Thai Pharmacopoeia II
Volume I Part 1

SUPPLEMENT 2020

กรมวิทยาศาสตร์การแพทย์
DEPARTMENT OF MEDICAL SCIENCES

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Volume I Part 1
SUPPLEMENT 2020

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and the Food and Drug Administration of Thailand

by

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PREFACE

Medical uses of preparations derived from cannabis have long been controversial, especially after being listed in the United Nations Single Convention on Narcotic Drugs, 1961 as a drug with restricted uses and for scientific purposes. However, cannabis has regained global interest in its health benefits as numerous scientific studies have suggested that cannabinoids, substances found in cannabis, may be used for treatments of a spectrum of conditions.

Aligned with the global trend, the demand for medical cannabis in Thailand has been evolving over the past few years as people have been seeking alternative treatments for several diseases, especially those of which effective cures or treatments are scarce or unavailable. Realizing the medical benefits of cannabis-derived preparations, the Ministry of Public Health, following the Thai Government's initiation to legalize the production, import, export, possession, and use of cannabis products for medicinal purposes, as well as for research studies relevant to medical cannabis. The Collaborating Center for Cannabis Testing Laboratories was subsequently established to provide analytical services of medical cannabis. Meanwhile, the Department of Medical Sciences, Ministry of Public Health published this supplement to the Thai Pharmacopoeia II, Volume I Part 1 to introduce the monographs of Cannabis Extract and Cannabis Sublingual Drops to support the implementation of relevant regulations.

The publication offers the national standards to ensure the quality, safety and efficacy of cannabis-containing products for medicinal use in Thailand, as well as to encourage domestic research, production and utilization of medical cannabis, creating conditions for the country's self-reliance and sustainable health care.

(Mr. Anutin Charnvirakul)
Deputy Prime Minister
and Minister of Public Health

THE THAI PHARMACOPOEIA COMMITTEE AND SUBCOMMITTEES

The Thai Pharmacopoeia Committee was first appointed by the Ministerial Cabinet in 1979. The functions of the Pharmacopoeia Commission are to

1. select therapeutically useful drugs for inclusion in the Pharmacopoeia;
2. establish compendial limits, tolerances and specifications for selected drugs;
3. specify appropriate tests and methods for quality control of the drugs;
4. compile complete text of the Thai Pharmacopoeia for publication;
5. issue supplements in compliance with the text;
6. revise the Thai Pharmacopoeia periodically;
7. appoint appropriate subcommittees to undertake the functions as entrusted;
8. perform any other tasks assigned by the Minister.

The Thai Pharmacopoeia Committee recruited assistance from the following subcommittees in carrying out its aforementioned functions:

1. The Subcommittee on Editorial Style

This subcommittee is responsible for:

1.1 designing the format and style for printing;

1.2 editing the text;

1.3 keeping conformity of the molecular formulae, chemical names, molecular weights, and expressions of the symbols of units throughout the text;

1.4 organizing the information compiled by the subcommittees into a pharmacopoeial form and completing the final draft of the Thai Pharmacopoeia;

1.5 attending to all matters related to editing the Pharmacopoeia.

2. The Subcommittee on Standards for Thai Herbal Drug Preparations

This subcommittee is responsible for:

2.1 producing draft specifications for Thai herbal drug preparations preselected by the Thai Pharmacopoeia Committee and compiling these specifications in monographs in the Thai Herbal Pharmacopoeia;

2.2 submitting the drafts to the Subcommittee on the Establishment of the Thai Herbal Pharmacopoeia for approval;

2.3 attending to all matters related to establishing the specifications for Thai herbal drug preparations;

2.4 preparing appendices of the tests related to the Thai herbal monographs.

3. The Subcommittee on Standards and Analytical Methods

This subcommittee is responsible for:

3.1 selecting drugs to be included in or excluded from the Thai Pharmacopoeia;

3.2 preparing draft specifications, including analytical procedure, for drug monographs in the Thai Pharmacopoeia;

3.3 preparing the appendices regarding testing methods and reagents;

3.4 performing any other assigned tasks.

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INTRODUCTION

The main objectives of this Thai Pharmacopoeia II Volume I Part 1 Supplement 2020 are to introduce the monographs of Cannabis Extract and Cannabis Sublingual Drops and to provide the standards to ensure the quality, safety and efficacy of cannabis-containing products for medicinal use in Thailand.

Most of the reagents, test solutions and methods of analysis are referred to those specified in the Thai Pharmacopoeia (TP). The appendices are revised to serve the need of those involved. Any appendices not reproduced in this edition can be found in the Thai Pharmacopoeia II 2011.

However, the appendices bearing "H" after their numerical values in the Thai Herbal Pharmacopoeia (THP), a companion book to the TP, are also adopted to facilitate the users in performing testings of crude drugs. It is to be noted that the words "crude drug" in the TP and "herbal drug" in the THP can be used interchangeably to refer to a medicinal plant-based drug.

To avoid ambiguity, the more concise tests in the monographs are introduced; the details of which are grouped under the corresponding subheadings. Another noteworthy change is the grouping of non-mandatory topics, such as safety, stability and additional information, under "Other Relevant Information".

GENERAL NOTICES

The main objective of the Thai Pharmacopoeia is to establish the Thai National Standards of selected pharmaceutical substances and preparations. Apart from their standards and requirements set forth in the monographs, the non-mandatory information given includes their main classes of actions and applications (categories) and their safety (contra-indications, warnings, etc.). Subjected to other pertinent laws and ministerial regulations and notifications promulgated by Thai Minister of Public Health, the various standards of articles set up in this book, together with their pharmacopoeial requirements, are legally recognized by the Royal Thai Government.

The General Notices and general requirements appearing in appendices provide the basic guidelines for the interpretation and application of the standards, tests, assays, and other specifications of the Pharmacopoeia. Where exceptions to the General Notices or Appendices are made, the expressions such as “unless otherwise specified”, “unless otherwise directed”, or “unless otherwise indicated” are employed.

Title

The full title of this book, including its supplements, is the Pharmacopoeia of the Kingdom of Thailand, second edition. This title may be abbreviated to Thai Pharmacopoeia, second edition, or to TP II. Where the term TP is used, without further qualification, during the period in which this Pharmacopoeia is officially effective, it refers to TP II.

“Official”, “Official Name” and “Official Standards”

The word “official”, as used in this Pharmacopoeia or with reference hereto, is synonymous with “Pharmacopoeial”, with “TP”, and with “compendial”.

The “official name” implies the “main title” of each Pharmacopoeial substance or preparation which is given in English and printed with capital letters. Any other names or subsidiary titles, where included, have the same status as the main title. They are mostly derived from the main title of that substance or preparation as they are used in the National List of Essential Medicine and the other recognized pharmacopoeiae such as the International Pharmacopoeia, the British Pharmacopoeia, and the United States Pharmacopeia. In some cases, the spelling “sulph-” may be substituted for “sulf-” in the main title. Similarly, the spelling “aluminum” may also be substituted for “aluminium”.

For crude drugs, Thai names are also included and transcribed into English following the Royal Institute’s official transliteration system. Subsidiary titles for crude drugs are their most commonly recognized English names.

In a monograph of pharmaceutical preparations, “other names” or “subsidiary titles” is nevertheless excluded.

The terminology “article” is an item for which a monograph is provided, whether a substance or a preparation.

The requirements stated in the monographs of the Pharmacopoeia apply to articles that are intended only for medicinal use. An article intended or labelled for medicinal use that is described by means of an official title must comply with the requirements of the relevant monograph. A formulated preparation must comply throughout its assigned shelf-life (period of validity). The subject of any other monograph must comply throughout its period of use.

Unless otherwise indicated in the General Notices or in the monographs, statements in monographs constitute mandatory requirements. The chemical formula and solubility statements are presented for informative purposes only and are not to be considered in the same category as the standards or tests for purity. The tests or methods in general appendices become mandatory when referred to in a monograph, unless such reference is made in a way to indicate that it is not the intention to make the text referred to mandatory but rather to cite it for information.

An article is not of pharmacopoeial quality unless it complies with all of the requirements stated. The general notice on Assays and Tests indicates that analytical methods other than those described in the Pharmacopoeia may be employed for routine purposes.

Where a monograph on a biological substance or preparation refers to a strain, a test, a method, a substance, etc., using the qualifications “suitable” or “appropriate” without further definition in the text, the choice of such strain, test, method, substance, etc., is made in accordance with any international agreements or national regulations affecting the subject concerned.

The designation "TP" in conjunction with the official title or elsewhere on the label of article indicates that a monograph is included in the Thai Pharmacopoeia and that the article must comply with all applicable TP standards.

Printing Types

In the text, words which refer to reagents, which conform to the requirements specified in the appendices of other parts of the book, and the systematic names of plants, animals and micro-organisms are usually printed in italics to distinguish them from the other words in that portion of the text.

Significant Figures and Tolerances

Where limits are expressed numerically herein, the upper and lower limits of a range include the two values themselves and all intermediate values, but no values outside the limits. The limits expressed in monograph definitions and tests, regardless of whether the values are expressed as percentages or as absolute numbers, are considered significant to the last digit shown.

EQUIVALENCE STATEMENTS IN TITRIMETRIC PROCEDURES In the equivalence statement of titrimetric procedures, the number of significant figures in the concentration of the titrant corresponds to the number of significant figures in the weight of the analyte.

TOLERANCES The limits specified in the monographs for Pharmacopoeial articles are established with a view to the use of these articles as drugs, except where it is indicated otherwise. The use of the molecular formula for the active ingredient(s) named in defining the required strength of a Pharmacopoeial article is intended to designate the chemical entity or entities, as given in the complete chemical name of the article, having absolute (100 per cent) purity.

A dosage form shall be formulated with the intent to provide 100 per cent of the quantity of each ingredient declared on the label. The tolerances and limits stated in the Definitions in the monographs for Pharmacopoeial articles allow for analytical error, for unavoidable variations in manufacturing and compounding, and for deterioration to an extent considered acceptable under practical conditions. No further tolerances should be applied to the values obtained in an assay to determine whether the product being examined complies with the requirements of monograph.

The specified tolerances are based upon such attributes of quality as might be expected to characterize an article produced from suitable raw materials under recognized principles of good manufacturing practice.

INTERPRETATION OF REQUIREMENTS Analytical results in the laboratory (observed or calculated from experimental measurements) are compared with stated limits to determine whether there is conformance with compendial assay or test requirements. The observed or calculated values usually will contain more significant figures than there are in the stated limit, and a reportable result is to be rounded off to the number of places that is in agreement with the limit expression by the following procedure. Intermediate calculations (e.g., slope for linearity in validation of compendial method) may be rounded for reporting purposes, but the original value (not rounded) should be used for any additional required calculations. Rounding off should not be done until the final calculations for the reportable value have been completed. (**Note** Limits, which are fixed numbers, are not rounded off.) When rounding off is required, consider only one digit in the decimal place to the right of the last place in the limit expression. If this digit is smaller than 5, it is eliminated and the preceding digit is unchanged. If this digit is equal to 5 or more than 5, it is eliminated and the preceding digit is increased by one.

Illustration of Rounding Numerical Values for Comparison with Requirements

Compendial Requirement	Unrounded Value		Rounded Result		Conforms
Assay limit ≥99.0 per cent	98.97	per cent	99.0	per cent	Yes
	98.93	per cent	98.9	per cent	No
	98.95	per cent	99.0	per cent	Yes
Assay limit ≤102.5 per cent	102.55	per cent	102.6	per cent	No
	102.54	per cent	102.5	per cent	Yes
	102.56	per cent	102.6	per cent	No
Limit test ≤0.03 per cent	0.035	per cent	0.04	per cent	No
	0.034	per cent	0.03	per cent	Yes
	0.037	per cent	0.04	per cent	No
Limit test ≤2 ppm	2.4	ppm	2	ppm	Yes
	2.5	ppm	3	ppm	No
	2.6	ppm	3	ppm	No

Limit of Content

When limits of content are prescribed in a monograph, they are determined by the method described under "Assay".

When the result of a test or assay is referred to the "dried", "anhydrous" or "ignited" substance, the determination of loss on drying, water content or loss on ignition is carried out by the method prescribed under the heading "Loss on drying", "Water" or "Loss on ignition", respectively, in the monograph concerned.

Freshly and Recently Prepared

The direction that a preparation must be freshly prepared indicates that it must be made not more than 24 hours before it is issued for use. The direction that a preparation should be recently prepared indicates that deterioration is likely if the preparation is stored for longer than about 4 weeks at 15° to 25°.

Added Substances

The word "added substances" as used in this Pharmacopoeia or with reference hereto is synonymous with "auxiliary substances".

An official substance, as distinguished from an official preparation, contains no added substances except where specifically permitted in the individual monograph. Where such addition is permitted, the label indicates the name(s) and amount(s) of any added substance(s).

Unless otherwise specified in the individual monograph, or elsewhere in the General Notices, suitable substances such as antimicrobial agents, bases, carriers, coatings, colours, flavours, preservatives, stabilizers, and vehicles may be added to an official preparation to enhance its stability, usefulness, or elegance or to facilitate its preparation. Such substances are regarded as unsuitable and are prohibited unless (a) they are harmless in the amounts used, (b) they do not exceed the minimum quantity required to provide their intended effect, (c) their presence does not impair the bioavailability or the therapeutic efficacy or safety of the official preparation, and (d) they do not interfere with the assays and tests prescribed for determining compliance with the Pharmacopoeial standards.

Colouring Agents

Added substances employed solely to impart colour may be incorporated into official preparations, except those intended for parenteral or ophthalmic use, in accordance with the regulations pertaining to the use of colouring agents issued by the Thai Food and Drug Administration provided such added substances are otherwise appropriate in all respects.

Description

Information on the "description" pertaining to an article, which is relatively general in nature, is provided for those who use, prepare, and dispense drugs, solely to indicate the properties of an article complying with monograph standards. The properties are not in themselves standards or tests for purity even though they may indirectly assist in the preliminary evaluation of the integrity of an article.

In the case of crude drugs, the macroscopical description of a drug includes those features which can be seen by the unaided eye or by the use of a hand lens. The diagnostic characters given under a powdered crude drug are to be read in conjunction with the microscopical description given under the whole drug.

Statements of the distinctive microscopic structural elements in powdered crude drugs may be included in the individual monograph as a means of determining identity, quality, or purity.

Solubility

Statements given in the monographs under "Solubility" are intended as information on the approximate solubility only and are not regarded as part of the standard for a substance. Where no temperature is given, statements of solubility apply at ordinary room temperature.

Statements given under "Solubility test" express exact requirements and constitute part of the standards for the substances under which they occur.

When the term "parts" is used in expressing the solubility of a substance, it means parts by weight (grams) of a solid in parts by volume (millilitres) of the solvent, or parts by volume (millilitres) of a liquid in parts by volume (millilitres) of the solvent. The following table indicates the meanings of such terms:

Descriptive Term	Parts of Solvent Required for 1 Part 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 or insoluble	more than 10,000

Category

A statement of Category usually is provided for each pharmacopoeial substance. This indicates the class of its main pharmacological action and its application. Where there are several main actions or applications, usually not more than the two most important are selected, with due consideration to the prevailing medical situation. No attempt has been made to describe in detail the actions and uses of a drug nor to specify its indications. The statement of Category is thus not intended to limit in any way the choice or use of the drug nor to indicate that it has no other activity or utility.

Strength(s) Available

Statements under the side-headings Strength or Strengths Available are included as a guide and are not necessarily comprehensive. For solid dosage forms such as Capsules and Tablets, the strength is given as the amount of active ingredient in each unit. For liquid dosage forms such as Injections and semi-solid dosage forms such as Creams, the strength is given as a concentration. For Powders for Injections, the strength is given as the amount of active ingredient in each sealed container. Unless otherwise stated, the strength is given in terms of the weight or concentration of the official medicinal substance used in making the formulation.

Dose

The statements given under "Dose" in the monographs of the Pharmacopoeia are primarily intended to serve only as a guide to physicians or pharmacists who may vary it in the best interests of patients and in accordance with the variables that affect the action of the drug.

The specific terms used to classify the age of the patients are as follows:
- Adults: over 12 years of age
- Adolescents: 12 to 15 years of age
- Children: 1 to 12 years of age
- Infants: 5 weeks to 1 year of age
- Neonates: from newborn to 1 month of age

Unless otherwise specified, the route of administration is oral.

The statements of dosage in the case of Capsules and Tablets are expressed in terms of the content of active ingredient and seldom represent the total weight of the capsule contents or of the tablets.

In some instances, the dosage may be stated in terms of the pharmacologically active portion (moiety) of the molecule in order to permit the prescriber or dispenser to correlate the weight equivalent for salts, esters, or other chemical forms of the same drug moiety. However, it is not to be inferred that all chemical forms in which the active moiety may be presented are therapeutically equivalent.

Where the body surface area is stated as a basis for the specified dose, the "Table of Body Surface Area from Height and Weight (m^2)" in Appendix 1.17 may be employed to simplify the calculation of square metres of body surface.

The dose given in each monograph is that which may ordinarily be expected to produce in the patients with normal renal/hepatic function, following administration in the manner indicated, at such time intervals as may be stated, the diagnostic, therapeutic or prophylactic effect for which the monograph is recognized.

Other Relevant Information

The statements under "Other Relevant Information" provide information that does not constitute standards, but enable better understanding of the drug. This includes, but not limited to, safety information such as contra-indications, warnings and precautions; specific routes of administration to be used for a particular drug; any pertinent personal observation or care for specific patient groups, and other related information such as stability data. Selection of information is based on what is considered practical and significant in an effective and safe use of a drug, minimizing risks and maximizing benefits from its use.

Packaging and Storage

The substances and preparations described in the Pharmacopoeia are stored in such a way as to prevent contamination and, as far as possible, deterioration. Precautions that should be taken in relation to the effects of the atmosphere, moisture, heat, and light are indicated, where appropriate, in the monographs.

CONTAINERS

The container is the device that holds the substance, either in the form of the raw material or of the finished dosage form. The closure of the container, including the stopper, the cap, the attached dropper, etc., is considered as a part of the container.

The *immediate container* is the one which is in direct contact with the substance.

The container should be cleaned before use, and no extraneous matter should be introduced into it or into the substance placed in it. It must, likewise, not interact physically or chemically with the substance which it holds so as to alter the latter's quality, purity, or therapeutic potency to a level below its Pharmacopoeial requirements.

Well-closed container

A well-closed container must protect the contents from extraneous matter or from loss of the substance under ordinary or customary conditions of handling, shipment, storage, or sale.

Tightly closed container

A tightly closed container must protect the contents from contamination by extraneous matter or moisture, from loss of the substance, and from efflorescence, deliquescence, or evaporation under the ordinary or customary conditions of handling, shipment, storage, or sale, and shall be capable of tight reclosure. Where a tightly closed container is specified, it may be replaced by a hermetically closed container for a single-dose of the substance.

Hermetically closed container

A hermetically closed container must be impervious to air or any other gas under the ordinary customary conditions of handling, shipment, storage, or sale.

Light-resistant container

A light-resistant container is the one which prevents transmission of light, such as an opaque container or a bottle of black, dark red or dark brown glass.

STORAGE

The following expressions are used in monographs under Packaging and storage with the meaning shown.

Protected from light means that the product is to be stored either in a light-resistant container or in a container enclosed in an outer cover that provides such protection or stored in a place from which all such light is excluded.

STORAGE TEMPERATURES

When special conditions of storage are necessary, including limits of temperature, they are prescribed in the monograph. Where, in a monograph, the storage conditions are mentioned using the general expressions "at room temperature", "in a cold place", and the like, these terms are generally defined as follows.

Very cold temperature Any temperature above –10° but not higher than 8°. A *refrigerator* is a very cold place in which the temperature is maintained thermostatically between 2° and 8°.

Cold temperature Any temperature above 8° but not higher than 16°.

Cool temperature Any temperature above 16° but not higher than 23°.

Room temperature Any temperature above 23° but not higher than 35°.

Labelling

Every article in this Pharmacopoeia shall bear one or more labels indicating its identity and certain pertinent characteristics. This label(s) shall be affixed or firmly fastened to, or indelibly written on, the article's immediate container and, if any, its package. The label shall comply, in addition to the requirements prescribed in the Pharmacopoeia, with those labelling requirements currently promulgated by the Thai Ministry of Public Health. In cases where contradiction might arise between these requirements the latter bears more legal authority.

A *shipping* container, or a shipping package, unless such container or package is also essentially the immediate container or the outside of the consumer package, is exempt from the labelling requirements of this Pharmacopoeia.

Labelling requirements, also, do not necessarily apply when the article is supplied in compliance with a medical prescription.

The label on the container and the label on the package of every official article state:

(1) the name (commercial and/or generic) of the article, together with its dosage form;

(2) for herbal drugs and herbal drug preparations, where applicable, the Latin binomial and, following the official name, the part of the plant source from which the article was derived;

(3) the name and address of the manufacturer;

(4) amount(s) of active ingredient(s) per dosage unit (capsule, tablet, or other unit dosage form), per gram (or 100 grams), per millilitre (or 100 millilitres), or per other measurement, as the case may be;

(5) total amount of the article in the immediate container;

(6) batch or lot, or quality control, number or code of the product;

(7) the expiration date;

(8) where applicable, the name(s) of any antimicrobial preservative(s);

(9) for herbal drug preparations, "Consult a physician or pharmacist prior to use if you are pregnant or breastfeeding";

(10) other statements specifically required under "Labelling" in that article's monograph.

Where an expiration date is required to be stated on the label, this date identifies the time during which the article may be expected to meet the requirements of the Pharmacopoeial monograph, provided it is kept under the prescribed storage conditions. The expiration date limits the time during which the product may be dispensed or used. Where an expiration date is stated only in terms of the month and the year, it is

a representation that the intended expiration date is the last day of the stated month. For articles requiring constitution prior to use, a suitable beyond-use date for the constituted product shall be identified on the label. The beyond-use date placed on the label shall not be later than the expiration date on the manufacturer's container.

Identification

Identification tests are provided for the verification of the identity of the substance described in the monograph. A judgment by the analyst is needed as to the extent of testing required, taking into account the available instrumentation.

It is generally recognized that the infrared spectrum provides the best method of identification because of the uniqueness of a well-developed "fingerprint" region of the spectrum for a given drug substance.

Wherever possible, infrared spectrum characteristics are used as the primary test of identification. Usually this can stand by itself without any additional test. However, where the article is a salt, it is necessary to include a "specific ion test". Further identification tests provided in an individual monograph, taken together, are intended to provide verification of identity, should the use of an infrared spectrophotometer be precluded. Such identification tests, however specific, are not necessarily sufficient to establish proof of identity; but failure of an article taken from a labelled container to meet the requirements of a prescribed identification test indicates that the article may be mislabelled. Other tests and specifications in the monograph often contribute to establishing or confirming the identity of the article under examination.

It should further be noted that whenever a melting temperature is provided under the heading "Identification", an approximate value is usually given, since no exact reproduction of the quoted temperature is necessary.

Arsenic and Heavy Metals

With regard to vegetable drugs, the toxic elements which may be present in sufficient quantity to pose potential risk vary from plant to plant. The amount of these elements depends on the location, the quality of the soil, or environmental pollution. Because of their toxic natures, arsenic and heavy metals are of major concern. Although not specifically required in the monograph, it is suggested that the maximum amounts of the toxic elements, based on the acceptable daily intake (ADI) values, in final dosage forms of plant materials be as follows:

Arsenic	4	ppm
Cadmium	0.3	ppm
Lead	10	ppm
Mercury	0.5	ppm

Unless otherwise indicated, the test procedures are provided in the "Limit Tests for Heavy Metals in Herbal Drugs and Herbal Drug Preparations" (Appendix 5.2).

Microbial Contamination

Although not specifically required in the monographs, possible microbial contamination should be controlled to such an extent that the preparations derived from them meet the requirements as described in the "Limits for Microbial Contamination" (Appendix 10.5).

Water and Loss on Drying

Where the water of hydration or adsorbed water of a Pharmacopoeial article is determined by the titrimetric method, the test is generally given under the heading "Water". Where the determination is made by drying under specified conditions, the test is generally given under the heading "Loss on drying". However, "Loss on drying" is most often given as the heading where the loss in weight is known to represent residual volatile constituents including organic solvents as well as water.

Assays and Tests

The Assays and Tests described are the official methods upon which the standards of the Pharmacopoeia depend. Assay and test procedures are provided for determining compliance with the Pharmacopoeial standards of identity, strength, quality and purity. The analysts are not precluded from employing alternative methods, including methods of micro-analysis, in any Assay or Test if they are satisfied that the method which they use will give the results of equivalent accuracy. In the event of doubt or dispute, the methods of analysis described in the Pharmacopoeia alone are authoritative.

When the solvent used for a solution is not named, the solvent is Purified Water.

The use of a proprietary designation to identify a material used in an Assay or Test does not imply that another equally suitable material may not be used.

APPARATUS

A specification for a definite size or type of container or apparatus in a test or assay is given solely as a recommendation. Where volumetric flasks or other exact measuring, weighing, or sorting devices are specified, this or other equipment of at least equivalent accuracy shall be employed. Where low-actinic or light-resistant containers are specified, clear containers that have been rendered opaque by application of a suitable coating or wrapping may be used.

Where an instrument for physical measurement, such as a spectrophotometer, is specified in a test or assay by its distinctive name, another instrument of equivalent or greater sensitivity and accuracy may be used. In order to obtain solutions having concentrations that are adaptable to the working range of the instrument being used, solutions of proportionately higher or lower concentrations may be prepared according to the solvents and proportions thereof that are specified for the procedure. Where a particular brand or source of a material, instrument, or piece of equipment, or the name and address of a manufacturer or distributor, is mentioned, this identification is furnished solely for informational purposes as a matter of convenience, without implication of approval, endorsement, or certification. Items capable of equal or better performance may be used if these characteristics have been validated.

Where the use of a centrifuge is indicated, unless otherwise specified, the directions are predicated upon the use of apparatus having an effective radius of about 20 cm (8 inches) and driven at a speed sufficient to clarify the supernatant layer within 15 minutes.

Unless otherwise specified, for chromatographic tubes and columns the diameter specified refers to internal diameter; for other types of tubes and tubing the diameter specified refers to outside diameter.

While one of the primary objectives of the Pharmacopoeia is to assure the user of official articles of their identity, strength, quality, and purity, it is manifestly impossible to include in each monograph a test for every impurity, contaminant, or adulterant that might be present, including microbial contamination. These may arise from a change in the source of material or from a change in the processing, or may be introduced from extraneous sources. Tests suitable for detecting such occurrences, the presence of which is inconsistent with applicable good manufacturing practice or good pharmaceutical practice, should be employed in addition to the tests provided in the individual monograph.

PROCEDURE

In performing the assay or test procedures in this Pharmacopoeia, it is expected that safe laboratory practices will be followed. This includes the use of precautionary measures, protective equipment, and work practices consistent with the chemicals and procedures used. Prior to undertaking any assay or procedure described in this Pharmacopoeia, the individual should be aware of the hazards associated with the chemicals and the procedures and means of protecting against them. This Pharmacopoeia is not designed to describe such hazards or protective measures.

Automated procedures employing the same basic chemistry as those assay and test procedures given in the monograph are recognized as being equivalent in their suitability for determining compliance. Conversely, where an automated procedure is given in the monograph, manual procedures employing the same basic chemistry are recognized as being equivalent in their suitability for determining compliance.

In the performance of assay or test procedures, not less than the specified number of dosage units should be taken for analysis. Proportionately larger or smaller quantities than the specified weights and volumes of assay or test substances and Reference Standards may be taken, provided the measurement is made with at least equivalent accuracy and provided that any subsequent steps, such as dilutions, are adjusted accordingly

to yield concentrations equivalent to those specified and are made in such manner as to provide at least equivalent accuracy. To minimize environmental impact or contact with hazardous materials, apparatus and chemicals specified in Pharmacopoeial procedures also may be proportionally changed.

Where it is directed in an assay or a test that a certain quantity of substance or a counted number of dosage units is to be examined, the specified quantity or number is a minimal figure (the singlet determination) chosen only for convenience of analytical manipulation; it is not intended to restrict the total quantity of substance or number of units that may be subjected to the assay or test or that should be tested in accordance with good manufacturing practices.

Where it is directed in the assay of Tablets to "weigh and finely powder not less than" a given number, usually 20, of the Tablets, it is intended that a counted number of Tablets shall be weighed and reduced to a powder. The portion of the powdered tablets taken for assay is representative of the whole Tablets and is, in turn, weighed accurately. The result of the assay is then related to the amount of active ingredient per Tablet by multiplying this result by the average Tablet weight and dividing by the weight of the portion taken for the assay.

Similarly, where it is directed in the assay of Capsules to remove, as completely as possible, the contents of not less than a given number, usually 20, of the Capsules, it is intended that a counted number of Capsules should be carefully opened and the contents quantitatively removed, combined, mixed, and weighed accurately. The portion of mixed Capsules contents taken for the assay is representative of the contents of the Capsules and is, in turn, weighed accurately. The result of the assay is then related to the amount of active ingredient per Capsule by multiplying this result by the average weight of Capsule content and dividing by the weight of the portion taken for the assay.

Where the definition in a monograph states the tolerances as being "calculated on the dried (or anhydrous or ignited) basis," the directions for drying or igniting the sample prior to assaying are generally omitted from the *Assay* procedure. Assay and test procedures may be performed on the undried or unignited substance and the results calculated on the dried, anhydrous, or ignited basis, provided a test for Loss on drying, or Water, or Loss on ignition, respectively, is given in the monograph. Results are calculated on an "as-is" basis unless otherwise specified in the monograph. Where the presence of moisture or other volatile material may interfere with the procedure, previous drying of the substance is specified in the individual monograph and is obligatory.

Throughout a monograph that includes a test for Loss on drying or Water, the expression "previously dried" without qualification signifies that the substance is to be dried as directed under Loss on drying or Water (gravimetric determination).

In stating the appropriate quantities to be taken for assays and tests, the use of the word "about" indicates a quantity within 10 per cent of the specified weight or volume. However, the weight or volume taken is accurately determined, and the calculated result is based upon the exact amount taken. The same tolerance applies to specified dimensions.

Where the use of a pipette is directed for measuring a specimen or an aliquot in conducting a test or an assay, the pipette conforms to the standards set forth under "Volumetric Apparatus" (Appendix 1.9), and is to be used in such manner that the error does not exceed the limit stated for a pipette of its size. Where a pipette is specified, a suitable burette, conforming to the standards set forth under "Volumetric Apparatus" (Appendix 1.9), may be substituted. Where a "to contain" pipette is specified, a suitable volumetric flask may be substituted.

Expressions such as "25.0 mL" and "25.0 mg," used with respect to volumetric or gravimetric measurements, indicate that the quantity is to be "accurately measured" or "accurately weighed" within the limits stated under "Volumetric Apparatus" (Appendix 1.9) or under "Weights and Balances" (Appendix 1.10).

The term "transfer" is used generally to specify a quantitative manipulation.

The term "concomitantly," used in such expressions as "concomitantly determine" or "concomitantly measured," in directions for assays and tests, is intended to denote that the determinations or measurements are to be performed in immediate succession.

Blank Determination Where it is directed that "any necessary correction" be made by a blank determination, the determination is to be conducted using the same quantities of the same reagents treated in the same manner as the solution or mixture containing the portion of the substance under assay or test, but with the substance itself omitted.

Desiccator The expression "in a desiccator" specifies the use of a tightly closed container of suitable size and design that maintains an atmosphere of low moisture content by means of self-indicating silica gel or other suitable desiccant.

Drying to Constant Weight The specification "dried to constant weight" means that the drying shall be continued until two consecutive weighings do not differ by more than 0.5 mg per g of substance taken, (2.5 mg per g in case of crude drug), the second weighing following an additional hour of drying at the prescribed conditions.

Ethanol The term "ethanol" used without other indication means ethanol 95 per cent v/v. Where other strengths are intended, the term "ethanol" is used followed by the statement of the strength.

Filtration Where it is directed to "filter", without further qualification, the intent is that the liquid be filtered through suitable filter paper or equivalent device until the filtrate is clear.

Ignition to Constant Weight The specification "ignite to constant weight" means that the ignition shall be continued until two consecutive weighings do not differ by more than 0.5 mg per g of substance taken, the second weighing following an additional 15-minute ignition period.

Indicators Unless otherwise directed, where starch is specified in a test or an assay as an indicator, 3 mL of *starch TS* shall be used; in cases of the other test solutions, approximately 0.2 mL, or 4 drops, of the specified solution shall be added.

Logarithms Logarithms used in this Pharmacopoeia are to the base 10 and are denoted by the abbreviation "log". Natural logarithms, if used, are denoted by the abbreviation "ln".

Negligible This term indicates a quantity not exceeding 0.5 mg.

Odour A suitable quantity is left open to the air in an evaporating dish for 15 minutes, after which any odour is defined. An odour designation is descriptive only and is not to be regarded as a standard of purity for a particular lot of an article, except in those cases where a particular odour is specifically prohibited in the individual monograph.

Percentage Expressions Percentage concentrations are expressed as follows:

Per cent weight in weight (w/w) expresses the number of g of a constituent in 100 g of solution or mixture.

Per cent weight in volume (w/v) expresses the number of g of a constituent in 100 mL of solution, and is used regardless of whether water or another liquid is the solvent. Per cent volume in volume (v/v) expresses the number of mL of a constituent in 100 mL of solution. The term "per cent" used without qualification means, for mixtures of solids and semisolids, per cent weight in weight; for solutions or suspensions of solids in liquids, per cent weight in volume; for solutions of liquids in liquids, per cent volume in volume; and for solutions of gases in liquids, per cent weight in volume. For example, a 1 per cent solution is prepared by dissolving 1 g of a solid or semisolid, or 1 mL of a liquid, in sufficient solvent to make 100 mL of the solution. In the dispensing of prescription medications, slight changes in volume owing to variations in room temperatures may be disregarded.

Pressure Pressure is expressed in kPa.

- 1 kPa is 7.5006 Torr.
- 1 Torr is the pressure exerted by a column 1 mm of mercury.

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, including the indicators and solutions required for the Assay and Tests of the Pharmacopoeia, are defined in the Appendices.

Reference Standards Reference Standards are authentic specimens that have been verified for suitability for use as comparison standards in Pharmacopoeial tests and assays. (See "Reference Substances", Appendix 1.8).

Where a Reference Substance is referred to in an assay or a test in this pharmacopoeia, the words "Reference Substance" are abbreviated to "RS".

Where a test or an assay calls for the use of a Pharmacopoeial article rather than a Reference Substance, as a material standard of reference, a substance meeting all of the requirements of the monograph for that article is to be used.

Any information necessary for proper use of the reference substance or reference preparation is given on the label or in the accompanying leaflet or brochure. Where no drying conditions are stated in the leaflet or on the label, the substance is to be used as received. The stability of the contents of opened containers cannot be guaranteed.

Solutions Unless otherwise specified in the individual monograph, all solutions called for in tests and assays are prepared with *water*.

An expression such as "(1 in 10)" means that 1 part *by volume* of a liquid is to be diluted with, or 1 part *by weight* of a solid is to be dissolved in, sufficient amount of the diluent or solvent to make the volume of the finished solution 10 parts *by volume*.

