and reference solutions (a) and (b).

Relative retention with reference to ribavirin (retention time = about 6 min): impurity A = about 0.8.

System suitability: reference solution (a):

– *resolution*: minimum 4.0 between the peaks due to impurity A and ribavirin.

Limits:

– *correction factor*: for the calculation of content, multiply the peak area of impurity A by 2.3;
– *impurity A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
– *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
– *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
– *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 5 h.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection: test solution and reference solution (c).

Calculate the percentage content of $C_8H_{12}N_4O_5$ from the declared content of *ribavirin CRS*.

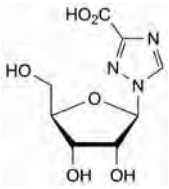
STORAGE

Protected from light.

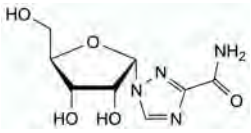
IMPURITIES

Specified impurities: *A*.

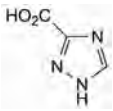
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *B, C, D, F, G*.



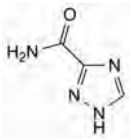
A. 1-β-D-ribofuranosyl-1*H*-1,2,4-triazole-3-carboxylic acid,



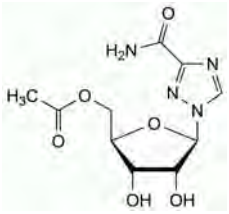
B. 1-α-D-ribofuranosyl-1*H*-1,2,4-triazole-3-carboxamide (anomer),



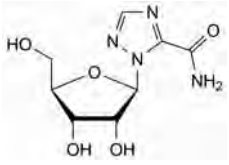
C. 1*H*-1,2,4-triazole-3-carboxylic acid,



D. 1*H*-1,2,4-triazole-3-carboxamide,



F. 1-(5-*O*-acetyl-β-D-ribofuranosyl)-1*H*-1,2,4-triazole-3-carboxamide (5′-*O*-acetylribavirin),

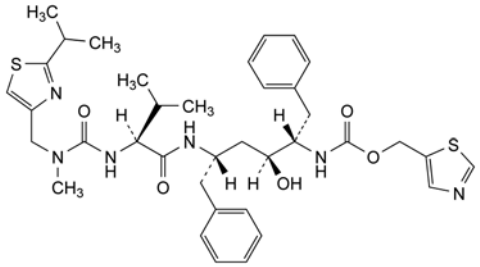


G. 1-β-D-ribofuranosyl-1*H*-1,2,4-triazole-5-carboxamide (*N*-isomer).

01/2017:2136

RITONAVIR

Ritonavirum



$C_{37}H_{48}N_6O_5S_2$ M_r 721
[155213-67-5]

DEFINITION

Thiazol-5-ylmethyl [(1*S*,2*S*,4*S*)-1-benzyl-2-hydroxy-4-[[(2*S*)-3-methyl-2-[[methyl[[2-(1-methylethyl)thiazol-4-yl]methyl]carbamoyl]amino]butanoyl]amino]-5-phenylpentyl]carbamate.

Content: 97.0 per cent to 102.0 per cent (anhydrous substance).

PRODUCTION

The production method is validated to demonstrate suitable enantiomeric purity of the final product.

CHARACTERS

Appearance: white or almost white powder.

Solubility: practically insoluble in water, freely soluble in methanol and in methylene chloride, very slightly soluble in acetonitrile.

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *ritonavir CRS*.

If the spectra obtained in the solid state show differences dissolve the substance to be examined and the reference substance separately in *methylene chloride R*, evaporate to dryness and record new spectra using the residues.

TESTS

Related substances. Liquid chromatography (*2.2.29*).

Solvent mixture. Mix equal volumes of *acetonitrile R* and a 4.1 g/L solution of *potassium dihydrogen phosphate R*.

Test solution (a). Dissolve 10.0 mg of the substance to be examined in the solvent mixture and dilute to 10.0 mL with the solvent mixture. Sonicate if necessary.

Test solution (b). Dilute 5.0 mL of test solution (a) to 10.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 20.0 mL with the solvent mixture.

Reference solution (a). Dissolve 5.0 mg of *ritonavir for peak identification CRS* (containing impurities E, F, L, O and T) in the solvent mixture and dilute to 5.0 mL with the solvent mixture. Sonicate if necessary.

Reference solution (b). Dilute 1.0 mL of test solution (a) to 10.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 100.0 mL with the solvent mixture.

Reference solution (c). Dissolve 10.0 mg of *ritonavir CRS* in the solvent mixture and dilute to 10.0 mL with the solvent mixture. Sonicate if necessary. Dilute 5.0 mL of this solution to 10.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 20.0 mL with the solvent mixture.

Column:

- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped butylsilyl silica gel for chromatography R* (3 µm);
- *temperature*: 60 °C.

Mobile phase:

- *mobile phase A*: mix 5 volumes of *butanol R*, 8 volumes of *tetrahydrofuran R*, 18 volumes of *acetonitrile R* and 69 volumes of a 4.1 g/L solution of *potassium dihydrogen phosphate R* filtered through a 0.45 µm nylon membrane;
- *mobile phase B*: mix 5 volumes of *butanol R*, 8 volumes of *tetrahydrofuran R*, 40 volumes of a 4.1 g/L solution of *potassium dihydrogen phosphate R* filtered through a 0.45 µm nylon membrane and 47 volumes of *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 60	100	0
60 - 120	100 → 0	0 → 100
120 - 120.1	0 → 100	100 → 0
120.1 - 155	100	0

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 240 nm.

Injection: 50 µL of test solution (a) and reference solutions (a) and (b).

Identification of impurities: use the chromatogram supplied with *ritonavir for peak identification CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities E, F, L, O and T.

Relative retention with reference to ritonavir (retention time = about 34 min): impurity E = about 0.39; impurity F = about 0.40; impurity L = about 0.8; impurity O = about 1.1; impurity T = about 2.6.

System suitability: reference solution (a):

- *peak-to-valley ratio*: minimum 1.2, where H_p = height above the baseline of the peak due to impurity E and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity F.

Limits:

- *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity F = 1.4; impurity L = 1.9; impurity T = 1.4;
- *impurities E, O*: for each impurity, not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
- *impurity T*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
- *impurities F, L*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
- *total*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water (*2.5.12*): maximum 0.5 per cent, determined on 0.500 g.

Sulfated ash (*2.4.14*): maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection: test solution (b) and reference solution (c).

Calculate the percentage content of $C_{37}H_{48}N_6O_5S_2$ from the declared content of *ritonavir CRS*.

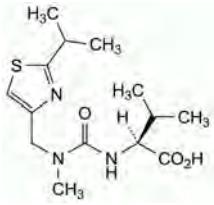
STORAGE

Protected from light.

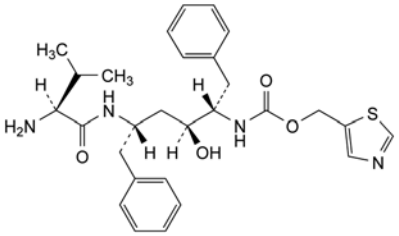
IMPURITIES

Specified impurities: E, F, L, O, T.

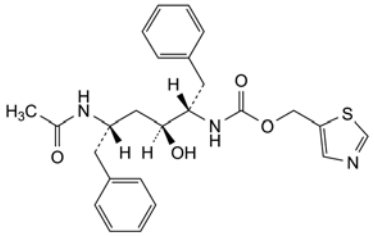
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, B, C, D, G, H, I, J, K, M, N, P, Q, R, S, U.*



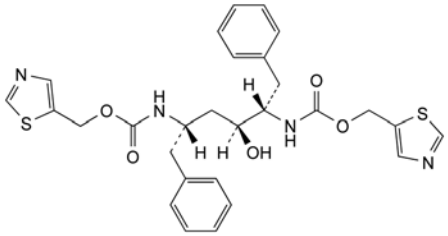
A. (2*S*)-3-methyl-2-[[methyl[[2-(1-methylethyl)thiazol-4-yl]methyl]carbamoyl]amino]butanoic acid,



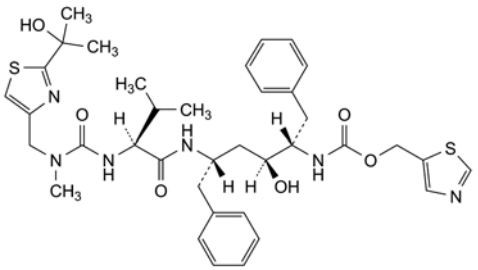
B. thiazol-5-ylmethyl [(1*S*,2*S*,4*S*)-4-[[(2*S*)-2-amino-3-methylbutanoyl]amino]-1-benzyl-2-hydroxy-5-phenylpentyl]carbamate,



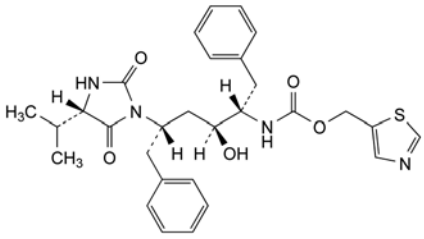
C. thiazol-5-ylmethyl [(1*S*,2*S*,4*S*)-4-(acetylamino)-1-benzyl-2-hydroxy-5-phenylpentyl]carbamate,