An expression such as "(20:5:2)" means that the respective numbers of parts, by volume, of the designated liquids are to be mixed, unless otherwise indicated.

Steam-Bath Where the use of a steam-bath is directed, exposure to actively flowing steam or to another form of regulated heat, corresponding in temperature to that of flowing steam, may be used.

Temperatures Unless otherwise specified, all temperatures in this Pharmacopoeia are expressed in Celsius degrees, and all measurements are made at 25°. Where "room temperature" is stated, a temperature from 20° to 30° is intended. Absolute temperatures are expressed in Kelvin degrees; the absolute temperature is related to a Celsius temperature by the equation $^oK = ^oC + 273.16$.

Vacuum The term "in vacuum" denotes exposure to a pressure not exceeding 0.7 kPa (about 5 Torr), unless otherwise indicated.

Water Where the use of *water* is specified or implied in identifications, tests and assays, for the preparation of reagents, or as a diluent in any of these circumstances, water complying with the requirements of the monograph on Purified Water is used. The term "distilled water" indicates purified water prepared by distillation.

Water-Bath Where the use of a water-bath is directed without qualification with respect to temperature, a bath of vigorously boiling water is intended.

Weights and Measures The metric system of weights and measures is employed in the Pharmacopoeia; the International System of Units (SI) has been adopted wherever practicable. Unless otherwise stated, all measurements involved in the analytical operations of the Pharmacopoeia are intended to be made at 25°.

Crude Drugs

Crude drugs in the monographs include medicinal parts obtained from plants or animals, their cell inclusions, secretions, and extracts, or minerals.

MONOGRAPHS

CANNABIS EXTRACT

Thai name สารสกัดกัญชา (SAN SAKAT KANCHA)

Category Anti-emetic; anti-epileptic; analgesic.

Cannabis Extract is the soft extract prepared from the extraction of the powdered, dried and mature pistillate inflorescences of *Cannabis sativa* L. by means of supercritical fluid (carbon dioxide) extraction or ethanol extraction. It contains not less than 80.0 per cent and not more than 120.0 per cent of the labelled amounts of tetrahydrocannabinol ($C_{21}H_{30}O_2$) and cannabidiol ($C_{21}H_{30}O_2$); the labelled amounts of tetrahydrocannabinol and cannabidiol are not less than 30.0 per cent and 1.0 per cent, respectively, calculated on the dried basis.

Description Yellowish brown to dark brown paste or viscous liquid; odour, characteristic.

Other relevant information Cannabinol may be found in some samples as it is a degraded product of tetrahydrocannabinol due to improper storage.

Packaging and storage Cannabis Extract shall be kept in tightly closed containers, preferably in amber glass containers, protected from light, and stored at a temperature not exceeding 25°.

Labelling The label on the container states the amounts of tetrahydrocannabinol and cannabidiol.

Identification

A. To 50 mg of the sample in a screw-capped, amber test-tube, add 10 mL of a mixture of 9 volumes of *methanol* and 1 volume of *chloroform*, sonicate for 30 minutes and allow to cool to room temperature. Filter through a 0.45-µm polyvinylidene fluoride filter (Vertical® or equivalent is suitable.) until a clear solution is obtained. Fold a filter paper into a quarter and open it partly to form a funnel, place a few drops of the solution into the centre of the paper and allow the filter paper to dry. Add a very small amount of a 1 per cent w/w mixture of *Fast Blue B salt* and *anhydrous sodium sulfate* and a few drops of a 10 per cent w/v solution of *sodium bicarbonate*: a purple red colour develops at the centre of the filter paper.

(**Note** This colour is a combination of the colours of the different cannabinoids which are the major components of cannabis: tetrahydrocannabinol is red, cannabidiol is orange and cannabinol is purple.)

B. Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1).

Standard solution A A solution containing 500 µg per mL of Tetrahydrocannabinol RS in *methanol.*

Standard solution B A solution containing 1 mg per mL of Cannabidiol RS in *methanol*.

Standard solution C A solution containing 1 mg per mL of Cannabinol RS in *methanol*.

Test solution To 30 mg of the sample in a screw-capped, amber test-tube, add 2 mL of *methanol*. Filter through a 0.45-µm polyvinylidene fluoride filter (Vertical® or equivalent is suitable.).

Adsorbent *Octadecylsilyl silica gel F254* (a precoated plate by Sorbent Technologies, Inc. or equivalent is suitable).

Mobile phase *Methanol*, *water* and *glacial acetic acid* (75:25:0.1).

Application Apply 1 µL of each of *Standard solutions* and *Test solution*, as 10-mm bands.

Development and drying Allow the solvent front to ascend 7 cm above the line of application. Dry the developed plate in air.

Detection Examine the plate under ultraviolet light (254 nm). Spray the plate with *Fast Blue B salt TS* and observe under visible light.

Results When examined under ultraviolet light (254 nm), the test solution shows two quenching bands (tetrahydrocannabinol and cannabidiol) or three quenching bands (tetrahydrocannabinol, cannabidiol and cannabinol) at the lower third of the chromatogram corresponding in R_f to the bands shown by the standard solutions.

When examined under visible light, the test solution shows a red band due to tetrahydrocannabinol, an orange band due to cannabidiol and/or a purple band due to cannabinol, corresponding in colour and R_f to the bands obtained from the standard solutions A, B and C, respectively.

C. The chromatogram of the Assay preparation shows several peaks, two of which correspond to the tetrahydrocannabinol peak and the cannabidiol peak of the Standard preparations, as obtained in the *Assay*.

Loss on drying Not more than 10.0 per cent w/w after drying at 105° for 2 hours (Appendix 4.15).

Heavy metals Complies with the requirements described under "Limit Test for Heavy Metals in Herbal Drugs and Herbal Drug Preparations" (Appendix 5.2); see also under "Arsenic and Heavy Metals" in the General Notices.

Pesticide residues Complies with the requirements in the "Pesticide Residues" (Appendix 7.22H).

Assay Carry out the determination as described in the "Liquid Chromatography" (Appendix 3.5).

Diluent Prepare a mixture of the equal volumes of *acetonitrile* and *water*.

Standard stock preparation A Dissolve an accurately weighed quantity of Tetrahydrocannabinol RS in *Diluent* to obtain a stock solution having a known concentration of about 1 mg per mL.

Standard stock preparation B Dissolve an accurately weighed quantity of Cannabidiol RS in *Diluent* to obtain a stock solution having a known concentration of about 500 µg per mL.

Internal standard preparation Dissolve an accurately weighed quantity of Diazepam RS in *Diluent* to obtain a stock solution having a known concentration of about 500 µg per mL. Dilute this solution with *Diluent* to obtain a solution having a final concentration of about 50 µg per mL.

Standard preparations Dilute *Standard stock preparation A* and *Standard stock preparation B*, quantitatively, and stepwise with *Diluent* to obtain five solutions having known concentrations of about 2, 25, 50, 75, and 100 µg each of tetrahydrocannabinol and cannabidiol per mL. Upon dilution, add sufficient amount of *Internal standard preparation* so that each final solution contains diazepam at a concentration of about 50 µg per mL.

System suitability preparation Dilute *Standard stock preparation A* and *Standard stock preparation B*, quantitatively, and stepwise with *Diluent* to obtain a solution having known concentrations of about 50 µg each of tetrahydrocannabinol and cannabidiol per mL. Upon dilution, add sufficient amount of *Internal standard preparation* so that each final solution contains diazepam at a concentration of about 50 µg per mL.

Assay preparation Transfer about 50 mg of Cannabis Extract, accurately weighed, to a screw-capped, amber test-tube, add 10 mL of a mixture of 9 volumes of *methanol* and 1 volume of *chloroform*, sonicate for 30 minutes and allow to cool to room temperature. Filter through a 0.45-µm polyvinylidene fluoride filter (Vertical® or equivalent is suitable.) until a clear solution is obtained. Evaporate 75.0 µL of the filtrate to dryness under a fume hood. Dissolve the residue in 2.0 mL of *Internal standard preparation*, sonicate for 2 minutes and filter through a polyvinylidene fluoride membrane having a 0.45-µm porosity.

(**Note** In case the concentrations of tetrahydrocannabinol and cannabidiol in the Assay preparation are beyond the concentration ranges of standard curve, allow to adjust the volume of the filtrate taken for evaporation.)

Mobile phase A Dissolve 3.153 g of *ammonium formate* in a 10 per cent v/v solution of *acetonitrile* and dilute with the same solvent to 1000.0 mL. Adjust with *formic acid* to a pH of 3.75±0.05.

Mobile phase B Prepare a 90 per cent v/v solution of *acetonitrile*.

The step gradient of mobile phases is as follows:

Time (Minutes)	*Mobile Phase A* (Per Cent V/V)	*Mobile Phase B* (Per Cent V/V)
0	30	70
15	10	90
30	10	90
31	30	70
40	30	70

Chromatographic system

Detector Ultraviolet light (228 nm)

Column A stainless steel column (15 cm × 4.6 mm), packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (3.5 µm) equipped with a similarly packed guard column (5 mm × 3.9 mm)

Column temperature 30°±1°

Flow rate 1.0 mL per minute.

(**Note** Adjustments are not recommended for gradient elutions, as stated in "Chromatographic Separation Techniques" (Appendix 3.9), unless it is necessary to obtain the relative retention times of about 0.2, 0.6 and 1 for diazepam, cannabiol and tetrahydrocannabinol, respectively.)

System suitability

Sample *System suitability preparation*

Suitability requirements

Symmetry factor Not more than 2.0 for the tetrahydrocannabinol peak and the cannabidiol peak.

Relative standard deviations Chromatograph five injections of *System suitability preparation*, and record peak areas. The analytical system is suitable for conducting this Assay if the relative standard deviation for the area ratios of tetrahydrocannabinol to diazepam and cannabidiol to diazepam are not more than 2.0 per cent for the tetrahydrocannabinol peak and the cannabidiol peak, respectively.

Procedure Separately inject about 30 µL each of *Standard preparations* into the chromatograph, record the chromatograms and measure the areas of the diazepam, cannabidiol and tetrahydrocannabinol peaks, respectively. Calculate the area ratios of cannabidiol to diazepam and the area ratios of tetrahydrocannabinol to diazepam. Plot the standard curves of the calculated area ratios of cannabidiol and tetrahydrocannabinol against their respective concentrations. Inject about 30 µL of *Assay*

preparation into the chromatograph, record the chromatogram and measure the peak areas and calculate the area ratios, as with the Standard preparation.

Calculation By reference to the standard curve, calculate the contents of tetrahydrocannabinol ($C_{21}H_{30}O_2$) and cannabidiol ($C_{21}H_{30}O_2$) in a portion of the Extract taken.

Other requirements Complies with the requirements described under "Extracts" (Appendix 1.16).

CANNABIS SUBLINGUAL DROPS

Thai name ยาน้ำมันกัญชาหยอดใต้ลิ้น (YA NAM MAN KANCHA YOT TAI LIN)

Category Anti-emetic; anti-epileptic; analgesic.

Cannabis Sublingual Drops are the Cannabis Extract in a suitable vegetable oil such as sesame oil, olive oil or coconut oil. They contain not less than 90.0 per cent and not more than 110.0 per cent of the labelled amounts of tetrahydrocannabinol ($C_{21}H_{30}O_2$) or cannabidiol ($C_{21}H_{30}O_2$) or a mixture thereof.

Description Pale yellow to brownish yellow oily liquid; odour, characteristic.

Strengths available Cannabidiol 100 mg per mL; tetrahydrocannabinol 10 and 17 mg per mL; 27 mg of tetrahydrocannabinol and 25 mg of cannabidiol per mL.

Dose Instill under the tongue, as directed by a physician.

Other relevant information

1. Cannabis sublingual drops must be used under strict supervision of a physician as they are still considered narcotics under Category 5 of the Narcotics Act B.E. 2522 (1979).
2. It is contra-indicated in patients with a known history of hypersensitivity to cannabis extract or any ingredients in the formulation, as well as those with unstable cardio-pulmonary diseases or having risks for cardiovascular diseases.
3. Risk-benefit should be considered if it is to be used in pregnant or nursing women, adolescents and patients with a known history of mental illnesses or compromised cardiovascular and/or respiratory symptoms.
4. It may cause dizziness, vomiting, urinary tract infections, relapsing multiple sclerosis, bradycardia, and/or abnormal blood pressure. Therefore, dose reduction may be required.
5. Drowsiness or light-headedness may occur which may impair the ability to drive or perform other tasks requiring alertness.
6. Cannabis therapy should be promptly discontinued if disorientation, agitation, anxiety, hallucination, and/or psychosis occur.

Packaging and storage Cannabis Sublingual Drops shall be kept in tightly closed containers, preferably in amber glass containers, protected from light, and stored at a temperature not exceeding 25°.

Labelling The label on the container states the amounts of tetrahydrocannabinol and/or cannabidiol.

Identification

A. To 500 mg of the sample in a screw-capped, amber test-tube, add 10 mL of a mixture of 9 volumes of *methanol* and 1 volume of *chloroform*, sonicate for 30 minutes and allow to stand for 90 minutes in a freezer. Immediately filter through a pledget of cotton, a solid phase extraction kit consisting of a hydrophilic-lipophilic balanced reversed-phase sorbent (Water® or equivalent is suitable) and a 0.45-µm polyvinylidene fluoride filter (Vertical® or equivalent is suitable.), successively, until a clear solution is obtained. Fold a filter paper into a quarter and open it partly to form a funnel, place a few drops of the solution into the centre of the paper and allow the filter paper to dry. Add a very small amount of a 1 per cent w/w mixture of *Fast Blue B salt* and *anhydrous sodium sulfate* and a few drops of a 10 per cent w/v solution of *sodium bicarbonate*: a purple red colour develops at the centre of the filter paper.

(**Note** This colour is a combination of the colours of the different cannabinoids which are the major components of cannabis: tetrahydrocannabinol is red, cannabidiol is orange, and cannabinol is purple.)

B. Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1).

Standard solution A, Standard solution B, Standard solution C, Adsorbent, **Mobile phase, Application, Development and drying,** and **Detection** Proceed as directed in the Identification under *Cannabis Extract*.

Test solution Transfer 2 mL of the sample to a screw-capped, amber test-tube, add 8 mL of a mixture of 9 volumes of *methanol* and 1 volume of *chloroform*, sonicate for 30 minutes and allow to stand for 90 minutes in a freezer. Immediately filter through a pledget of cotton, a solid phase extraction kit consisting of a hydrophilic-lipophilic balanced reversed-phase sorbent (Water® or equivalent is suitable) and a 0.45-µm polyvinylidene fluoride

filter (Vertical® or equivalent is suitable.), successively, until a clear solution is obtained. Evaporate the filtrate to dryness under a fume hood. Dissolve the residue in 2.0 mL of *methanol.*

Results As described in the Identification under *Cannabis Extract*.

C. The chromatogram of the Assay preparation shows several peaks, one or two of which correspond to the tetrahydrocannabinol peak and the cannabidiol peak of the Standard preparations, as obtained in the *Assay*.

Microbial limit Comply with the requirements for Category 1A in the "Limits for Microbial Contamination" (Appendix 10.5).

Assay Carry out the determination as described in the "Liquid Chromatography" (Appendix 3.5).

Diluent, Standard stock preparation A, Standard stock preparation B, Standard preparations, Internal standard preparation, System suitability preparation, Mobile phase A, Mobile phase B, Chromatographic system, System suitability, Suitability requirements and **Procedure** Proceed as directed in the *Assay* under *Cannabis Extract*.

Assay preparation Transfer about 500 mg of Cannabis Sublingual Drops, accurately weighed, to a screw-capped amber test-tube, add 10 mL of a mixture of 9 volumes of *methanol* and 1 volume of *chloroform*, sonicate for 30 minutes and allow to stand for 90 minutes in a freezer. Immediately filter through a pledget of cotton, a solid phase extraction kit consisting of a hydrophilic-lipophilic balanced reversed-phase sorbent (Water® or equivalent is suitable) and a 0.45-µm polyvinylidene fluoride filter (Vertical® or equivalent is suitable.), sucessively, until a clear solution is obtained. Evaporate 150.0 µL of the filtrate to dryness under a fume hood. Dissolve the residue in 2.0 mL of *Internal standard preparation*, sonicate for 2 minutes and filter through a polyvinylidene fluoride membrane having a 0.45-µm porosity.

(**Note** In case the concentrations of tetrahydrocannabinol and cannabidiol in the Assay preparation are beyond the concentration ranges of standard curve, allow to adjust the volume of the filtrate taken for evaporation.)

Calculation By reference to the standard curve, calculate the contents of tetrahydrocannabinol ($C_{21}H_{30}O_2$) and cannabidiol ($C_{21}H_{30}O_2$) in a portion of the Sublingual Drops taken.

Other requirements Comply with the requirements described under "Oromucosal Preparations" (Appendix 1.16).

APPENDICES

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APPENDIX 1 GENERAL INFORMATION

The specifications given below are strictly for the use of the materials as reagents. The inclusion of a material in this Appendix does not imply that it is suitable for use in medicines. Exceptionally, a trademark or supplier may be indicated for certain reagents whose availability is limited. It is however acceptable to use reagents from another source provided that they comply with the standards of the Pharmacopoeia.

1.1 REAGENTS

The name of a substance or solution indicates a reagent included in the following list. The specifications given for reagents do not necessarily guarantee their quality for use in medicines.

Some of the reagents included may be injurious to health. Important cautions have been stated for these reagents. They should be handled in accordance with good laboratory practice and any relevant regulations.

Reagents in aqueous solution are prepared using *water.* Where the name of the solvent is not stated, an aqueous solution is intended.

Unless otherwise specified, the reagents and reagent solutions are to be stored in well-closed containers. The labelling should comply with the relevant national legislation.

Absorbent Cotton

Use a suitable grade.

Acetic Acid, Glacial $C_2H_4O_2$ = 60.05

Use an analytical reagent grade of commerce containing not less than 99.5 per cent w/w and not more than 100.5 per cent w/w of $C_2H_4O_2$.

DESCRIPTION Clear, colourless liquid; odour, pungent and characteristic. Boils at about 118°.

Acetonitrile (Methyl Cyanide) C_2H_3N = 41.05

DESCRIPTION Clear, colourless liquid.

SOLUBILITY Miscible with *water*.

ACIDITY OR ALKALINITY A 10 per cent v/v solution is neutral to *litmus*.

BOILING RANGE Not less than 95 per cent distils between 80° and 82° (Appendix 4.5).

WEIGHT PER MILLILITRE 0.780 to 0.783 g (Appendix 4.9).

Ammonium Dihydrogenphosphate (Ammonium Phosphate, Monobasic) $(NH_4)H_2PO_4$ = 115.03

Use analytical reagent grade of commerce.

pH About 4.2, in a 2.3 per cent w/v solution (Appendix 4.11).

Ammonium Formate (Formic Acid Ammonium Salt) CH_5NO_2 = 63.06

Use an analytical reagent grade of commerce containing not less than 97.0 per cent w/w of CH_5NO_2.

DESCRIPTION Colourless, hygroscopic, crystalline solid.

Anisaldehyde (4-Methoxybenzaldehyde) $C_8H_8O_2$ = 136.15

Use a general reagent grade of commerce.

DESCRIPTION Colourless or pale yellow, oily liquid; odour, aromatic.

SOLUBILITY Slightly soluble in *water*; miscible with *ethanol* and with *ether*.

BOILING TEMPERATURE About 248° (Appendix 4.6).

WEIGHT PER MILLILITRE About 1.125 g (Appendix 4.9).

Chloroform $CHCl_3$ = 119.38

> ***Caution*** Care should be taken not to vaporize chloroform in the presence of a flame, because of the production of harmful gases.

Use an analytical reagent grade of commerce containing 0.4 to 1.0 per cent w/v of *ethanol*.

DESCRIPTION Colourless, volatile liquid; odour, characteristic.

SOLUBILITY Slightly soluble in *water*; miscible with *absolute ethanol,* with *ether*, with fixed and volatile oils, and with most organic solvents.

RELATIVE DENSITY 1.475 to 1.481 (Appendix 4.9).

BOILING TEMPERATURE About 60° (Appendix 4.6).

Store protected from light.

Cyclohexane C_6H_{12} = 84.16

DESCRIPTION Clear, colourless liquid.

BOILING RANGE Not less than 95 per cent distils between 80° and 82° (Appendix 4.5).

REFRACTIVE INDEX 1.4262 to 1.4265, at 20° (Appendix 4.7).

WEIGHT PER MILLILITRE 0.776 to 0.780 g (Appendix 4.9).

Cyclohexane UV *Cyclohexan*e which complies with the following additional test.

ABSORBANCE Determine the absorbance of the sample throughout the range of 220 to 250 nm (Appendix 2.2) against *water* as the blank. The absorbance should not exceed 0.346 at 220 nm, 0.155 at 235 nm, 0.046 at 240 nm, and 0.009 at 250 nm.

Dichloromethane (Methylene Chloride) CH_2Cl_2 = 84.93

DESCRIPTION Clear, colourless, mobile liquid.

SOLUBILITY Soluble in 50 parts of *water*; miscible with *ethanol* and with *ether*.

BOILING RANGE Not less than 95 per cent distils between 39° and 41° (Appendix 4.5).

WEIGHT PER MILLILITRE 1.323 to 1.325 g (Appendix 4.9).

NON-VOLATILE MATTER When evaporated on a water-bath and dried at 105° to constant weight, leaves not more than 0.05 per cent w/v of residue.

Ethanol C_2H_6O = 46.07

Use Ethanol (95 Per Cent) (see under "Reagents").

Ethanol (95 Per Cent)

A mixture of ethanol and water. Contains not less than 92.3 per cent w/w and not more than 93.8 per cent w/w, corresponding to not less than 94.9 per cent v/v and not more than 96.0 per cent v/v, at 15.56°, of C_2H_6O.

DESCRIPTION Colourless, clear, mobile and volatile liquid; odour, characteristic and spirituous. Flammable, burning with a blue smokeless flame. Boils at about 78°.

SOLUBILITY Miscible with *water*, with *chloroform* and with *ether*.

IDENTIFICATION

A. Mix 5 drops in a small beaker with 1 mL of *potassium permanganate TS* and 5 drops of *dilute sulfuric acid* and cover the beaker immediately with a filter paper moistened with a solution recently prepared by dissolving 100 mg of *sodium nitroferricyanide* and 500 mg of *piperazine hydrate* in 5 mL of *water*: an intense blue colour is produced on the filter paper, the colour becoming paler after a few minutes.

B. To 5 mL of a 0.5 per cent v/v solution, add 1 mL of 0.1 M *sodium hydroxide*; then slowly add 2 mL of *iodine TS*: the odour of iodoform develops and a yellow precipitate is produced.

ACIDITY OR ALKALINITY To 20 mL add 5 drops of *phenolphthalein TS*: the solution remains colourless and requires not more than 0.20 mL of 0.10 M *sodium hydroxide* to produce a pink colour.

CLARITY OF SOLUTION Dilute 5 mL to 100 mL with *water* in a glass cylinder: the solution remains clear when examined against a black background.

ALDEHYDES AND KETONES Heat 100 mL of *hydroxylamine TS* in a loosely stoppered flask on a water-bath for 30 minutes, cool, and, if necessary, add sufficient 0.050 M *sodium hydroxide* to restore the green colour. To 50 mL of this solution add 25 mL of the sample and heat on a water-bath for 10 minutes in a loosely stoppered flask. Cool, transfer to a Nessler cylinder, and titrate with 0.050 M *sodium hydroxide* until the colour matches that of the remainder of the hydroxylamine solution contained in a similar cylinder, both solutions being viewed down the axis of the cylinder. Not more than 0.90 mL or 0.050 M sodium hydroxide is required.

OXIDIZABLE SUBSTANCES To 20 mL add 1 mL of 0.002 M *potassium permanganate.* Allow the solution to stand at 20° for 10 minutes protected from light: the colour is not completely discharged.

NON-VOLATILE MATTER A 100-mL sample, when evaporated and dried at 100° to 105° to constant weight, leaves not more than 2.5 mg of residue.

SPECIFIC GRAVITY 0.805 to 0.821, at 25° (Appendix 4.9), using this result to ascertain the percentage of C_2H_6O contained in the liquid examined by reference to the Alcoholometric Table.

VOLATILE IMPURITIES Carry out the test as described in the "Gas Chromatography" (Appendix 3.4).

Reference solution (a) Dilute 100 mL of *anhydrous methanol* to 50.0 mL with the test substance. Dilute 5.0 mL of the solution to 50.0 mL with the test substance.

Reference solution (b) Dilute 50 mL of *anhydrous methanol* and 50 mL of *acetaldehyde* to 50.0 mL with the test substance. Dilute 100 mL of the solution to 10.0 mL with the test substance.

Reference solution (c) Dilute 150 mL of *acetal* to 50.0 mL with the test substance. Dilute 100 mL of the solution to 10.0 mL with the test substance.

Reference solution (d) Dilute 100 mL of *benzene* to 100.0 mL with the test substance. Dilute 100 mL of the solution to 50.0 mL with the test substance.

Test solution (a) The test substance.

Test solution (b) Add 150 mL of *4-methyl-2-pentanol* to 500.0 mL of the test substance.

Chromatographic system A gas chromatograph equipped with (a) a glass (fused silica) column (30 m × 0.32 mm) packed with porous poly[(cyanopropyl)

(phenyl)][dimethyl]-siloxane (1.8 mm), maintained as the following table, (b) a flame ionization detector maintained at 280°, and (c) *helium* as the carries gas.

	Time (Minutes)	Temperature (°)
Column	0-12	40
	12-32	40 ⟶ 240
	32-42	240
Injection port		200

System suitability Chromatograph *Reference solution (b)* and record the peak response as directed for *Procedure*: the resolution between the first peak (acetaldehyde) and the second peak (methanol) is not less than 1.5.

Procedure Inject separately suitable volumes of each of *Reference solution (a)*, *Reference solution (b)*, *Reference solution (c)*, *Reference solution (d)*, *Test solution (a),* and *Test solution (b).*

Limits:

- methanol in the chromatogram obtained from test solution (a): not more than half the area of the corresponding peak in the chromatogram obtained from reference solution (a) (200 ppm v/v),
- acetaldehyde + acetal: maximum 10 ppm v/v, expressed as acetaldehyde.

Calculation Calculate the sum of the contents of acetaldehyde and acetal in parts per million (v/v) using the following expression:

$$\frac{10 \times A_E}{A_T - A_E} + \frac{30 \times C_E}{C_T - C_E}$$

where A_E = area of the acetaldehyde peak in the chromatogram obtained from test solution (a),

A_T = area of the acetaldehyde peak in the chromatogram obtained from reference solution (b),

C_E = area of the acetal peak in the chromatogram obtained from test solution (a), and

C_T = area of the acetal peak in the chromatogram obtained from reference solution (c).

- benzene: maximum 2 ppm v/v.

Calculation Calculate the content of benzene in parts per million (v/v) using the following expression:

$$\frac{2B_E}{B_T - B_E}$$

where B_E = area of the benzene peak in the chromatogram obtained from the test solution (a), and

B_T = area of the benzene peak in the chromatogram obtained from reference solution (d).

If necessary, the identity of benzene can be confirmed using another suitable chromatographic system (stationary phase with a different polarity).

- total of other impurities in the chromatogram obtained from test solution (b): not more than the area of the peak due to 4-methyl-2-pentanol in the chromatogram obtained from test solution (b) (300 ppm v/v),
- disregard limit: 0.03 times the area of the peak corresponding to 4-methyl-2-pentanol in the chromatogram obtained from test solution (b) (9 ppm v/v).

Ethanol, Absolute C_2H_6O = 46.07

Use an analytical reagent grade of commerce containing not less than 99.5 per cent v/v of C_2H_6O.

DESCRIPTION Colourless, clear, mobile and volatile liquid; odour, characteristic and spirituous. Flammable, burning with a blue, smokeless flame. Hygroscopic.

SOLUBILITY Miscible with *water*, with *chloroform* and with *ether*.

BOILING RANGE 78° to 79° (Appendix 4.5).

RELATIVE DENSITY 0.791 to 0.794 (Appendix 4.9).

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

Ethanols, Diluted Prepare by diluting the volumes of *ethanol* indicated in the following table with *water* to 1000 mL.

Strength (Per Cent V/V)	Volume of *Ethanol* (mL)	Weight per mL (approx.) g
90	947	0.83
85	894	0.85
80	842	0.86
70	737	0.89
60	632	0.91
50	526	0.93
45	474	0.94
25	263	0.97
20	210	0.975

Ether $C_4H_{10}O$ = 74.12

> ***Caution*** Ether tends to form explosive peroxides, especially when anhydrous.

Use an analytical reagent grade of commerce.

DESCRIPTION Clear, colourless, volatile, very mobile liquid; odour, characteristic. Highly flammable; mixtures of its vapour with oxygen, air, or nitrous oxide in certain concentrations are explosive.

SOLUBILITY Soluble in 10 parts of *water*, miscible with *benzene*, with *chloroform*, with *dichloromethane*, with *ethanol*, with fixed oils, with *petroleum ether*, and with volatile oils.

PEROXIDES Transfer 8 mL of *potassium iodide and starch TS* to a stoppered tube of about 12-mL capacity and about 1.5 cm in diameter. Fill completely with the test substance, shake vigorously, and allow to stand in the dark for 30 minutes. No colour is produced.

Store protected from light at a temperature not exceeding 15°. The name and concentration of any added stabilizer are stated on the table.

Ethyl Acetate $C_4H_8O_2$ = 88.11

Use an analytical reagent grade of commerce.

DESCRIPTION Colourless liquid; odour, fruity-like.

BOILING RANGE 76° to 78°(Appendix 4.5).

WEIGHT PER MILLILITRE 0.901 to 0.904 g (Appendix 4.9).

Fast Blue B Salt $C_{14}H_{12}Cl_4N_4O_2$ = 339.18 Stabilized by the addition of zinc chloride.

DESCRIPTION Dark green powder.

Store in tightly closed containers at a temperature between 2° and 8°.

Formic Acid CH_2O_2 = 46.03

Use an analytical reagent grade of commerce containing about 90 per cent w/w of CH_2O_2 and about 23.6 M in strength.

DESCRIPTION Colourless, corrosive liquid; odour, pungent.

WEIGHT PER MILLILITRE About 1.20 g (Appendix 4.9).

Formic Acid, Anhydrous CH_2O_2 = 46.03

> ***Caution*** Show decomposition of this reagent may produce pressure in the bottle. Loosen cap occasionally to vent the gas.

Use an analytical reagent grade of commerce containing not less than 98.0 per cent w/w of CH_2O_2.

DESCRIPTION Colourless, corrosive liquid; odour, pungent.

RELATIVE DENSITY About 1.22 (Appendix 4.9).

D-Galactose $C_6H_{12}O_6$ = 180.16

Use an analytical reagent grade of commerce.

DESCRIPTION White crystalline or finely granulated powder.

SOLUBILITY Soluble in *water*; very slightly soluble in *ethanol*.

MELTING TEMPERATURE About 164°, with decomposition (Appendix 4.3).

SPECIFIC ROTATION About +80° at 20°, determined in a 10 per cent w/v solution containing about 0.05 per cent v/v of *ammonia* (Appendix 4.8).

Gelatin

Use a general reagent grade of commerce.

DESCRIPTION Colourless or slightly yellow, transparent, brittle, tasteless sheets, flakes, or powder; odourless.

SOLUBILITY Soluble in hot *water*, *acetic acid* and *glycerol*; insoluble in organic solvents.

Helium He = 4.00

Use a suitable laboratory cylinder grade of commerce containing not less than 99.995 per cent v/v of He.

Hexane C_6H_{14} = 86.18

The hexane fraction from petroleum.

DESCRIPTION Colourless, mobile, highly flammable liquid.

BOILING RANGE Not less than 95 per cent distils between 67° and 70° (Appendix 4.5).

WEIGHT PER MILLILITRE 0.670 to 0.677 g (Appendix 4.9).

NON-VOLATILE MATTER When evaporated on a water-bath and dried at 105° to constant weight, leaves not more than 0.01 per cent w/v of residue.

***n*-hexane** C_6H_{14} = 86.18

Use an analytical reagent grade of commerce usually containing not less than 99 per cent of the pure isomer, n-C_6H_{14}.

DESCRIPTION Colourless, flammable liquid.

BOILING RANGE Distils completely over a range of 1°

between 67.5° and 69.5° (Appendix 4.5).

REFRACTIVE INDEX 1.374 to 1.375 (Appendix 4.7).

WEIGHT PER MILLILITRE 0.658 to 0.659 g (Appendix 4.9).

Holmium Oxide Ho_2O_3 = 377.86

Use general reagent grade of commerce.

DESCRIPTION Yellowish powder.

SOLUBILITY Insoluble in *water*.

Hydrochloric Acid HCl = 36.46

Use an analytical reagent grade of commerce.

When no molarity is indicated, use analytical reagent grade of commerce with a relative density of about 1.18, containing not less than 35 per cent w/w and not more than 38 per cent w/w of HCl and about 11.5 M in strength.

DESCRIPTION Clear, colourless, fuming liquid; odour, pungent.

SOLUBILITY Miscible with *water*.

Solutions of molarity *x*M should be prepared by diluting 85*x* mL of *hydrochloric acid* to 1000 mL with *water*.

Store in a container of polyethylene or other non-reacting material at a temperature not exceeding 30°.

Hydrochloric Acid, Dilute A 10 per cent w/v solution. Prepare by mixing 226 mL of *hydrochloric acid* with sufficient *water* to produce 1000 mL.

Hydrochloric Acid, Heavy Metal-Free

Use a suitable reagent grade of commerce. Complies with the requirements prescribed for *hydrochloric acid* with the following maximum contents of heavy metals in ppm: Arsenic 0.005, Cadmium 0.003, Copper 0.003, Iron 0.05, Mercury 0.005, Nickel 0.004, Lead 0.001, and Zinc 0.005.

Hydrochloric Acid, Dilute, Heavy Metal-Free

Use a suitable reagent grade of commerce. Complies with the requirements prescribed for *hydrochloric acid* with the following maximum contents of heavy metals in ppm: Arsenic 0.005, Cadmium 0.003, Copper 0.003, Iron 0.05, Mercury 0.005, Nickel 0.004, Lead 0.001, and Zinc 0.005.

Iron(II) Sulfate (Ferrous Sulfate) $FeCl_4 \cdot 7H_2O$ = 278.01

Use a general reagent grade of commerce.

DESCRIPTION Bluish green crystals or pale, crystalline powder; odourless. Efflorescent in air. Oxidizes in moist air, becoming brown.

Store in well-closed containers

Methanol (Methyl Alcohol) CH_4O = 32.04

Use an analytical reagent grade of commerce.

DESCRIPTION Colourless liquid.

SOLUBILITY Miscible with *water*, forming a clear colourless liquid.

BOILING RANGE 64° and 65° (Appendix 4.5).

RELATIVE DENSITY 0.791 to 0.793° (Appendix 4.9).

Nitric Acid HNO_3 = 63.01

When no molarity is indicated, use analytical reagent grade of commerce containing about 70.0 per cent w/w of HNO_3 and about 16 M in strength.

DESCRIPTION Corrosive, fuming liquid.

WEIGHT PER MILLILITRE About 1.42 g (Appendix 4.9).

When solutions of molarity *x*M are required, they should be prepared by diluting 63*x* of *nitric acid* with *water* to 1000 mL.

Store protected from light.

Nitric Acid, Dilute, Heavy Metal-Free

Use a suitable reagent grade of commerce. Complies with the requirements prescribed for *hydrochloric acid* with the following maximum contents of heavy metals in ppm: Arsenic 0.005, Cadmium 0.005, Copper 0.001, Iron 0.02, Mercury 0.002, Nickel 0.005, Lead 0.001, and Zinc 0.01.

Nitric Acid, Heavy Metal-Free

Use a suitable reagent grade of commerce. Complies with the requirements prescribed for *hydrochloric acid* with the following maximum contents of heavy metals in ppm: Arsenic 0.005, Cadmium 0.005, Copper 0.001, Iron 0.02, Mercury 0.002, Nickel 0.005, Lead 0.001, and Zinc 0.01.

Nitrogen N_2 = 28.01

Use a laboratory cylinder grade of commerce, washed with *water* and dried.

Olive Oil

Use a general reagent grade of commerce.

FREEZING TEMPERATURE 110° (Appendix 4.4).

REFRACTIVE INDEX 1.4680 (Appendix 4.7).

WEIGHT PER MILLILITRE About 0.910 g (Appendix 4.9).

Perchloric Acid $HClO_4$ = 100.46

When no molarity is indicated, use analytical reagent grade of commerce containing not less than 70.0 per cent and not more than 73.0 per cent w/w of $HClO_4$ and about 12 M in strength.

DECRIPTION Corrosive liquid.

WEIGHT MILLILITRE About 1.7 g (Appendix 4.9).

Phosphorus Pentoxide Desiccant (Diphosphorus Pentoxide) P_2O_5 = 141.94

Use a grade specially supplied for use in desiccators.

DESCRIPTION White, amorphous, deliquescent powder hydrated by water with the evolution of heat.

Store in well-closed containers.

Potassium Chloride KCl = 74.55

Use analytical reagent grade of commerce.

DESCRIPTION Colourless crystals or white, granular powder, odourless.

SOLUBILITY Very soluble in *water*; slightly soluble in *ethanol*.

Potassium chloride used for infrared absorption sepctrophotometry complies with the following requirement.

INFRARED ABSORPTION The spectrum of a disc prepared from the material, previously dried at 250° for 1 hour, has a substantially flat baseline over the range of 4000 to 620 cm^{-1}; it exhibits no maxima with an absorbance greater than 0.02 above the baseline with the exception of maxima due to water at 3440 and 1630 cm^{-1} (Appendix 2.1).

Potassium Dichromate (Dipotassium Dichromate) $K_2Cr_2O_7$ = 294.18

Use analytical reagent grade of commerce.

DESCRIPTION Orange-red crystals or crystalline powder.

SOLUBILITY Soluble in *water*; practically insoluble in *ethanol*.

Potassium dichromate used for the calibration of spectrophotometers contains not less than 99.9 per cent w/w of $K_2Cr_2O_7$, *calculated with reference to the substance dried at 130° for 1 hour*.

Potassium Hydroxide KOH = 56.11

Use an analytical reagent grade of commerce.

DESCRIPTION White or practically white, fused masses, or small pellets, or flakes, or sticks, or other forms.

SOLUBILITY Freely soluble in *water*, in *ethanol* and in *glycerol*; very soluble in boiling *ethanol*.

Store in tightly closed containers.

Potassium Iodide KI = 166.0

Use an analytical reagent grade of commerce.

DESCRIPTION White crystalline powder.

1-Propanol (Isopropanol) C_3H_8O = 60.10

Use an analytical reagent grade of commerce.

DESCRIPTION Colourless liquid; odour, characteristic.

BOILING RANGE 81° to 83° (Appendix 4.5).

WEIGHT PER MILLILITRE About 0.785 g (Appendix 4.9).

Pyridine C_5H_5N = 79.10

Use an analytical reagent grade of commerce.

DESCRIPTION Clear, colourless liquid; odour, characteristic and unpleasant. Hygroscopic.

BOILING TEMPERATURE About 115° (Appendix 4.6).

Store in well-closed containers.

Sodium Acetate $C_2H_3NaO_2 \cdot 3H_2O$ = 136.08

Use an analytical reagent grade of commerce.

Sodium Borohydride (Sodium Tetrahydroborate) $NaBH_4$ = 37.83

Use analytical reagent grade of commerce.

DESCRIPTION Colourless, hygroscopic crystals.

SOLUBILITY 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.

Store in tightly closed containers, in a cool and dry place, protected from light.

Sodium Hydrogencarbonate (Sodium Bicarbonate) $NaHCO_3$ = 84.01

Use an analytical reagent grade of commerce.

Sodium Hydroxide NaOH = 40.00

Use an analytical reagent grade of commerce.

DESCRIPTION White, or practically white, fused masses, in small pellets, in flakes, or sticks, and in other forms.

SOLUBILITY Freely soluble in *water* and in *ethanol*.

Store in tightly closed containers.

Sodium Sulfate (Glauber's Salt) $Na_2SO_4 \cdot 10H_2O$ = 322.19

DESCRIPTION Colourless crystals or white granules; odourless. Efflorescent. Melts at 32.5°.

SOLUBILITY Soluble in 1.5 parts of *water*; soluble in *glycerol*; insoluble in *ethanol*.

INSOLUBLE MATTER Not more than 0.01 per cent w/w (Appendix 4.13); use 10.0 g.

pH 5.2 to 8.2, in a 5.0 per cent w/v solution in *ammonia-* and *carbon dioxide-free water* (Appendix 4.11).

CHLORIDE Not more than 20 ppm (Appendix 5.2). Dissolve 2.1 g in 50.0 mL of *water*, and filter if necessary. A 25.0-mL portion of the resulting solution shows no more chloride than that corresponds to 0.03 mL of 0.020 M *hydrochloric acid*.

ARSENIC Not more than 1 ppm (Appendix 5.2); use a mixture of 3.0 g and 35 mL of *water* as the Test Preparation.

CALCIUM, MAGNESIUM, AND TRIVALENT OXIDE PRECIPITATE Not more than 0.02 per cent w/w.

Dissolve about 5 g, accurately weighed, in 75 mL of *water*, filter, and add 7 mL of *ammonium oxalate TS*, 2 mL of *ammonium phosphate TS,* and 10 mL of *strong ammonia solution.* Stir well, and allow to stand overnight. If any precipitate forms, filter, wash with a 25 per cent v/v solution of *dilute ammonia solution*, ignite at 800°±25° to constant weight.

HEAVY METALS Not more than 5 ppm (Appendix 5.2); use 2.0 g. For the Standard Preparation, use *lead standard solution (1 ppm Pb).*

IRON Not more than 10 ppm. Dissolve 1.0 g in 47 mL of *water*; add 2 mL of *hydrochloric acid.* The solution complies with the "Limit Test for Iron" (Appendix 5.2).

NITROGEN COMPOUNDS Not more than 5 ppm. Dissolve 2.0 g in 60 mL of *ammonia-free water* in a flask connected through a spray trap to a condenser, the end of which dips beneath the surface of 10 mL of 0.1 M *hydrochloric acid*. Add to the contents of the flask 10 mL of freshly boiled *sodium hydroxide TS* and 500 mg of *aluminium wire* in small pieces, and allow to stand for 1 hour. Distil about 35 mL and dilute the distillate with *water* to 50 mL. Add 2 mL of *sodium hydroxide TS*, mix, and add 2 mL of *alkaline mercuric-potassium iodide TS*; the colour produced is not darker than that produced by 0.1 mL of *nitrogen standard solution (100 ppm N)* when similarly treated.

Sulfuric Acid H_2SO_4 = 98.07

When no molarity is indicated, use an analytical reagent grade of commerce containing about 96 per cent w/w of *sulfuric acid* and about 18 M in strength.

DESCRIPTION Colourless, oily, corrosive liquid.

WEIGHT PER MILLILITRE About 1.84 g (Appendix 4.9).

When solutions of molarity *x*M are required, they should be prepared by carefully adding 54*x* mL of *sulfuric acid* to an equal volume of *water* and diluting to 1000 mL with *water*.

When "*sulfuric acid*" is followed by a percentage figure, an instruction to add, carefully, *sulfuric acid* to *water* to produce the specified percentage v/v (or, if required, w/w) proportion of sulfuric acid is implied.

Toluene (Methylbenzene) C_7H_8 = 92.14

Use an analytical reagent grade of commerce.

DESCRIPTION Clear, colourless liquid; odour, characteristic. Flammable.

SOLUBILITY Miscible with *water* and with *ethanol*.

BOILING TEMPERATURE About 110° (Appendix 4.6).

WEIGHT PER MILLILITRE 0.865 to 0.870 g (Appendix 4.9).

Water H_2O = 18.02

Use Purified Water of the Official Pharmacopoeia.

Xylose $C_5H_{10}O_5$ = 150.13

For microbiological purposes, use a suitable grade.

1.2 VOLUMETRIC SOLUTIONS

In this Pharmacopoeia, all concentrations of volumetric solutions are expressed in terms of molarity. The molarity of a solution is stated as the number of moles of substance contained in 1000 mL of the solution. A solution which contains x moles of substance per 1000 mL is designated as *x* molar (*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).

Throughout the Pharmacopoeia, molar solutions to be standardized before use in assays and other quantitative tests are designated by appending the letters *VS* to the name of the reagent.

Preparation and Standardization

For each solution the preparation and standardization of the most commonly used strength is described. Stronger or weaker solutions than those described are prepared and standardized using proportionate amounts of the reagents or by making an exact dilution of a stronger solution with *carbon dioxide-free water*. Solutions of molarity below 0.01 M are freshly prepared using *carbon dioxide-free water*.

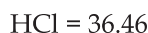
The water used in preparing volumetric solutions complies with the requirements of the monograph on Purified Water. When used for the preparation of unstable solutions such as potassium permanganate and sodium thiosulfate, it should be freshly boiled and cooled. When a solution is to be used in an assay in which the end-point is determined by an electro-chemical process, the solution must be standardized in the same way. All volumetric solutions, if practicable, are to be prepared, standardized and used at the same temperature.

Blank Determinations

Where it is directed that "any necessary correction" be made by a blank determination, the determination is to be conducted with the use of the same quantities of the same reagents treated in the same manner as the solution or mixture containing the portion of the substance under assay or test, but with the substance itself omitted. Appropriate blank corrections are to be made for all Pharmacopoeial titrimetric assays.

All Pharmacopoeial assays that are volumetric in nature indicate the weight of the substance being assayed to which each mL of the primary volumetric solution is equivalent. In general, these equivalents may be derived by simple calculation from the respective molecular formulae and weights.

Hydrochloric Acid, Molar (1 M)

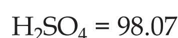


HCl = 36.46

Dilute 85 mL of *hydrochloric acid* with *water* to produce 1000 mL.

Standardization: Weigh accurately about 1.5 g of *anhydrous sodium carbonate* that previously has been heated at about 270° for 1 hour. Dissolve it in 100 mL of *water*, and add 0.1 mL of *methyl red TS*. Titrate with the *hydrochloric acid*, until the solution becomes faintly pink. Heat the solution to boiling, cool, and continue the titration. Heat again to boiling, and titrate further as necessary until the faint pink colour is no longer affected by continued boiling. Each mL of 1 M *hydrochloric acid* is equivalent to 52.99 mg of Na_2CO_3.

Sulfuric Acid, Half-Molar (0.5 M)



H_2SO_4 = 98.07

Carefully add 28 mL of *sulfuric acid* to *water*, and dilute to 1000 mL with the same solvent.

Standardization: Standardize the solution as described under *Hydrochloric Acid, Molar (1 M)*.

1.4 pH INDICATORS

Methyl Red $C_{15}H_{16}ClN_3O_2$ = 305.76

Dark red powder or violet crystals. Sparingly soluble in *water*; soluble in *ethanol*. Transition interval: from pH 4.2 to 6.3. Colour change: from red to yellow.

Methyl Red TS Dissolve 10 mg of *methyl red* in 100 mL of *ethanol*, and filter if necessary.

1.6 TEST SOLUTION

Fast Blue B Salt TS Dissolve 50 mg of *fast blue B salt* in 10 mL of *water*.

Prepare freshly before use.

1.7 MATERIALS FOR CHROMATOGRAPHY

Gas Chromatography

SUPPORTS

Diatomaceous Support (Diatomaceous Earth; Siliceous Earth) White or almost white, fine granular powder made up of siliceous frustules of fossil diatoms or debris of fossil diatoms. It may be identified by microscopic examination with a magnification of ×500. (**Note** Use a suitable grade available such as Celite 545.)

Diatomaceous Support, Acid-Washed Diatomaceous support that has been purified by treatment with hydrochloric acid and washed with *water* to remove metallic impurities, and to reduce surface activity and peak-tailing. (**Note** Use a suitable grade available such as Celite 545-AW.)

Diatomaceous Support, Alkali-Washed Diatomaceous support that has been treated with potassium hydroxide solution to reduce peak-tailing of basic compounds.

Diatomaceous Support, Silanized Diatomaceous earth for gas chromatography, silanized Acid-washed diatomaceous support that has been silanized with dimethyldichlorosilane or other suitable silanizing agents. (**Note** Use suitable grades available such as Anachrome Q, Gas-Chrom Q and Varaport 30.)

STATIONARY PHASES

A wide range of chemical substances is used, including polyethylene glycols, high-molecular weight esters and amides, hydrocarbons, silicone gums and fluids (polysiloxanes often substituted by methyl, phenyl, nitriol, vinyl, or fluoroalkyl groups, or mixtures of these), and microporous cross-linked

polyaromatic beads. Care should be taken to select grades specifically intended for use in gas chromatography. In most cases reference is made to a particular commercial brand which has been found to be suitable for the determination in question, but such statements do not imply that a different but equivalent commercial brand may not be used.

INTERNAL STANDARDS

Reagents used as internal standards should not contain any impurity which would produce a peak likely to interfere in the determination described in the monograph.

Thin-Layer Chromatography

The coating substances described below are used to prepare thin-layer chromatoplates in accordance with the procedure described in Appendix 3.1. Prepare suspensions of the coating substances as recommended by the manufacturer unless otherwise prescribed. Commercial pre-coated chromatoplates may be used for Pharmacopoeial tests provided they comply with the test for chromatographic separation described for the corresponding coating substance.

Kieselguhr G A fine, greyish white powder, the grey colour becoming more pronounced on triturating with water. The average particle size is between 10 and 40 µm. It consists of natural kieselguhr which has been extracted with hydrochloric acid and calcined, and to which about 15 per cent w/w of calcium sulfate hemihydrate has been added. It complies with the following requirements.

CONTENT OF CALCIUM SULFATE Carry out the test described under Silica gel G.

SEPARATING POWER Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1), using a mixture of 65 volumes of *ethyl acetate*, 23 volumes of *2-propanol* and 12 volumes of *water* as the mobile phase. Prepare the chromatoplates using a slurry of the sample in 0.02 M *sodium acetate*. Apply to the plate, 5 µl of a solution in *pyridin*e containing 0.01 per cent w/v of each of *lactose, sucrose, fructose*, *D-glucose*, and *D-galactose*. After removal of the plate, dry it at 105° to 110°, and allow to cool. Spray the plate with about 10 mL of *anisaldehyde TS* and heat at 105° to 110° for 5 to 10 minutes; the chromatogram shows five, well-defined, well-separated spots with no tailing.

ALKALINITY pH of a suspension prepared by shaking 1 g with 10 mL of *carbon dioxide-free water* for 5 minutes, 7.0 to 8.0 (Appendix 4.11).

Silica gel G A fine, white, homogeneous powder of an average particle size between 10 and 40 µm containing about 13 per cent w/w of calcium sulfate hemihydrate, and complying with the following requirements.

CONTENT OF CALCIUM SULFATE To about 250 mg, accurately weighed, add 3 mL of 2 M *hydrochloric acid* and 100 mL of *water* and shake vigorously for 30 minutes. Filter, wash the residue with *water* and carry out the "Complexometric Titration of Calcium" (Appendix 6.3) on the combined filtrate and washings. Each mL of 0.1 M disodium edetate VS is equivalent to 14.51 mg of $CaSO_4 \cdot ½H_2O$.

SEPARATING POWER Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1), using *toluene* as the mobile phase. Apply to the plate, 10 µL of a solution in *dichloromethane* containing 0.1 mg per mL of each of *indophenols blue*, *Sudan red G* and *dimethyl yellow* in *toluene*. Allow the mobile phase to ascend 10 cm. The chromatogram shows three clearly separated spots of the indophenols blue, Sudan red G and dimethyl yellow in order of increasing R_f value.

ALKALINITY pH of a suspension prepared by shaking 1 g with 10 mL of *carbon dioxide-free water* for 5 minutes, about 7 (Appendix 4.11).

Silica gel GF254 A fine, white, homogeneous powder of an average particle size between 10 and 40 µm containing about 13 per cent w/w of *calcium sulfate hemihydrate* and about 1.5 per cent w/w of a fluorescent indicator having a maximum intensity at 254 nm. It complies with the tests for Content of Calcium Sulfate, Alkalinity and Separating Power stated under Silica gel G and with the following test.

FLUORESCENCE Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1), using a mixture of 90 volumes of *2-propanol* and 10 volumes of *anhydrous formic acid* as the mobile phase, but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate, ten portions from 1 to 10 µL of a 1 mg per mL solution of *benzoic acid* in the same solvent mixture. After removal of the plate, dry in a current of warm air. Examine the chromatogram under ultraviolet light at 254 nm. The benzoic acid appears as dark spots on a fluorescent background in the upper third of the chromatogram at levels of 2 µg and greater.

Silica gel H A fine, white, homogenous powder of an average particle size between 10 and 40 µm. It is free from calcium sulfate hemihydrate and complies with the tests for Separating Power and Alkalinity stated under Silica gel G.

Silica gel HF254 A fine, white homogenous powder of an average particle size between 10 and 40 µm containing about 1.5 per cent w/w of a fluorescent indicator having a maximum intensity at about 254 nm. It is free from calcium sulfate hemihydrates and complies with the tests for Alkalinity and Separating Power stated under Silica gel G, and with the test for Fluorescence stated under Silica gel GF254.

Silica gel F254 A fine, white, homogeneous powder of an average particle size of about 15 µm containing a suitable binding agent and about 1.5 per cent w/w of a fluorescent indicator having a maximum intensity at 254 nm. It complies with the following requirement.

FLUORESCENCE Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1), using a mixture of 90 volumes of *2-propanol* and 10 volumes of *anhydrous formic acid* as the mobile phase, but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate, ten portions from 1 to 10 µL of a 1 mg per mL solution of *benzoic acid* in the mobile phase. After removal of the plate, dry in a current of warm air. Examine the chromatogram under ultraviolet light at 254 nm. The benzoic acid appears as dark spots on a fluorescent background in the upper third of the chromatogram at levels of 2 µg and greater.

Silica gel, Octadecylsilyl A very finely divided silica gel, chemically modified at the surface by the bonding of octadecylsilyl groups.

1.8 REFERENCE SUBSTANCE

A Reference Substance is an authenticated uniform material that is intended for use in specified chemical, physical and biological tests, by which the properties of the product under examination are compared.

Reference Substances are needed for certain analytical procedures in many monographs of the Thai Pharmacopoeia including:

(1) infrared identification;

(2) spectrophotometric or photometric methods of assay;

(3) chromatographic tests and assays;

(4) biological tests and assays including microbiological assays; and

(5) others.

Where a test or an assay calls for the use of a Reference Substance, the Department of Medical Sciences Reference Substances (DMScRS), ASEAN Reference Substances (ARS) or other recognized reference substances may be used. The DMScRS and ARS are available from Bureau of Drug and Narcotic (BDN), Department of Medical Sciences (DMSc), Nonthaburi, Thailand.

DMSc Reference Substance and ASEAN Reference Substance

The ARS project was established in 1980 under the Technical Cooperation among ASEAN countries on Pharmaceuticals in order to produce reference substances for utilization within ASEAN region. The project was supported by United Nations Development Program (UNDP) and World Health Organization (WHO) from 1982 to 1991 and Japan Pharmaceutical Manufacturers Association (JPMA) from 1992 to 2018. The DMScRS and ARS are recognized as secondary reference standards according to the definition of WHO Technical Report Series No. 943, 2007.

The DMScRS was initiated in 1993 following the requirements stated in the International Standard ISO 17034. To date, 67 DMScRS have been produced and distributed for utilization in pharmaceutical quality control laboratories. A model of the testing protocol is set up by the DMSc. The testing protocol may comprise identification tests (e.g., infrared absorption, UV-absorption), determination of physico-chemical constant (e.g., melting range, specific rotation, specific gravity), chromatographic purity test, inorganic contaminants determination, volatile substances test (e.g., water, solvents), functional group analysis (e.g., titrations, UV absorptivity), thermal analysis (e.g., differential scanning calorimetry), and assay against another well-characterized standard (e.g., USPRS, BPCRS and EPCRS).

Label Text

The label text is designed to provide the user with the information needed for the correct storage and usage of the reference substances in monograph application(s). The label includes an assigned value for purity of reference substances with quantitative applications as well as storage conditions. DMScRS and ARS are accompanied by certificate of analysis with more details of testing results, intended use, direction for use, and storage conditions.

Packaging and Storage

DMScRS and ARS are packaged in individual units designed to maintain the integrity of reference substances. The most common containers for solid material are colourless or amber glass vial with plastic inner and screw cap. The packaging environment is determined by the sensitivity of the material

to light, oxidation, or atmospheric humidity. Where appropriate, containers are filled in a glove box under inert gas and in conditions of controlled low residual humidity.

To serve its intended purpose, the DMScRS and ARS must be properly stored, handled and used. Generally, reference substances should be stored in their original containers away from heat and protected from light. Avoid humid storage areas in particular.

Continued Suitability for Use Program

To ensure that the reference substances maintain the properties determined at the initial evaluation, the DMSc maintains a continued suitability for use program. The retesting intervals and protocols are a function of the uses and properties of the substance and of the information available about its stability. Abbreviated protocols use the stability-indicating methodology employed in the initial characterization of the material to confirm the consistency of attributes such as appearance, chromatographic purity, or volatiles content.

Proper Use

The reference substances are not intended for use as drugs. DMScRS and ARS do not carry an expiration date, but a retest date, as long as they are in distribution. An updated version of catalogue can be found on the BDN website at https://bdn.go.th. Whenever the directions for use specified in the certificate of analysis require a preliminary drying or a correction for volatiles, it should be performed "at the time" of use. Further experimental details should be controlled by the user's Standard Operating Procedures and Good Laboratory Practices.

1.9 VOLUMETRIC APPARATUS

Most of the volumetric apparatus used in the analytical operation is calibrated at 20°, although the temperature specified generally for Pharmacopoeial tests and assays is 25°. This discrepancy is inconsequential provided the room temperature is reasonably constant.

Use To attain the degree of precision required in many Pharmacopoeial assays involving accurate volumetric measurements, the apparatus must be chosen and used with care. A burette should be of such size that the titrant volume represents not less than 30 per cent of the nominal volume. Where less than 10 mL of titrant is to be measured, a 10-mL burette or a microburette generally is required. The design of volumetric apparatus is an important factor in assuring accuracy. For example, the length of the graduated portions of graduated cylinders should be not less than five times the inside diameter, and the tips of burettes and pipettes should restrict the outflow rate to not more than 500 µL per second.

Standard of accuracy The capacity tolerances for volumetric flasks, transfer pipettes, and burettes are those accepted by the International Organization for Standardization (ISO) as indicated in the accompanying tables.

Volumetric Flask

Nominal Capacity (mL)	Capacity Tolerances (±mL)
5	0.025
10	0.025
25	0.040
50	0.060
100	0.100
200	0.150
250	0.150
500	0.250
1000	0.400
2000	0.600

From ISO 1042:1999

Transfer pipettes calibrated "to deliver" should be drained in a vertical position and then touched against the wall of the receiving vessel to drain the tips. Volume readings on burettes should be estimated to the nearest 0.01 mL for 25- and 50-mL burettes, and to the nearest 0.005 mL for 5- and 10-mL burettes. Pipettes calibrated "to contain" are called for in special cases, generally for measuring viscous fluids like syrups; however, a volumetric flask may be substituted for a "to contain" pipette. In such cases, the pipette or flask should be washed clean, after draining, and the washings added to the measured portion.

Transfer Pipettes

Nominal Capacity (mL)	Capacity Tolerances (±mL)
1	0.008
2	0.010
5	0.015
10	0.02
20	0.03
25	0.03
50	0.05
100	0.08

From ISO 648:2008

Burettes

Nominal Capacity (mL)	Capacity Tolerances (±mL)
10	0.02
25	0.03
50	0.05
100	0.10

From ISO 385:2005

1.10 WEIGHTS AND BALANCES

Pharmacopoeial tests and assays require the use of balance of capacity and sensitivity corresponding to the degree of accuracy sought. When substances must be "accurately weighed", the weighing shall be performed using a balance that is calibrated over the operating range and meets the requirements defined for repeatability and accuracy. For balances used for other applications, the balance repeatability and accuracy should be commensurate with the requirements for its use.

Apparatus

Analytical balances should possess adequate capacity and sensitivity. The analytical balance should be so constructed as to support its full capacity without developing undue stress and its sensitivity should not be altered by repeated weighing of the full-capacity load. The type of analytical balance having constant sensitivity over the whole capacity range is the constant-load, single-pan balance. It has a set of weights suspended from a counterpoised beam; in the process of weighing, these are removed from the beam by a manually operated mechanical device until equilibrium is reached.

The analytical balance should be constructed in a proper housing with suitable openings to permit the placement of the material to be weighed and to exclude air currents. Desiccants may be placed inside the housing for the maintenance of a relatively dry atmosphere.

Sets of calibrated weights used with balances that require manual placement of weights and sets of weights used to check the sensitivity of balances of another type should be kept in a case made of suitable material and properly lined.

Placement of Balance

The analytical balance should be placed upon a firm foundation that is as free from mechanical vibration as possible, preferably on an antivibration table of proper design. Alternatively, it may be placed on a concrete slab resting upon piers that are either sunk into the ground or connected to the construction elements of the building; or it may be placed upon a stout table or shelf protected by shock absorbers, such as cork mats or sheet rubber.

The balance should also be protected from humidity and acid fumes, preferably by placing it in a separate room of the laboratory. It should not be placed in a current of air or in direct sunlight.

The balance should be equipped with a levelling device and an indicator of proper position. Proper adjustment of levelling should be frequently checked.

Checking of Sensitivity

The sensitivity of the balance should be periodically checked by a qualified expert.

Checking the Stability of the Equilibrium Position

Before the balance is used, its equilibrium position without load should be checked several times. After each test, the balance has to be arrested.

The equilibrium position of the balance under load should also be determined from time to time, for example, with one-tenth of the full load and with the full load. The difference between equilibrium positions found in two successive determinations made with equal loads should not exceed 0.1 mg for analytical balances and 0.0001 mg for analytical microbalances.

Operation of the Balance

When the balance is not in use, the balance beam and pan supports should be raised. The doors of the housing should always be kept closed.

To release the balance, the beam and pans should be lowered very carefully. Objects to be weighed must be allowed to attain the temperature of the balance before weighing is started. The object to be weighed, as well as the weights, should always be placed on the pan as centrally as possible. During a weighing or on any occasion when objects are being added to or removed from the pans, both the beam arrests and the pan supports must be raised. Substances must be weighed in suitable containers such as beakers, weighing bottles, or crucibles. Liquids and volatile or hygroscopic solids must be weighed in tightly closed vessels, such as stoppered weighing bottles. No chemicals or objects that might injure the balance pans should be placed directly upon them.

When small quantities of a substance (for example, the sulfated ash) must be weighed in a large vessel and a fairly long period elapses between the two

weighings, atmospheric pressure and temperature may alter sufficiently to affect the buoyancy and thus cause an appreciable error. In two-pan balances, this error may be eliminated by using another vessel of similar shape and weight for taring.

The pans of the balance should be periodically lightly brushed with a camel-hair or similar brush to remove any dust that may have collected.

The weights should be handled only by means of a pair of forceps, which should possess tips covered with suitable material.

Calibration

If necessary, turn on the power, and allow the balance to equilibrate for at least 1 hour before proceeding with the calibration. (Microbalances may require up to 24 hours to reach equilibrium.)

REPEATABILITY

Repeatability is assessed by weighing one test weight not less than 10 times. (**Note** The test weight must be within the balance's operating range, but the weight need not be calibrated. Because the standard deviation is virtually independent of sample mass within the balance's capacity, use of a small test weight, which may be difficult to handle, is not required.)

Repeatability is satisfactory if twice the standard deviation of the weighed value, s, divided by the desired smallest net weight (i.e., smallest net weight that the users plan to use on that balance), does not exceed 0.10 per cent. The repeatability measurement establishes the smallest net amount of material that may be weighed on the balance in conformance with the 0.10 per cent limit. The minimum weight, M_{min}, is described by the inequality $M_{min} \geq 2000s$. For example, if s is found to be 0.00015, then M_{min} must be ≥ 0.30000 g or 300.00 mg. If the standard deviation, s, obtained is less than 0.41d, where d is the scale interval of the balance, then the inequality becomes $M_{min} \geq 2000\ (0.41d)$. For example, for a 4-place analytical balance, d is 0.0001 so that M_{min} must be ≥ 0.0820 g or 82.0 mg.

ACCURACY

The accuracy of a balance is satisfactory if its weighing value, when tested with a suitable weight(s), is within 0.10 per cent of the test weight value.

A test weight is suitable if it has a mass between 5 per cent and 100 per cent of the balance's capacity. The test weight's maximum permissible error (mpe), or alternatively its calibration uncertainty, shall be not more than one-third of the applied test limit of the accuracy test.

(**Note** Applicable standards are the following: International Organization of Legal Metrology (OIML) R 111 and American Society for Testing and Materials (ASTM) E617.)

1.14 WEIGHTS AND MEASURES: SI UNITS

The International System of Units (SI) is used in this Pharmacopoeia and comprises three categories of units, namely basic units, derived units and supplementary units. The basic units are set out in Table 1.

The derived units may be formed by combining the basic units according to certain algebraic relationships between the corresponding quantities. Some of these derived units have special names and symbols. The SI units used in this Pharmacopoeia are shown in Table 2.

Certain units of the SI have not yet been classified as basic or derived; they are known as supplementary units and are shown in Table 3. Some important and widely used units outside the international system are shown in Table 4.

The prefixes shown in Table 5 are used to form the names and symbols of the decimal multiples and submultiples of SI units.

Table 1 Basic Units

Quantity	Name of Basic SI Unit	Symbol
Length	metre	m
Mass	kilogram	kg
Time	second	s
Electric current	ampere	A
Thermodynamic temperature	kelvin	K
Amount of substance	mole	mol
Luminous intensity	candela	cd

Table 2 Derived SI Units Used in the Thai Pharmacopoeia and Their Equivalence with Other Units

Quantity	Name of derived SI Units	Symbol	Expression in Basic SI Units	Equivalence with Other Units
Absorbed dose of ionizing radiation	gray	Gy	m^2s^{-2}	1 Gy = 1 joule per kg = 100 rad
Energy, work, quantity of heat	joule	J	kgm^2s^{-2}	1 J = 10^7 ergs
Electrical potential, potential difference, electromotive force	volt	V	$kgm^2A^{-1}s^{-3}$	
Electric resistance	ohm	Ω	$kgm^2A^{-2}s^{-3}$	
Quantity of electricity	coulomb	C	As	
Force	newton	N	$kgms^{-2}$	1 N = 10^5 dynes
Frequency	hertz	Hz	s^{-1}	1 Hz = 1 cycle per second
Power	watt	W	kgm^2s^{-3}	
Pressure	pascal	Pa	$kgm^{-1}s^{-2}$	1 kPa = 7.5 mm Hg = 7.5 Torr = 1.45 lb/in^2
Radioactivity	becquerel	Bq	s^{-1}	1 Bq = 2.703×10^{-11} curies

Table 3 Supplementary Units

Quantity	Name of Supplementary SI Unit	Symbol
Plane angle	radian	rad
Solid angle	steradian	sr

Table 4 Units Used with the International System

Quantity	Unit		Value in SI Units
	Name	Symbol	
Time	minute	min	1 min = 60 s
	hour	h	1 h = 60 min = 3,600 s
	day	d	1 d = 24 h = 86,400 s
Plane angle	degree	°	$1° = (\pi/180)$ rad
	minute	′	$1' = (1/60)° = (\pi/10{,}800)$ rad
	second	″	$1'' = (1/60)' = (\pi/648{,}000)$ rad
Volume	litre	L	1 L = 1 dm^3 = $10^{-3}m^3$
Light	lux	lx	1 lx = 1 lumen m^2
	lumen	lm	1 lm = luminous flux emitted per unit solid angle from a uniform source of 1 candela
Mass	tonne	t	1 t = 10^3 kg
	gram	g	1 g = 10^{-3} kg

Note In the Pharmacopoeia, conditions of centrifugation are defined by reference to the acceleration due to gravity (g): $g = 9.80665\ ms^{-2}$

Table 5 Decimal Multiples and Sub-multiples

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

1.16 PHARMACEUTICAL DOSAGE FORMS

Dosage forms are provided for most of the Pharmacopoeial drug substances, but the processes for the preparation of many of them are, in general, beyond the scope of the Pharmacopoeia. In addition to defining the dosage forms, this section presents general requirements of some of them. Besides these requirements, the pharmaceutical products should be designed to possess certain desirable properties of bioavailability and stability.

Bioavailability

Bioavailability[1] is the rate and extent of absorption of a drug from a dosage form as determined by its concentration/time curve in the systemic circulation or by its excretion in urine. A variety of factors are knownto affect absorption. They are, for example, the method of manufacture or method of compounding; the particle size and crystal form or polymorph of the drug substance; and the diluents and excipients used in formulating the dosage form, including fillers, binders, disintegrating agents, lubricants, coatings, solvents, suspending agents, and dyes. Lubricants and coatings are foremost among these. The maintenance of a demonstrably high degree of bioavailability requires particular attention to all aspects of production and quality control that may affect the nature of the finished dosage form.

Stability

Stability of a pharmaceutical dosage form[2] refers to the capability of the dosage unit, in a specific container/closure system, to remain within its physical, chemical, microbiological, therapeutic, and toxicological specifications. The shelf-life of the dosage form is the period of time during which a product is expected, if stored under recommended conditions, to remain within the specification as determined by stability studies on a number of

[1]Further information on bioavailability may be obtained from (1) "ASEAN Guidelines for the Conduct of Bioavailability and Bioequivalence Studies", issued by ASEAN Pharmaceutical Product Working Group (ASEAN PPWG), 2005; (2) "Instruction for the *In Vivo* Bioequivalence Study Protocol Development", notified by Drug Control Division, Food and Drug Administration, Ministry of Public Health, Thailand, 2006.