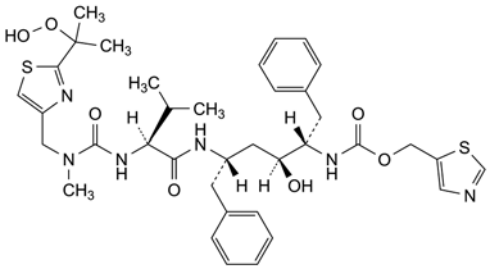
D. thiazol-5-ylmethyl [(1*S*,2*S*,4*S*)-1-benzyl-2-hydroxy-5-phenyl-4-[[(thiazol-5-ylmethoxy)carbonyl]amino]pentyl]-carbamate,



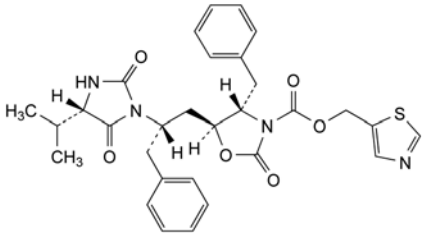
E. thiazol-5-ylmethyl [(1*S*,2*S*,4*S*)-1-benzyl-2-hydroxy-4-[[(2*S*)-2-[[[[2-(1-hydroxy-1-methylethyl)thiazol-4-yl]methyl]methylcarbamoyl]amino]-3-methylbutanoyl]-amino]-5-phenylpentyl]carbamate,



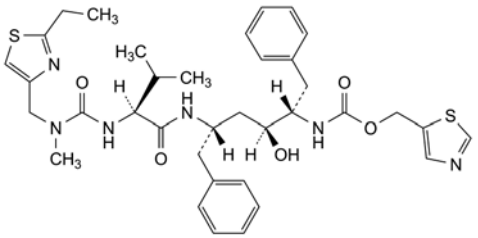
F. thiazol-5-ylmethyl [(1*S*,2*S*,4*S*)-1-benzyl-4-[[(2*S*)-1-benzyl-2-hydroxy-4-[(4*S*)-4-(1-methylethyl)-2,5-dioxoimidazolidin-1-yl]-5-phenylpentyl]carbamate,



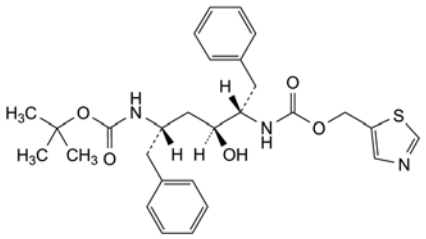
G. thiazol-5-ylmethyl [(1*S*,2*S*,4*S*)-1-benzyl-4-[[(2*S*)-2-[[[[2-(1-hydroperoxy-1-methylethyl)thiazol-4-yl]methyl]methylcarbamoyl]amino]-3-methylbutanoyl]-amino]-2-hydroxy-5-phenylpentyl]carbamate,



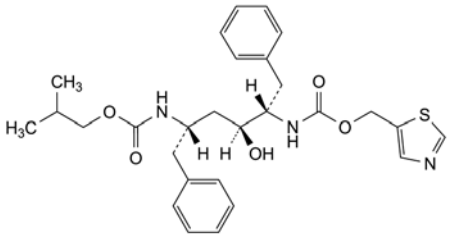
H. thiazol-5-ylmethyl (4*S*,5*S*)-4-benzyl-5-[(2*S*)-2-[(4*S*)-4-(1-methylethyl)-2,5-dioxoimidazolidin-1-yl]-3-phenylpropyl]-2-oxooxazolidine-3-carboxylate,



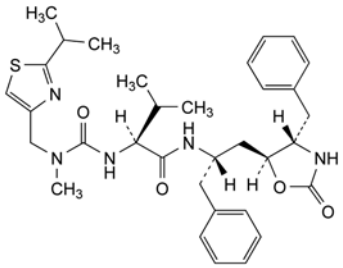
I. thiazol-5-ylmethyl [((1*S*,2*S*,4*S*)-1-benzyl-4-[[(2*S*)-2-[[[[2-ethylthiazol-4-yl]methyl]methylcarbamoyl]-amino]-3-methylbutanoyl]amino]-2-hydroxy-5-phenyl-pentyl]carbamate,



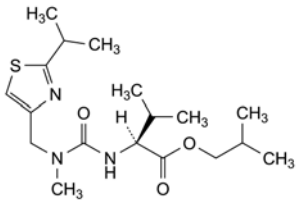
J. thiazol-5-ylmethyl [(1*S*,2*S*,4*S*)-1-benzyl-4-[[(1,1-dimethylethoxy)carbonyl]amino]-2-hydroxy-5-phenylpentyl]carbamate,



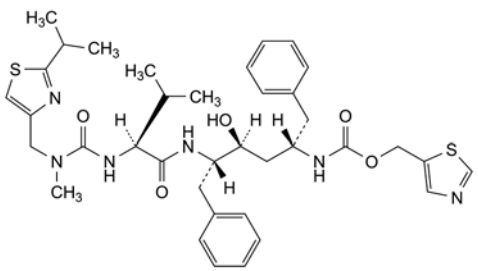
K. thiazol-5-ylmethyl (1*S*,2*S*,4*S*)-1-benzyl-2-hydroxy-4-[[(2-methylpropoxy)carbonyl]amino]-5-phenylpentyl]carbamate,



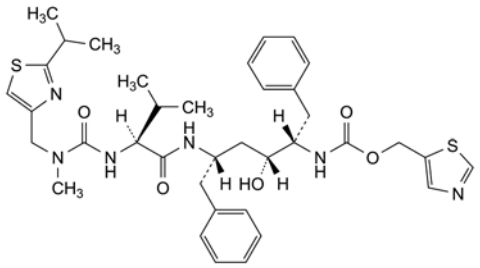
L. (4*S*,5*S*)-4-benzyl-5-[(2*S*)-2-[[(2*S*)-3-methyl-2-[[methyl[[2-(1-methylethyl)thiazol-4-yl]methyl]carbamoyl]amino]-butanoyl]amino]-3-phenylpropyl]oxazolidin-2-one,



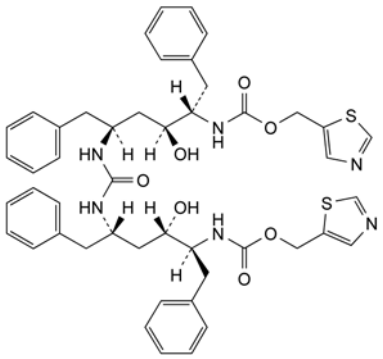
M. 2-methylpropyl (2*S*)-3-methyl-2-[[methyl[[2-(1-methyl-ethyl)thiazol-4-yl]methyl]carbamoyl]amino]butanoate,



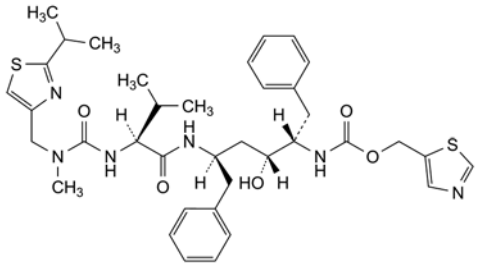
N. thiazol-5-ylmethyl [(1*S*,3*S*,4*S*)-1-benzyl-3-hydroxy-4-[[(2*S*)-3-methyl-2-[[methyl[[2-(1-methylethyl)thiazol-4-yl]methyl]carbamoyl]amino]butanoyl]amino]-5-phenylpentyl]carbamate,



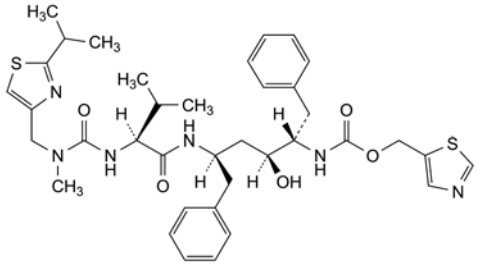
O. thiazol-5-ylmethyl [(1*S*,2*R*,4*S*)-1-benzyl-2-hydroxy-4-[[(2*S*)-3-methyl-2-[[methyl[[2-(1-methylethyl)thiazol-4-yl]methyl]carbamoyl]amino]butanoyl]amino]-5-phenylpentyl]carbamate,



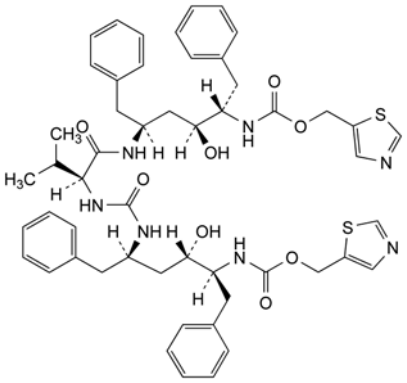
P. bis(thiazol-5-ylmethyl) [carbonylbis[imino[(2*S*,3*S*,5*S*)-3-hydroxy-1,6-diphenylhexane-5,2-diyl]]]dicarbamate,



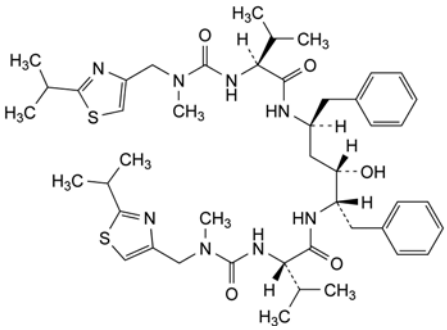
Q. thiazol-5-ylmethyl [(1*S*,2*R*,4*R*)-1-benzyl-2-hydroxy-4-[[(2*S*)-3-methyl-2-[[methyl[[2-(1-methylethyl)-thiazol-4-yl]methyl]carbamoyl]amino]butanoyl]amino]-5-phenylpentyl]carbamate,