[2]The stability study requirements may be obtained from (1) "ASEAN Guideline on Stability Study on Drug Product", issued by ASEAN Pharmaceutical Product Working Group (ASEAN PPWG), 2005; (2) "Guidelines for Stability Testing of Pharmaceutical Products", published by Food and Drug Administration and Department of Medical Sciences, Ministry of Public Health, Thailand, 2004.

batches of the product. The shelf-life is used to establish the expiration date. The stability parameters of a pharmaceutical dosage form can be influenced by environmental conditions of storage (temperature, light, air, and humidity), as well as the package components. Pharmacopoeial articles should include required storage conditions on their labelling. These are the conditions under which the expiration date shall apply. The storage requirements specified in the labelling for the article must be observed throughout the distribution of the article (for example, beyond the time it leaves the manufacturer up and including its handling by the dispenser or seller of the article to the consumer).

AEROSOLS

Aerosols are presented in special containers under pressure of a gas and contain one or more active ingredients. The preparations are released from the container, upon actuation of an appropriate valve, in the form of an aerosol (dispersion of solid or liquid particles in a gas, the size of the particles being adapted to the intended use). The pressure for the release is generated by suitable propellants. The preparations consist of a solution, an emulsion or a suspension and are intended for local application to the skin or the mucous membranes of various body orifices or for inhalation. The term "aerosol" refers to the fine mist of spray that results from most pressurized systems. However, the term has been broadly misapplied to all self-contained pressurized products, some of which deliver foams or semisolid fluids. The basic components of an aerosol system are the container, the propellant, the concentrate containing the active ingredient(s), the valve, and the actuator. Suitable auxiliary substances may also be used, for example solvents, solubilizers, emulsifying agents, suspending agents and lubricants for the valve to prevent clogging.

Propellants The propellants are either gases liquefied under pressure or compressed gases or low-boiling liquids. Liquefied gases are, for example, halogenated hydrocarbons (especially chloro-fluoro-derivatives of methane and ethane) and low-molecular-mass hydrocarbons (such as propane and butane). Compressed gases are, for example, carbon dioxide, nitrogen and nitrous oxide. Mixtures of these propellants may be used to obtain optimal solution properties and desirable pressure, delivery and spray characteristics.

Valves A suitable valve keeps the container tightly closed when not in use and regulates the delivery of the contents during use. The spray characteristics are influenced by the type of spraying device, in particular by the dimensions, number and location of orifices. Some valves provide a continuous release, others ("metering dose valves") deliver a defined quantity of product upon each valve actuation. The various valve materials in contact with the contents are compatible with them.

Actuators An actuator is the fitting attached to an aerosol valve stem which, when depressed or moved, opens the valve, and directs the spray containing the drug preparation to the desired area. The actuator usually indicates the direction in which the preparation is dispensed and protects the hand or finger from the refrigerant effects of the propellant. Actuators incorporate an orifice which may vary widely in size and shape. The size of this orifice, the expansion chamber design, and the nature of the propellant and formulation influence the physical characteristics of the spray foam, or stream of solid particles dispensed. For inhalation or oral dose aerosols, an actuator capable of delivering the medication in the proper particle size range is utilized.

Production Aerosols are prepared by one of two general processes. In the "cold-fill" process, the concentrate (generally cooled to a temperature below 0°) and the refrigerated propellant are measured into open containers (usually chilled). The valve-actuator assemblyis then crimped onto the container to form a pressure-tight seal. During the interval between propellant addition and crimping, sufficient volatilization of propellant occurs to displace air from the container. In the "pressure-fill" method, the concentrate is placed in the container, and either the propellant is forced under pressure through the valve orifice after the valve is sealed, or the propellant is allowed to flow under the valve cap and then the valve assembly is sealed ("under-the-cap" filling). In both cases of the "pressure-fill" method, provision must be made for evacuation of air by means of vacuum or displacement with a small amount of propellant. Manufacturing process controls usually include monitoring or proper formulation and propellant fill weight, and pressure testing and leak testing and valve function testing of the finished aerosol. Microbiological attributes should also be controlled.

Leak testing Aerosols comply with the Leak testing under "Test for Aerosols" (Appendix 4.19).

Minimum fill Aerosols comply with the test described in the "Minimum Fill" (Appendix 4.26).

Packaging and storage The containers are tight and resistant to the internal pressure and may be made of metal, glass, plastic or combinations of these materials. They are compatible with their

contents. Suitable metals include stainless steel, aluminium, and tin-plated steel. Glass containers are protected with a plastic coating. Aerosols shall be stored at a temperature not exceeding 50º and protected from frost.

Labelling The label of aerosols states (1) the method of use, and, if necessary, that the container should be shaken before use; (2) if necessary, the precautions to be taken, for example, avoid inhaling, avoid contact with the eyes and other mucous membranes; (3) that the container should not be exposed to or stored at a temperature above 50º and should not be exposed to direct sunlight; (4) for a container with a metering dose valve, the amount of active ingredient in a unit spray; (5) that the container should not be punctured or incinerated.

AROMATIC WATERS

Aromatic waters are clear saturated solutions of volatile oils or other aromatic substances in water, usually employed for their flavouring rather than their medicinal properties. Aromatic waters prepared as described below contain a small amount of Ethanol.

Production Aromatic waters are prepared by (1) dilution of a concentrated, ethanolic solution of the aromatic substance with water; (2) solution of the aromatic substance, with or without the use of a dispersing agent; or (3) distillation of the aromatic substance.

Packaging and storage Aromatic waters should be kept in tightly closed containers, protected from intense light and excessive heat.

CAPSULES

Capsules are solid dosage forms with hard or soft shells. They are of various shapes and sizes, and contain a single dose of one or more active ingredients. They are intended for oral administration, but preparations for alternative applications may require a special formulation, method of manufacture, or form of presentation, appropriate to their particular use. For this reason they may not comply with certain sections of this monograph. Starch capsules (often known as cachets) are not described in this monograph. The different categories of capsules that exist include hard, soft, and modified-release capsules. Their surfaces may bear symbols or other markings. They should be sufficiently robust to withstand handling, including packaging, storage, and transportation, without cracking or breaking. They should be packaged and stored in a manner that protects them from microbial contamination.

Capsule shells are made of gelatin or other substances, the consistency of which may be modified by the addition of substances such as glycerol and sorbitol. Preservatives may also be necessary. The shell should disintegrate in the presence of digestive fluids so that the contents are released. The contents of capsules may be solid, liquid or of a paste-like consistency. Capsule shells and contents may contain excipients such as diluents, sweeteners, colouring matters, flavouring substances, disintegrating agents, glidants, lubricants, and substances capable of modifying the behaviour of the active ingredient(s) in the gastrointestinal tract. The contents should not cause deterioration of the shell. When excipients are used, it is necessary to ensure that they do not adversely affect the stability, dissolution rate, bioavailability, safety, or efficacy of the active ingredient(s); there must be no incompatibility between any of the components of the dosage form.

Disintegration Capsules comply with the “Disintegration Test for Tablets and Capsules” (Appendix 4.23). For those capsules for which a dissolution requirement is included in the individual monograph, omission of the requirement for disintegration is considered justifiable and is therefore authorized.

Uniformity of dosage units Unless otherwise prescribed in the individual monographs, capsules comply with the “Uniformity of Dosage Units” (Appendix 4.28).

Visual inspection Unpack and inspect at least 20 capsules. They should be smooth and undamaged. Evidence of physical instability is demonstrated by gross changes in physical appearance, including hardening or softening, cracking, swelling, mottling, or discoloration of the shell.

Packaging and storage Capsules should be kept in well-closed containers at a temperature not exceeding 30º and protected from light, excessive moisture, or dryness.

Hard Capsules

Hard capsules have shells consisting of two prefabricated cylindrical sections that fit together. One end of each section is rounded and closed, and the other is open. The contents of hard capsules are usually in solid form (powder or granules); in certain cases the contents may be in the form of encapsulated powders or micropellets.

Production Hard capsules are prepared by mixing the active ingredient(s) with a number of

excipients. Sometimes, the physical characteristics of the mixture allow it to be directly filled into the shell, but it may occasionally be necessary to granulate before filling. Normally the granulate needs to be mixed with lubricants and/or disintegrating agents.

A uniform mass of the capsule mixture is volumetrically fed into the narrower lower section of the shell body which is then closed by slipping the larger section or cap over it. The security of the closure may be ensured by suitable means.

Soft Capsules

Soft capsules have thicker shells than hard capsules, and preservatives are usually added. The shells are of one piece and various shapes. Partial migration of the contents into the shell may occur (and vice versa) depending on the nature of the materials used.

Production Soft capsules are prepared by mixing the active ingredient(s) with a number of excipients. Soft gelatin capsules are usually formed, filled, and sealed in one operation. However, shells for extemporaneous use are sometimes prefabricated. Liquids may be incorporated directly. Solids are usually dissolved or dispersed in a suitable excipient(s) to give a solution or dispersion of thick consistency.

Modified–Release Capsules

Modified-release capsules are hard or soft capsules in which the contents or the shell or both contain additives or are prepared by special procedures such as micro-encapsulation which, separately or together, are designed to modify the rate of release of the active ingredient(s) in the gastro-intestinal tract.

DELAYED-RELEASE CAPSULES (ENTERIC CAPSULES) Delayed-release capsules are hard or soft capsules prepared in such a manner that either the shell or the contents resist the action of the gastric fluid but release the active ingredient(s) in the presence of the intestinal fluid.

All requirements for these specialized dosage forms are given in the individual monographs.

EXTENDED-RELEASE CAPSULES Extended-release capsules are designed to slow the rate of release of the active ingredient(s) in the gastro-intestinal tract.

All requirements for these specialized dosage forms are given in the individual monographs.

Production Delayed-release capsules are prepared by providing hard or soft capsules with a gastro resistant shell (enteric capsules) or by filling capsules with either granules or particles covered with a gastro-resistant coating. See also under Hard Capsules or Soft Capsules.

EAR PREPARATIONS

Ear preparations are liquid, semi-solid or powder preparations usually containing one or more active ingredients in a suitable vehicle. They are intended for instillation, for spraying, for insufflation, for application to the auditory meatus or as an ear wash. Ear preparations may contain auxiliary substances, for example, to adjust tonicity or viscosity, to adjust or stabilize the pH, to increase the solubility of the active ingredients, to stabilize the preparation or to provide adequate antimicrobial properties. Such additives should not adversely affect the intended medicinal action of the preparation, nor, at the concentrations used, cause toxicity or undue local irritation.

Preparations for application to the injured ear, particularly where the ear-drum is perforated, or prior to surgery are sterile, free from antimicrobial preservatives and supplied in single-unit containers.

Unless otherwise justified and authorized, ear preparations supplied in multiple-unit containers contain a suitable antimicrobial preservative in appropriate concentration, except when the preparation itself has adequate antimicrobial properties. The antimicrobial preservatives should be compatible with the other ingredients of the preparation and should remain effective throughout the period of use of the ear preparations. Five categories of ear preparations may be distinguished: (1) ear drops; (2) ear sprays; (3) semisolid ear preparations; (4) ear powders; (5) ear washes.

Minimum fill Ear preparations comply with the test described in the "Minimum Fill" (Appendix 4.26).

Sterility Where the ear preparations are labelled as sterile, unless otherwise directed in the individual monograph, they comply with the "Sterility Test" (Method I, Appendix 10.1).

Ear Drops

Ear drops are suspensions, emulsions or solutions of one or more active ingredients suspended, dispersed or dissolved in liquids such as water, glycols or fatty oils, suitable for application to the auditory meatus without exerting harmful pressure on the ear-drum. They may also be placed in the auditory meatus by means of a plug impregnated with the liquid. Suspended solids may separate slowly on standing but are easily redispersed on shaking. The size of the dispersed particles should be controlled.

Ear drops are usually supplied in multiple-unit containers fitted with an appropriate applicator.

Containers Ear drops are supplied in containers of glass or suitable plastic that are fitted with an integral dropper or with a screw cap of suitable materials incorporating a dropper and rubber or plastic teat. Alternatively, such a cap assembly is supplied separately.

Labelling The label of ear drops states (1) the name(s) and concentration(s) of the active ingredient(s); (2) the name(s) and concentration(s) of any antimicrobial preservative(s); (3) that they are intended for external use only; (4) where appropriate, that the preparation is sterile.

Ear Sprays

(**Note** When ear sprays are supplied in aerosol containers, these comply with the appropriate requirements for Aerosols.)

Ear sprays are suspensions, emulsions or solutions of one or more active ingredients suspended, dispersed or dissolved in liquids suitable for spraying to auditory meatus without exerting harmful pressure on the eardrum. The special requirements may be necessary for the selection of propellants, for particle size for the single-dose delivered by the metering valves.

See also under Ear Drops.

Packaging and storage Ear sprays are supplied in multiple-unit containers fitted with an appropriate applicator.

Labelling When ear sprays are supplied in aerosol containers, the label shall state (1) the method of use and, if necessary, that the container should be shaken before use; (2) if necessary, the precautions to be taken, for example, avoid inhaling; (3) that the container shall not be exposed to or stored at a temperature above 50º and should not be exposed to direct sunlight; (4) for a container with a metering dose valve, the amount of active ingredient in a unit-spray; (5) that the container should not be punctured or incinerated.

See also under Ear Drops.

Semi-Solid Ear Preparations

(**Note** Semi-solid ear preparations comply with the appropriate requirements for Topical Semi-solid Preparations.)

Semi-solid ear preparations are semi-solid dosage forms such as creams, gels, or ointments, etc. Intended for application to the external auditory meatus, if necessary by means of a plug impregnated with the preparation. Semi-solid ear preparations are supplied in containers fitted with a suitable applicator.

Labelling See under Ear Drops.

Ear Powders

(**Note** Ear powders comply with the appropriate requirements for powders.)

Ear powders are fine powders intended for application or insufflation to the external auditory meatus.

Containers Ear powders are supplied in containers fitted with a suitable device for application or insufflation.

Labelling See under Ear Drops.

Ear Washes

Ear washes are solutions intended to cleanse the external auditory meatus. They are usually aqueous solutions with a pH within physiological limits.

See also under Ear Drops.

Containers Ear washes are supplied in containers fitted with a suitable applicator.

Labelling See under Ear Drops.

EXTRACTS

Extracts are preparations of liquid, solid or semisolid consistency, obtained from herbal or animal matter, which is usually dried. Extracts may be subjected to purification processes that increase the content of characterized constituents with respect to the content of dry extractable matter from that which would be expected from extraction with the stated solvent: such extracts are termed "enriched".

Three types of extract can be distinguished:

Type A Type A extracts (standardized extracts) are adjusted to a defined range of therapeutically active constituents. Standardization is achieved by adjustment of the extract with inert material or by blending extracts.

Type B Type B extracts (quantified extracts) are adjusted to a defined range of active constituents. Adjustments are made either by blending batches of extracts or by blending batches of herbal or animal matter prior to extraction.

Type C Type C extracts are essentially defined by the production process (state of the matter to be extracted, solvent, extraction conditions). Constituents considered to be relevant markers may be determined.

Production Extracts are prepared by maceration, percolation or other suitable validated methods using ethanol or other suitable solvent. The matter to be extracted may undergo a preliminary treatment, for example, inactivation of enzymes, grinding or defatting. In addition, unwanted matter may be removed, if necessary, after extraction. Herbal drugs, animal matters and organic solvents used for the preparation of extracts comply with any relevant monograph of the Pharmacopoeia. For soft and dry extracts where the organic solvent is removed by evaporation, recovered or recycled solvent may be used, provided that the recovery procedures are controlled and monitored to ensure that solvents meet appropriate standards before reuse or admixture with other approved materials.

Water used for the preparation of extracts is of suitable quality. Except for the test for bacterial endotoxins, water complying with the section on Purified Water in bulk of the monograph on Purified Water is suitable. Potable water may be suitable if it complies with a defined specification that allows the consistent production of a suitable extract. Where applicable, concentration to the intended consistency is carried out using suitable methods, usually under reduced pressure, and at a temperature at which deterioration of the constituents is reduced to a minimum. Volatile oils that have been distilled during processing may be restored to the extracts at an appropriate stage in the manufacturing process. Suitable inert excipients may be added at the various stages of the manufacturing process to improve technological qualities like homogeneity, consistency or stability of active constituents. Where applicable, as a result of analysis of the herbal or animal matter used for the production of extracts, tests for microbiological quality, heavy metals, aflatoxins, and pesticide residues in the extracts have to be carried out.

Labelling For Type A, the label on the container states (1) the herbal or animal matter used; (2) whether the extract is dry, soft or liquid; (3) the composition of the extraction solvent; (4) where applicable, that fresh herbal or animal matter has been used; (5) where applicable, that the extract is "enriched"; (6) the name and amount of any excipient used. For Types B and C, the label on the container states (1) to (6) as for type A; (7) the content of constituents (markers) used for quantification and (8) the range of starting material: final extract (Drug: Extract Ratio or DER).

Liquid Extracts

Liquid extracts are liquid preparations of which, in general, one part by mass or volume is equivalent to one part by mass of the original dried herbal or animal matter. These preparations are adjusted, if necessary, so that they satisfy the requirements for content of solvent, and, where applicable, for constituents or dry residue.

Production Liquid extracts are prepared by using ethanol of suitable concentration or water to extract the stated herbal or animal matter or by dissolving a soft or dry extract (which has been produced using the same strength of extraction solvent as is used in preparing the liquid extract by direct extraction) of the stated herbal or animal matter in either ethanol of suitable concentration or water and filtering, if necessary. A slight sediment may form on standing, which is acceptable as long as the composition of the liquid extract is not changed significantly. Liquid extracts may contain suitable antimicrobial preservatives.

Relative density Where applicable, the liquid extract complies with the limits prescribed in the monograph.

Ethanol content For ethanolic liquid extracts, carry out the "Determination of Ethanol" (Appendix 6.5). The preparation complies with the limits prescribed in the monograph.

Methanol and 2-propanol Not more than 0.05 per cent v/v of methanol and not more than 0.05 per cent v/v of 2-propanol for ethanolic liquid extracts unless otherwise prescribed.

Dry residue In a flat-bottomed dish about 50 mm in diameter and about 30 mm in height, introduce rapidly 2.0 g or 2.0 ml of the extract to be examined. Evaporate to dryness on a water-bath and dry at 105° for 3 hours. Allow to cool in a desiccator over *phosphorus pentoxide desiccant* or *self-indicating silica gel* and weigh. Calculate the result as a percentage or in grams per litre.

Packaging and storage Liquid extracts should be kept in well-closed containers, protected from light.

Labelling In addition to the requirements listed above the label on the container states the final extract; (2) the concentration of any added antimicrobial preservative.

Soft Extracts

Soft extracts are semi-solid preparations obtained by evaporation of the solvent used for preparation. Soft extracts generally have a dry residue of not less than 70 per cent w/w. They may contain suitable

antimicrobial preservatives.

Dry residue In a flat-bottomed dish about 50 mm in diameter and about 30 mm in height, weigh rapidly 2.0 g of the extract to be examined. Heat to dryness on a water-bath and dry at 105º for 3 hours. Allow to cool in a desiccator over *phosphorus pentoxide desiccant* or *self-indicating silica gel* and weigh. Calculate the result as a percentage weight in weight. Where applicable, a monograph on a soft extract prescribes a limit test for the solvent used for extraction.

Packaging and storage Soft extracts should be keptin well-closed containers, protected from light.

Labelling In addition to the requirements listed above. The label on the container states the concentration of any added antimicrobial preservative.

Dry Extracts

Dry extracts are solid preparations obtained by evaporation of the solvent used for their production.

Dry extracts generally have a dry residue of not less than 95 per cent w/w.

Loss on drying Where applicable, the dry extract complies with the limits prescribed in the monograph. In a flat-bottomed dish about 50 mm in diameter and about 30 mm in height, weigh rapidly 500 mg of the extract to be examined, finely powdered. Dry at 105º for 3 hours. Allow to cool in a desiccator over *phosphorus pentoxide desiccant* or *self-indicating silica gel* and weigh. Calculate the result as a percentage weight in weight. Where applicable, a monograph on a drug extract prescribes a limit test for the solvent used for extraction.

Packaging and storage Dry extracts should be kept in well-closed containers, protected from light.

EYE PREPARATIONS

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. Several categories of eye preparations may be distinguished: (1) eye drops; (2) eye lotions; (3) powders for eye drops and powders for eye lotions; (4) semi-solid eye preparations; (5) ocular systems.

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 "Efficacy of Antimicrobial Preservation" (Appendix 10.6).

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 "Sterilization and Sterility Assurance" (Appendix 12).

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.

For multiple-unit containers of eye preparations, the period after opening the container, after which the contents must not be used, does not exceed 4 weeks.

Sterility Eye preparations comply with the "Sterility Test" (Appendix 10.1). Applicators supplied separately also comply with the test for sterility. 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 for sterility.

Minimum fill Eye preparations except eye strips and ocular systems comply with the test described in the "Minimum Fill" (Appendix 4.26).

Packaging and storage Eye preparations should be kept in a sterile, tightly closed, tamper-evident container.

Labelling The label of eye preparations states the name(s) of any added antimicrobial preservative(s).

Eye Drops

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 stabilize the pH, to increase the solubility of the active substance, or to stabilize 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 are prescribed without antimicrobial

preservatives, they are supplied wherever possible in single-dose containers. Eye drops intended for use in surgical procedures do not contain antimicrobial preservatives and are supplied in single-dose containers.

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.

Limit of particle size Unless otherwise specified in the individual monograph, 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 be 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 of eye drops states the storage condition.

Eye Lotions

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 stabilize 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 are prescribed without an antimicrobial preservative, 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 singledose 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 specified in the individual monograph.

Labelling See under Eye Drops.

Powders for Eye Drops and Eye Lotions

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 stabilize the pH or to stabilize the preparation.

After dissolution or suspension in the prescribed liquid, they comply with the requirements for eye drops or eye lotions, as appropriate.

Uniformity of dosage units Single-unit powders for eye-drops and eye lotions comply with the "Uniformity of Dosage Units" (Appendix 4.28).

Semi-solid Eye Preparations

Semi-solid eye preparations are sterile ointments, creams or gels intended for application to the conjunctiva. 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 on Topical Semi-solid Preparations. The basis is non-irritant to the conjunctiva.

Semi-solid eye preparations are packed in small, sterilized collapsible tubes fitted or provided with a sterilized cannula and having a content of not more than 10 g of the preparation. 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.

Limit of 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 See under Eye Drops.

Ocular Systems

See under Systems.

GRANULES

Granules are preparations consisting of solid, dry aggregates of powder particles sufficiently resistant to withstand handling. They are intended for oral administration. Some are swallowed as such, some are chewed and some are dissolved or dispersed in water or another suitable liquid before being administered. Granules contain one or more active ingredients with or without added substances including, where necessary, authorized colouring matter and flavouring agents. Granules are presented as single-unit or multiple-unit preparations. For single-unit preparations each dose is enclosed in an individual container, for example, a sachet, a paper packet or a vial. Each dose of a multiple-unit preparation is administered by means of a device suitable for measuring the quantity prescribed.

Several categories of granules may be distinguished: (1) uncoated granules; (2) granules for the preparation for oral liquids (see under Oral Liquids); (3) coated granules; (4) modified-release granules.

Uniformity of dosage units Unless otherwise prescribed in the individual monographs, granules comply with the "Uniformity of Dosage Units" (Appendix 4.28). The test for Content Uniformity is not required for multivitamin and trace element granules.

Packaging and storage Unless otherwise specified the individual monograph. Granules shall be kept in tightly closed containers.

Labelling For single-unit containers the label states the name(s) and amount(s) of active ingredient(s) per container and for multiple-unit containers the label states the name(s) and amount(s) of active ingredient(s) in a suitable quantity by weight.

Uncoated Granules

Uncoated granules may be plain or effervescent granules.

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

Disintegration Place a single dose of the granules in a beaker containing 200 mL of *water* at 15º to 25º; numerous gas bubbles are evolved. When the evolution of gas around the individual grains has ceased, the granules have disintegrated, being either dissolved or dispersed in the water. Repeat the operation on a further five doses. The granules comply with the test if each of the six doses used in the test disintegrates within 5 minutes.

Coated Granules

Coated granules are granules covered with one or more layers of mixtures of various substances. Substances used as coatings are usually applied as a solution or suspension in conditions in which evaporation of the vehicle occurs. Coated granules are usually presented as multiple-unit preparations.

Modified-release Granules

DELAYED-RELEASE GRANULES (ENTERIC-COATED GRANULES) Delayed-release granules are intended to resist the gastric fluid and to release the active ingredient(s) in the intestinal fluid. These properties are achieved by covering the granules with a gastro-resistant material (enteric-coated granules) or by other suitable means.

EXTENDED-RELEASE GRANULES Extended-release granules are coated or uncoated granules prepared by using added substances or procedures which, separately or together, are designed to modify the rate or the place at which the active ingredient(s) are released.

HERBAL DRUG PREPARATIONS

Herbal drug preparations are obtained by subjecting herbal drugs to treatments such as extraction, distillation, expression, fractionation, purification, concentration or fermentation. These include comminuted or powdered herbal drugs, tinctures, extracts, essential oils, expressed juices and processed exudates.

Herbal teas comply with the monograph on Herbal teas.

Instant herbal teas consist of powder or granules of one or more herbal drug preparation(s) intended for the preparation of an oral solution immediately before use.

HERBAL TEAS

Herbal teas consist exclusively of one or more herbal drugs intended for oral aqueous preparations by means of decoction, infusion or maceration. The preparation is prepared immediately before use. Herbal teas are usually supplied in bulk form or in sachets. The herbal drugs used comply with the appropriate individual monographs.

Recommendations on the microbiological quality of herbal teas under the "Limits for Microbial Contamination" (Category 2 in Table 2, Appendix 10.5) taking into account the prescribed preparation method (use of boiling or non-boiling water).

Identification The identity of herbal drugs present in herbal teas is checked by botanical examinations. The proportion of herbal drugs present in herbal teas is checked by appropriate methods. Herbal teas in sachets comply with the following test:

Weight variation Determine the average weight of twenty randomly chosen units as follows: weigh a single full sachet of herbal tea, open it without losing any fragments. Empty it completely using a brush. Weigh the empty sachet and calculate the mass of the contents by subtraction. Repeat the operation on the nineteen remaining sachets. Unless otherwise justified not more than two of the twenty individual masses of the contents deviate from the average mass of the contents by more than the percentage deviation shown in the table below and none deviates by more than twice that percentage.

Average Weight	Percentage Deviation
Less than 1.5 g	15 per cent
1.5 g to 2.0 g	10 per cent
More than 2.0 g	7.5 per cent

Packaging and storage Herbal teas should be protected from light.

INFUSIONS

Infusions are dilute solutions that contain the readily soluble constituents of crude drugs. Fresh infusions are made by pouring boiling water onto the drug, in a suitable state of comminution, and macerating for a short time, or they are usually prepared by diluting one volume of a concentrated infusion to ten volumes with water. Concentrated infusions are usually made by maceration of the drug with Ethanol (25 Per Cent).

For dispensing purposes, Infusions should be used within 12 hours of preparation from concentrated infusions.

Packaging and storage Infusions should be kept in well-closed containers.

Labelling The label on the container states the storage conditions.

IRRIGATION SOLUTIONS

Irrigation solutions are sterile solutions of one or more active ingredients intended for irrigation. If the solution is intended to be used for the irrigation of body cavities, for the flushing of wounds or operation cavities or for the irrigation of the urogenital system, it is sterile and apyrogenic. Such solutions are prepared using Water for Irrigation. Irrigation solutions may contain added substances such as suitable substances to make the preparation isotonic with blood. They are supplied in containers holding sufficient of the solution for use on one occasion only. When viewed under suitable conditions of visibility, they are practically clear and practically free from particles.

Sterility Unless otherwise directed in the individual monograph, irrigation solutions comply with the "Sterility Test" (Method I, Appendix 10.1).

Packaging and storage Irrigation solutions are supplied in containers made from materials that are sufficiently transparent to permit the visual inspection of the contents and that do not cause deterioration of the preparation as a result of diffusion into or across the material of the container or by yielding foreign substances into the preparation. The containers should be readily distinguishable from containers for preparations intended for parenteral administration. The containers are tamper-evident and are sealed so as to exclude micro-organisms. Unless otherwise specified in the individual monograph, Irrigation Solutions should be stored at a temperature not exceeding 25°.

Labelling The label of irrigation solutions states (1) that the irrigation solution is sterile and, where applicable, that the irrigation solution is apyrogenic; (2) that the irrigation solution is not to be used for injection; (3) that the irrigation solution should be used on one occasion only and that any remainder should be discarded.

LOZENGES

See under Oromucosal Preparations.

MEDICATED FOAMS

Medicated foams are preparations consisting of large volumes of gas dispersed in a liquid generally containing one or more active ingredients, a surfactant ensuring their formation and various other excipients. Medicated foams are usually intended for application to the skin or mucous membranes. Medicated foams are usually formed at the time of administration from a liquid preparation in a pressurized container. The container is equipped with a device consisting of a valve and a push button suitable for the delivery of the foam. Medicated foams intended for use on severely injured skin and on large open wounds are sterile. Medicated foams supplied in pressurized containers comply with the requirements in the monograph for "Aerosols" (Appendix 1.16).

Production Sterile medicated foams are prepared using materials and methods designed to ensure sterility and to avoid the introduction of contaminants and the growth of micro-organisms.

Relative foam density Maintain the container at about 25º for at least 24 hours. Taking care not to warm the container, fit a rigid tube 70 mm to 100 mm long and about 1 mm in internal diameter onto the push button. Shake the container to homogenize the liquid phase of the contents and dispense 5 ml to 10 ml of foam to waste. Tare a flat-bottomed dish of about 60 ml volume and about 35 mm high. Place the end of the rigid tube attached to the push button in the corner of the dish, press the push button and fill the dish uniformly, using a circular motion. After the foam has completely expanded, level off by removing the excess foam with a slide. Weigh. Determine the weight of the same volume of *water* by filling the same dish with *water*. The relative foam density is equivalent to the ratio:

$$\frac{m}{e},$$

where m is the weight of test sample of foam in g and e is the weight of same volume of *water* in g. Carry out three measurements. None of the individual values deviate by more than 20 per cent from the mean value.

Duration of expansion The apparatus (Fig. 1) consists of a 50-mL burette, 15 mm in internal diameter, with 0.1-mL graduations and fitted with a 4-mm single bore stopcock. The graduation corresponding to 30 mL is at least 210 mm from the axis of the stopcock. The lower part of the burette is connected by means of a plastic tube not longer than 50 mm and 4 mm in internal diameter to the foam-generating container equipped with a push button fitted to this connection. Maintain the container at about 25º for at least 24 hours. Shake the container, taken care not to warm it, to homogenize the liquid phase of the contents and dispense 5 mL to 10 mL of the foam to waste.

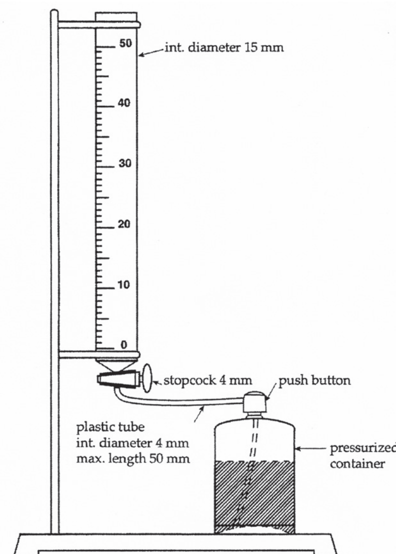


Fig. 1 The Apparatus for Duration of Expansion

Connect the push button to the outlet of the burette. Press the button and introduce about 30 mL of foam in a single delivery. Close the stopcock and at the same time start the chronometer and read the volume of foam in the burette. Every 10 seconds read the growing volume until the maximum volume is reached. Carry out three measurements. None of the times needed to obtain the maximum volume is more than 5 minutes.

Sterility Comply with the "Sterility Test" (Appendix 10.1), when the label indicates that the preparation is sterile.

Labelling The label on the container states (1) that the Medicated Foam is intended for external use only and (2) the storage conditions.

MEDICATED TAMPONS

Medicated tampons are solid, single-dose preparations intended to be inserted into the body cavities for a limited period of time. They consist of a suitable material such as cellulose, collagen or silicone impregnated with one or more active ingredients.

Production In manufacturing, packaging, storage and distribution of medicated tampons, suitable means are taken to ensure their microbial quality.

Microbial limit Comply with the "Limits for Microbial Contamination" (Appendix 10.5).

NASAL PREPARATIONS

Nasal preparations are liquid, semi-solid or solid preparations containing one or more active ingredients. They are intended for administration to the nasal cavities (nostrils) for local or systemic effects. Nasal preparations should as far as possible be non-irritating and should not adversely affect the functions of the nasal mucosa and its cilia. Aqueous Nasal preparations are usually isotonic. Nasal preparations are supplied in multiple-unit or single-unit containers provided, if necessary, with a suitable administration device.

Unless otherwise justified and authorized, aqueous nasal preparations supplied in multiple-unit containers contain a suitable antimicrobial preservative in appropriate concentration, except when the preparation itself has adequate antimicrobial properties.

Five categories of nasal preparations may be distinguished: (1) nasal drops; (2) liquid nasal sprays; (3) nasal powders; (4) semi-solid nasal preparations; (5) nasal washes.

Minimum fill Nasal preparations comply with the test described in the “Minimum Fill” (Appendix 4.26).

Labelling The label of nasal preparations states the instructions for use.

Nasal Drops

Nasal drops are solutions, emulsions or suspensions intended for instillation into the nostrils. Emulsions should not show evidence of phase separation; they have a uniform appearance after shaking. Suspensions may show a sediment which is readily redispersible on shaking to give a suspension which remains sufficiently stable to enable the correct dose to be delivered.

Production Single doses of nasal drops intended for systemic absorption of active ingredients through the nasal mucosa are tested for uniformity of volume, content or weight.

Containers Nasal drops are usually supplied in glass or plastic containers provided with a suitable applicator.

Uniformity of weight Nasal drops that are solutions comply with the following test: weigh individually the contents of ten containers emptied as completely as possible, and determine the average mass. Not more than two of the individual masses deviate by more than 10 per cent from the average mass and none deviates by more than 20 per cent.

Uniformity of content Nasal drops that are suspensions comply with the “Content Uniformity” (Appendix 4.28).

Liquid Nasal Sprays

(**Note** Where liquid nasal sprays are supplied in aerosol containers, these comply with the appropriate requirements for Aerosols.)

Liquid nasal sprays are solutions, emulsions or suspensions intended for spraying into the nostrils.

See also under Nasal Drops.

Production For liquid nasal sprays that are suspensions, the size of the dispersed particles of the spray should be such as to localize their deposition in the nostril.

Single doses of liquid nasal sprays intended for systemic absorption of active ingredients through the nasal mucosa are tested for uniformity of volume, content or weight.

Packaging and storage Liquid nasal sprays are supplied in glass or plastic containers with atomizing devices or in aerosol containers fitted with a suitable adapter and with or without a metering dose valve. They may also be administered by means of suitable inhalers. Aerosol preparations shall be stored at a temperature not exceeding 50° and protected from frost.