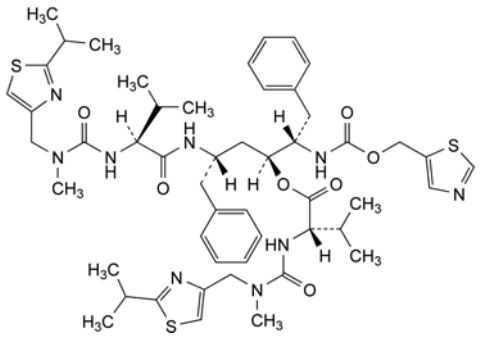
R. thiazol-5-ylmethyl [(1*S*,2*S*,4*R*)-1-benzyl-2-hydroxy-4-[[(2*S*)-3-methyl-2-[[methyl[[2-(1-methylethyl)thiazol-4-yl]methyl]carbamoyl]amino]butanoyl]amino]-5-phenylpentyl]carbamate,



S. thiazol-5-ylmethyl [(1*S*,2*S*,4*S*)-1-benzyl-4-[[(2*S*)-2-[[[(1*S*,3*S*,4*S*)-1-benzyl-3-hydroxy-5-phenyl-4-[[(thiazol-5-ylmethoxy)carbonyl]amino]pentyl]carbamoyl]amino]-3-methylbutanoyl]amino]-2-hydroxy-5-phenylpentyl]-carbamate,



T. (2*S*)-*N*-[(1*S*,2*S*,4*S*)-1-benzyl-2-hydroxy-4-[[(2*S*)-3-methyl-2-[[methyl[[2-(1-methylethyl)thiazol-4-yl]methyl]carbamoyl]amino]butanoyl]amino]-5-phenylpentyl]-3-methyl-2-[[methyl[[2-(1-methylethyl)-thiazol-4-yl]methyl]carbamoyl]amino]butanamide,

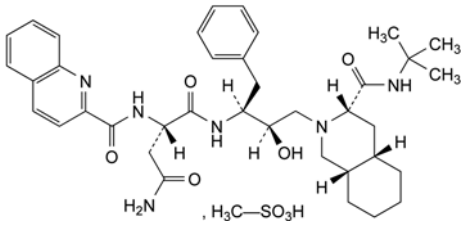


U. (1*S*,3*S*)-3-[[(2*S*)-3-methyl-2-[[methyl[[2-(1-methylethyl)-thiazol-4-yl]methyl]carbamoyl]amino]butanoyl]amino]-4-phenyl-1-[(1*S*)-2-phenyl-1-[[(thiazol-5-ylmethoxy)-carbonyl]amino]ethyl]butyl (2*S*)-3-methyl-2-[[methyl[[2-(1-methylethyl)thiazol-4-yl]methyl]carbamoyl]amino]-butanoate.

01/2017:2267

SAQUINAVIR MESILATE

Saquinaviri mesilas



$C_{39}H_{54}N_6O_8S$ M_r 767
[149845-06-7]

DEFINITION

(2*S*)-*N*[1]-[(1*S*,2*R*)-1-Benzyl-3-[(3*S*,4a*S*,8a*S*)-3-[(1,1-dimethylethyl)carbamoyl]octahydroisoquinolin-2(1*H*)-yl]-2-hydroxypropyl]-2-[(quinolin-2-ylcarbonyl)amino]-butanediamide methanesulfonate.

Content: 97.5 per cent to 102.0 per cent (anhydrous substance).

PRODUCTION

It is considered that alkyl methanesulfonate esters are genotoxic and are potential impurities in saquinavir mesilate. The manufacturing process should be developed taking into consideration the principles of quality risk management, together with considerations of the quality of starting materials, process capability and validation. The general methods *2.5.37. Methyl, ethyl and isopropyl methanesulfonate in methanesulfonic acid*, *2.5.38. Methyl, ethyl and isopropyl methanesulfonate in active substances* and *2.5.39. Methanesulfonyl chloride in methanesulfonic acid* are available to assist manufacturers.

CHARACTERS

Appearance: white or almost white, slightly hygroscopic powder.

Solubility: practically insoluble in water, sparingly soluble in methanol, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *saquinavir mesilate CRS*.

TESTS

Specific optical rotation (*2.2.7*): – 42.0 to – 35.0 (anhydrous substance).

Dissolve 0.25 g in *anhydrous methanol R* and dilute to 50.0 mL with the same solvent.

Related substances. Liquid chromatography (*2.2.29*).

Solvent mixture: *water for chromatography R*, *acetonitrile R1* (47:53 *V/V*).

Test solution. Dissolve 30.0 mg of the substance to be examined in the solvent mixture, using sonication, and dilute to 100.0 mL with the same solvent.

Reference solution (a). Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b). Dissolve the contents of a vial of *saquinavir for system suitability CRS* (containing impurities A, B, C and D) in 1.0 mL of the solvent mixture and sonicate for 2 min.

Reference solution (c). Dissolve 30.0 mg of *saquinavir mesilate CRS* in the solvent mixture, using sonication, and dilute to 100.0 mL with the same solvent.

Column:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (3.5 µm).

Mobile phase:
- *mobile phase A*: to 2.5 mL of *strong sodium hydroxide solution R* add 900 mL of *water for chromatography R*, adjust to pH 1.8 with *perchloric acid R* and dilute to 1000 mL with *water for chromatography R*;
- *mobile phase B*: mobile phase A, *acetonitrile R1* (38:62 *V/V*);

Time (min)	Mobile phase A (per cent)	Mobile phase B (per cent)
0 - 1	50	50
1 - 31	50 → 0	50 → 100

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 210 nm.

Injection: 10 µL of the test solution and reference solutions (a) and (b).

Identification of impurities: use the chromatogram supplied with *saquinavir for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B, C and D.

Relative retention with reference to saquinavir (retention time = about 17 min): impurity A = about 0.2; impurity B = about 0.3; impurity C = about 0.5; impurity D = about 0.9.

System suitability: reference solution (b):
- *peak-to-valley ratio*: minimum 3, where H_p = height above the baseline of the peak due to impurity D and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to saquinavir.

Limits:
- *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 0.5; impurity B = 0.5; impurity C = 2.5;
- *impurities A, B, C*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent);
- *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent);
- *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *disregard limit*: not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.03 per cent).

Water (*2.5.12*): maximum 1.0 per cent, determined on 0.250 g.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection: 10 µL of the test solution and reference solution (c).

Calculate the percentage content of saquinavir mesilate from the assigned content of *saquinavir mesilate CRS*.

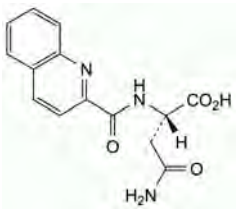
STORAGE

In an airtight container, protected from light.

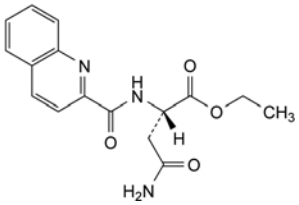
IMPURITIES

Specified impurities: A, B, C.

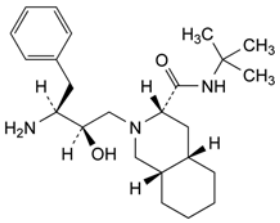
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *D, E, F, G, H.*



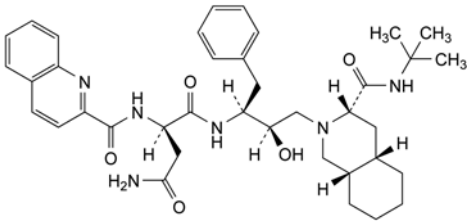
A. (2*S*)-4-amino-4-oxo-2-[(quinolin-2-ylcarbonyl)amino]-butanoic acid,



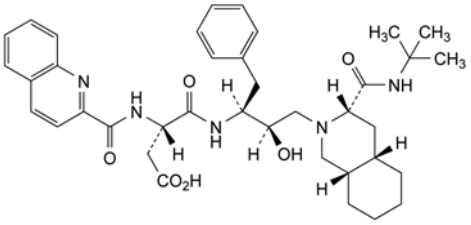
B. ethyl (2*S*)-4-amino-4-oxo-2-[(quinolin-2-ylcarbonyl)-amino]butanoate,



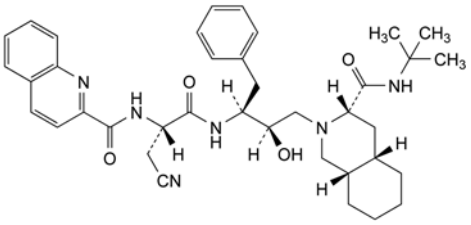
C. (3*S*,4a*S*,8a*S*)-2-[(2*R*,3*S*)-3-amino-2-hydroxy-4-phenyl-butyl]-*N*-(1,1-dimethylethyl)decahydroisoquinoline-3-carboxamide,