Uniformity of weight Metered dose nasal sprays that are solutions comply with the following test: discharge once to waste. Wait for not less than 5 seconds and discharge again to waste. Repeat this procedure for a further three actuations. Weigh the mass of the container, discharge once to waste and weigh the remaining mass of the container. Calculate the difference between the two masses. Repeat the procedure for a further nine containers. They comply with the test if not more than two of the individual values deviate by more than 25 per cent from the average value and none deviates by more than 35 per cent.

Uniformity of delivered dose Metered dose nasal sprays that are suspensions or emulsions comply with the following test: use an apparatus capable of quantitatively retaining the dose leaving the actuator of the atomising device.

Shake a container for 5 seconds and discharge once to waste. Wait for not less than 5 seconds, shake for 5 seconds and discharge again to waste. Repeat this procedure for a further three actuations. After 2 seconds, fire one dose of the metered dose nasal spray into the collecting vessel by actuating the atomizing device. Collect the contents of the

collecting vessel by successive rinses. Determine the content of active ingredient in the combined rinses.

Repeat the procedure for a further nine containers. Unless otherwise justified and authorized, the preparation complies with the test if not more than one of the individual contents is outside the limits of 75 per cent to 125 per cent and none is outside the limits of 65 per cent and 135 per cent of the average content. If two or three individual contents are outside the limits of 75 per cent to 125 per cent but within the limits of 65 per cent to 135 per cent, repeat the test for twenty more containers. The preparation complies with the test if not more than three individual contents of the thirty individual contents are outside the limits of 75 per cent to 125 per cent and none is outside the limits of 65 per cent to 135 per cent of the average content.

Nasal Powders

(**Note** Nasal powders comply with the appropriate requirements for Powders.)

Nasal powders are powders intended for insufflation into the nostrils by means of a suitable device.

Production The size of the particles should be such as to localize their deposition in the nostril. Particle size should be verified by adequate methods of particle-size determination.

Containers See under Nasal Drops.

Semi-solid Nasal Preparations

(**Note** Semi-solid nasal preparations comply with the appropriate requirements for Topical Semi-solid Preparations.)

Semi-solid nasal preparations are semi-solid dosage forms such as creams, gels, or ointments, etc. intended for application into the nostrils.

Containers The containers for semi-solid nasal preparations should be adapted to deliver the product to the site for application.

Nasal Washes

Nasal washes are generally aqueous solutions intended for irrigation of the nostrils. Nasal washes intended for application to injured parts or prior to a surgical operation are sterile.

Sterility Where the nasal washes are labelled as sterile, unless otherwise directed in the individual monograph, they comply with the "Sterility Test" (Method I, Appendix 10.1).

Containers See under Nasal Drops.

Labelling The label of nasal washes shall state, where applicable, a statement that the preparations are sterile.

ORAL LIQUIDS

Oral liquids usually consist of solutions, suspensions or emulsions of one or more active ingredients in a suitable vehicle; some oral liquids may consist of liquid active ingredients as such. They are intended to be swallowed either undiluted or after dilution. Oral liquids may contain suitable antimicrobial preservatives, antioxidants and other auxiliary substances such as dispersing, suspending, thickening, emulsifying, buffering, wetting, solubilizing, stabilizing, flavouring, sweetening agents, and authorized colouring agents. The vehicle for any particular oral liquids should be chosen having regard to the nature of the active ingredient(s) and providing organoleptic characteristics appropriate to the intended use of the preparation.

Oral liquids other than oral emulsions may be supplied as liquids or prepared just before issue for use by diluting concentrated liquid preparations or dissolving or dispersing granules or powder in the liquid stated on the label.

Suspensions may show a sediment that is readily dispersible on shaking. Emulsions may show evidence of phase separation but are easily reformed on shaking. The preparation remains sufficiently stable to enable a homogeneous dose to be withdrawn.

Several categories of oral liquids may be distinguished: (1) elixirs; (2) linctuses; (3) mixtures; (4) oral drops; (5) oral emulsions; (6) oral solutions; (7) oral suspensions; (8) preparations for oral liquids; (9) syrups.

Deliverable volume Oral liquids comply with the test described in the "Deliverable Volume" (Appendix 4.21).

Uniformity of dosage units Oral liquids that are suspensions or solids in single-unit containers comply with the "Uniformity of Dosage Units" (Appendix 4.28).

Packaging and storage Oral liquids should be kept in well-closed containers. They are supplied in multiple-unit or single-unit containers. They are administered either in volumes such as 5 mL, or multiples of 5 mL, or in small volumes (drops). Each dose of a multiple-unit preparation is administered by means of a device suitable for measuring the prescribed volume.

Oral liquids are supplied in containers that comply with the appropriate requirements given in "Containers" (Appendix 11).

Labelling The label of oral liquids states for Oral Emulsions, Oral Suspensions and, where appropriate, for Mixtures, that the bottle should be shaken before use.

Elixirs

Elixirs are clear, flavoured oral liquids containing one or more active ingredients dissolved in a vehicle that usually contains a high proportion of Sucrose or a suitable polyhydric alcohol or alcohols and may also contain Ethanol (95 Per Cent) or Dilute Ethanols.

Linctuses

Linctuses are viscous oral liquids that may contain one or more active ingredients in solution. The vehicle usually contains a high proportion of Sucrose, other sugars or a suitable polyhydric alcohol or alcohols. Linctuses are intended for use in the treatment or relief of cough, and are sipped and swallowed slowly without the addition of water.

Mixtures

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

Oral Drops

Oral drops are oral liquids that are intended to be administered in small volumes with the aid of a suitable measuring device (Appendix 1.15).

Oral Emulsions

Oral emulsions are oral liquids containing one or more active ingredients. They are stabilized oil-in-water dispersions, either or both phases of which may contain dissolved solids. Solids may also be suspended in oral emulsions.

Containers When issued for use, oral emulsions should be supplied in wide-mouthed bottles.

Oral Solutions

Oral solutions are oral liquids containing one or more active ingredients dissolved in a suitable vehicle.

Oral Suspensions

Oral suspensions are oral liquids containing one or more active ingredients suspended in a suitable vehicle. Suspended solids may slowly separate on standing but are easily redispersed.

Preparations for Oral Liquids

Preparations for oral liquids are solids or mixtures of solids intended for the preparations of solutions or suspensions by dissolving or dispersing them in a suitable vehicle. They may contain auxiliary substances in particular to facilitate dispersion or dissolution and to prevent caking. They are designated "for Oral Solution" or "for Oral Suspension" (e.g., Ampicillin for Oral Suspension).

Packaging and storage Preparations for oral liquids shall be kept in tightly closed containers.

Labelling The label of preparations for oral liquids states (1) the directions for preparing the oral liquids including the nature and quantity of liquid to be used; (2) the storage condition; (3) the conditions and the duration of storage after constitution.

Syrups

Syrups are concentrated aqueous solutions of sucrose, other sugars or sweetening agents, to which small quantities of suitable polyhydric alcohols may be added to retard crystallization or to increase the solubility of the other ingredients. Syrups usually contain aromatic or other flavouring materials and may also contain active ingredient(s). They should be recently prepared unless they contain suitable antimicrobial preservatives.

Packaging and storage Syrups should be kept in well-closed containers and stored at temperatures not exceeding 30°.

OROMUCOSAL PREPARATIONS

Oromucosal preparations are solid, semi-solid or liquid preparations, containing one or more active substances intended for administration to the oral cavity and/or the throat to obtain a local or systemic effect. Preparations intended for a local effect may be designed for application to a specific site within the oral cavity such as the gums (gingival preparations) or the throat (oropharyngeal preparations). Preparations intended for a systemic effect are designed to be absorbed primarily at one or more sites on the oral mucosa (e.g., sublingual preparations). Mucoadhesive preparations are intended to be retained in the oral cavity by adhesion to the mucosal epithelium and may modify systemic drug absorption at the site of application. For many oromucosal preparations, it is likely that some proportion of the active substance(s) will be swallowed and may be absorbed via the gastrointestinal tract.

Oromucosal preparations may contain suitable antimicrobial preservatives and other excipients such as dispersing, suspending, thickening, emulsifying, buffering, wetting, solubilizing, stabilizing, flavouring and sweetening agents. Solid

preparations may in addition contain glidants, lubricants and excipients capable of modifying the release of the active substance(s).

Several categories of preparations for oromucosal use may be distinguished: (1) gargles; (2) mouthwashes; (3) gingival solutions; (4) oromucosal solutions and oromucosal suspensions; (5) semi-solid oromucosal preparations (including for example gingival gel, gingival paste, oromucosal gel, oromucosal paste); (6) oromucosal drops, oromucosal sprays, sublingual drops, and sublingual sprays (including oropharyngeal sprays); (7) lozenges and pastilles; (8) compressed lozenges; (9) sublingual tablets and buccal tablets; (10) oromucosal capsules; (11) mucoadhesive preparations.

Production During the development of an oromucosal preparation containing 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 the criteria for judging the preservative properties of the formulation are provided under “Efficacy of Antimicrobial Preservation” (Appendix 10.6). In the manufacture, packaging, storage and distribution of oromucosal preparations, suitable means are taken to ensure their microbiological quality; recommendations on this aspect are provided under “Limit for Microbial Contamination” (Appendix 10.5). In the manufacture of semi-solid and liquid oromcosal preparations containing dispersed particles, measures are taken to ensure a suitable and controlled particle size with regard to the intended use.

Uniformity of dosage units Single-unit oromucosal preparations comply with the “Uniformity of dosage units” (Appendix 4.28).

Gargles

Gargles are aqueous solutions intended for gargling to obtain a local effect. They are not to be swallowed. They are supplied as ready-to-use solutions or concentrated solutions to be diluted. They may also be prepared from powders or tablets to be dissolved in water before use. Gargles may contain excipients to adjust the pH which, as far as possible, is neutral.

Mouthwashes

See under Topical Preparations.

Gingival Solutions

Gingival solutions are intended for administration to the gingivae by means of a suitable applicator.

Oromucosal Solutions and Oromucosal Suspensions

Oromucosal solutions and oromucosal suspensions are liquid preparations intended for administration to the oral cavity by means of a suitable applicator.

Oromucosal suspensions may show a sediment which is readily dispersible on shaking to give a suspension which remains sufficiently stable to enable the correct dose to be delivered.

Semi-solid Oromucosal Preparations

Semi-solid oromucosal preparations are hydrophilic gels or pastes intended for administration to the oral cavity or to a specific part of the oral cavity such as the gingivae (gingival gel, gingival paste). They may be provided as single-dose preparations. Semi-solid oromucosal preparations comply with the requirements of the monograph for “Topical semisolid Preparations” (Appendix 1.16).

Oromucosal Drops, Oromucosal Sprays, Sublingual Drops and Sublingual Sprays

Oromucosal drops, oromucosal sprays, sublingual drops and sublingual sprays are solutions, emulsions or suspensions intended for local or systemic effect. They are applied by instillation or spraying into the oral cavity or onto a specific part of the oral cavity such as instilling or spraying under the tongue (sublingual drops or sprays) or into the throat (oropharyngeal sprays).

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 which remains sufficiently stable to enable the correct dose to be delivered.

Liquid oromucosal sprays are supplied in containers with atomizing devices or in pressurized containers having a suitable adaptor, with or without a metering dose valve, which comply with the requirements of the monograph for “Aerosols” (Appendix 1.6).

The size of the droplets of the spray is such as to localize their deposition in the oral cavity or the throat as intended.

Unless otherwise prescribed or justified and authorized, oromucosal drops supplied in single-dose containers, single doses of metered-dose oromucosal sprays and sublingual sprays, all intended for systemic action, comply with the following requirement.

Oromucosal Drops in Single-dose Containers

Uniformity of dosage units Oromucosal drops in single-unit containers comply with the "Uniformity of Dosage Units" (Appendix 4.28).

Metered-dose Oromucosal Sprays and Sublingual Sprays

Uniformity of dosage units Metered-dose oromucosal sprays and sublingual sprays comply with the "Uniformity of Dosage Units" (Appendix 4.28) or, where justified and authorized, with the test for uniformity of weight or the test for uniformity of delivered dose shown below.

IN THE CASE OF METERED-DOSE OROMUCOSAL SPRAYS AND SUBLINGUAL SPRAYS THAT ARE SOLUTIONS, PROCEED AS FOLLOWS Discharge once to waste. Wait for a minimum of 5 seconds, shake for 5 seconds 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 weights. Repeat the procedure for a further 9 containers. Determine the weight variation (Appendix 4.28).

IN THE CASE OF METERED-DOSE OROMUCOSAL SPRAYS AND SUBLINGUAL SPRAYS THAT ARE SUSPENSIONS OR EMULSIONS PROCEED AS FOLLOWS Use an apparatus capable of quantitatively retaining the dose leaving the actuator of the atomizing device. Shake the container for 5 seconds and discharge once to waste. Wait for a minimum of 5 seconds, shake for 5 seconds and discharge again to waste. Repeat this procedure for a further 3 actuations. After 2 seconds, fire 1 dose of the metered-dose spray into the collecting vessel by actuating the atomizing device. 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 (Appendix 4.28).

Weight variation Metered-dose oromucosal sprays and sublingual sprays that are solutions comply with the following test. Discharge once to waste. Wait for a minimum of 5 seconds, shake for 5 seconds 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 weights. Repeat the procedure for a further 9 containers. The preparation complies with the test if maximum 2 of the individual values deviate by more than 25 per cent from the average value and none deviates by more than 35 per cent.

Uniformity of delivered dose Metered-dose oromucosal sprays and sublingual sprays that are suspensions or emulsions comply with the following test. Use an apparatus capable of quantitatively retaining the dose leaving the actuator of the atomizing device. Shake the container for 5 seconds and discharge once to waste. Wait for a minimum of 5 seconds, shake for 5 seconds and discharge again to waste. Repeat this procedure for a further 3 actuations. After 2 seconds, fire 1 dose of the metered-dose spray into the collecting vessel by actuating the atomizing device. 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. Unless otherwise justified and authorized, the preparation complies with the test if maximum 1 of the individual contents is outside the limits of 75 per cent to 125 per cent and none is outside the limits of 65 per cent to 135 per cent of the average content. If 2 or maximum 3 individual contents are outside the limits of 75 per cent to 125 per cent but within the limits of 65 per cent to 135 per cent, repeat the test for 20 more containers. The preparation complies with the test if maximum 3 individual contents of the 30 individual contents are outside the limits of 75 per cent to 125 per cent and none is outside the limits of 65 per cent to 135 per cent of the average content.

Lozenges and Pastilles

Lozenges and pastilles are solid, single-dose preparations intended to be sucked to obtain, usually, a local effect in the oral cavity and the throat. They contain one or more active substances, usually in a flavoured and sweetened base, and are intended to dissolve or disintegrate slowly in the mouth when sucked. Lozenges are hard preparations prepared by moulding. Pastilles are soft, flexible preparations prepared by moulding of mixtures containing natural or synthetic polymers or gums and sweeteners.

Compressed Lozenges

Compressed lozenges are solid, single-dose preparations intended to be sucked to obtain a local or systemic effect. They are prepared by compression and are often rhomboid in shape. Compressed lozenges conform with the general definition of tablets.

Production In the manufacture of compressed lozenges, measures are taken to ensure that they possess suitable mechanical strength to resist handling without crumbling or breaking. This may

be demonstrated by examining the "Friability of Uncoated Tablet" (Appendix 4.30) and the "Resistance to Crushing of Tablets" (Appendix 4.31).

Dissolution For compressed lozenges intended for a systemic effect, a suitable test is carried out to demonstrate the appropriate release of the active substance(s).

Sublingual Tablets and Buccal Tablets

Sublingual tablets and buccal tablets are solid, single-dose preparations to be applied under the tongue or to the buccal cavity, respectively, to obtain a systemic effect. They are prepared by compression of mixtures of powders or granulations into tablets with a shape suited for the intended use. Sublingual tablets and buccal tablets conform to the general definition of tablets.

Production In the manufacture of sublingual tablets and buccal tablets, measures are taken to ensure that they possess suitable mechanical strength to resist handling without crumbling or breaking. This may be demonstrated by examining the "Friability of Uncoated Tablets" (Appendix 4.30) and the "Resistance to Crushing of Tablets" (Appendix 4.31).

Dissolution Unless otherwise justified and authorized, a suitable test is carried out to demonstrate the appropriate release of the active substance(s).

Oromucosal Capsules Oromucosal capsules are soft capsules to be chewed or sucked.

Mucoadhesive Preparations

Mucoadhesive preparations contain one or more active substances intended for systemic absorption through the buccal mucosa over a prolonged period of time. They may be supplied as mucoadhesive buccal tablets or as other mucoadhesive solid or semi-solid preparations. Mucoadhesive buccal tablets are prepared by compression of mono- or multi-layered tablets. They usually contain hydrophilic polymers, which on wetting with the saliva produce a flexible hydrogel that adheres to the buccal mucosa.

Production In the manufacture of mucoadhesive buccal tablets, measures are taken to ensure that they possess suitable mechanical strength to resist handling without crumbling or breaking. This may be demonstrated by examining the "Friability of Uncoated Tablet" (Appendix 4.30) and the "Resistance to Crushing of Tablets" (Appendix 4.31).

Dissolution Unless otherwise justified and authorized, a suitable test is carried out to demonstrate the appropriate release of the active substance(s).

PARENTERAL PREPARATIONS

Parenteral preparations are sterile preparations intended for administration by injection, infusion or implantation into the body. There are five main forms of these preparations defined as follows: (1) medicaments or solutions or emulsions thereof suitable for injection, bearing titles of the form, ____ Injection; (2) dry solids or liquid concentrates containing active ingredient(s) with or without buffer(s), diluent(s) or other added substances, and which, upon the addition of suitable solvents, yield solutions conforming in all respects to the requirements for Injections, and which are distinguished by titles of the form, ____ for Injection; (3) solids which are suspended in a suitable fluid medium and which are not to be injected intravenously or into the spinal canal, distinguished by titles of the form, Sterile ____ Suspension; (4) dry solids which, upon the addition of suitable vehicles, yield preparations conforming in all respects to the requirements for sterile suspensions; and which are distinguished by titles of the form, Sterile ____ for Suspension; and (5) sterile solid preparations, of which size and shape are suitable for implantation into body tissues, distinguished by titles of the form, ____ Implant. The term "Injections" may be used for preparations (1) to (4).

Where used in this Pharmacopoeia, the designation *Large-volume intravenous solution* applies to a single-dose injection that is intended for intravenous use and is packaged in containers labelled as containing more than 100 mL. The designation *Small-volume Injection* applies to an injection that is packaged in containers labelled as containing 100 mL or less.

Production Parenteral preparations are prepared by methods designed to ensure their sterility and to avoid the introduction of contaminants, the presence of pyrogens and the growth of micro-organisms. Water used in the manufacture of injections complies with the requirements for Water for Injections in bulk.

Aqueous vehicles The vehicles for aqueous parenteral preparations comply with the "Pyrogen Test" (Appendix 8.2) or the "Test for Bacterial Endotoxins" (Appendix 8.5), whichever is specified. Water for Injection generally is used as the vehicle, unless otherwise specified in the individual monograph. Sodium Chloride may be added in amounts sufficient to render the resulting solution isotonic; Sodium Chloride Injection, or Ringer's Injection, may be used in whole or in part instead of Water for Injection unless otherwise specified in

the individual monograph. For conditions applying to other adjuvants, see Added substances, in this appendix.

Other vehicles Fixed oils used as vehicles for nonaqueous injections are of vegetable origin, are odourless or nearly so, and have no odour or taste suggesting rancidity. They comply with the test for solid paraffin under Liquid Paraffin, the cooling bath being maintained at 10º, have a saponification value of between 185 and 200 (Appendix 5.7), have an iodine value of between 79 and 128 (Iodine Bromide Method, Appendix 5.6), and comply with the requirements of the following tests.

UNSAPONIFIABLE MATTER Reflux on a water-bath 10 mL of the oil with 15 mL of a 16.7 per cent w/v solution of *sodium hydroxide* and 30 ml of *ethanol*, with occasional shaking until the mixture becomes clear. Transfer the solution to a shallow dish, evaporate the ethanol on a water-bath, and mix the residue with 100 mL of *water*: a clear solution results.

ACID VALUE Not more than 0.22 (Appendix 5.4); using 0.020 M *potassium hydroxide* as titrant.

Synthetic mono- or di-glycerides of fatty acids may be used as vehicles, provided they are liquid and remain clear when cooled to 10º and have an iodine value of not more than 140 (Iodine Bromide Method, Appendix 5.6).

These and other nonaqueous vehicles may be used, provided they are safe in the volume of parenteral preparations administered, and also provided they do not interfere with the therapeutic efficacy of the preparation or with its response to prescribed assays and tests.

Added substances Suitable substances may be added to parenteral preparations to increase stability or usefulness, unless prescribed in the individual monograph, provided they are harmless in the amounts administered and do not interfere with the therapeutic efficacy or with the responses to the specified assays and tests. No colouring agent may be added, solely for the purpose of colouring the finished preparation, to a solution intended for parenteral administration [see also Added Substances, under General Notices, and "Efficacy of Antimicrobial Preservation" (Appendix 10.6)].

Observe special care in the choice and use of added substances in injections that are administered in a volume exceeding 5 mL. The following maximum limits prevail unless otherwise directed: for agents containing mercury and the cationic, surface-active compounds, 0.01 per cent; for those of the types of chlorobutanol, cresol, and phenol, 0.5 per cent; and for sulfur dioxide, or an equivalent amount of the sulfite, bisulfite, or metabisulfite of potassium or sodium, 0.2 per cent.

A suitable substance or mixture of substances to prevent the growth of micro-organisms must be added to preparations intended for injection that are packaged in multiple-dose containers, regardless of the method of sterilization employed, unless otherwise specified in the individual monograph, or unless the active ingredients are themselves antimicrobial. Such substances are used in concentrations that will prevent the growth of or kill micro-organisms in the preparations. Such substances also comply with the "Efficacy of Antimicrobial Preservation" (Appendix 10.6) and "Content of Antimicrobial Agents" (Appendix 6.22). Sterilization processes are employed even though such substances are used [see also Added Substances, under General Notices, and "Sterilization and Sterility Assurance" (Appendix 12)]. The air in the container may be evacuated or be displaced by a chemically inert gas. If the injection is oxygen-sensitive, that information must appear in the labelling.

Containers for parenteral preparations Containers, including the closures, for parenteral preparations do not interact physically or chemically with the preparations in any manner to alter the strength, quality, or purity beyond the official requirements under the ordinary or customary conditions of handling shipment, storage, sale, and use. The container is made of material that permits inspection of the contents. Parenteral preparations are supplied in glass ampoules, bottles or vials or in other containers such as plastic bottles or bags and in prefilled syringes the integrity of which is ensured by suitable means. The type of glass preferable for each parenteral preparation is usually stated in the individual monograph.

For definitions of single-dose and multiple-dose containers, see Containers under General Notices, Containers comply with the "Containers" (Appendix 11). Containers are closed by fusion, or by application of suitable closures, in such manner as to prevent contamination or loss of contents. Closures for multipledose containers permit the withdrawal of the contents without removal or destruction of the closure. The closure permits penetration by a needle, and, upon withdrawal of the needle, at once recloses the container against contamination.

Containers for sterile solids Containers, including the closures, for dry solids intended for parenteral use do not interact physically or chemically

with the preparation in any manner to alter the strength, quality, or purity beyond the official requirements under the ordinary or customary conditions of handling, shipment, storage, sale and use.

A container for a sterile solid permits the addition of a suitable solvent and withdrawal of portions of the resulting solution or suspension in such manner that the sterility of the product is maintained.

Where the Assay in a monograph provides a procedure for Assay preparation in which the total withdraw able contents are to be withdrawn from a single-dose container with a hypodermic needle and syringe, the contents are to be withdrawn as completely as possible into a dry hypodermic syringe of a rated capacity not exceeding three times the volume to be withdrawn and fitted with a 21-gauge needle not less than 2.5 cm in length, care being taken to expel any air bubbles, and discharged into a container for dilution and assay.

Volume in containers Each container of an injection is filled with a volume in slight excess of the labelled size or the volume which is to be withdrawn. The excess volumes recommended in the accompanying table are usually sufficient to permit withdrawal and administration of the labelled volumes.

DETERMINATION OF VOLUME OF INJECTIONS IN CONTAINERS Select one or more containers if the volume is 10 mL or more, three or more if the volume is more than 3 mL and less than 10 mL, or five or more if the volume is 3 mL or less. Take up individually the contents of each container selected into a dry hypodermic syringe of a rated capacity not exceeding three times the volume to be measured, and fitted with a 21-gauge needle not less than 2 cm (1 inch) in length. Expel any air bubbles from the syringe and needle, and then discharge the contents of the syringe, without emptying the needle, into a standardized, dry cylinder (graduated to contain rather than to deliver the designated volumes) of such size that the volume to be measured occupies at least 40 per cent of its rated volume. Alternatively, the contents of the syringe may be discharged into a dry, tared beaker, the volume, in mL, being calculated as the weight, in g, of injections taken divided by its density. The contents of two or three 1-mL or 2-mL containers may be pooled for the measurement, provided that a separate, dry syringe assembly is used for each container. The content of containers holding 10 mL or more may be determined by means of opening them and emptying the contents directly into the graduated cylinder or tared beaker.

The volume is not less than the labelled volume in the case of containers examined individually or, in the case of 1-mL and 2-mL containers, is not less than the sum of the labelled volumes of the containers taken collectively.

For injections in multiple-dose containers labelled to yield a specific number of doses of a stated volume, proceed as directed in the foregoing, using the same number of separate syringes as the number of doses specified. The volume is such that each syringe delivers not less than the stated dose.

For injections containing oil, warm the containers, if necessary, and thoroughly shake them immediately before removing the contents. Cool to 25º before measuring the volume.

Constituted solutions Sterile dosage forms from which constituted solutions are prepared for injection bearing titles of the form, for Injection comply with the "Constituted Solutions" (Appendix 4.20).

Particulate matter All large-volume injections for single-dose infusion, and those small-volume injections for which the monographs specify such requirements, are subject to the particulate matter limits set forth in the "Particulate Matter in Injections" (Appendix 4.27). An article packaged as both a large-volume and a small olume injection meets the requirements set forth for "Small-volume Injections" where the container is labelled as containing 100 mL or less if the individual monograph includes a test for "Particulate matter"; it meets the requirements set forth for "Large-volume Injections for Single-dose Infusion" where the container is labelled as containing more than 100 mL. Injections packaged and labelled for use as irrigating solutions are exempt from requirements for "Particulate matter".

Pyrogen test; Bacterial endotoxins test Parenteral preparations comply with the "Pyrogen Test" (Appendix 8.2) or the "Test for Bacterial Endotoxins" (Appendix 8.5) as specified in the individual monograph.

Sterility Parenteral preparations comply with the "Sterility Test" (Appendix 10.1). Unless otherwise specified in the individual monograph, carry out the test using Method I.

Uniformity of dosage units Parenteral preparations that are packaged in single-unit containers comply with the "Uniformity of Dosage Units" (Appendix 4.28). The test for Content Uniformity is required for sterile solids containing a unit weight equal to or less than 40 mg but not required for multivitamin and trace element parenteral preparations.

Packaging and storage The volume of the parenteral preparations in single-dose containers provides the amount specified for parenteral administration at one time and in no case is more than sufficient to permit the withdrawal and administration of 1 litre.

Preparations intended for intraspinal, intracisternal, or peridural administration are packaged only in single dose containers.

Unless otherwise specified in the individual monograph, no multiple-dose container contains a volume of parenteral preparation more than sufficient to permit the withdrawal of 30 mL.

Injections packaged for use as irrigation solutions or for hemofiltration or dialysis or for parenteral nutrition are exempt from the 1-litre restriction of the foregoing requirements relating to packaging. Containers for injections packaged for use as irrigation solutions or for hemofiltration may be designed to empty rapidly and may contain a volume of more than 1 litre.

Labelling The label of injection complies with the Labelling, under General Notices. The label also states the name of any added substances and the storage conditions.

The container is so labelled that a sufficient area of the container remains uncovered for its full length or circumference to permit inspection of the contents. In case the area of the container is not sufficient to be labelled as mentioned above, unless otherwise specified in the individual monograph, the label shall state (1) the name of the drug product and (2) the batch or lot number assigned by the manufacturer.

The label of a single-dose parenteral preparation states that any portion of the contents remaining should be discarded.

In the case of a dry preparation or other preparation to which a diluent is intended to be added before use, the label includes the following information: the amount of each ingredient, the composition of recommended diluent(s) [the name(s) alone, if the formula is specified in the individual monograph], the amount to be used to attain a specific concentration of active ingredient and the final volume of solution so obtained, a brief description of the physical appearance of the constituted solution, directions for proper storage of the constituted solution, and an expiration date limiting the period during which the constituted solution may be expected to have the required or labelled potency if it has been stored as directed.

POWDERS

Powders are preparations consisting of solid, loose, dry particles of varying degrees of fineness that contain one or more active ingredients with or without added substances including, where necessary, flavouring agents and authorized colouring matter. Two categories of powders may be distinguished: (1) oral powders; (2) topical powders.

Minimum fill Powders comply with the test described in the "Minimum Fill" (Appendix 4.26).

Uniformity of dosage units Powders comply with the "Uniformity of Dosage Units" (Appendix 4.28). The test for Content Uniformity is not required for multivitamin and trace element powders.

Packaging and storage Powders should be kept in tightly closed containers.

Oral Powders

Oral powders are generally administered in or with water or another suitable liquid. They may also be swallowed directly.

Oral powders are presented as single-unit or multiple-unit preparations. For single-unit powders each dose is enclosed in a separate container, for example, a sachet, a paper packet or a vial. Multiple-unit powders require the provision of a measuring device capable of delivering the quantity prescribed.

Effervescent oral powders are presented as single unit or multiple-unit powders and generally contain acid substances and either carbonates or bicarbonates that react rapidly in the presence of water to release carbon dioxide. They are intended to be dissolved or dispersed in water before administration.

Labelling For single-unit containers the label states the name(s) and quantity(ies) of active ingredient(s) per container. For multiple-unit containers the label states the name(s) and quantity(ies) of active ingredient(s) in a suitable amount by weight.

Topical Powders

Topical powders are free from grittiness. They are presented as single-unit or multiple-unit preparations. If a topical powder is specifically intended for use on large open wounds or on severely injured skin, it must be sterile.

Multiple-unit topical powders should preferably be dispensed in sifter-top containers or in aerosol containers.

Dusting powders are topical powders consisting of finely divided powders that are intended to be applied to the skin for therapeutic, prophylactic or lubricant purposes. In general, they should be passed through at least a *No. 150 sieve* to assure freedom from grit that could irritate traumatized areas.

Labelling The label of topical powders states, where applicable, that the topical powder is sterile.

PREPARATIONS FOR INHALATION

Preparations for inhalation are solid or liquid preparations that contain one or more active ingredients. They are intended for administration to the lower respiratory tract for local or systemic effect.

Preparations for inhalation should not adversely affect the functions of the mucosa of the respiratory tract and its cilia. Preparations for inhalation are supplied in multiple-unit or single-unit containers provided with a suitable administration device, if necessary.

Four categories of preparations for inhalation may be distinguished: (1) inhalations; (2) powders for inhalation; (3) inhalation aerosols; (4) inhalants. Preparations for inhalation that are converted into an aerosol are generally administered by nebulizers, by pressurized metered-dose inhalers or by dry-powder inhalers.

Production During the development of a preparation for inhalation which contains an antimicrobial preservative, the effectiveness of the chosen preservative shall be demonstrated to the satisfaction of the competent authority. A suitable method of test together with the criteria for judging the preservative properties of the formulation are described in the "Efficacy of Antimicrobial Preservation" (Appendix 10.6). The size of particles to be inhaled should be adjusted so as to localize their deposition in the lower respiratory tract and verified by suitable methods of particle-size determination.

For preparations for inhalation that are supplied in single-unit containers provided with a suitable administration device, the single doses released by the administration device are tested for uniformity of volume, weight or content. The content of a unit spray of suspensions for inhalation dispensed in multiple-unit aerosol containers is verified using a suitable number of unit sprays for quantification.

Minimum fill Preparations for inhalation comply with the test described in the "Minimum Fill" (Appendix 4.26).

Packaging and storage Preparations for inhalation should be kept in well-closed containers. Aerosol preparations shall be stored at a temperature not exceeding 50° and should be protected from frost.

Labelling The label of preparations for inhalation states the name(s) and quantity(ies) of any antimicrobial preservative(s).

Inhalations

Inhalations are solutions or suspensions or emulsions of one or more active ingredients administered by the nasal or oral respiratory route for local or systemic effect.

As far as possible, liquids not dispensed in aerosol containers are aqueous, isotonic liquids. The pH of the liquid should be not lower than 3 and not higher than 8.5. Co-solvents or suitable solubilizers may be used to increase the solubility of the active ingredients. Aqueous inhalations supplied in multiple-unit containers should contain a suitable antimicrobial preservative at a suitable concentration except where the preparation itself has adequate antimicrobial properties.

Suspensions are readily dispersible on shaking and the suspension remains sufficiently stable to enable the correct dose to be delivered by the device. Suitable suspension stabilizers may be added.

Inhalations are usually supplied in glass or plastic containers. If inhalations are provided in concentrated form, they should be diluted in the nebulizers to the prescribed volume with the prescribed liquid before use.

Powders for Inhalation

Powders for inhalation are powders containing medicinal substances usually diluted with suitable diluents. These powders are normally dispensed in hard gelatin capsules. They may also be administered by mechanical devices that require manually produced pressure or a deep inhalation by the patient, e.g., *Cromoglycate Sodium for Inhalation*.

Uniformity of dosage units Powders for inhalation comply with the "Uniformity of Dosage Units" (Appendix 4.28).

Labelling The label on the container states that the capsules are intended for use in an inhaler and are not to be swallowed.

Inhalation Aerosols

(**Note** Inhalation aerosols comply with the appropriate requirements for aerosols.)

Inhalation aerosols are metered-dose preparations. They are drugs or solutions or suspensions or

emulsions intended to be inhaled in controlled amounts and are delivered by the actuation of an appropriate metering valve. They contain multiple doses, often exceeding several hundreds. The most common single-dose volumes delivered are from 25 to 100 µL (also expressed as mg) per actuation.

Production The formulation of inhalation aerosols and the components of the delivery device (i.e., the aerosol container with its integral metering valve and the actuator) should be designed and, where appropriate, the particle size of the active ingredients should be controlled so that, when the inhalation aerosols are used in accordance with the manufacturer's recommendations, an adequate proportion of the active ingredients is made available for inhalation. A proportion of the active ingredient is deposited on the inner surface of the actuator; the amount available for inhalation is therefore less than the amount released by actuation of the valve. Inhalation aerosols should be manufactured in conditions designed to minimize microbial and particulate contaminations.

Packaging and storage Inhalation aerosols are supplied in suitable containers fitted with an appropriate metering valve that forms an integral part of the container. The containers are usually supplied with an appropriate actuator. Inhalation aerosols should be protected from extremes of temperature and from undue fluctuations in temperature.