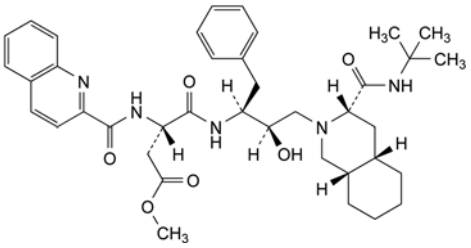
D. (2*R*)-*N*[1]-[(1*S*,2*R*)-1-benzyl-3-[(3*S*,4a*S*,8a*S*)-3-[(1,1-dimethylethyl)carbamoyl]octahydroisoquinolin-2(1*H*)-yl]-2-hydroxypropyl]-2-[(quinolin-2-ylcarbonyl)amino]-butanediamide (2-*epi*-saquinavir),



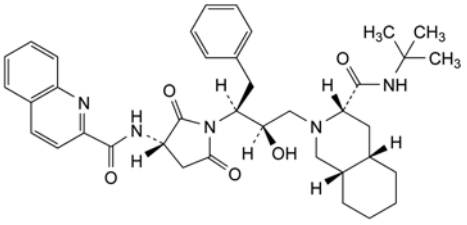
E. (3*S*)-4-[[(1*S*,2*R*)-1-benzyl-3-[(3*S*,4a*S*,8a*S*)-3-[(1,1-dimethylethyl)carbamoyl]octahydroisoquinolin-2(1*H*)-yl]-2-hydroxypropyl]amino]-4-oxo-3-[(quinolin-2-ylcarbonyl)amino]butanoic acid,



F. *N*-[(1*S*)-2-[[(1*S*,2*R*)-1-benzyl-3-[(3*S*,4a*S*,8a*S*)-3-[(1,1-dimethylethyl)carbamoyl]octahydroisoquinolin-2(1*H*)-yl]-2-hydroxypropyl]amino]-1-(cyanomethyl)-2-oxoethyl]quinoline-2-carboxamide,



G. methyl (3*S*)-4-[[(1*S*,2*R*)-1-benzyl-3-[(3*S*,4a*S*,8a*S*)-3-[(1,1-dimethylethyl)carbamoyl]octahydroisoquinolin-2(1*H*)-yl]-2-hydroxypropyl]amino]-4-oxo-3-[(quinolin-2-ylcarbonyl)amino]butanoate,

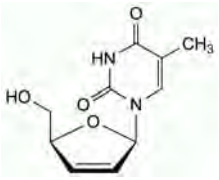


H. *N*-[(3*S*)-1-[(1*S*,2*R*)-1-benzyl-3-[(3*S*,4a*S*,8a*S*)-3-[(1,1-dimethylethyl)carbamoyl]octahydroisoquinolin-2(1*H*)-yl]-2-hydroxypropyl]-2,5-dioxopyrrolidin-3-yl]quinoline-2-carboxamide.

01/2015:2130

STAVUDINE

Stavudinum



$C_{10}H_{12}N_2O_4$ M_r 224.2
[3056-17-5]

DEFINITION

1-(2,3-Dideoxy-β-D-*glycero*-pent-2-enofuranosyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione.

Content: 97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white powder.

Solubility: soluble in water, sparingly soluble in ethanol (96 per cent), slightly soluble in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Specific optical rotation (*2.2.7*): – 45.9 to – 39.5 (anhydrous substance).

Dissolve 0.100 g in *water R* and dilute to 10.0 mL with the same solvent.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *stavudine CRS*.

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *anhydrous ethanol R*, evaporate to dryness and record new spectra using the residues.

TESTS

Related substances. Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use or store them at 2-8 °C until use.*

Test solution. Dissolve 25.0 mg of the substance to be examined in *water R* and dilute to 50.0 mL with the same solvent.

Reference solution (a). Dilute 0.5 mL of the test solution to 100.0 mL with *water R*.

Reference solution (b). Dilute 20.0 mL of reference solution (a) to 100.0 mL with *water R*.

Reference solution (c). Dissolve 5 mg of *stavudine for system suitability CRS* (containing impurities A, B, C, E and G) in *water R* and dilute to 10.0 mL with the same solvent.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm;
– *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

– *mobile phase A*: mix 35 volumes of *acetonitrile for chromatography R* and 965 volumes of a 0.77 g/L solution of *ammonium acetate R*;
– *mobile phase B*: mix 250 volumes of *acetonitrile for chromatography R* and 750 volumes of a 0.77 g/L solution of *ammonium acetate R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	100	0
10 - 20	100 → 0	0 → 100
20 - 30	0	100

Flow rate: 2.0 mL/min.

Detection: spectrophotometer at 254 nm.

Injection: 10 µL.

Identification of impurities: use the chromatogram supplied with *stavudine for system suitability CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B, C, E and G.

Relative retention with reference to stavudine (retention time = about 10 min): impurity A = about 0.3; impurity B = about 0.50; impurity C = about 0.53; impurity E = about 1.1; impurity G = about 1.9.

System suitability: reference solution (c):

– *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity C and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity B; minimum 1.5, where H_p = height above the baseline of the peak due to impurity E and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to stavudine.

Calculation of percentage contents:

– *correction factor*: multiply the peak area of impurity A by 0.7;
– for impurity A, use the concentration of stavudine in reference solution (a);
– for impurities other than A, use the concentration of stavudine in reference solution (b).

Limits:

– *impurity A*: maximum 0.5 per cent;
– *impurity G*: maximum 0.2 per cent;
– *unspecified impurities*: for each impurity, maximum 0.10 per cent;
– *total*: maximum 1.0 per cent;
– *reporting threshold*: 0.05 per cent.

Impurity I. Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use or store them at 2-8 °C until use.*

Test solution. Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (a). Dissolve 2 mg of *stavudine impurity I CRS* in the mobile phase and dilute to 50.0 mL with the mobile phase. Dilute 1.0 mL of the solution to 20.0 mL with the mobile phase.

Reference solution (b). Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm;
– *stationary phase*: *end-capped octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: mix 30 volumes of *acetonitrile for chromatography R* and 70 volumes of a 1.15 g/L solution of *ammonium dihydrogen phosphate R* previously adjusted to pH 6.8 with *triethylamine R*.

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 266 nm.

Injection: 20 µL.

Run time: 7 times the retention time of stavudine.

Identification of impurities: use the chromatogram obtained with reference solution (a) to identify the peak due to impurity I.

Relative retention with reference to stavudine (retention time = about 3 min): impurity I = about 6.0.

System suitability: reference solution (b):

– *signal-to-noise ratio*: minimum 40 for the principal peak.

Calculation of percentage content:

– *correction factor*: multiply the peak area of impurity I by 1.7;

– for impurity I, use the concentration of stavudine in reference solution (b).

Limit:

– *impurity I*: maximum 0.15 per cent.

Water (*2.5.12*): maximum 0.5 per cent, determined on 0.500 g.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use or store them at 2-8 °C until use.*

Test solution. Dissolve 10.0 mg of the substance to be examined in *water R* and dilute to 100.0 mL with the same solvent. Dilute 10.0 mL of the solution to 50.0 mL with *water R*.

Reference solution (a). Dissolve 10.0 mg of *stavudine CRS* in *water R* and dilute to 100.0 mL with the same solvent. Dilute 10.0 mL of the solution to 50.0 mL with *water R*.

Reference solution (b). Dissolve 5 mg of *thymine R* (impurity A) and 7.5 mg of *thymidine R* (impurity C) in *water R* and dilute to 100.0 mL with the same solvent. Dilute 10.0 mL of the solution to 50.0 mL with *water R*.

Column:

– *size*: *l* = 0.033 m, Ø = 4.0 mm;

– *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase: mix 5 volumes of *acetonitrile for chromatography R* and 95 volumes of a 0.77 g/L solution of *ammonium acetate R*.

Flow rate: 0.7 mL/min.

Detection: spectrophotometer at 254 nm.

Injection: 25 µL.

Run time: twice the retention time of stavudine.

Identification of impurities: use the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A and C.

Relative retention with reference to stavudine (retention time = about 4 min): impurity A = about 0.4; impurity C = about 0.6.

System suitability:

– *resolution*: minimum 3.5 between the peaks due to impurities A and C in the chromatogram obtained with reference solution (b);

– *symmetry factor*: maximum 1.6 for the peak due to stavudine in the chromatogram obtained with reference solution (a).

Calculate the percentage content of $C_{10}H_{12}N_2O_4$ using the chromatograms obtained with the test solution and reference solution (a) and taking into account the assigned content of *stavudine CRS*.

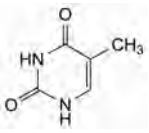
STORAGE

Protected from light and humidity.

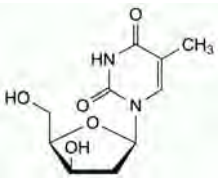
IMPURITIES

Specified impurities: A, G, I.