Labelling The label of inhalation aerosols states (1) the quantity(ies) of active ingredient(s) delivered by each actuation of the valve; (2) the instructions for using the inhalation aerosols; (3) any special precautions associated with the use of the inhalation aerosols.

Inhalants

Inhalants are drugs or combination of drugs which may contain an inert, suspended, diffusing agent. They are intended to release volatile constituents for inhalation either when placed on a pad or when added to hot, but not boiling, water.

Labelling The label on the container states that the inhalants are not to be taken by mouth.

RECTAL PREPARATIONS

Rectal preparations are intended for rectal use in order to obtain a systemic or local effect, or they may be intended for diagnostic purposes. Several categories of rectal preparations may be distinguished: (1) rectal suppositories; (2) rectal capsules; (3) rectal solutions and suspensions (enemas); (4) powders and tablets for rectal solutions and suspensions; (5) rectal creams; (6) rectal gels; (7) rectal ointments; (8) rectal foams; and (9) rectal tampons.

Production During the development of a rectal 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 method of testing together with criteria for judging the preservative of the formulation is provided under "Efficacy of Antimicrobial Preservation" (Appendix 10.6).

In the manufacture, packaging, storage and distribution of rectal preparations, suitable means are taken to ensure their microbial quality; recommendations on this aspect are provided under "Limit for Microbial Contamination" (Appendix 10.5).

In the manufacture of semi-solid and liquid rectal preparations containing dispersed particles, measures are taken to ensure a suitable and controlled particle size with regard to the intended use.

Dissolution A suitable test may be required to demonstrate the appropriate release of the active ingredient(s) from solid, single dose preparations, for example the dissolution test for suppositories and soft capsules (Appendix 4.24). Where a dissolution test is prescribed, a disintegration test may not be required.

Uniformity of dosage units Rectal preparations comply with the "Uniformity of Dosage Units" (Appendix 4.28).

Rectal Suppositories

Rectal suppositories are solid, single-dose preparations. The shape, volume and consistence of suppositories are suitable for rectal administration.

They contain one or more active ingredients dispersed or dissolved in a simple or compound excipient which may be soluble or dispersible in water or may melt at body temperature. Excipients such as diluents, adsorbents, surface-active agents, lubricants, antimicrobial preservatives and colouring matter, authorized by the competent authority, may be added if necessary.

Production Rectal suppositories are prepared by compression or moulding. If necessary, the active ingredient(s) are previously ground and sieved through a suitable sieve. When prepared by moulding, the medicated mass, sufficiently liquified by heating, is poured into suitable moulds. The suppository solidifies on cooling. Various excipients are avail-

able for this process, such as hard fat, macrogols, cocoa butter, and various gelatinous mixtures consisting, for example, of gelatin, water and glycerol.

A suitable test is carried out to demonstrate the appropriate release of the active ingredient(s) from suppositories intended for modified release or for prolonged local action.

Disintegration Unless intended for modified release or for prolonged local action, rectal suppositories comply with the "Disintegration Test for Suppositories and Pessaries" (Appendix 4.22). For suppositories with a fatty base, examine after 30 minutes and for suppositories with a water-soluble base after 60 minutes, unless otherwise justified and authorized.

Packaging and storage Rectal suppositories shall be kept in well-closed containers.

Labelling The label on the container states (1) that they are intended for external use only; (2) the storage conditions.

Rectal Capsules

Rectal capsules (shell suppositories) are solid, single-dose preparations generally similar to soft capsules as defined in capsules except that they may have lubricating coatings. They are of elongated shape, are smooth and have a uniform external appearance.

Production A suitable test is carried out to demonstrate the appropriate release of the active ingredient(s) from rectal capsules intended for modified release or prolonged local action.

Disintegration Unless intended for modified release or for prolonged local action, rectal capsules comply with the "Disintegration Test for Suppositories" (Appendix 4.22). Examine the state of the capsules after 30 minutes, unless otherwise justified and authorized.

Rectal Solutions and Suspensions (Enemas)

Rectal solutions and suspensions (enemas) are liquid preparations intended for rectal use in order to obtain a systemic or local effect, or they may be intended for diagnostic purposes.

They are single-dose preparations containing one or more active ingredients dissolved or dispersed in water, glycerol, macrogols or other suitable solvents. Suspensions may show a sediment which is readily dispersible on shaking to give a suspension which remains sufficiently stable to enable the correct dose to be delivered.

Rectal solutions and suspensions may contain excipients intended, for example, to adjust the viscosity of the preparation, to adjust or stabilize pH, to increase the solubility of the active ingredient(s) or to stabilize the preparation. These substances do not adversely affect the intended medical action or, at the concentrations used, cause undue local irritation.

Rectal solutions and suspensions are supplied in containers containing a volume in the range of 2.5 mL to 2000 mL. The container is adapted to deliver the preparation to the rectum or it is accompanied by a suitable applicator.

Powders and Tablets for Rectal Solutions and Suspensions

Powders and tablets intended for the preparation of rectal solutions or suspensions are single-dose preparations which are dissolved or dispersed in water at the time of administration. They may contain excipients to facilitate dissolution or dispersion or to prevent aggregation of the particles.

After dissolution or suspension, they comply with the requirements for rectal solutions or rectal suspensions, as appropriate.

Disintegration Tablets for rectal solutions or suspensions disintegrate within 3 minutes when tested as described under "Disintegration Test for Suppositories and Pessaries" (Appendix 4.22), but using *water* at 15° to 25°.

Labelling The label on the container states (1) the directions for preparing the rectal solution or suspension; (2) the storage conditions; (3) the period during which the constituted rectal solutions or suspensions may be expected to remain satisfactory for use when prepared and stored in accordance with the manufacturer's recommendations.

Rectal Creams

See under Topical Semi-solid Preparations.

Rectal Gels

See under Topical Semi-solid Preparations.

Rectal Ointments

See under Topical Semi-solid Preparations.

Rectal Foams

See under Medicated Foams.

Rectal Tampons

Rectal tampons are solid, single-dose preparations intended to be inserted into the lower part of the rectum for a limited time.

See also under Medicated Tampons.

SPIRITS

Spirits are ethanolic or hydroethandic solutions of volatile substances usually prepared by simple solution or by admixture of the ingredients. Some spirits serve as flavouring agents while others have medicinal value. Reduction of the high ethanolic content of spirits by admixture with aqueous preparations often causes turbidity.

Packaging and storage Spirits shall be kept in tightly closed containers, protected from light to prevent loss by evaporation and to limit oxidative changes.

SYSTEMS

Systems are preparations that allow for the uniform release or targeting of drugs to the body. These preparations are commonly called drug delivery systems or delivery systems. The most widely used of these are transdermal systems.

Transdermal Systems

Transdermal drug delivery systems are self-contained, discrete dosage forms that, when applied to intact skin, are designed to deliver the drug(s) through the skin to the systemic circulation. Systems typically comprise an outer covering (barrier), a drug reservoir, which may have a rate controlling membrane, a contact adhesive applied to some or all parts of the system and the system/ skin interface, and a protective liner that is removed before applying the system. The activity of these systems is defined in terms of the release rate of the drug(s) from the system. The total duration of drug release from the system and the system surface area may also be stated.

Labelling The label of transdermal systems states (1) the dose released per unit time; (2) the storage conditions.

Ocular Systems

Ocular systems are sterile, solid or semi-solid preparations of suitable size and shape, designed to be inserted in the conjunctival cul-de-sac, to produce an ocular effect. They generally consist of a reservoir of active ingredient(s) embedded in a matrix or bounded by a rate-controlling membrane. The active ingredient, which is more or less soluble in physiological fluids, is released over a predetermined period of time. Ocular systems are individually distributed into sterile containers.

Sterility Unless otherwise specified in the individual monograph, ocular systems comply with the "Sterility Test" (Method I, Appendix 10.1).

Drug release pattern Ocular systems comply with the test for drug release pattern in the individual monograph.

Labelling The label of ocular systems states (1) the dose released per unit time; (2) the storage conditions.

Intrauterine Systems

An intrauterine system, based on a similar principle but intended for release of drug over a much longer period of time, i.e. one year, is also available.

TABLETS

Tablets are solid dosage forms containing one or more active ingredients. They are obtained by single or multiple compression (in certain cases they are moulded) and may be uncoated or coated. They are usually intended for oral administration.

The different categories of tablets that exist include soluble tablets, effervescent tablets, tablets for use in the mouth, and modified-release tablets. Tablets are normally circular in shape and their surfaces are flat or convex. Tablets may have lines or break-marks, symbols, or other markings. They should be sufficiently hard to withstand handling, including packaging, storage, and transportation, without crumbling or breaking.

Tablets may contain excipients such as diluents, binders, disintegrating agents, glidants, lubricants, substances capable of modifying the behaviour of the dosage forms and the active ingredient(s) in the gastrointestinal tract, colouring matter, and flavouring substances. When such excipients are used, it is necessary to ensure that they do not adversely affect the stability, dissolution rate, bioavailability, safety, or efficacy of the active ingredient(s); there must be no incompatibility between and of the components of the dosage form.

Preparations for alternative applications, such as solution-tablets for injections, irrigations, or for external use, etc., are also available in this presentation. These preparations may require a special formulation, method of manufacture, or form of presentation, appropriate to their particular use. For this reason they may not comply with certain sections of this monograph.

Production The following information is intended to provide very broad guidelines concerning the main steps to be followed during production.

Tablets may be prepared by three general methods: wet granulation, dry granulation (roll compaction or slugging), and direct compression. The purpose of both wet and dry granulation is to improve flow of the mixture and/or to enhance its compressibility. Tablets are compressed by the application of high pressures, utilizing steel punches and dies, to powders or granulations. However, throughout manufacturing, certain procedures should be validated and monitored by carrying out appropriate inprocess controls.

For modified-release tablets, a suitable test is carried out to demonstrate the appropriate release of the active ingredient(s).

Delayed-release tablets are prepared by covering tablets with a gastro-intestinal coating or from granules or particles already covered with a gastro-resistant coating.

In the manufacture of tablet cores, means are taken to ensure that they possess a suitable mechanical strength to withstand handling without crumbling or breaking. This may be demonstrated by examining the "Friability of Uncoated Tablets" (Appendix 4.30) and the "Resistance to Crushing of Tablets" (Appendix 4.31). Chewable tablets are prepared to ensure that they are easily crushed by chewing.

Disintegration Comply with the "Disintegration Test for Tablets and Capsules" (Appendix 4.23). For those tablets for which a dissolution requirement is included in the individual monograph, omission of the requirement for disintegration is considered justifiable and is therefore authorized.

Dissolution Comply with the "Dissolution Test" (Appendix 4.24). Where a dissolution test is specified in the individual monograph, a disintegration test may not be required.

Uniformity of dosage units Comply with the "Uniformity of Dosage Units" (Appendix 4.28). The test for Content Uniformity is not required for multivitamin and trace element tablets.

Visual inspection Unpack and inspect at least 20 tablets. They should be undamaged, smooth and usually of uniform colour. Evidence of physical instability is demonstrated by: (1) presence of excessive powder and/or pieces of tablets at the bottom of the container (from abraded, crushed, or broken tablets); (2) cracks or capping, chipping in the tablet surfaces or coating, swelling, mottling, discoloration, fusion between tablets; (3) the appearance of crystals on the container walls or on the tablets.

Packaging and storage Tablets should be kept in well-closed containers at a temperature not exceeding 30º and protected from crushing and mechanical shock.

Moisture-sensitive forms such as effervescent tablets should be kept in tightly closed containers or moisture proof packs and may require the use of separate packages containing water-adsorbent agents, such as silica gel.

Uncoated Tablets

The majority of uncoated tablets are made in such a way that the release of active ingredients is unmodified. 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.

TABLETS FOR SOLUTIONS (SOLUBLE TABLETS) Tablets for solutions are uncoated tablets that dissolve in water to give a clear solution.

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

Labelling The label should state "Not to be swallowed directly".

DISPERSIBLE TABLETS Dispersible tablets are uncoated tablets that produce a uniform dispersion in water.

Uniformity of dispersion Place two tablets in 100 ml of *water* and stir until completely dispersed. A smooth dispersion is produced which passes through a sieve screen with a nominal mesh aperture of 710 µm.

TABLETS FOR USE IN THE MOUTH Tablets for use in the mouth are usually uncoated tablets. They are formulated to effect a slow release and local action of the active ingredient (for example, compressed lozenges) or the release and systemic absorption of the active ingredient under the tongue (sublingual tablets) or in the other parts of the mouth. (Certain tablets for use in the mouth may be referred to as "Lozenges".)

Chewable tablets Chewable tablets are tablets intended to be chewed, producing a pleasant tasting residue in the oral cavity that is easily swallowed and does not leave a bitter or unpleasant after-taste. These tablets have been used in tablet formulations for children, especially multivitamin formulations,

and for the administration of antacids and selected antibiotics. Chewable tablets are prepared by compression, usually utilizing mannitol, sorbitol, or sucrose as binders and fillers, and containing colours and flavours to enhance their appearance and taste.

Buccal and sublingual tablets Buccal tablets are tablets intended to be inserted in the buccal pouch, and sublingual tablets are intended to be inserted beneath the tongue, where the active ingredient is absorbed directly through the oral mucosa. Few drugs are readily absorbed in this way, but for those that are (such as nitroglycerin and certain steroid hormones), a number of advantages may result.

Coated Tablets

Coated tablets are tablets covered with one or more layers of mixtures of substances such as natural or synthetic resins, polymers, gums, fillers, sugars, plasticizers, polyhydric alcohols, waxes, colouring matters, flavouring substances, and sometimes also active ingredients. A broken section, when examined under a lens, shows a core which is surrounded by a continuous layer of a different texture.

The tablets may be coated for a variety of reasons such as protection of the active ingredients from air, moisture, or light, masking of unpleasant tastes and odours, or improvement of appearance. The substance used for coating is usually applied as a solution or suspension.

SUGAR-COATED TABLETS Sugar-coated tablets are tablets containing a sugar coating.

FILM-COATED TABLETS Film-coated tablets are tablets covered with a thin layer of resins, polymers, and/or plasticizers capable of forming a film.

Modified-release Tablets

Modified-release tablets are coated, uncoated, or matrix tablets containing excipients or prepared by procedures which, separately or together, are designed to modify the rate of release of the active ingredient(s) in the gastro-intestinal tract.

DELAYED-RELEASE TABLETS Delayed-release tablets are tablets intended to resist gastric fluid but to release the active ingredient(s) in the intestinal fluid. This is achieved by using coating substances such as cellacefate (cellulose acetate phthalate) and anionic copolymers of methacrylic acid and its esters. It is sometimes necessary to apply more than one layer.

All requirements for these specialized dosage forms are given in the individual monographs.

EXTENDED-RELEASE TABLETS Extended-release tablets are tablets designed to slow the rate of release of the active ingredient(s) in the gastro-intestinal tract.

All requirements for these specialized dosage forms are given in the individual monographs.

TINCTURES

Tinctures are ethanolic or hydroethanolic solutions usually obtained from dried vegetable or animal matter or from chemical substances. They are usually obtained either using 1 part of drug and 10 parts of extraction solvent or 1 part of drug and 5 parts of extraction solvent. Tinctures are usually clear; a slight sediment may be produced on standing provided that the composition is not changed significantly.

Production For some preparations, the matter to be extracted must undergo a preliminary treatment, for example, inactivation of enzymes, grinding or defatting.

Tinctures are prepared by maceration, percolation or other suitable, justified methods, using Ethanol of suitable concentration. Tinctures may also be obtained by dissolving or diluting extracts in Ethanol of suitable concentration.

PRODUCTION BY PERCOLATION If necessary, the drug is reduced to pieces of suitable size, mixed thoroughly with a portion of extraction solvent and allowed to stand for an appropriate time. The mixture is transferred to a percolator and the percolate allowed to flow slowly ensuring that the drug is always covered with the remaining extraction solvent. The drug residue may be pressed out and the expressed fluid combined with the percolate.

PRODUCTION BY MACERATION Unless otherwise prescribed, the drug is reduced to pieces of suitable size, mixed thoroughly with the prescribed extraction solvent and allowed to stand in a closed container for anappropriate time. The drug residue is separated from the extraction solvent and, if necessary, pressed out. In the latter case, the two liquids obtained are combined.

PRODUCTION FROM EXTRACTS The tincture is prepared by dissolving or diluting an extract, using Ethanol of appropriate concentration. The content of solvent and constituents or, where applicable, the contents of solvent and of dry residue correspond to that of tinctures obtained by maceration or percolation.

When the content of constituents has to be adjusted, such adjustment may be carried out, if necessary, either by adding the extraction solvent of suitable concentration or by adding another tincture

of the vegetable or animal matter used for the preparation.

Ethanol Comply with the limits prescribed in the monograph (Appendix 6.5).

Methanol and 2-propanol Not more than 0.05 per cent v/v of methanol or 2-propanol (Appendix 5.15), unless otherwise prescribed in the monograph.

Total solids Comply with the limits prescribed in the monograph (Appendix 7.21).

Weight per millilitre Comply with the limits prescribed in the monograph (Appendix 4.9).

Packaging and storage Tinctures should be kept in tightly closed containers, protected from light, in a cool place.

Labelling The label on the container states (1) the vegetable, animal matter or chemical substances used; (2) where applicable, that fresh vegetable or animal matter was used; (3) the name and concentration of the solvent used for the preparation; (4) the concentration of ethanol in the final tincture; (5) the content of active principle and/or the ratio of starting material to extraction fluid and of starting material to final tincture.

TOPICAL PREPARATIONS

Topical preparations are drugs intended for topical application in a wide variety of dosage forms.

Minimum fill Topical preparations except plasters comply with the test described in the "Minimum Fill" (Appendix 4.26).

Applications Applications are liquid or semi-liquid preparations containing one or more active ingredients intended for application to the skin. They may contain suitable antimicrobial preservatives, unless the active ingredients or vehicles have sufficient intrinsic antibacterial and antifungal activity. They may contain other suitable auxiliary substances such as stabilizers. They have traditionally been used to administer antiparasitic medications.

Collodions

Collodions are liquid preparations, usually containing pyroxylin in a mixture of ether and ethanol, that are intended for application to the skin. When they are allowed to dry, a flexible film is formed at the site of application.

Packaging and storage Collodions should be kept in well-closed containers, at a temperature not exceeding 25° and remote from fire.

Jellies

See hydrophilic gels under Topical Semi-solid Preparations.

Liniments

Liniments are liquid or semi-liquid preparations containing one or more active ingredients in suitable vehicles. They are intended to be applied to the unbroken skin with friction. They may contain suitable antimicrobial preservatives.

Packaging and storage Liniments should be kept in well-closed containers. Certain plastic containers, such as those made from polystyrene, are unsuitable for liniments.

Lotions

Lotions are liquid or semi-liquid preparations containing one or more active ingredients in suitable vehicles. They are usually intended to be applied to the unbroken skin without friction. Lotions may contain suitable antimicrobial preservatives, unless the active ingredients or vehicles have sufficient intrinsic antibacterial and antifungal activity. They may contain other suitable added substances such as stabilizers. The term "lotion" is applied to solutions or suspensions or emulsions applied topically.

TOPICAL SOLUTIONS Topical solutions are solutions, usually aqueous but often containing other solvents, such as alcohol and polyols, intended for topical application to the skin, or to the oral mucosal surface.

TOPICAL SUSPENSIONS Topical suspensions are liquid preparations containing solid particles dispersed in a liquid vehicle, intended for application to the skin.

TOPICAL EMULSIONS Topical emulsions are liquid preparations in which one liquid is dispersed throughout another liquid in the form of small droplets.

Packaging and storage Lotions should be kept in well-closed containers.

Mouthwashes

Mouthwashes are aqueous solutions containing one or more active ingredients. They are intended for use in contact with the mucous membranes of the oral cavity, usually after dilution with warm water. They may contain added substances such as suitable antimicrobial preservatives.

Packaging and storage Mouthwashes should be kept in well-closed containers.

Labelling The label of mouthwashes states the directions for the dilution of the mouthwash for use, if appropriate.

Paints

Paints are solutions or dispersions of one or more active ingredients. They are intended for application to the skin or, in some cases, mucous membranes.

Packaging and storage Paints should be kept in tightly closed containers.

Plasters

Plasters are usually solid at ordinary temperature, and are intended for external application. They are used by spreading on cloth, paper, or plastic film and adhering to the skin.

Medicated plaster, long used for local or regional drug delivery, are the prototypical transdermal system.

Topical Emulsions

See under Lotions.

Topical Powders

See under Powders.

Topical Semi-solid Preparations

Topical semi-solid preparations are intended to be applied to the skin or to certain mucous surfaces for local action or percutaneous penetration of medicaments, or for their emollient or protective action. They are of homogeneous appearance. Topical semi-solid preparations consist of a simple or compound base in which, usually, one or more active substances are dissolved or dispersed. According to its composition, the base may influence the action of the preparation and the release of the active substance(s).

The bases may consist of natural or synthetic substances and may be single-phase or multi-phase systems. According to the nature of the base the preparation may have hydrophilic or hydrophobic (lipophilic) properties; it may contain suitable additives such as antimicrobial preservatives, antioxidants, stabilizers, emulsifiers and thickeners.

If a preparation is specifically intended for use on large open wounds or on severely injured skin, it should be sterile. Preparations required to be sterile must comply with the test for sterility.

If the particle size of the ingredients is of importance for the therapeutic purpose of a topical semi-solid preparation, the test to be applied should be specified. Topical semi-solid preparations can be distinguished into four categories: (1) creams (hydrophobic or hydrophilic); (2) gels (hydrophobic or hydrophilic); (3) ointments (hydrophobic, water-emulsifying or hydrophilic); (4) pastes.

Sterility Where the preparation is labelled as sterile and unless otherwise directed in the individual monograph, it complies with the "Sterility Test" (Method I, Appendix 10.1).

Packaging and storage Topical semi-solid preparations should be stored in well-closed containers or, if the preparation contains water or other volatile constituents, in a tightly closed container. The containers are preferably collapsible metal tubes from which the preparation may be readily extruded. Other types of container may be used. Containers for preparations for nasal, aural, vaginal, or rectal use should be adapted to deliver the product to the site of application or should be accompanied by a suitable applicator. They should be stored at a temperature not exceeding 30° unless otherwise prescribed. For creams and gels, they shall not be frozen.

Labelling The label of topical semi-solid preparations states (1) the name and concentration of any added antimicrobial preservative(s); (2) where applicable, that the preparation is sterile.

CREAMS Creams are homogeneous, semi-solid preparations consisting of opaque emulsion systems. They are multiphase preparations composed of a lipophilic phase and an aqueous phase.

Hydrophobic creams Hydrophobic creams have the lipophilic phase as the continuouss phase. They contain water-in-oil emulsifying agents such as wool fat, sorbitan esters and monoglycerides.

Hydrophilic creams Hydrophilic creams have the aqueous phase as the continuous phase. They contain oil-in-water emulsifying agents such as sodium or triethanolamine soaps, sulfated fatty alcohols and polysorbates, combined, if necessary, with water-in-oil emulsifying agents.

GELS Gels are usually homogeneous, clear, semisolid preparations consisting of a liquid phase within a three-dimensional polymeric matrix with physical or sometimes chemical cross-linkage by means of suitable gelling agents.

Hydrophobic gels Hydrophobic gel (oleogel) bases usually consist of liquid paraffin with polyethylene or fatty oils gelled with colloidal silica or aluminium or zinc soaps.

Hydrophilic gels Hydrophilic gel (hydrogel) bases usually consist of water, glycerol, or propylene

glycol gelled with suitable agents such as tragacanth, starch, cellulose derivatives, carboxyvinyl polymers and magnesium aluminium silicates.

OINTMENTS Ointments are homogeneous, semi-solid preparations intended for external application to the skin or mucous membranes. They are formulated using hydrophobic, hydrophilic, or water-emulsifying bases to provide preparations that are immiscible, miscible, or emulsifiable with skin secretions. They can also be derived from hydrocarbon (fatty), absorption, water-removable, or water-soluble bases.

Hydrophobic ointments Hydrophobic (lipophilic) ointments are usually anhydrous and can absorb only small amounts of water. Typical bases used for their formulation are water-insoluble hydrocarbons such as hard, soft, and liquid paraffin, vegetable oil, animal fats, waxes, synthetic glycerides, and polyalkylsiloxanes.

Water-emulsifying ointments Water-emulsifying ointments can absorb large amounts of water. They typically consist of a hydrophobic fatty base in which a water-in-oil agent, such as wool fat, wool alcohols, sorbitan esters, monoglycerides, or fatty alcohols can be incorporated to render them hydrophilic. They may also be water-in-oil emulsions that allow additional quantities of aqueous solutions to be incorporated. Such ointments are used especially when formulating aqueous liquids or solutions.

Hydrophilic ointments Hydrophilic ointment bases are either water-removable or water-soluble bases. They are oil-in-water emulsions such as hydrophilic ointment or greaseless ointment bases comprised of water soluble constituents such as mixtures of liquid and solid polyethyleneglycols (macrogols).

PASTES Pastes are homogeneous, semi-solid preprations usually containing high concentrations of insoluble powdered substances (commonly not less than 20 per cent) dispersed in a suitable base. One class is made from a single phase aqueous gel (e.g., zinc oxide gelatin paste). The other class, the fatty pastes (e.g., zinc oxide paste) consists of thick, stiff ointments that do not ordinarily flow at body temperature. The pastes should adhere well to the skin. In many cases they form a protective film that controls the evaporation of water.

Topical Solutions

See under Lotions.

Topical Suspensions

See under Lotions.

VAGINAL PREPARATIONS

Vaginal preparations are liquid, semi-solid preparations intended for administration to the vagina usually in order to obtain a local effect. They contain one or more active ingredients in a suitable basis. Several categories of vaginal preparations may be distinguished: (1) moulded pessaries; (2) vaginal tablets; (3) vaginal capsules; (4) vaginal foams; and (5) vaginal tampons.

Production In the manufacturing, packaging, storage and distribution of vaginal preparations, suitable means are taken to ensure their microbial quality; recommendations on this aspect are provided under "Limits for Microbial Contamination" (Appendix 10.5).

Disintegration Vaginal preparations comply with the "Disintegration Test for Suppositories" (Appendix 4.22).

Uniformity of dosage units Vaginal preparations comply with the "Uniformity of Dosage Units" (Appendix 4.28).

Moulded Pessaries

Moulded pessaries are solid, single-dose preparations. They have various shapes, usually ovoid, with a volume and consistence suitable for insertion into the vagina. Apart from their shape they conform to rectal suppositories.

Moulded pessaries are prepared using the method and excipients described for rectal suppositories. The active ingredient(s) are dispersed or dissolved in a simple or compound basis, which may be soluble, insoluble but melting at body temperature or dispersible in water.

Vaginal preparations which conform to the definition of moulded pessaries may be prepared by compression. They comply with the requirements for moulded pessaries.

Production A suitable test is carried out to demonstrate the appropriate release of the active ingredient(s) from pessaries intended for modified release or prolonged local action.

Packaging and storage Moulded pessaries shall be kept in well-closed containers.

Labelling The label on the container states (1) that they are intended for external use only; (2) the storage conditions.

Vaginal Tablets

Vaginal tablets (compressed pessaries) are solid single-dose preparations. They generally conform to the definitions of uncoated or film-coated tablets

in Tablets.

Production A suitable test is carried out to demonstrate the appropriate release of the active ingredient(s) from vaginal tablets intended for modified release or prolonged local action.

Vaginal Capsules

Vaginal capsules (shell pessaries) are solid, single dose preparations. They are generally similar to soft capsules, differing only in their shape and size. Vaginal capsules have various shapes, usually ovoid. They are smooth and have a uniform external appearance.

Production A suitable test is carried out to demonstrate the appropriate release of the active ingredients(s) from vaginal capsules intended for modified release or prolonged local action.

Vaginal Foams

See under Medicated Foams.

Vaginal Tampons

Vaginal tampons are solid, single-dose preparations intended to be inserted in the vagina for a limited time. See also under Medicated Tampons.

APPENDIX 2 SPECTROSCOPY

2.2 ULTRAVIOLET AND VISIBLE SPECTROPHOTOMETRY

Absorption spectroscopy in the ultraviolet and visible region is one of the most useful tools for qualitative and quantitative analysis.

When a beam of monochromatic radiation traverses a solution containing an absorbing substance, its radiant power is reduced in relation to the distance that it travels through. It also decreases in relation to the concentration of absorbing molecules or ions encountered in that medium. These two factors determine the proportion of the total incident energy that emerges. The decrease in power of monochromatic radiation is stated quantitatively by Beer's law:

$$\log (1/T) = A = abc.$$

The terms used in connection with spectrophotometric tests are defined as follows:

Absorbance (A) is the logarithm, to the base 10, of the reciprocal of the transmittance (T).

(**Note** Descriptive terms used formerly include optical density, absorbancy and extinction.)

Absorptivity (a) is the quotient of the absorbance (A) per litre, of the substance (c) and the absorption path length in cm (b). (**Note** It is not to be confused with specific absorbance, specific extinction or extinction coefficient [A (1 per cent, 1 cm) or E (1 per cent, 1 cm)]. These are generally used in different pharmacopoeia, as the quotient of the absorbance divided by the product of the concentration, expressed in g per 100 mL, of the substance, and the absorption path length in cm, therefore: A (1 per cent, 1 cm) = 10 a.)

Molar absorptivity (ε) is the quotient of the absorbance (A) divided by the product of the concentration, expressed in moles per litre, of the substance (c) and the absorptivity (a) and the molecular weight of the substance. (**Note** Terms formerly used include molar absorbancy index, molar extinction coefficient and molar absorption coefficient.)

Transmittance (T) is the quotient of the radiant power transmitted by a sample (I) divided by the radiant power incident upon the sample (I_0).

Absorption spectrum is a graphic representation of absorbance, or any function of absorbance, plotted against wavelength or function of wavelength.

Where a monograph gives a single value for the position of an absorption maximum, it is understood that the value obtained may differ by not more than ±2 nm.

Apparatus

All types of spectrophotometer are designed to permit substantially monochromatic radiant energy to be passed through the test substance in a suitable form and to allow measurement of the fraction of energy that is transmitted. The spectrophotometer comprises an energy source, a dispersing device with slits for selecting the wavelength band, a cell for holding the test substance, a detector of radiant energy, associated amplifiers, and measuring and recording devices. Some instruments are manually operated, while others are equipped for automatic operation. Instruments are available for use in the visible region of the spectrum, usually 380 nm to about 700 nm, and in the ultraviolet and visible regions of the spectrum, usually 190 nm to about 700 nm.

Both double-beam and single-beam instruments are commercially available and either is suitable. Depending on the type of apparatus used, the results may be displayed on a scale, on a digital counter, or by a recorder or printer.

The apparatus should be maintained in proper working condition. The housing of the optical system should minimize any possibility of errors due to stray light; this is particularly relevant in the short-wave region of the spectrum.

CONTROL OF WAVELENGTHS Verify the wavelength scale using the absorption maxima of Holmium Perchlorate Solution, the line of a hydrogen or deuterium discharge lamp or the lines of a mercury vapour as shown below. The permitted tolerance is ±1 nm for the range 200 to 400 nm and ±3 nm for the range 400 to 600 nm.

241.15 nm (Ho)	404.66 nm (Hg)
253.70 nm (Hg)	435.83 nm (Hg)
287.15 nm (Ho)	486.00 nm (Dβ)
302.25 nm (Hg)	486.10 nm (Hβ)
13.16 nm (Hg)	536.30 nm (Ho)
334.15 nm (Hg)	546.07 nm (Hg)
361.50 nm (Ho)	576.96 nm (Hg)
365.48 nm (Hg)	579.07 nm (Hg)

The wavelength scale may also be calibrated by means of suitable glass filters that have useful absorption bands through the visible and ultraviolet regions. Standard glass containing didymium (a mixture of praseodymium and neodymium)

has been widely used. Glass containing holmium is considered superior. The exact values for the position of characteristic maxima in holmium glass filters are 241.5±1, 287.5±1, 360.9±1, and 536.2±3 nm. Holmium glass filters are obtainable from some national institutions and from commercial sources. The performance of an uncertified filter should be checked against one that has been properly certified.

CONTROL OF ABSORBANCE Check the absorbance using Potassium Dichromate Solution UV at the wavelengths indicated in the following table, which gives for each wavelength the exact value of *A* (1 per cent, 1 cm) and the permitted limits.

Wavelength (nm)	*A* (1 Per Cent, 1 cm)	Maximum Tolerance
235	124.5	122.9 to 126.2
257	144.0	142.4 to 145.7
313	48.6	47.0 to 50.3
350	106.6	104.9 to 108.2

LIMIT OF STRAY LIGHT Stray light may be detected at a given wavelength with suitable filters or solutions. For example, the absorbance of a 1.2 per cent w/v solution of *potassium chloride* at a path-length of 1 cm should be greater than 2 at 200 nm when compared with *water* as reference liquid.

SPECTRAL SLIT WIDTH When measuring the absorbance at an absorption maximum, the spectral slit width must be small compared with the half-width of the absorption band, or erroneously low absorbances will be measured. Particular care is needed for certain substances and the instrumental slit width used should always be such that further reduction does not result in an increased absorbance reading.

CELLS Cells usually in the spectral range discussed are 1-cm absorption cells with glass or silica windows. Other path lengths may also be used. The cells used for the test solution and the blank should be matched, and must have the same spectral transmittance when containing only the solvent. If this is not the case, an appropriate correction must be applied.

SOLVENTS In measuring the absorbance of a solution at a given wavelength, the absorbance of the solvent cell and its contents shall not exceed 0.4 and is preferably less than 0.2 when measured with reference to air at the same wavelength. The solvent in the solvent cell shall be of the same batch as that used to prepare the solution and must be free from fluorescence at the wavelength of measurement. *Ethanol, absolute ethanol, methanol* and *cyclohexane UV* used as solvents shall have an absorbance, measured in a 1-cm cell at 240 nm with reference to *water*, not exceeding 0.10. Statements of concentration and thickness of the solution to be used in the determination of light absorption apply to measurements made with photoelectric instruments.

SOLUTIONS FOR USE IN THE CALIBRATION OF WAVELENGTHS AND ABSORBANCE

Holmium Perchlorate Solution Dissolve 40 g of *holmium oxide* in sufficient 1.4 M *perchloric acid* to produce 1000 mL.

Potassium Dichromate Solution UV Dry a quantity of *potassium dichromate* by heating to constant weight at 130°. Weigh accurately a quantity not less than 57.0 mg and not more than 63.0 mg and dissolve it in sufficient 0.005 M *sulfuric acid* to produce 1000.0 mL.

Determination of Absorbance

Unless otherwise prescribed, measure the absorbance, *A*, at the prescribed wavelength using a path length of 1 cm, and the measurements are carried out with reference to the solvent used to prepare the solution being examined. In certain cases measurements are carried out with reference to a mixture of reagents, details of which are prescribed in the monograph.