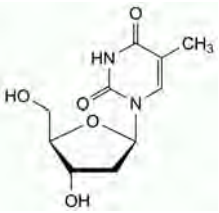
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *B, C, D, E, F, H.*



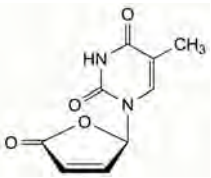
A. 5-methylpyrimidine-2,4(1*H*,3*H*)-dione (thymine),



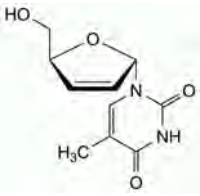
B. 1-(2-deoxy-β-D-*threo*-pentofuranosyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (3′-epithymidine),



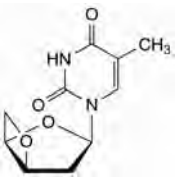
C. 1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (thymidine),



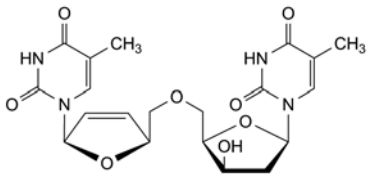
D. 1-[(2*R*)-5-oxo-2,5-dihydrofuran-2-yl]-5-methylpyrimidine-2,4(1*H*,3*H*)-dione,



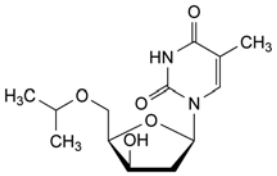
E. 1-(2,3-dideoxy-α-D-*glycero*-pent-2-enofuranosyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (stavudine anomer α),



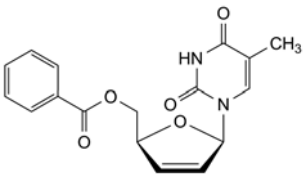
F. 1-(3,5-anhydro-2-deoxy-β-D-*threo*-pentofuranosyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione,



G. 1-[2-deoxy-5-*O*-[[(2*S*,5*R*)-5-[5-methyl-2,4-dioxo-3,4-dihydropyrimidine-1(2*H*)-yl]-2,5-dihydrofuran-2-yl]methyl]-β-D-*threo*-pentofuranosyl]-5-methylpyrimidine-2,4(1*H*,3*H*)-dione,



H. 1-[2-deoxy-5-*O*-(1-methylethyl)-β-D-*threo*-pentofuranosyl]-5-methylpyrimidine-2,4(1*H*,3*H*)-dione,

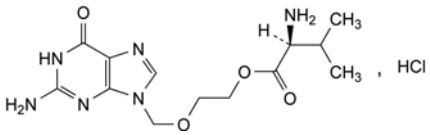


I. 1-(5-*O*-benzoyl-2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione.

07/2019:1768

VALACICLOVIR HYDROCHLORIDE

Valacicloviri hydrochloridum



$C_{13}H_{21}ClN_6O_4$ M_r 360.8
[124832-27-5]

DEFINITION

2-[(2-Amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)-methoxy]ethyl L-valinate hydrochloride.

Content: 95.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white powder.

Solubility: freely soluble in water, slightly soluble in anhydrous ethanol.

It shows polymorphism (*5.9*).

IDENTIFICATION

Carry out either tests A, B, C, E or tests A, B, D, E.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *anhydrous valaciclovir hydrochloride CRS*.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *anhydrous ethanol R* and evaporate to dryness in a desiccator, under high vacuum, over *diphosphorus pentoxide R*. Record new spectra using the residues.

B. It gives reaction (a) of chlorides (*2.3.1*).

C. It complies with the limit for impurity R given in test A for related substances.

D. Optical rotation (*2.2.7*): laevorotatory.

Dissolve 2.50 g in *water R* and dilute to 50.0 mL with the same solvent.

E. Water (see Tests).

TESTS

Impurities E, F and G. Thin-layer chromatography (*2.2.27*).

Test solution. Dissolve 0.250 g of the substance to be examined in 2 mL of *water R* and dilute to 5.0 mL with *ethanol (96 per cent) R*.

Reference solution (a). Dissolve 5 mg of *valaciclovir impurity D CRS*, 5.0 mg of *valaciclovir impurity E CRS*, 5.0 mg of *valaciclovir impurity G CRS* and 8.4 mg of *valaciclovir impurity F para-toluenesulfonate CRS* in a mixture of 2 mL of *water R* and 6 mL of *ethanol (96 per cent) R*, and dilute to 10.0 mL with *ethanol (96 per cent) R*.

Reference solution (b). Dilute 3.0 mL of reference solution (a) to 10.0 mL with *ethanol (96 per cent) R*.

Reference solution (c). Dilute 2.0 mL of reference solution (a) to 10.0 mL with *ethanol (96 per cent) R*.

Reference solution (d). Dilute 0.5 mL of reference solution (a) to 10.0 mL with *ethanol (96 per cent) R*.

Plate: *TLC silica gel F_{254} plate R* (2-10 µm).

Pretreatment: wash the plate with *methanol R* until the solvent front has migrated over at least 4/5 of the plate; allow the plate to dry.

Mobile phase: *concentrated ammonia R*, *tetrahydrofuran R*, *methanol R*, *methylene chloride R* (3:12:34:54 *V/V/V/V*); use freshly prepared mobile phase.

Application: 4 µL of the test solution and reference solutions (b), (c) and (d).

Development: over 4/5 of the plate.

Drying: in a current of air.

Detection: examine in ultraviolet light at 254 nm for impurities E and G; spray with a 0.1 g/L solution of *fluorescamine R* in *ethylene chloride R* and examine in ultraviolet light at 365 nm for impurity F.

Retardation factors: impurity A = about 0; impurity B = about 0.2; valaciclovir = about 0.3; impurity C = about 0.5; impurity D = about 0.6; impurity E = about 0.7; impurity F = about 0.75; impurity G = about 0.79; impurity C is masked by the leading edge of the spot due to valaciclovir; impurities F and G may co-elute, but this does not adversely affect their quantification because they are visualised differently.

System suitability: the chromatograms obtained with reference solutions (b), (c) and (d) each show 3 clearly separated spots when examined under ultraviolet light at 254 nm, due to impurities D, E and G.

Limits:

- *impurity E*: any spot due to impurity E is not more intense than the corresponding spot in the chromatogram obtained with reference solution (c) (0.2 per cent);
- *impurity F*: any spot due to impurity F is not more intense than the corresponding spot in the chromatogram obtained with reference solution (b) (0.3 per cent calculated as hydrochloride salt);
- *impurity G*: any spot due to impurity G is not more intense than the corresponding spot in the chromatogram obtained with reference solution (d) (0.05 per cent).

Related substances.

A. Impurities A, B, I and R. Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution. Dissolve 50.0 mg of the substance to be examined in a 0.5 per cent *V/V* solution of *hydrochloric acid R* and dilute to 100.0 mL with the same solution.

Reference solution (a). Dissolve 2.5 mg of *valaciclovir for system suitability CRS* (containing impurities A, B, C, D, H, I, J, M and R) in a 0.5 per cent *V/V* solution of *hydrochloric acid R* and dilute to 5.0 mL with the same solution.

Reference solution (b). Dissolve 50.0 mg of *anhydrous valaciclovir hydrochloride CRS* in a 0.5 per cent *V/V* solution of *hydrochloric acid R* and dilute to 100.0 mL with the same solution.

Reference solution (c). Dilute 3.0 mL of the test solution to 100.0 mL with a 0.5 per cent *V/V* solution of *hydrochloric acid R*. Dilute 1.0 mL of this solution to 100.0 mL with a 0.5 per cent *V/V* solution of *hydrochloric acid R*.

Column:

- *size*: *l* = 0.15 m, Ø = 4.0 mm;
- *stationary phase*: *crown-ether silica gel for chiral separation R* (5 µm);
- *temperature*: 10 °C.

Mobile phase: *perchloric acid R*, *methanol R*, *water for chromatography R* (0.5:5:95 *V/V/V*).

Flow rate: 0.75 mL/min.

Detection: spectrophotometer at 254 nm.

Injection: 10 µL of the test solution and reference solutions (a) and (c).

Run time: 1.5 times the retention time of valaciclovir.

Identification of impurities: use the chromatogram supplied with *valaciclovir for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A + B, C + R, D, I and M.

Relative retention with reference to valaciclovir (retention time = about 21 min): impurities A and B = about 0.2; impurity I = about 0.4; impurities C and R = about 0.6; impurity D = about 0.7; impurity M = about 1.3.

System suitability: reference solution (a):

- *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity D and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurities C and R.

Limits:

- *correction factor*: for the calculation of content, multiply the peak area of impurities A and B by 0.7;
- *impurity R*: maximum 3.0 per cent; for the calculation, subtract the content of impurity C as determined in test B for related substances from the content of the coeluting impurities C and R as determined in this test;
- *sum of impurities A and B*: maximum 2.0 per cent;
- *impurity I*: maximum 0.2 per cent;
- *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c) (0.03 per cent); disregard any peaks due to impurities other than A + B, C + R or I.

B. Liquid chromatography (*2.2.29*): use the normalisation procedure. *Use the solutions within 24 h of preparation.*

Solvent mixture: *ethanol (96 per cent) R*, *water R* (20:80 *V/V*).

Test solution. Dissolve 40 mg of the substance to be examined in the solvent mixture and dilute to 100.0 mL with the solvent mixture.

Reference solution (a). Dissolve 2.5 mg of *valaciclovir for system suitability CRS* (containing impurities A, B, C, D, H, I, J, M and R) in the solvent mixture and dilute to 5.0 mL with the solvent mixture.

Reference solution (b). Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 20.0 mL with the solvent mixture.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped phenylhexylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 15 °C.

Mobile phase:

- *mobile phase A*: *trifluoroacetic acid R*, *water for chromatography R* (0.2:100 *V/V*);
- *mobile phase B*: *trifluoroacetic acid R*, *methanol R2* (0.2:100 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	90	10
5 - 35	90 → 60	10 → 40

Flow rate: 0.8 mL/min.