When the absorbance is being measured for a quantitative determination, for example, an assay or a limit test, a manually-scanning instrument is used. In tests for identification, it is more convenient to use a recording instrument and the concentration of the solution and the pathlength are specified accordingly. If these conditions are not appropriate for a particular instrument, the thickness and the concentration of the solution should be varied. A statement in an assay or test of the wavelength at which maximum absorption occurs implies the maximum occurring either precisely at or in the vicinity of the given wavelength.

When an assay or test prescribes the use of a reference substance, the spectrophotometric measurements are made first with the solution prepared from the reference substance and second with the corresponding solution prepared from the substance being examined. The second measurement is carried out as quickly as possible after the first, using the matched cell and the same experimental conditions.

The requirements for light absorption in the Pharmacopoeia apply to the dried, anhydrous, or solvent-free material in all those monographs in which standards for loss on drying, water or solvent content are given. In calculating the result, the loss on drying, water, or solvent content determined by the method specified in the monograph is used.

SECOND DERIVATIVE SPECTROPHOTOMETRY

Derivative spectrophotometry involves the transformation of absorption spectra (zero order) into first, second or higher order derivative spectra. A first derivative spectrum is a plot of the gradient of the absorption curve (rate of change of the absorbance with wavelength, $dA/d\lambda$) against wavelength. A second derivative spectrum is a plot of the curvature of the absorption spectrum ($d^2A/d\lambda^2$) against wavelength.

If the absorbance follows the Beer-Lambert relationship, the second derivative at any wavelength, λ, is related to concentration by the following equation:

$$\frac{d^2A}{d\lambda^2} = \frac{d^2A\,(1\%,\,1\text{ cm})}{d\lambda^2} \times cd,$$

where A = the absorbance at wavelength l,
A (1%, 1 cm) = the specific absorbance at wavelength l,
c = the concentration of the absorbing solute expressed as a percentage w/v, and
d = the thickness of the absorbing layer in cm.

Apparatus

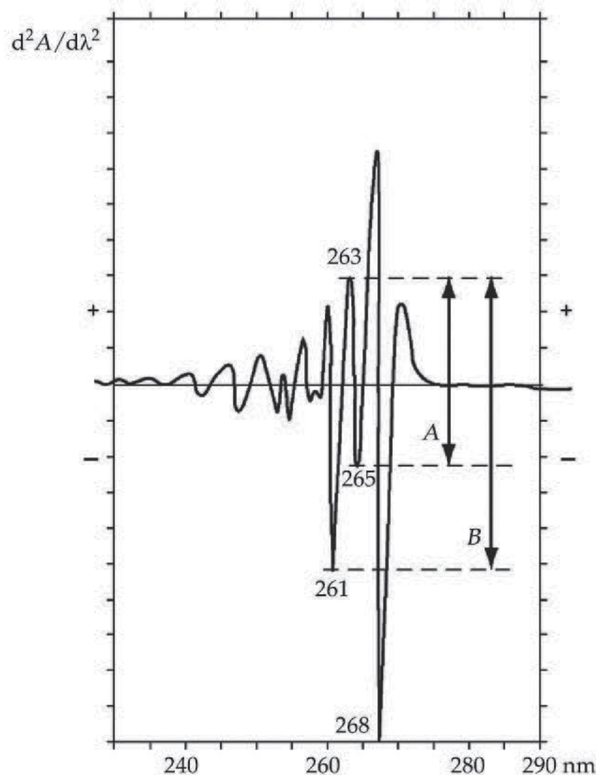
A spectrophotometer complying with the requirements prescribed above and equipped with an analogue resistance-capacitance differentiation module or a digital differentiator or another means of producing second derivative spectra should be used in accordance with the manufacturer's instructions. Some methods of producing second derivative spectra lead to a wavelength shift relative to the zero order spectrum and this should be taken into account, when necessary. Unless otherwise stated in the monograph, the spectral slit width of the spectrophotometer, where variable, should be set as described under Spectral slit width above. The cells used should comply with the statements given under the heading Cells.

Resolution

When prescribed in a monograph, record the second derivative spectrum in the range 255 to 275 nm of a 0.020 per cent v/v solution of *toluene* in *methanol* using *methanol* in the reference cell. The spectrum shows a small negative extremum (or trough) located between two large negative extrema at 261 nm and 268 nm, respectively as shown in the figure. Unless otherwise prescribed in the monograph, the ratio *A/B* (see the figure) is not less than 0.2.

Procedure

Prepare a solution of the substance being examined, adjust the various instrument settings according to the manufacturer's instructions and calculate the amount of the substance being determined as prescribed in the monograph.



2.3 ATOMIC SPECTROPHOTOMETRY: EMISSION AND ABSORPTION

These techniques are used to determine the concentration of certain metallic ions by measuring the intensity of emission or absorption of light at a particular wavelength by the atomic vapour of the element generated from the substance, for example, by introducing a solution of the substance into a flame.

FOR ATOMIC EMISSION SPECTROMETRY

Atomic emission is a process that occurs when electromagnetic radiation is emitted by excited atoms or ions. In atomic emission spectrometry the sample is subjected to temperatures high enough to cause not only dissociation into atoms, but also to cause significant amounts of collisional excitation and ionization of the sample atoms to take place. Once the atoms and ions are in the excited states, they can decay to lower states through thermal or radiative (emission) energy transitions and electromagnetic radiation is emitted. An emission spectrum of an element contains several more lines than the corresponding absorption spectrum. Atomic emission spectrometry is a technique for determining the concentration of an element in a sample by measuring the intensity of one of the emission lines of the atomic vapour of the element generated from the sample. The determination is carried out at the wavelength corresponding to this emission line.

Apparatus

The apparatus essentially consists of the following:

(a) a sample introduction and nebulization system;

(b) a flame to generate the atoms to be determined;

(c) a monochromator;

(d) a detector;

(e) a data-acquisition unit.

Oxygen, air and a combustible gas such as hydrogen, acetylene, propane or butane may be used in flames. The atomization source is critical, since it must provide sufficient energy to excite and atomize the atoms. The atomic spectra emitted from flames have the advantage of being simpler than those emitted from other sources, the main limitation being that the flames are not powerful enough to cause emission for many elements allowing their determination. Acidified water is the solvent of choice for preparing test and standard preparations, although organic solvents may also be used if precautions are taken to ensure that the solvent does not interfere with the stability of the flame.

Interferences

Spectral interference is reduced or eliminated by choosing an appropriate emission line for measurement or by adjusting the slit for spectral band-width. Physical interference is corrected by diluting the sample preparation, by matching the matrix or by using the method of standard additions. Chemical interference is reduced by using chemical modifiers or ionization buffers.

Memory effect

The memory effect caused by deposit of analyte in the apparatus may be limited by thoroughly rinsing between runs, diluting the solutions to be measured if possible and thus reducing their salt content, and by aspirating the solutions through as swiftly as possible.

Method

(**Note** Evaluate and select the type of material of construction, pretreatment, and cleaning of analytical labware used in the Atomic Absorption Spectrophotometric analyses. The material must be inert and, depending on the specific application, resistant to caustics, acids, and/or organic solvents, i.e., plastic.)

Operate an atomic emission spectrometer in accordance with the manufacturer's instructions at the prescribed wavelength. Optimize the experimental conditions (flame temperature, burner adjustment, use of an ionic buffer, concentration of solutions) for the specific element to be analyzed and in respect of the sample matrix. Introduce a blank solution into the atomic generator and adjust the instrument reading to zero or to its blank value. Introduce the most concentrated standard preparation and adjust the sensitivity to obtain a suitable reading. It is preferable to use concentrations which fall within the linear part of the calibration curve. If this is not possible, the calibration plots may also be curved and are then to be applied with appropriate calibration software.

Determinations are made by comparison with standard preparations with known concentrations of the element to be determined either by the method of direct calibration (Method I) or the method of standard additions (Method II).

METHOD I: METHOD OF DIRECT CALIBRATION

For routine measurements three standard preparations of the element to be determined and a blank are prepared and examined. Prepare the solution of the substance to be examined (test preparation) as prescribed in the monograph. Prepare not fewer than three standard preparations of the element to be determined, the concentrations

of which span the expected value in the test preparation. For assay purposes, optimal calibration levels are between 0.7 and 1.3 times the expected content of the element to be determined or the limit prescribed in the monograph. For purity determination, calibration levels are between the limit of detection and 1.2 times the limit specified for the element to be determined. Any reagents used in the preparation of the test preparation are added to the standard preparations and to the blank solution at the same concentration. Introduce each of the solutions into the instrument using the same number of replicates for each solution, to obtain a steady reading.

Calculation Prepare a calibration curve from the mean of the readings obtained with the standard preparation by plotting the means as a function of concentration. Determine the concentration of the element in the test preparation from the curve obtained.

METHOD II: METHOD OF STANDARD ADDITIONS

Add to at least three similar volumetric flasks equal volumes of the solution of the substance to be examined (test preparation) prepared as prescribed. Add to all but one of the flasks progressively larger volumes of a standard preparation containing a known concentration of the element to be determined to produce a series of solutions containing steadily increasing concentrations of that element known to give responses in the linear part of the curve, if at all possible. Dilute the contents of each flask to volume with solvent. Introduce each of the solutions into the instrument using the same number of replicates for each solution, to obtain a steady reading.

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

Validation of the method

Satisfactory performance of methods prescribed in monographs is verified at suitable time intervals.

LINEARITY

Prepare and analyze not fewer than four standard preparations over the calibration range and a blank solution. Perform not fewer than five replicates. The calibration curve is calculated by least-square regression from all measured data. The regression curve, the means, the measured data and the confidence interval of the calibration curve are plotted.

The operating method is valid when:

— the correlation coefficient is at least 0.99,

— the residuals of each calibration level are randomly distributed around the calibration curve.

Calculate the mean and relative standard deviation for the lowest and highest calibration level. When the ratio of the estimated standard deviation of the lowest and the highest calibration level is less than 0.5 or greater than 2.0, a more precise estimation of the calibration curve may be obtained using weighted linear regression. Both linear and quadratic weighting functions are applied to the data to find the most appropriate weighting function to be employed. If the means compared to the calibration curve show a deviation from linearity, two-dimensional linear regression is used.

ACCURACY

Verify the accuracy preferably by using a certified standard material. Where this is not possible, perform a test for recovery.

RECOVERY

For assay determinations a recovery of 90 per cent to 110 per cent is to be obtained. For other determinations, for example for trace element determination, the test is not valid if recovery is outside of the range 80 per cent to 120 per cent at the theoretical value. Recovery may be determined on a suitable standard preparation (matrix solution) which is spiked with a known quantity of analyte (middle concentration of the calibration range).

REPEATABILITY

The repeatability is not greater than 3 per cent for an assay and not greater than 5 per cent for an impurity test.

LIMIT OF QUANTIFICATION

The limit of quantification can be estimated by calculating the standard deviation of not less than six replicate measurements of a blank solution, divided by the slope of a standard curve, and multiplying by 10. If validating a procedure using the method of standard additions, the slope of standards applied to a solution of the substance being examined (test preparation) is used. Other suitable approaches can be used.

FOR ATOMIC ABSORPTION SPECTROMETRY

Atomic absorption is a process that occurs when a ground state-atom absorbs electromagnetic radiation of a specific wavelength and is elevated to an excited state. The atoms in the ground state absorb energy at their resonant frequency and the electromagnetic radiation is attenuated due to resonance absorption. The energy absorption is virtually a direct function of the number of atoms present.

This appendix provides general information and defines the procedures used in element determinations by atomic absorption spectrometry, either atomization by flame, by electrothermal vaporization in a graphite furnace, by hydride generation or by cold vapour technique for mercury.

Atomic absorption spectrometry is a technique for determining the concentration of an element in a sample by measuring the absorption of electromagnetic radiation by the atomic vapour of the element generated from the sample. The determination is carried out at the wavelength of one of the absorption (resonance) lines of the element concerned. The amount of radiation absorbed is, according to the Beer's law, proportional to the element concentration.

Apparatus

The apparatus consists essentially of the following:

(a) a source of radiation;
(b) a sample introduction device;
(c) a sample atomizer;
(d a monochromator or polychromator;
(e) a detector;
(f) a data-acquisition unit.

The apparatus is usually equipped with a background correction system. Hollow-cathode lamps and electrodeless discharge lamps (EDL) are used as radiation source. The emission of such lamps consists of a spectrum showing very narrow lines with half-width of about 0.002 nm of the element being determined.

There are three types of sample atomizers:

FLAME TECHNIQUE

A flame atomizer is composed of a nebulization system with a pneumatic aerosol production accessory, a gas-flow regulation and a burner. Although other flame types have been documented, the most commonly used flame is an air–acetylene flame. Because the temperature of the air–acetylene flame is not sufficient to destroy oxides that might form or are present, a nitrous oxide–acetylene flame often is used, depending on the analyte and nature of the sample. The air–acetylene flame burns within a temperature range of 2125° to 2400°, but the nitrous oxide–acetylene flame burns within a temperature range of 2650° to 2800°. The configuration of the burner is adapted to the gases used and the gas flow is adjustable. Samples are nebulized, acidified water being the solvent of choice for preparing test and standard preparations. Organic solvents may also be used if precautions are taken to ensure that the solvent does not interfere with the stability of the flame.

ELECTROTHERMAL ATOMIZATION TECHNIQUE

An electrothermal atomizer is generally composed of a graphite tube furnace and an electric power source. Electrothermal atomization in a graphite tube furnace atomizes the entire sample and retains the atomic vapour in the light path for an extended period. This improves the detection limit. Samples, liquid as well as solid, are introduced directly into the graphite tube furnace, which is heated in a programmed series of steps to dry the sample and remove major matrix components by pyrolysis and to then atomize all of the analyte. The furnace is cleaned using a final temperature higher than the atomization temperature. The flow of an inert gas during the pyrolysis step in the graphite tube furnace allows a better performance of the subsequent atomization process.

COLD VAPOUR AND HYDRIDE GENERATION TECHNIQUE

The atomic vapour may also be generated outside the spectrometer. This is notably the case for the cold-vapour method for mercury or for certain hydride-forming elements such as arsenic, antimony, bismuth, selenium and tin. For mercury, atoms are generated by chemical reduction with stannous chloride or sodium borohydride and the atomic vapour is swept by a stream of an inert gas into a cold quartz cell mounted in the optical path of the instrument. Hydrides thus generated are swept by an inert gas into a heated cell in which they are dissociated into atoms. Both cold vapour and hydride generation techniques are very sensitive and have detection limits in the part per billion (ppb) or part per trillion (ppt) range.

Interference

Chemical, physical, ionization, and spectral interferences are encountered in atomic absorption measurements. Chemical interference is compensated by addition of matrix modifiers, of releasing agents or by using high temperature produced by a nitrous oxide-acetylene flame; the use of specific ionization buffers (for example, lanthanum and caesium) compensates for ionization interference; by dilution of the sample, through the method of standard additions or by matrix matching, physical interference due to high salt content or viscosity is eliminated. Spectral interference results from the overlapping of resonance lines and can be avoided by using a different resonance line. The use of Zeeman background correction also compensates for spectral interference and interferences from molecular absorption, especially when using the electrothermal atomization technique. The use of multi-element

hollow-cathode lamps may also cause spectral interference. Specific or non-specific absorption is measured in a spectral range defined by the bandwidth selected by the monochromator (0.2 to 2 nm).

Background correction

Scatter and background in the flame or the electrothermal atomization technique increase the measured absorbance values. Background absorption covers a large range of wavelengths, whereas atomic absorption takes place in a very narrow wavelength range of about 0.005 to 0.02 nm. Background absorption can in principle be corrected by using a blank solution of exactly the same composition as the sample, but without the specific element to be determined, although this method is frequently impracticable. With the electrothermal atomization technique the pyrolysis temperature is to be optimized to eliminate the matrix decomposition products causing background absorption. Background correction can also be made by using two different light sources, the hollow-cathode lamp that measures the total absorption (element and background) and a deuterium lamp with a continuum emission from which the background absorption is measured. Background is corrected by subtracting the deuterium lamp signal from the hollow-cathode lamp signal. This method is limited in the spectral range on account of the spectra emitted by a deuterium lamp from 190 to 400 nm. Background can also be measured by taking readings at a non-absorbing line near the resonance line and then subtracting the results from the measurement at the resonance line. Another method for the correction of background absorption is the Zeeman effect (based on the Zeeman splitting of the absorption line in a magnetic field). This is particularly useful when the background absorption shows fine structure. It permits an efficient background correction in the range of 185 to 900 nm.

Choice of the operating conditions

After selecting the suitable wavelength and slit width for the specific element, the need for the following has to be ascertained:

— correction for non-specific background absorption,

— chemical modifiers or ionization buffers to be added to the sample as well as to blank and standard solutions,

— dilution of the sample to minimize, for example, physical interferences,

— details of the temperature programme, preheating, drying, pyrolysis, post-atomization with ramp and hold times,

— inert gas flow,

— matrix modifiers for electrothermal atomization (furnace),

— chemical reducing reagents for measurements of mercury or other hydride-forming elements along with cold vapour cell or heating cell temperature,

— specification of furnace design.

Method

(**Note** Evaluate and select the type of material of construction, pretreatment, and cleaning of analytical labware used in the Atomic Absorption Spectrophotometric analyses. The material must be inert and, depending on the specific application, resistant to caustics, acids, and/or organic solvents, i.e., plastic.)

The preparation of the sample may require a dissolution, a digestion (mostly microwave-assisted), an ignition step or a combination thereof in order to clear up the sample matrix and/or to remove carbon-containing material. If operating in an open system, the ignition temperature should not exceed 600°, due to the volatility of some metals, unless otherwise stated in the monograph.

Operate an atomic absorption spectrometer in accordance with the manufacturer's instructions at the prescribed wavelength. Introduce a blank solution into the atomic generator and adjust the instrument reading so that it indicates maximum transmission. The blank value may be determined by using solvent to zero the apparatus. Introduce the most concentrated standard preparation and adjust the sensitivity to obtain a maximum absorbance reading. Rinse in order to avoid contamination and memory effects. After completing the analysis, rinse with *water* or acidified *water*.

If a solid sampling technique is applied, full details of the procedure are provided in the monograph.

Ensure that the concentrations to be determined fall preferably within the linear part of the calibration curve. If this is not possible, the calibration plots may also be curved and are then to be applied with appropriate calibration software.

Determinations are made by comparison with standard preparations with known concentrations of the element to be determined either by the method of direct calibration (Method I) or the method of standard additions (Method II).

METHOD I: DIRECT CALIBRATION

For routine measurements three standard preparations and a blank solution are prepared and examined.

Prepare the solution of the substance to be examined (test preparation) as prescribed in the monograph. Prepare not fewer than three standard preparations of the element to be determined, the concentrations of which span the expected value in the test preparation. For assay purposes, optimal calibration levels are between 0.7 and 1.3 times the expected content of the element to be determined or the limit prescribed in the monograph. For purity determination, calibration levels are the limit of detection and 1.2 times the limit specified for the element to be determined. Any reagents used in the preparation of the test preparation are added to the standard and blank solutions at the same concentration. Introduce each of the solutions into the instrument using the same number of replicates for each of the solutions to obtain a steady reading.

Calculation Prepare a calibration curve from the mean of the readings obtained with the standard preparations by plotting the means as a function of concentration. Determine the concentration of the element in the test preparation from the curve obtained.

METHOD II: STANDARD ADDITIONS

Add to at least three similar volumetric flasks equal volumes of the solution of the substance to be examined (test preparation) prepared as prescribed. Add to all but one of the flasks progressively larger volumes of a standard preparation containing a known concentration of the element to be determined to produce a series of solutions containing steadily increasing concentrations of that element known to give responses in the linear part of the curve, if possible. Dilute the contents of each flask to volume with solvent.

Introduce each of the solutions into the instrument, using the same number of replicates for each of the solutions, to obtain a steady reading.

Calculation Calculate the linear equation of the graph using a least-squares fit and derive from it the concentration of the element to be determined in the test preparation.

Validation of the method

Satisfactory performance of methods prescribed in monographs is verified at suitable time intervals.

LINEARITY

Prepare and analyze not fewer than four standard preparations over the calibration range and a blank solution. Perform not fewer than five replicates.

The calibration curve is calculated by least-square regression from all measured data. The regression curve, the means, the measured data and the confidence interval of the calibration curve are plotted. The operating method is valid when:

— the correlation coefficient is at least 0.99,

— the residuals of each calibration level are randomly distributed around the calibration curve.

Calculate the mean and relative standard deviation for the lowest and highest calibration level.

When the ratio of the estimated standard deviation of the lowest and the highest calibration level is less than 0.5 or greater than 2.0, a more precise estimation of the calibration curve may be obtained using weighted linear regression. Both linear and quadratic weighting functions are applied to the data to find the most appropriate weighting function to be employed. If the means compared to the calibration curve show a deviation from linearity, two-dimensional linear regression is used.

ACCURACY

Verify the accuracy preferably by using a certified reference material. Where this is not possible, perform a test for recovery.

RECOVERY

For assay determinations a recovery of 90 per cent to 110 per cent is to be obtained. For other determinations, for example, for trace element determination the test is not valid if recovery is outside of the range 80 per cent to 120 per cent at the theoretical value. Recovery may be determined on a suitable standard preparation (matrix solution) which is spiked with a known quantity of analyte (middle concentration of the calibration range).

REPEATABILITY

The repeatability is not greater than 3 per cent for an assay and not greater than 5 per cent for an impurity test.

LIMIT OF QUANTIFICATION

The limit of quantification can be estimated by calculating the standard deviation of not less than six replicate measurements of a blank solution, divided by the slope of a standard curve, and multiplying by 10. If validating a procedure using the method of standard additions, the slope of standards applied to a solution of the substance being examined (test preparation) is used. Other suitable approaches can be used.

2.10 PLASMA SPECTROCHEMISTRY

Inductively Coupled Plasma-Atomic Emission Spectrometry

Inductively coupled plasma-atomic emission spectrometry (ICP-AES); also referred to as inductively

coupled plasma-optical emission spectroscopy (ICP-OES), is one of the plasma-based instrumental methods. The inductively coupled plasma (ICP) is a high-temperature excitation source that desolvates, vapourizes, and atomizes aerosol samples and ionizes the resulting atoms. The excited analyte ions and atoms can then subsequently be detected by observing their emission lines, a method termed inductively coupled plasma-atomic emission spectroscopy, or the excited or ground state ions can be determined by a technique known as inductively coupled plasma-mass spectrometry (ICP-MS). The ICP can use either an atomic emission (optical emission) or a mass spectral detection system. In ICP-AES, analyte detection is achived at an emission wavelength of the analyte in question. In ICP-MS, analytes are detected directly at their atomic masses because these masses must be charged to be detected in ICP-MS, the method relies on the ability of the plasma source to both atomize and ionize sample constituents. ICP-AES and ICP-MS may be used for either a single- or multi-element analysis and used for either sequential or simultaneous analyses with good sensitivity over an extended linear range.

ICP formation

The components that make up the ICP excitation source include the argon gas supply, torch, radio frequency (RF) induction coil, impedance-matching unit, and RF generator. Argon gas is almost universally used in the ICP. The plasma torch consists of three concentric tubes designated as the inner, the intermediate, and the outer tube. The intermediate and outer tubes are almost universally made of quartz. The inner tube can be made of quartz or alumina if the analysis is conducted with solutions containing hydrofluoric acid. The nebulizer gas flow carries the aerosol of the sample solution into and through the inner tube of the torch and into the plasma. The intermediate tube carries the intermediate (sometimes referred to as the auxiliary) gas. The intermediate gas flow helps to lift the plasma off the inner and intermediate tubes to prevent their melting and the deposition of carbon and salts on the inner tube. The outer tube carries the outer (sometimes referred to as the plasma or coolant) gas, which is used to form and sustain the toroidal plasma. The toroidal plasma is referred to as the induction region through which the sample is introduced into the centre of the plasma.

An RF induction coil, also called the load coil, surrounds the torch and produces an oscillating magnetic field, which in turn sets up an oscillating current in the ions and electrons produced from the argon. The impedance-matching unit serves to efficiently couple the RF energy from the generator to the load coil. Within the load coil of the RF generator, the energy transfer between the coil and the argon creates a self-sustaining plasma. Collisions of the ions and electrons liberated from the argon ionize and excite the analyte atoms in the high-temperature plasma. The plasma operates at temperatures of 6000 K to 10,000 K (about 5727° to 9727°), so most covalent bonds and analyte-to-analyte interactions are eliminated.

Apparatus

The apparatus consists essentially of the following elements:

(a) a sample-introduction system consisting of a peristaltic pump delivering the solution at constant flow rate into a nebulizer;

(b) an RF generator;

(c) a plasma torch;

(d) transfer optics focusing the image of the plasma at the entrance slit of the spectrometer; radial viewing is better for difficult matrices (alkalis, organics), whereas axial viewing gives more intensity and better detection limits in simple matrices;

(e) wavelength dispersive devices consisting of diffraction gratings, prisms, filters or interferometers;

(f) detectors converting radiant energy into electrical energy;

(g) a data-acquisition unit.

Interference

Interference is anything that causes the signal from an analyte in a sample to be different from the signal for the same concentration of that analyte in a calibration solution. The well-known chemical interference that is encountered in flame atomic absorption spectrometry is usually weak in ICP-AES. In rare cases where interference occurs, it may be necessary to increase the RF power or to reduce the inner support-gas flow to eliminate it. The interference in ICP-AES can be of spectral origin or even the result of high concentrations of certain elements or matrix compounds. Physical interference (due to differences in viscosity and surface tension of the sample and calibration standards) can be minimized by dilution of the sample, matrix matching, use of internal standards, or through application of the method of standard additions. Another type of interference occasionally encountered in ICP-AES is the so-called “easily ionized elements (EIEs) effect”. The EIEs are those elements that are ionized much more easily, for example alkaline metals and alkaline earths. In samples that contain high concentrations of EIEs (more than 0.1 per cent), suppression or enhancement of emission signals is likely to occur.

SPECTRAL INTERFERENCE This interference may be due to other lines or shifts in background intensity. These lines may correspond to argon (observed above 300 nm), OH bands due to the decomposition of water (at about 300 nm), NO bands due to the interaction of the plasma with the ambient air (between 200 nm and 300 nm), and other elements in the sample, especially those present at high concentrations. The interference falls into four different categories: simple background shift, sloping background shift, direct spectral overlap, and complex background shift.

ABSORPTION INTERFERENCE This interference arises when part of the emission from an analyte is absorbed before it reaches the detector. This effect is observed particularly when the concentration of a strongly emitting element is so high that the atoms or ions of that element that are in the lower energy state of transition absorb significant amounts of the radiation emitted by the relevant excited species. This effect, known as self-absorption, determines the upper end of the linear working range for a given emission line.

MULTICOMPONENT SPECTRAL FITTING Multiple emission-line determinations are commonly used to overcome problems with spectral interferences. A better, more accurate method for performing spectral interference corrections is to use the information obtained with advanced detector systems through multicomponent spectral fitting. This quantifies not only the interference, but also the background contribution from the matrix, thereby creating a correction formula. Multicomponent spectral fitting utilizes a multiple linear-squares model based on the analysis of pure analyte, the matrix and the blank, creating an interference corrected mathematical model. This permits the determination of the analyte emission in a complex matrix with improved detection limits and accuracy.

Procedure

SAMPLE PREPARATION AND SAMPLE INTRODUCTION The basic goal for the sample preparation is to ensure that the analyte concentration falls within the working range of the instrument through dilution or preconcentration, and that the sample-containing solution can be nebulized in a reproducible manner. Several sample-introduction systems tolerate high acid concentrations, but the use of sulfuric and phosphoric acids can contribute to background emission observed in the ICP spectra. Therefore, nitric and hydrochloric acids are preferable. The availability of hydrofluoric acid-resistant (for example, perfluoroalkoxy polymer) sample-introduction systems and torches also allows the use of hydrofluoric acid. In selecting a sample-introduction method, the requirements for sensitivity, stability, speed, sample size, corrosion resistance and resistance to clogging have to be considered. The use of a cross-flow nebulizer combined with a spray chamber and torch is suitable for most requirements. The peristaltic pumps used for ICP-AES usually deliver the standard and sample solutions at a rate of 1 mL per minute or less. In the case of organic solvents being used, the introduction of oxygen must be considered to avoid organic layers.

CHOICE OF OPERATING CONDITIONS The standard operating conditions prescribed by the manufacturer are to be followed. Usually, different sets of operating conditions are used for aqueous solutions and for organic solvents. Suitable operating parameters are to be properly chosen:

(1) wavelength selection;

(2) support-gas flow rates (outer, intermediate and inner tubes of the torch);

(3) RF power;

(4) viewing position (radial or axial);

(5) pump speed;

(6) conditions for the detector (gain/voltage for photomultiplier tube detectors, others for array detectors);

(7) integration time (time set to measure the emission intensity at each wavelength).

Control of instrument performance

SYSTEM SUITABILITY The following tests may be carried out with a multi-element control solution to ensure the adequate performance of the ICP-AES system:

(1) energy transfer (generator, torch, plasma); measurement of the ratio Mg II (280.270 nm)/Mg I (285.213 nm) may be used;

(2) sample transfer, by checking nebulizer efficiency and stability;

(3) resolution (optical system), by measuring peak widths at half height, for example, As (189.042 nm), Mn (257.610 nm), Cu (324.754 nm) or Ba (455.403 nm);

(4) analytical performance, by calculating detection limits of selected elements over the wavelength range.

Validation of the method

Satisfactory performance of methods prescribed in monographs is verified at suitable time intervals.

LINEARITY Prepare and analyze not fewer than four standard solutions over the calibration range plus a blank. Perform not fewer than five replicates.

The calibration curve is calculated by least-square regression from all measured data of the calibration test. The regression curve, the means, the measured data and the confidence interval of the calibration curve are plotted. The operating method is valid when:

(1) the correlation coefficient is at least 0.99;

(2) the residuals of each calibration level are randomly distributed around the calibration curve.

Calculate the mean and relative standard deviation for the lowest and for the highest calibration level.

When the ratio of the estimated standard deviations of the lowest and the highest calibration level is less than 0.5 or greater than 2.0, a more precise estimation of the calibration curve may be obtained using weighted linear regression. Both linear and quadratic weighting functions are applied to the data to find the most appropriate weighting function to be employed. If the means compared to the calibration curve show a deviation from linearity, two-dimensional linear regression is used.

ACCURACY Verify the accuracy preferably by using a certified reference material. Where this is not possible, perform a test for recovery.

RECOVERY For assay determinations a recovery of 90 per cent to 110 per cent is to be obtained. The test is not valid if recovery, for example, for trace-element determination, is outside of the range 80 per cent to 120 per cent of the theoretical value. Recovery may be determined on a suitable standard solution (matrix solution) spiked with a known quantity of analyte (concentration range that is relevant to the samples to be determined).

REPEATABILITY The repeatability is not greater than 3 per cent for an assay and not greater than 5 per cent for an impurity test.

LIMIT OF QUANTIFICATION The limit of quantification is estimated by calculating the standard deviation of not less than ten replicate measurements of a blank solution and multiplying by 10. When validating a procedure using the method of standard additions, the slope of standards applied to a solution of the test material is used. Other suitable approaches can be used.

Inductively coupled plasma-mass spectrometry

Inductively coupled plasma-mass spectrometry (ICP-MS) is a plasma-based instrumental method that uses mass spectral detection system. When using the ICP-MS, analytes are detected directly at their atomic masses. Because these masses must be charged to be detected in ICP-MS, the method relies on the ability of the plasma source to both atomize and ionize sample constituents. The basic principles of ICP formation are described in ICP Formation under Inductively Coupled Plasma-Atomic Emission Spectrometry.

The sample-introduction system and data-handling techniques of an ICP-AES system are also used in ICP-MS.

Apparatus

The apparatus consists essentially of the following elements:

(a) a sample-introduction system, consisting of a peristaltic pump delivering the solution at constant flow rate into a nebulizer;

(b) an RF generator;

(c) a plasma torch;

(d) an interface region including cones to transport ions to the ion optics;

(e) a mass spectrometer;

(f) a detector;

(g) a data-acquisition unit.

Interference

Mass interference is the major problem, for example by isobaric species that significantly overlap the mass signal of the ions of interest, especially in the central part of the mass range (for example, 40 to 80 a.m.u.). The combination of atomic ions leads to polyatomic or molecular interferences (i.e., $^{40}Ar^{16}O$ with ^{56}Fe or $^{40}Ar^{40}Ar$ with ^{80}Se). Matrix interference may also occur with some analytes. Some samples have an impact on droplet formation or on the ionization temperature in the plasma. These phenomena may lead to the suppression of analyte signals. Physical interference is to be circumvented by using the method of internal standardization or by standard addition. The element used as internal standard depends on the element to be measured: ^{59}Co and ^{115}In, for example, can be used as internal standards. The prime characteristic of an ICP-MS instrument is its resolution, i.e. the efficiency of separation of two close masses. Quadrupole instruments are, from this point of view, inferior to magnetic-sector spectrometers.

Procedure

SAMPLE PREPARATIONS AND SAMPLE INTRODUCTION The sample preparation usually involves a step of digestion of the matrix by a suitable method, for example in a microwave oven. Furthermore, it is important to ensure that the analyte concentration falls within the working range of the instrument through dilution or preconcentration, and that the

sample-containing solution can be nebulized in a reproducible manner.

Several sample-introduction systems tolerate high acid concentrations, but the use of sulfuric and phosphoric acids can contribute to background emission. Therefore, nitric and hydrochloric acids are preferable. The availability of hydrofluoric acid-resistant (for example, perfluoroalkoxy polymer) sample-introduction systems and torches also allows the use of hydrofluoric acid. In selecting a sample-introduction method, the requirements for sensitivity, stability, speed, sample size, corrosion resistance and resistance to clogging have to be considered. The use of a cross-flow nebulizer combined with a spray chamber and torch is suitable for most requirements. The peristaltic pumps usually deliver the standard and sample solutions at a rate of 20 to 1000 µL per minute. In the case of organic solvents being used, the introduction of oxygen must be considered to avoid organic layers.

CHOICE OF OPERATING CONDITIONS The standard operating conditions prescribed by the manufacturer are to be followed. Usually, different sets of operating conditions are used for aqueous solutions and for organic solvents. Suitable operating parameters are to be properly chosen:

(1) selection of cones (material of sampler and skimmer);

(2) support-gas flow rates (outer, intermediate and inner tubes of the torch);

(3) RF power;

(4) pump speed;

(5) selection of one or more isotopes of the element to be measured (mass).

Isotope selection

Isotope selection is made using several criteria. The most abundant isotope for a given element is selected to obtain maximum sensitivity. Furthermore, an isotope with the least interference from other species in the sample matrix and from the support gas should be selected. Information about isobaric interferences and interferences from polyatomic ions of various types, for example, hydrides, oxides, chlorides, etc., is usually available in the software of ICP-MS instrument manufacturers.

Control of instrument performance

SYSTEM SUITABILITY

(1) Tuning of the instrument allows to monitor and adjust the measurement before running samples. ICP-MS mass accuracy is checked with a tuning solution containing several isotopes covering the whole range of masses, for example, ^{9}Be, ^{59}Co, ^{89}Y, ^{115}In, ^{140}Ce, and ^{209}Bi.