Detection: spectrophotometer at 254 nm.

Injection: 10 µL.

Identification of impurities: use the chromatogram supplied with *valaciclovir for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, C, D, H, I, J and M.

Relative retention with reference to valaciclovir (retention time = about 19 min): impurity A = about 0.3; impurity B = about 0.4; impurity H = about 0.5; impurity C = about 1.06; impurity I = about 1.09; impurity D = about 1.2; impurity J = about 1.3; impurity M = about 1.6.

System suitability: reference solution (a):

- *peak-to-valley ratio*: minimum 2.5, where H_p = height above the baseline of the peak due to impurity C and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to valaciclovir;
- the chromatogram obtained is similar to the chromatogram supplied with *valaciclovir for system suitability CRS*.

Limits:

- *impurity M*: maximum 1.5 per cent;
- *impurity D*: maximum 0.5 per cent;
- *impurity C*: maximum 0.3 per cent;
- *impurities H, J*: for each impurity, maximum 0.10 per cent;
- *unspecified impurities*: for each impurity, maximum 0.05 per cent;
- *disregard limit*: 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.03 per cent); disregard the peaks due to impurities A, B and I.

Limit:

- *total for tests A and B*: maximum 5.0 per cent.

Chloride: 9.4 to 9.9 per cent (anhydrous and solvent-free substance).

Dissolve 0.350 g in 100 mL of *water R* and add 0.2 mL of *nitric acid R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M silver nitrate*. Use a silver indicator electrode and a silver-silver chloride reference electrode or a combined silver electrode. Discard the result from the first titration, which is used to condition the electrodes. Carry out a blank titration.

1 mL of *0.1 M silver nitrate* is equivalent to 3.543 mg of Cl.

Water (*2.5.12*): maximum 2.0 per cent, determined on 0.250 g.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in test A for related substances with the following modification.

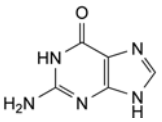
Injection: test solution and reference solution (b).

Calculate the percentage content of $C_{13}H_{21}ClN_6O_4$ taking into account the assigned content of *anhydrous valaciclovir hydrochloride CRS*.

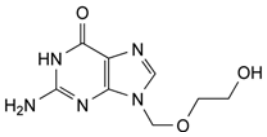
IMPURITIES

Specified impurities: A, B, C, D, E, F, G, H, I, J, M, R.

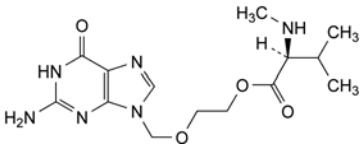
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *K, L, N, O, P, Q.*



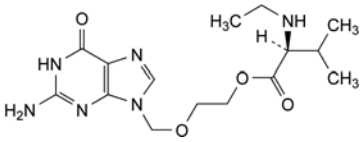
A. 2-amino-1,9-dihydro-6*H*-purin-6-one (guanine),



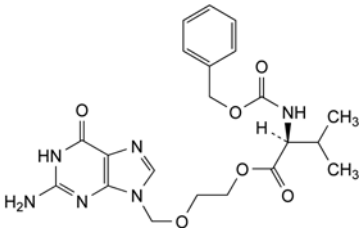
B. 2-amino-9-[(2-hydroxyethoxy)methyl]-1,9-dihydro-6*H*-purin-6-one (aciclovir),



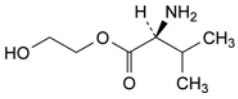
C. 2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl *N*-methyl-L-valinate,



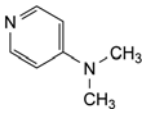
D. 2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl *N*-ethyl-L-valinate,



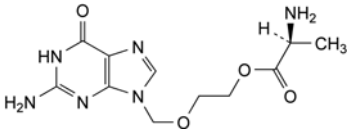
E. 2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl *N*-[(benzyloxy)carbonyl]-L-valinate,



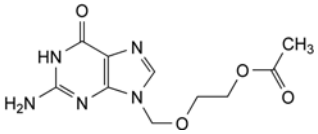
F. 2-hydroxyethyl L-valinate,



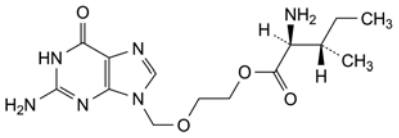
G. *N*,*N*-dimethylpyridin-4-amine,



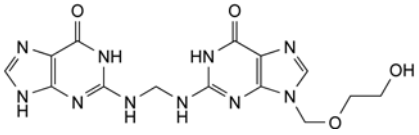
H. 2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl L-alaninate,



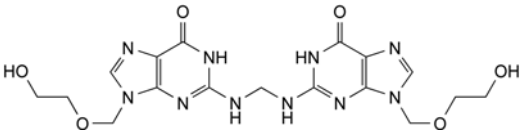
I. 2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl acetate,



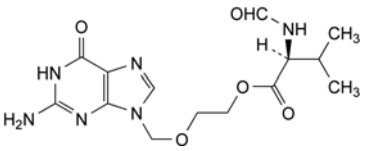
J. 2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl L-isoleucinate,



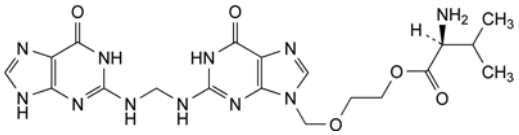
K. 9-[(2-hydroxyethoxy)methyl]-2-[[[(6-oxo-6,9-dihydro-1*H*-purin-2-yl)amino]methyl]amino]-1,9-dihydro-6*H*-purin-6-one,



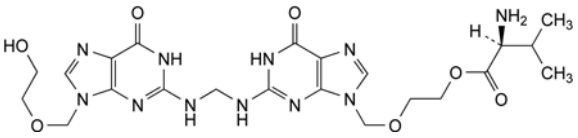
L. 2,2′-(methylenediazanediyl)bis[9-[(2-hydroxyethoxy)methyl]-1,9-dihydro-6*H*-purin-6-one],



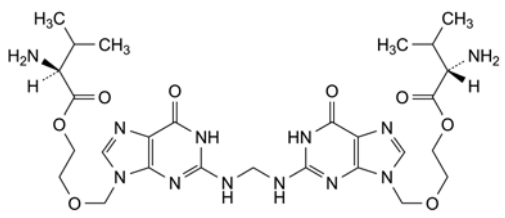
M. 2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl *N*-formyl-L-valinate,



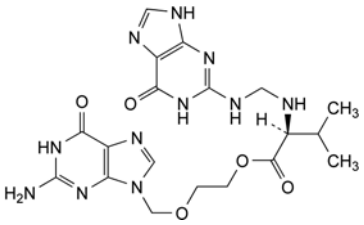
N. 2-[[6-oxo-2-[[[(6-oxo-6,9-dihydro-1*H*-purin-2-yl)amino]methyl]amino]-1,6-dihydro-9*H*-purin-9-yl]methoxy]ethyl L-valinate,



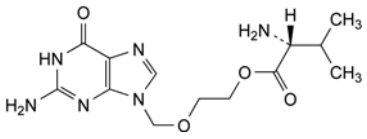
O. 2-[[2-[[[[9-[(2-hydroxyethoxy)methyl]-6-oxo-6,9-dihydro-1*H*-purin-2-yl]amino]methyl]amino]-6-oxo-1,6-dihydro-9*H*-purin-9-yl]methoxy]ethyl L-valinate,



P. [methylenebis[azanediyl(6-oxo-1,6-dihydro-9*H*-purine-2,9-diyl)methyleneoxyethan-2,1-diyl]] di-L-valinate,



Q. 2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl *N*-[[(6-oxo-6,9-dihydro-1*H*-purin-2-yl)amino]methyl]-L-valinate,

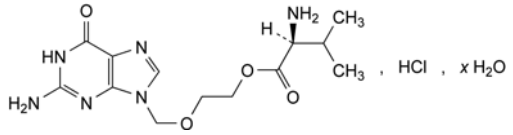


R. 2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl D-valinate.

07/2019:2751

VALACICLOVIR HYDROCHLORIDE HYDRATE

Valacicloviri hydrochloridum hydricum



$C_{13}H_{21}ClN_6O_4,xH_2O$ M_r 360.8 (anhydrous substance)
[1218948-84-5]

DEFINITION

2-[(2-Amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]-ethyl L-valinate hydrochloride hydrate.

Content: 95.0 per cent to 102.0 per cent (anhydrous substance).

It contains a variable quantity of water.

CHARACTERS

Appearance: white or almost white powder, hygroscopic.

Solubility: freely soluble in water, very slightly soluble in anhydrous ethanol, practically insoluble in acetonitrile.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation: dissolve the substance to be examined in the minimum volume of *water R*, evaporate to dryness at room temperature and record the spectrum using the residue.

Comparison: repeat the operations using *anhydrous valaciclovir hydrochloride CRS*.

B. It complies with the limit for impurity R (see test A for related substances).

C. Water (see Tests).

D. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Impurities G and S. Thin-layer chromatography (*2.2.27*).

Test solution. Dissolve 0.250 g of the substance to be examined in 2 mL of *water R* and dilute to 5.0 mL with *ethanol (96 per cent) R*.

Reference solution. Dissolve 5.0 mg of *valaciclovir impurity G CRS* and 5.0 mg of *valaciclovir impurity S CRS* in a mixture of 2 mL of *water R* and 6 mL of *ethanol (96 per cent) R* and dilute to 10.0 mL with *ethanol (96 per cent) R*. Dilute 0.5 mL of the solution to 10.0 mL with *ethanol (96 per cent) R*.

Plate: *TLC silica gel F_{254} plate R* (2-10 µm).

Pretreatment: wash the plate with *methanol R* until the solvent front has migrated over at least 4/5 of the plate; allow to dry in air.

Mobile phase: *concentrated ammonia R*, *tetrahydrofuran R*, *methanol R*, *methylene chloride R* (3:12:34:54 *V/V/V/V*); use freshly prepared mobile phase.

Application: 4 µL.

Development: over 4/5 of the plate.

Drying: in a current of air.

Detection: examine in ultraviolet light at 254 nm.

Retardation factors: valaciclovir = about 0.3; impurity S = about 0.7; impurity G = about 0.8.

System suitability: the chromatogram obtained with the reference solution shows 2 clearly separated spots due to impurities S and G.

Limits:

– *impurity G*: any spot due to impurity G is not more intense than the corresponding spot in the chromatogram obtained with the reference solution (0.05 per cent);

– *impurity S*: any spot due to impurity S is not more intense than the corresponding spot in the chromatogram obtained with the reference solution (0.05 per cent).

Related substances

A. Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution. Dissolve 50.0 mg of the substance to be examined in a 0.5 per cent *V/V* solution of *hydrochloric acid R* and dilute to 100.0 mL with the same solution.

Reference solution (a). Dissolve 2.5 mg of *valaciclovir for system suitability CRS* (containing impurities A, B, C, D, H, M and R) in a 0.5 per cent *V/V* solution of *hydrochloric acid R* and dilute to 5.0 mL with the same solution.

Reference solution (b). Dissolve 50.0 mg of *anhydrous valaciclovir hydrochloride CRS* in a 0.5 per cent *V/V* solution of *hydrochloric acid R* and dilute to 100.0 mL with the same solution.

Reference solution (c). Dilute 3.0 mL of the test solution to 100.0 mL with a 0.5 per cent *V/V* solution of *hydrochloric acid R*. Dilute 1.0 mL of this solution to 100.0 mL with a 0.5 per cent *V/V* solution of *hydrochloric acid R*.

Column:

– *size*: *l* = 0.15 m, Ø = 4.0 mm;

– *stationary phase*: *crown-ether silica gel for chiral separation R* (5 µm);

– *temperature*: 10 °C.

Mobile phase: *perchloric acid R*, *methanol R*, *water for chromatography R* (0.5:5:95 *V/V/V*).

Flow rate: 0.75 mL/min.

Detection: spectrophotometer at 254 nm.

Injection: 10 µL of the test solution and reference solutions (a) and (c).

Run time: 1.5 times the retention time of valaciclovir.

Identification of impurities: use the chromatogram supplied with *valaciclovir for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A + B, C + R, D and M.

Relative retention with reference to valaciclovir (retention time = about 17 min): impurities A and B = about 0.2; impurities C and R = about 0.6; impurity D = about 0.7; impurity M = about 1.3.

System suitability: reference solution (a):

– *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity D and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurities C and R.

Limits:

– *correction factor*: for the calculation of content, multiply the peak area of impurities A and B by 0.7;

– *impurity R*: maximum 3.0 per cent; for the calculation, subtract the content of impurity C as determined in test B for related substances from the content of the coeluting impurities C and R as determined in this test;

– *sum of impurities A and B*: maximum 2.0 per cent;

– *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c) (0.03 per cent); disregard any peaks due to impurities other than A + B and C + R.

B. Liquid chromatography (*2.2.29*): use the normalisation procedure. *Use the solutions within 24 h of preparation.*

Solvent mixture: *ethanol (96 per cent) R*, *water R* (20:80 *V/V*).

Test solution. Dissolve 80 mg of the substance to be examined in the solvent mixture and dilute to 100.0 mL with the solvent mixture.

Reference solution (a). Dissolve 1.6 mg of *valaciclovir for system suitability CRS* (containing impurities A, B, C, D, H, M and R) in the solvent mixture and dilute to 2.0 mL with the solvent mixture.

Reference solution (b). Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 20.0 mL with the solvent mixture.

Reference solution (c). Dissolve 2 mg of *valaciclovir impurity P CRS* in the solvent mixture and dilute to 25.0 mL with the solvent mixture. Dilute 1.0 mL of the solution to 100.0 mL with the solvent mixture.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped phenylhexylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 15 °C.

Mobile phase:

- *mobile phase A*: *trifluoroacetic acid R*, *water for chromatography R* (0.2:100 *V/V*);
- *mobile phase B*: *trifluoroacetic acid R*, *methanol R2* (0.2:100 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	90	10
5 - 35	90 → 60	10 → 40
35 - 45	60	40

Flow rate: 0.8 mL/min.

Detection: spectrophotometer at 254 nm.

Injection: 10 µL.

Identification of impurities: use the chromatogram supplied with *valaciclovir for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, C, D, H and M; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity P.

Relative retention with reference to valaciclovir (retention time = about 20 min): impurity A = about 0.3; impurity B = about 0.4; impurity H = about 0.5; impurity C = about 1.06; impurity D = about 1.2; impurity M = about 1.6; impurity P = about 2.0.

System suitability: reference solution (a):

- *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to impurity C and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to valaciclovir.

Limits:

- *impurity M*: maximum 0.6 per cent;
- *impurity D*: maximum 0.3 per cent;
- *impurity C*: maximum 0.2 per cent;
- *impurities H, P*: for each impurity, maximum 0.10 per cent;
- *unspecified impurities*: for each impurity, maximum 0.05 per cent;
- *disregard limit*: 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.03 per cent); disregard the peaks due to impurities A and B.

Limit:

- *total for tests A and B*: maximum 4.0 per cent.

Water (*2.5.12*): 4.5 per cent to 11.0 per cent, determined on 0.100 g.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in test A for related substances with the following modification.

Injection: test solution and reference solution (b).

Calculate the percentage content of $C_{13}H_{21}ClN_6O_4$ taking into account the assigned content of *anhydrous valaciclovir hydrochloride CRS*.

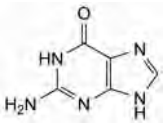
STORAGE

In an airtight container.

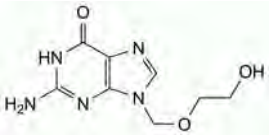
IMPURITIES

Specified impurities: A, B, C, D, G, H, M, P, R, S.

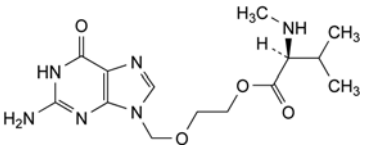
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *I, J, N.*



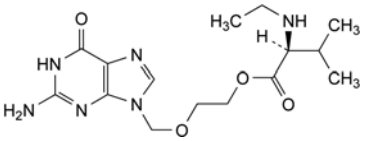
A. 2-amino-1,9-dihydro-6*H*-purin-6-one (guanine),



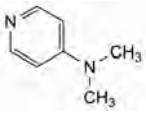
B. 2-amino-9-[(2-hydroxyethoxy)methyl]-1,9-dihydro-6*H*-purin-6-one (aciclovir),



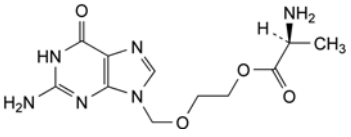
C. 2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl *N*-methyl-L-valinate,



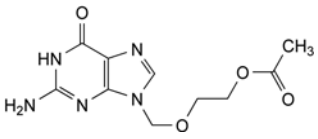
D. 2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl *N*-ethyl-L-valinate,



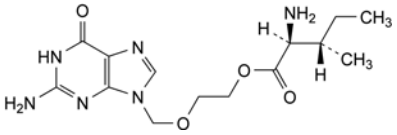
G. *N*,*N*-dimethylpyridin-4-amine,



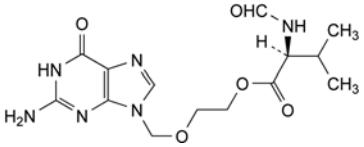
H. 2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl L-alaninate,



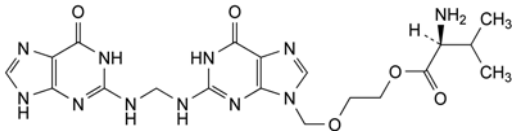
I. 2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl acetate,



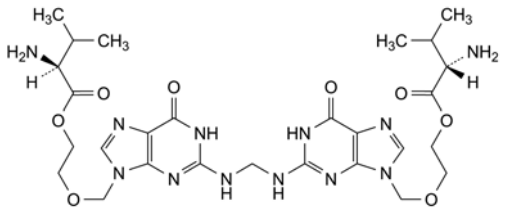
J. 2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl L-isoleucinate,



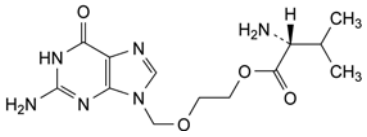
M. 2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl *N*-formyl-L-valinate,



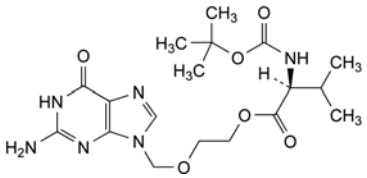
N. 2-[[6-oxo-2-[[[(6-oxo-6,9-dihydro-1*H*-purin-2-yl)amino]methyl]amino]-1,6-dihydro-9*H*-purin-9-yl]methoxy]ethyl L-valinate,



P. [methylenebis[azanediyl(6-oxo-1,6-dihydro-9*H*-purine-2,9-diyl)methyleneoxyethan-2,1-diyl]] di-L-valinate,



R. 2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl D-valinate,

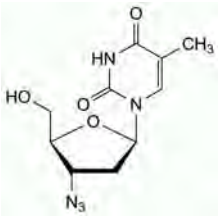


S. 2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl *N*-(*tert*-butoxycarbonyl)-L-valinate.

01/2017:1059

ZIDOVUDINE

Zidovudinum



$C_{10}H_{13}N_5O_4$ M_r 267.2
[30516-87-1]

DEFINITION

1-(3-Azido-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione.

Content: 97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance: white or slightly brownish powder.

Solubility: sparingly soluble in water, soluble in anhydrous ethanol, practically insoluble in heptane.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *zidovudine CRS*.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *water R*, evaporate to dryness in a desiccator, under high vacuum over *diphosphorus pentoxide R* and record new spectra using the residues.

TESTS

Appearance of solution. The solution is not more opalescent than reference suspension I (*2.2.1*) and not more intensely coloured than reference solution BY_5 (*2.2.2, Method II*).

Dissolve 0.5 g in 50 mL of *water R*, heating if necessary.

Specific optical rotation (*2.2.7*): + 60.5 to + 63.0 (dried substance), measured at 25 °C.

Dissolve 0.50 g in *anhydrous ethanol R* and dilute to 50.0 mL with the same solvent.

Related substances

A. Liquid chromatography (*2.2.29*).

Solvent mixture. Mix 4 volumes of *acetonitrile R*, 20 volumes of *methanol R* and 76 volumes of a 2 g/L solution of *ammonium acetate R* previously adjusted to pH 6.8 with *dilute acetic acid R*.

Test solution (a). Dissolve 20.0 mg of the substance to be examined in the solvent mixture and dilute to 20.0 mL with the solvent mixture.

Test solution (b). Dilute 10.0 mL of test solution (a) to 50.0 mL with the solvent mixture.

Reference solution (a). Dissolve 2 mg of *thymine R* (impurity C) and 2 mg of *zidovudine impurity B CRS* in the solvent mixture and dilute to 50.0 mL with the solvent mixture. Dilute 1.0 mL of the solution to 20.0 mL with the solvent mixture.

Reference solution (b). Dissolve 5 mg of *zidovudine for system suitability CRS* (containing impurities A, G and H) in reference solution (a) and dilute to 5.0 mL with reference solution (a).

Reference solution (c). Dilute 1.0 mL of test solution (a) to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (d). Dissolve 20.0 mg of *zidovudine CRS* in the solvent mixture and dilute to 20.0 mL with the solvent mixture. Dilute 10.0 mL of the solution to 50.0 mL with the solvent mixture.

Reference solution (e). Dissolve 1 mg of *zidovudine impurity D CRS* in a mixture of 4 volumes of *acetonitrile R*, 40 volumes of *methanol R* and 56 volumes of a 2 g/L solution of *ammonium acetate R* previously adjusted to pH 6.8 with *dilute acetic acid R* and dilute to 50.0 mL with the same mixture of solvents. Dilute 5.0 mL of the solution to 10.0 mL with the same mixture of solvents.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

- *mobile phase A*: 2 g/L solution of *ammonium acetate R* previously adjusted to pH 6.8 with *dilute acetic acid R*;
- *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	95	5
3 - 18	95 → 85	5 → 15
18 - 28	85 → 30	15 → 70
28 - 43	30	70

Flow rate: 1.5 mL/min.

Detection: spectrophotometer at 265 nm.

Injection: 20 µL of test solution (a) and reference solutions (b), (c) and (e).

Identification of impurities: use the chromatogram supplied with *zidovudine for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B, C, G and H; use the chromatogram obtained with reference solution (e) to identify the peak due to impurity D.

Relative retention with reference to zidovudine (retention time = about 16 min): impurity C = about 0.2; impurity A = about 0.5; impurity H = about 0.95; impurity B = about 1.05; impurity G = about 1.5; impurity D = about 2.0.

System suitability: reference solution (b):

- *resolution*: minimum 2.0 between the peaks due to impurity H and zidovudine; minimum 2.0 between the peaks due to zidovudine and impurity B.

Calculation of percentage contents:

- *correction factor*: multiply the peak area of impurity C by 0.6;
- for each impurity, use the concentration of zidovudine in reference solution (c).

Limits:

- *impurity G*: maximum 0.5 per cent;
- *impurities A and C*: for each impurity, maximum 0.15 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *reporting threshold*: 0.05 per cent; disregard any peak due to impurity D and any peak eluted after this impurity.

B. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 0.5 g of the substance to be examined in 10 mL of *acetonitrile R1* and dilute to 100.0 mL with the mobile phase.

Reference solution (a). Dissolve 5.0 mg of *zidovudine impurity D CRS* in *acetonitrile R1* and dilute to 10.0 mL with the same solvent.

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

Reference solution (c). Dilute 1.0 mL of reference solution (a) to 50.0 mL with the test solution.

Column:

– *size*: *l* = 0.15 m, Ø = 4.6 mm;

– *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: *water for chromatography R, acetonitrile R1* (30:70 *V/V*).

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 210 nm.

Injection: 20 µL of the test solution and reference solutions (b) and (c).

Run time: 10 times the retention time of zidovudine.

Identification of impurities: use the chromatogram obtained with reference solution (b) to identify the peak due to impurity D.

Relative retention with reference to zidovudine (retention time = about 1.5 min): impurity D = about 2.5.

System suitability: reference solution (c):

– *resolution*: minimum 5.0 between the peaks due to zidovudine and impurity D.

Calculation of percentage contents:

– for each impurity, use the concentration of impurity D in reference solution (b).

Limits:

– *unspecified impurities*: for each impurity, maximum 0.10 per cent;

– *reporting threshold*: 0.05 per cent; disregard any peak eluted before impurity D.

Limit:

– *total for tests A and B*: maximum 1.0 per cent.

Loss on drying (*2.2.32*): maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in test A for related substances with the following modification.

Injection: test solution (b) and reference solution (d).

Calculate the percentage content of $C_{10}H_{13}N_5O_4$ taking into account the assigned content of *zidovudine CRS*.

STORAGE

Protected from light.

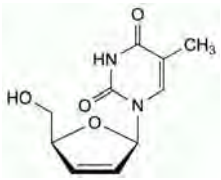
IMPURITIES

Test A for related substances: A, B, C, E, F, G, H.

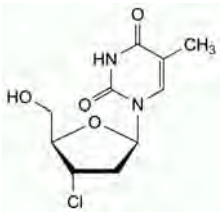
Test B for related substances: D, J, K.

Specified impurities: A, C, G.

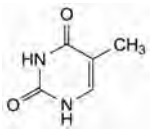
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *B, D, E, F, H, J, K.*



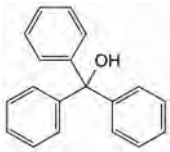
A. 1-[(2*R*,5*S*)-5-(hydroxymethyl)-2,5-dihydrofuran-2-yl]-5-methylpyrimidine-2,4(1*H*,3*H*)-dione,



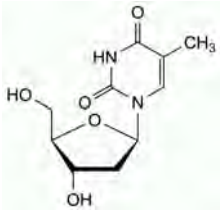
B. 1-(3-chloro-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione,



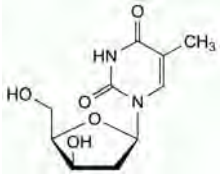
C. 5-methylpyrimidine-2,4(1*H*,3*H*)-dione (thymine),



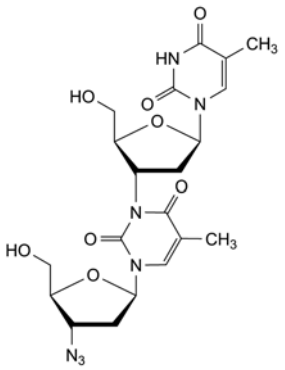
D. triphenylmethanol,



E. 1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (thymidine),

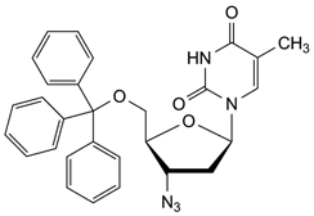


F. 1-(2-deoxy-β-D-*threo*-pentofuranosyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione,

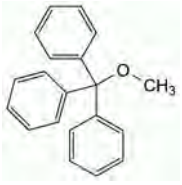


G. 1-[(2*R*,4*S*,5*S*)-4-azido-5-(hydroxymethyl)tetrahydrofuran-2-yl]-3-[(2*S*,3*S*,5*R*)-2-(hydroxymethyl)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-3-yl]-5-methylpyrimidine-2,4(1*H*,3*H*)-dione,

H. unknown structure,



J. 1-[3-azido-2,3-dideoxy-5-*O*-(triphenylmethyl)-β-D-*erythro*-pentofuranosyl]-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (trityl zidovudine),



K. 1,1′,1″-(methoxymethanetriyl)tribenzene (methyl trityl ether).