(2) Sensitivity and short- and long-term stability are recorded. The instrument parameters (plasma condition, ion lenses and quadrupole parameter) are to be optimized to obtain the highest possible number of counts.

(3) Tuning for resolution and mass axis is to be done with a solution of Li, Y and Tl to ensure an acceptable response over a wide range of masses.

(4) Evaluation of the efficiency of the plasma to decompose oxides has to be performed in order to minimize these interferences. The ratio Ce/CeO and/or Ba/BaO is a good indicator, and a level less than about 3 per cent is required.

(5) Reduction of the formation of double-charged ions is made with Ba and Ce. The ratio of the signal for double-charged ions to the assigned element should be less than 2 per cent.

(6) Long-term stability is checked by running a standard first and at the end of the sample sequence, controlling whether salt deposits on the cones have reduced the signal throughout the run.

Validation of the method

Satisfactory performance of methods prescribed in monographs is verified at suitable time intervals.

LINEARITY Prepare and analyze not fewer than four standard preparations over the calibration range plus a blank. Perform not fewer than five replicates. The calibration curve is calculated by least-square regression from all measured data of the calibration test. The regression curve, the means, the measured data and the confidence interval of the calibration curve are plotted. The operating method is valid when:

(1) the correlation coefficient is at least 0.99;

(2) the residuals of each calibration level are randomly distributed around the calibration curve.

Calculate the mean and relative standard deviation for the lowest and for the highest calibration level.

When the ratio of the estimated standard deviations of the lowest and the highest calibration level is less than 0.5 or greater than 2.0, a more precise estimation of the calibration curve may be obtained using weighted linear regression. Both linear and quadratic weighting functions are applied to the data to find the most appropriate weighting function to be employed.

If the means compared to the calibration curve show a deviation from linearity, two-dimensional linear regression is used.

ACCURACY Verify the accuracy preferably by using a certified reference material. Where this is not possible, perform a test for recovery.

RECOVERY For assay determinations a recovery of 90 per cent to 110 per cent is to be obtained. The test is not valid if recovery, for example for trace-element determination, is outside the range 80 per cent to 120 per cent of the theoretical value. Recovery may be determined on a suitable standard solution (matrix solution) spiked with a known quantity of analyte (concentration range that is relevant to the samples to be determined).

REPEATABILITY The repeatability is not greater than 3 per cent for an assay and not greater than 5 per cent for an impurity test.

LIMIT OF QUANTIFICATION The limit of quantification is estimated by calculating the standard deviation of not less than ten replicate measurements of a blank solution and multiplying by 10. When validating a procedure using the method of standard additions, the slope of standards applied to a solution of the test material is used. Other suitable approaches can be used.

APPENDIX 3 CHROMATOGRAPHY

3.1 THIN-LAYER CHROMATOGRAPHY

Thin-Layer Chromatography (TLC) is used for the rapid separation of compounds by means of a uniform layer of dry, finely powdered material applied to a glass, plastic, or metal sheet or plate. The coated plate can be considered as an "open chromatographic column". 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).

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. As R_f values may vary significantly with the experimental conditions, it is always necessary to prepare chromatograms of authentic specimens or reference substances; preferably in varied quantities, alongside the chromatogram of the sample. Positive identification may be effected by observation of two spots of identical R_f value and about equal magnitude. A visual comparison of the size of the spots may serve for semi-quantitative estimation. More accurate quantitative measurements can be made by densitometry, fluorescence, and fluorescence quenching, or careful removal of the spots from the plate, followed by elution with a suitable solvent and spectrophotometric measurement. For two-dimensional thin-layer chromatography, the chromatographed plate is turned at a right angle and again chromatographed, usually in another chamber equilibrated with a different solvent system.

Apparatus

Plate The chromatography is carried out using the TLC plate (typically 20 cm × 20 cm) of which the stationary phase has an average particle size of 10 to 15 µm, and that of high-performance thin-layer chromatography (HPTLC) plates (typically 10 cm × 10 cm) has an average particle size of 5 µm. Commercial plates with a pre-adsorbent zone can be used if they are specified in a monograph.

Spreader A spreader, which, when moved over the plate, will apply a uniform layer of adsorbant, 250 to 300 µm thick, over the entire surface of the plate. Other thicknesses might be desirable in some procedures, and an adjustable spreader would be particularly useful in such cases.

Preparation of the TLC plate Use flat glass plates of convenient size typically 20 cm × 20 cm.

The adsorbent consists of finely divided solid material, normally 10 to 15 µm in diameter, suitable for chromatography. It can be applied directly to the plate or can be bonded to the plate by means of plaster of Paris (hydrated calcium sulfate) at a ratio of 5 to 15 per cent, or with starch paste or other binders. The former will not yield as hard a surface as will the starch, but it is not affected by strongly oxidizing spray reagents. The adsorbent may contain fluorescing material to aid in the visualization of spots that absorb ultraviolet light. Clean the plates scrupulously, as by immersion in *chromic acid cleansing mixture*, rinsing them with copious quantities of *water* until the water runs off the plates without leaving any visible water or oily spots, and then dry. It is important that the plates be completely free from lint and dust when the adsorbent is applied.

Arrange the plate or plates on the aligning tray, place a 5-cm × 20-cm plate adjacent to the front edge of the first square plate and another 5-cm × 20-cm plate adjacent to the rear edge of the last square, and secure all of the plates so that they will not slip during the application of the adsorbant. Position the spreader on the end plate opposite to the raised end of the aligning tray. Mix 1 part of adsorbent with 2 parts of *water* (or in the ratio suggested by the supplier) by shaking vigorously for about 30 seconds in a glass-stoppered conical flask, and transfer the slurry to the spreader. Usually 30 g of adsorbent and 60 mL of water are sufficient for five 20-cm × 20-cm plates. Complete the application of adsorbents using plaster of Paris binder within 2 minutes of addition of the water, since thereafter the mixture begins to harden. Draw the spreader smoothly over the plates towards the raised end of the aligning tray, and remove the spreader when it is on the end plate next to the raised end of the aligning tray. (Wash away all traces of adsorbant from the spreader immediately after use). Allow the plates to remain undisturbed for 5 minutes, then transfer the square plates, layer side up, to the storage rack and dry at 105° for 30 minutes. Preferably place the rack at an angle in the drying oven to prevent the condensation of moisture on the back side of the plates in the rack. When the plates are dry, allow them to cool to room

temperature, and inspect the uniformity of the distribution and the texture of the adsorbant layer; transmitted light will show uniformity of texture. Store the satisfactory plates over *self-indicating silica gel* in a suitable chamber.

Pre-treatment of the plate 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° for 20 minutes.

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

Micropipette, microsyringe, calibrated disposable capillary A micropipette, microsyringe, calibrated disposable capillary or other application devices suitable for the proper application of the solutions are used.

Template A template (generally made of plastic) is used to aid in placing the test spots at definite intervals, to mark distances as needed, and to aid in labelling the plate.

Detection/Visualization device An ultraviolet (UV) light source suitable for observations under short- (254 nm) and long- (366 nm) wavelength UV light and a variety of other spray reagents to make spots visible are used.

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

Procedure

Sample application Apply the prescribed volume of the solutions at a distance of at least 15 mm (5 mm on HPTLC plates) 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 HPTLC plates) between the centres of circular spots and 5 mm (2 mm on HPTLC plates) between the edges of bands.

Apply the solutions in sufficiently small portions to obtain circular spots of 2 to 5 mm in diameter (1 to 2 mm on HPTLC plates) or bands of 10 to 20 mm (5 to 10 mm on HPTLC plates) by 1 to 2 mm (0.5 to 1 mm on HPTLC plates) and allow to dry. Avoid physical disturbance of the adsorbant during the spotting procedure (by the pipette or other applicator) or when handling the plates. The template will aid in determining the spot points and the specified distance through which the solvent front should pass.

Development Line the walls of the developing chamber with filter paper. Pour into the developing chamber a sufficient quantity of the mobile phase for the size of the chamber 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 developing chamber, replace the lid and allow to stand for 1 hour. Unless otherwise indicated in the monograph, the chromatographic separation is performed in a saturated chamber. Apply the prescribed volume of solutions as described above. When the solvent has evaporated from the applied solutions, place the plate in the developing chamber, 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 developing chamber. Remove the plate when the mobile phase has moved over 15 cm, or over three-quarters of the length of the plate, above the initial spots or bands, unless otherwise indicated in the monograph. Dry the plate and visualize the chromatograms as prescribed.

Horizontal development can be used in place of vertical development, if specified in the monograph. 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.

Detection/Visualization Observe the principal spot or band in the chromatogram first under short-wavelength ultraviolet light (254 nm) and then under long-wavelength ultraviolet light (366 nm). Measure and record the distance of each spot or band from the point of origin, and indicate for each spot or band the wavelength under which it was observed. If further directed, spray the spots or bands with the reagent specified, observe, and compare the sample with the standard chromatogram.

3.5 LIQUID CHROMATOGRAPHY

The term Liquid chromatography (LC), in this Pharmacopoeia, is referred to as HPLC, both high-pressure and high-performance. It is a separation technique based on a solid stationary phase and a liquid mobile phase. Separations are achieved

by partition (mass distribution), adsorption, ion-exchange, or stereochemical-interaction processes, depending upon the type of stationary phase used. Unless otherwise specified, all information below is valid for LC as well as for LC using reduced particle-size columns (e.g., sub-2 µm). The latter requires instrumentation characterized by the capability to apply higher pressures (typically up to 100 MPa, i.e., about 15,000 psi), lower extra-column band broadening, improved gradient mixing and a higher sampling rate in the detection system.

Apparatus

A liquid chromatograph consists of a reservoir containing the mobile phase, a pump to force the mobile phase through the system at high pressure, an injector to introduce the sample into the mobile phase, a chromatographic column, a detector, and a data acquisition system.

PUMPING SYSTEMS LC pumping systems deliver metered amounts of mobile phase from the solvent reservoirs to the column through high-pressure tubing and fittings. 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 After dissolution in mobile phase or other suitable solution, compounds to be chromatographed are injected into the mobile phase. Fixed-loop and variable volume devices operated manually or by an auto-sampler are used.

STATIONARY PHASES For most pharmaceutical analyses, separation is achieved by partition of compounds in the test solution between the mobile phase and the stationary phase. A system consisting of a polar stationary phase and a nonpolar mobile phase is described as normal-phase chromatography, while the opposite arrangement, a polar mobile phase and a nonpolar stationary phase, is called reversed-phase chromatography. There are many types of stationary phase materials employed in LC, including:

— silica, alumina or porous graphite, used in normal-phase chromatography, where the separation is based on differences in adsorption and/or mass distribution (partition chromatography),

— resins or polymers with acid 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, where separation is based on differences between the volumes of the molecules, corresponding to steric exclusion,

— a variety of chemically modified supports prepared from polymers, silica or porous graphite, used in normal-phase (adsorption chromatography) and reversed-phase LC, where the separation is based principally on partition of the molecules between the mobile phase and the stationary phase,

— special chemically modified stationary phases, e.g., cellulose or amylose derivatives, proteins or peptides, cyclodextrins, etc., for the separation of enantiomers (chromatography on chiral stationary phases).

Most separations are based upon partition mechanisms utilizing chemically modified silica as the stationary phase and polar solvents as the mobile phase. The surface of the support, e.g., 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.

Commonly used bonded phases are shown below:

octyl (C_8)	=	$Si-[CH_2]_7-CH_3$
octadecyl (C_{18})	=	$Si-[CH_2]_{17}-CH_3$
phenyl (C_6H_5)	=	$Si-[CH_2]_n-C_6H_5$
cyanopropyl (CN)	=	$Si-[CH_2]_3-CN$
aminopropyl (NH_2)	=	$Si-[CH_2]_3-NH_2$
diol	=	$Si-[CH_2]_3-O-CH(OH)-CH_2-OH$

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 of 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 chromatography with unmodified silica, porous graphite or polar chemically modified silica, e.g., cyanopropyl or diol, as the stationary phase with a nonpolar mobile phase is applicable in certain cases. For analytical separations, the particle size of the most commonly used stationary phases varies

between 2 µm and 10 µm. The particles may be spherical or irregular, 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 being 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.

COLUMNS Columns, made of stainless steel, lined stainless steel, and polymers of varying length and internal diameter 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 an analysis. Most separations are performed at room temperature, but some require a different temperature for optimal performance.

In LC procedures, a guard column may be used with the following requirements, unless otherwise indicated in the individual monograph: (a) the length of the guard column must be not more than 15 per cent of the length of the analytical column, (b) the internal diameter must be the same or smaller than that of the analytical column, and (c) the packing material should be the same as the analytical column and contain the same bonded phase. In any case, all system suitability requirements specified in the official procedure must be met with the guard column installed.

MOBILE PHASES

For normal-phase chromatography, less polar 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 phases, with or without organic solvents, are employed.

Components of the mobile phase are usually filtered to remove particles greater than 1.45 µm (or 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). Solvents are normally degassed before pumping by sparging with helium, sonication and/or using on-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 stabilizers 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. Adjustment of the pH, if necessary, is effected using only 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 5 per cent v/v mixture of the organic part of the mobile phase in water to prevent crystallization of salts after completion of the analysis. Mobile phases may contain other components, e.g., a counter-ion for ion-pair chromatography or a chiral selector for chromatography using an achiral stationary phase.

DETECTORS

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

Procedure

Criteria for assessing the suitability of the system are described in the "Chromatographic Separation Techniques" (Appendix 3.9). 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 Appendix.

In general, the steps involved in the liquid chromatographic technique are as follows:

1. Equilibrate the column and detector with the mobile phase at the specified flow rate until a stable baseline is achieved.
2. Prepare the sample and the standard solutions. The solutions must be free from solid particles.
3. Inject the standard/sample solution through the injector, or use an autosampler.
4. Perform the isocratic/gradient programme.
5. Record the chromatogram.
6. Analyze as specified in the monograph.

In quantitative work, particularly where the use of an internal standard is not specified in the monograph, the use of a fixed-volume loop injector is recommended. In certain exceptional cases the use of peak heights alone is prescribed in the monograph; where this is the case peak heights should be used irrespective of the symmetry factor. The column is usually made of stainless steel and its dimensions

are stated in the monograph as length × internal diameter. The nominal diameter of the particles of the stationary phase is stated in parentheses following the name of stationary phase prescribed in the monograph. In most cases reference is made to a particular commercial brand that has been found to be suitable for the purpose, but such statements do not imply that a different but equivalent commercial brand may not be used. The separation should be carried out at a constant room temperature unless otherwise specified in the monograph. When using mobile phases of high pH with a silica-based column, it is advisable to use a pre-column before the analytical column.

Unless otherwise specified in the monograph, the detector consists of a photometric detector fitted with a low-volume flow cell (about 10 µL is suitable); the wavelength setting is specified in the monograph.

3.9 CHROMATOGRAPHIC SEPARATION TECHNIQUES

Interpretation of Chromatograms

Figure 1 represents a typical chromatographic separation of two substances, 1 and 2, where t_1 and t_2 are the respective retention times. h, $h/2$, and $W_{h/2}$ are the height, the half-height, and the width at half-height, respectively, for peak 1. W_1 and W_2 are the respective widths of peaks 1 and 2 at the base-line. Air peaks are a feature of gas chromatograms and correspond to the solvent front in liquid chromatography.

Chromatographic retention times are characteristic of the compounds they represent but are not unique. Coincidence of retention times of a test and a reference substance can be used as a feature in construction of an identity profile but is insufficient on its own to establish identity. Absolute retention times of a given compound vary from one chromatogram to the next.

The elution time of a compound can be described by the retention factor, k, which depends on the chemical nature of the analyte, the composition and flow rate of the mobile phase, and the composition and surface area of the stationary phase.

The retention factor (also known as the capacity factor, k') is defined as:

$$k = \frac{\text{amount of substance in stationary phase}}{\text{amount of substance in mobile phase}} \%$$

or

$$k = \frac{\text{time spent by substance in stationary phase}}{\text{time spent by substance in mobile phase}} = \frac{t}{t_M} - 1,$$

where t is the retention time measured from time of injection to time of elution of peak maximum and t_M is the hold-up time–the time required for elution of a non-retained component, shown in Figure 1 as an air or non-retained solvent peak, with the baseline scale in minutes.

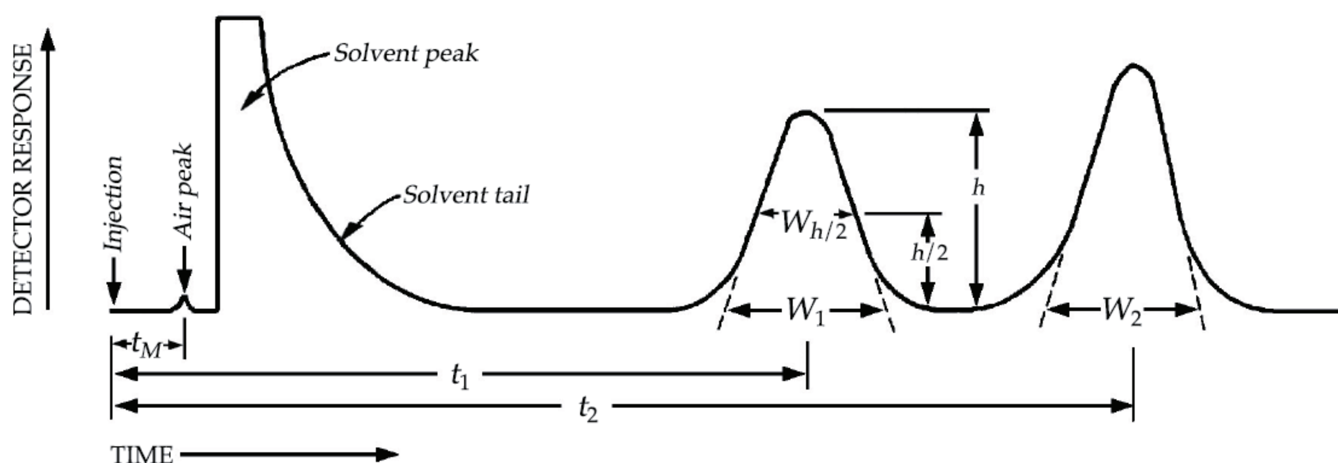


Fig. 1 Chromatographic Separation of Two Substances

The volume of mobile phase required for elution of a component is defined as retention volume, V. It may be calculated from the retention time and the flow rate, F, in mL/min:

$$V = t \times F$$

The volume of mobile phase required for elution of a non-retained component is defined as the hold-up volume, V_M. It may be calculated from the hold-up time, t_M, and the flow rate, F, in mL/min:

$$V_M = t_M \times F$$

Comparisons are normally made in terms of relative retention times, R_r, unless otherwise specified in the monograph:

$$R_r = \frac{t_2}{t_1},$$

where t_2 and t_1 are the retention times, measured from the point of injection, of the test and reference substances, respectively, determined under identical experimental conditions on the same column.

Other procedures may identify the peak position using the relative retention, r:

$$r = \frac{t_2 - t_M}{t_1 - t_M},$$

where t_M is the retention time of a non-retained component, which needs to be defined in the procedure.

The number of theoretical plates, N, is a measure of column efficiency. For Gaussian peaks, it is calculated by the equation:

$$N = 16\left(\frac{t}{W}\right)^2,$$

where t is the retention time of the substance and W is the width of the peak at its base, obtained by extrapolating the relatively straight sides of the peak to the baseline. The value of N depends on the substance being chromatographed as well as the operating conditions such as mobile phase or carrier gas flow rates and temperature, the quality of the packing, the uniformity of the packing within the column and, for capillary columns, the thickness of the stationary phase film, and the internal diameter and length of the column.

The separation of two components in a mixture, the resolution, R, is determined by the equation:

$$R = \frac{2(t_2 - t_1)}{W_1 - W_2},$$

where t_2 and t_1 are the retention times of the two components, and W_2 and W_1 are the corresponding widths at the bases of the peaks obtained by extrapolating the relatively straight sides of the peaks to the baseline.

Where electronic integrators are used, it may be convenient to determine the number of theoretical plates, N, by the equation:

$$N = 5.54(t/W_{2,h/2})^2$$

and to determine the resolution, R, by the equation:

$$R = \frac{2(t_2 - t_1)}{1.70(W_{1,h/2} + W_{2,h/2})},$$

where $W_{h/2}$ is the peak width at half-height, obtained directly by electronic integrators. However, in the event of dispute, only equations based on peak width at baseline are to be used.

Peak areas and peak heights are usually proportional to the quantity of compound eluting. Peak areas are generally used but may be less accurate if peak interference occurs. For accurate quantitative work, the components to be measured should be separated from any interfering components. Peak tailing and fronting and the measurement of peaks on solvent tails are to be avoided.

The peak-to-valley ratio, p/v, may be employed as a system suitability criterion in a test for related substances when baseline separation between two peaks is not achieved. Figure 2 represents a partial separation of two substances, where H_p is the height above the extrapolated baseline of the minor peak and H_v is the height above the extrapolated baseline at the lowest point of the curve separating the minor and major peaks:

$$p/v = H_p/H_v$$

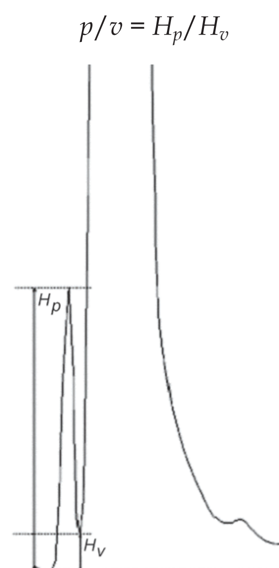


Fig. 2 Peak-to-Valley Ratio Determination

Chromatographic purity tests for drug raw materials are sometimes based on the determination of peaks due to impurities, expressed as a percentage of the area due to the drug peak. It is preferable, however, to compare impurity peaks to the chromatogram of a standard at a similar concentration. The standard may be the drug itself at a level corresponding to, for example, 0.5 per cent impurity, or in the case of toxic or signal impurities, a standard of the impurity itself.

System Suitability

System suitability tests are an integral part of gas and liquid chromatographic methods. They are used to verify that the detection sensitivity, resolution, and reproducibility of the chromatographic system are adequate for the analysis to be done. The tests are based on the concept that the equipment, electronics, analytical operations, and samples to be analyzed constitute an integral system that can be evaluated as such. 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, and 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 detection sensitivity is a measure used to ensure the suitability of a given chromatographic procedure for the complete detection of the impurities in the Chromatographic purity or Related compounds tests by injecting a volume of a quantitation limit solution equal to that of the Test solution. Unless otherwise specified in the individual monograph, the quantitation limit solution may be prepared by dissolving the Drug Reference Substance in the same solvent as that used for the Test solution at a 0.05 per cent concentration level relative to the amount of drug substance in the Test solution for drug substances, and a 0.1 per cent level relative to the amount of drug substance in the Test solution for drug products. The signal-to-noise ratio for the drug substance peak obtained with the quantitation limit solution should be not less than 10.

The signal-to-noise (S/N) ratio is determined by the equation:

$$\text{S/N ratio} = 2H/h,$$

where H is the height of the peak measured from the peak apex to a baseline extrapolated over a distance ≥5 times the peak width at its half-height, and h is the difference between the largest and smallest noise values observed over a distance ≥5 times the width at the half-height of the peak and, if possible, situated equally around the peak of interest (Fig. 3).

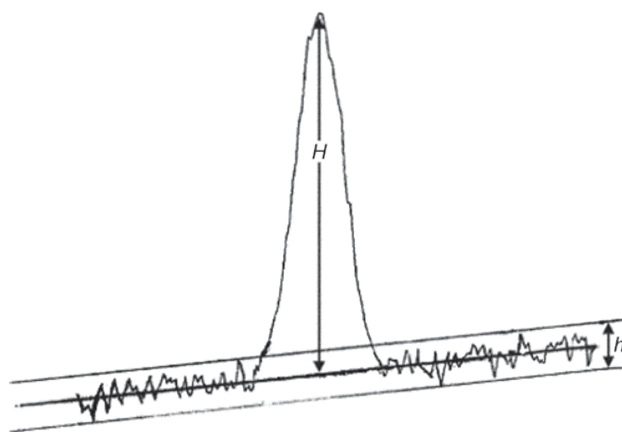


Fig. 3 Noise and Chromatographic Peak, Components of the S/N Ratio

The resolution, R, is a function of the number of theoretical plates, N (also referred to as column efficiency) and may be specified to ensure that closely eluting compounds are resolved from each other, to establish the general resolving power of the system, and to ensure that internal standards are resolved from the drug. Column efficiency may be specified also as a system suitability requirement, especially if there is only one peak of interest in the chromatogram; however, it is a less reliable means to ensure resolution than direct measurement. Column efficiency is a measure of peak sharpness, which is important for the detection of trace components.

Replicate injections of a standard preparation used in the assay or other standard solution are compared to ascertain whether requirements for precision are met. Unless otherwise specified in the individual monograph, data from five replicate injections of the analyte are used to calculate the relative standard deviation, RSD, if the requirement is 2.0 per cent or less; data from six replicate injections are used if the relative standard deviation requirement is more than 2.0 per cent.

RSD (%) relative standard deviation in percentage,

$$RSD(\%) = \frac{100}{\overline{X}} \left[\frac{\sum_{i=1}^{n} (X_i - \overline{X})^2}{n-1} \right]^{1/2},$$

where X_i is an individual measurement in a set of n measurements and is the arithmetic mean of the set.

The symmetry factor (or tailing factor), T, a measure of peak symmetry, is unity for perfectly symmetrical peaks and its value increases as tailing becomes more pronounced (Fig. 4):

$$T = \frac{W_{0.05}}{2f},$$

where T is the symmetry factor (or tailing factor), $W_{0.05}$ is the width of the peak at 5 per cent height and f is the distance from the peak maximum to the leading edge of the peak, the distance being measured at a point 5 per cent of the peak height from the baseline. In some cases, values less than unity may be observed. As peak asymmetry increases, integration and hence precision become less reliable.

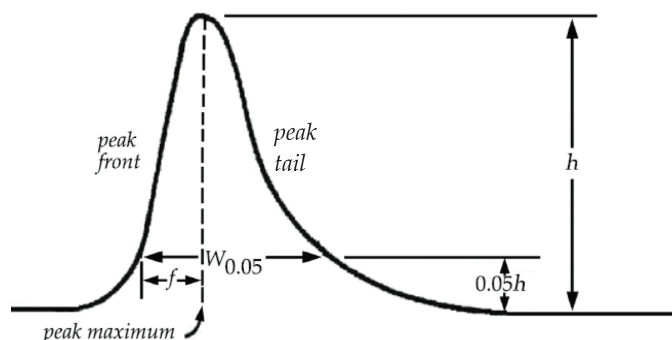


Fig. 4 Asymmetrical Chromatographic Peak

These system suitability tests are performed by collecting data from replicate injections of standard or other solutions as specified in the individual monograph. The specification of definitive parameters in a monograph does not preclude the use of other suitable operating conditions. Adjustments of operating conditions to meet system suitability requirements may be necessary.

Unless otherwise directed in the monograph, system suitability parameters are determined from the analyte peak.

Relative retention times may be provided in monographs for informational purposes only, to aid in peak identification. There are no acceptance criteria applied to relative retention times.

To ascertain the effectiveness of the final operating system, it should be subjected to suitability testing. Replicate injections of the standard solution required to demonstrate adequate system precision may be made before the injection of samples or may be interspersed among sample injections. System suitability must be demonstrated throughout the run by injection of an appropriate control preparation at appropriate intervals. The control preparation can be a standard preparation or a solution containing a known amount of analyte and any additional materials useful in the control of the analytical system, such as excipients or impurities. Whenever there is a significant change in equipment or in a critical reagent, suitability testing should be performed before the injection of samples. No sample analysis is acceptable unless the requirements of system suitability have been met. Sample analyses obtained while the system fails system suitability requirements are unacceptable.

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.

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; pH OF THE AQUEOUS COMPONENT OF THE MOBILE PHASE; CONCENTRATION OF SALTS The adjustments can be made as described under Liquid Chromatography (Appendix 3.5).

APPLICATION VOLUME The application volume can be adjusted to 10 to 20 per cent of the prescribed volume if using fine particle size plates (2 to 10 µm).

MIGRATION DISTANCE The migration distance of the solvent front is to be not less than 50 mm or 30 mm on high-performance plates.

Liquid Chromatography

COMPOSITION OF THE MOBILE PHASE The following adjustment limits apply to minor components of the mobile phase (specified at 50 per cent or less). The amount(s) of these component(s) can be adjusted by ±30 per cent relative. However, the change in any component cannot exceed ±10 per cent absolute (i.e., in relation to the total mobile phase), nor can the final concentration of any component be reduced to zero. Adjustment can be made to one minor component in a ternary mixture. Examples of adjustments are given below.

Specified ratio of 50:50 Thirty per cent of 50 is 15 per cent absolute, but this exceeds the maximum permitted change of ±10 per cent absolute in either component. Therefore, the mobile phase ratio may be adjusted only within the range of 40:60 to 60:40.

Specified ratio of 2:98 Thirty per cent of 2 is 0.6 per cent absolute. Therefore, the maximum allowed adjustment is within the range of 1.4:98.6 to 2.6:97.4.

Specified ratio of 60:35:5 For the second component, 30 per cent of 35 is 10.5 per cent absolute, which exceeds the maximum permitted change of ±10 per cent absolute in any component. Therefore, the second component may be adjusted only within the range of 25 per cent to 45 per cent absolute. For the third component, 30 per cent of 5 is 1.5 per cent absolute. In all cases, a sufficient quantity of the first component is used to give a total of 100 per cent. Therefore, mixture ranges of 50:45:5 to 70:25:5 or 58.5: 35:6.5 to 61.5:35:3.5 would meet the requirement.

pH OF THE AQUEOUS COMPONENT OF THE MOBILE PHASE The pH of the aqueous buffer used in the preparation of the mobile phase can be adjusted to within ±0.2 pH of the value or range specified, or ±1.0 pH when neutral substances are to be examined.

CONCENTRATION OF SALTS The concentration of the salts used in the preparation of the aqueous buffer used in the mobile phase can be adjusted to within ±10 per cent, provided the permitted pH variation is met.

DETECTOR WAVELENGTH No adjustment permitted.

STATIONARY PHASE

For isocratic separations, changes in column length, column internal diameter and particle size are allowed as follows:

- column length: ±70 per cent,
- column internal diameter: ±25 per cent,
- particle size: maximal reduction of 50 per cent, no increase permitted.

For gradient separations, only changes in column length and column internal diameter, are allowed as follows:

- column length: ±70 per cent,
- column internal diameter: ±25 per cent,
- particle size: no adjustment permitted.

FLOW RATE

For isocratic separations, the flow rate can be adjusted by ±50 per cent. When column dimensions are changed, the flow rate is determined by the equation:

$$F_2 = F_1 \frac{L_2 d_2^2}{L_1 d_1^2} ,$$

where F_1 is the flow rate specified in the monograph, in mL per minute; F_2 is adjusted flow rate, in mL per minute; L_1 is the length of the column specified in the monograph, in mm; L_2 is the length of the column used, in mm; d_2 is the internal diameter of the column specified in the monograph, in mm; d_2 is the internal diameter of the column used, in mm.

For gradient separations, the adjustment of the flow rate is allowed when changing the column dimensions. The flow rate is determined by the equation above.

COLUMN TEMPERATURE The column temperature can be adjusted as follows, unless otherwise specified:

- isocratic separations: ±10°,
- gradient separations: ±5°.

INJECTION VOLUME The injection volume can be reduced as far as is consistent with accepted precision and detection limits. No increase in the injection volume is permitted.

Gas Chromatography

STATIONARY PHASE
- column length: ±70 per cent,
- column internal diameter: ±50 per cent,
- particle size: maximal reduction of 50 per cent, no increase permitted,
- film thickness: –50 per cent to +100 per cent.

FLOW RATE The flow rate can be adjusted by as much as ±50 per cent.

OVEN TEMPERATURE The oven temperature can be adjusted by as much as ±10 per cent.

OVEN TEMPERATURE PROGRAM Adjustment of temperatures is permitted as stated above. For the times specified for the temperature to be maintained or for the temperature to be changed from one value to another, an adjustment of up to ±20 per cent is permitted.

INJECTION VOLUME The injection volume can be reduced as far as is consistent with accepted precision and detection limits.

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) quantified is determined by comparing the response(s) obtained with the sample solution to the response(s) obtained with a standard solution.

INTERNAL STANDARD METHOD Equal amounts of the internal standard are introduced into the sample solution and a standard solution. The internal standard is chosen so that it does not react with the test material and does not contain impurities with the same retention time as that of the analytes, and is stable and resolved from the component(s) quantified (analytes). The concentrations of the analytes are determined by comparing the ratios of their peak areas or peak heights and the internal standard in the sample solution with the ratios of their peak areas or peak heights and the internal standard in the standard solution.

NORMALIZATION PROCEDURE The per cent content of a component of the test substance 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 limit at which they can be disregarded.

CALIBRATION PROCEDURE The relationship between the measured or evaluated signal y and the quantity (e.g., concentration or mass) 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 and its position on the calibration curve.

In tests for impurities for both the external standard method, when a dilution of the sample solution is used for comparison, and the normalization procedure, any correction factors indicated in the monograph are applied (e.g., when the relative response factor is outside the range of 0.8 to 1.2).

When the impurity test prescribes the total of impurities or there is a quantitative determination of an impurity, choice of an appropriate threshold setting and appropriate conditions for the integration of the peak areas is important. In such tests the limit at or below which a peak is disregarded is generally 0.05 per cent. Thus, the threshold setting of the data collection system corresponds to at least half of this limit. Integrate the peak area of any impurity that is not completely separated from the principal peak, preferably by valley-to-valley extrapolation (tangential skim).

APPENDIX 4 PHYSICAL TESTS

4.15 LOSS ON DRYING

The procedure set forth in this section determines the amount of volatile matter of any kind that is driven off under the conditions specified. For substances appearing to contain water as the only volatile constituent, the procedure given in "Determination of Water" (Appendix 4.12), is appropriate, and is specified in the individual monograph.

Unless otherwise directed in the monograph, conduct the determination on 1 to 2 g of the substance (2 to 5 g in case of crude drugs), previously mixed and accurately weighed. If the test substance is in the form of large crystals, reduce the particle size to about 2 mm by quickly crushing. Tare a glass-stoppered, shallow weighing bottle that has been dried for 30 minutes under the same conditions to be employed in the determination. Put the test substance in the bottle, replace the cover, and accurately weigh the bottle and the contents. By gentle, sidewise shaking distribute the test substance as evenly as practicable to a depth of about 5 mm generally, and not over 10 mm in the case of bulky materials. Place the loaded bottle in the drying chamber, removing the stopper and leaving it also in the chamber. Dry the test substance at the temperature and for the time specified in the monograph. The temperature of heating is within the range of ±2° of the stated figure in the monograph. Upon opening the chamber, close the bottle promptly and allow it to come to room temperature in a desiccator before weighing.

If the substance melts at a lower temperature than that specified for the determination of Loss on drying, maintain the bottle with its contents for 1 to 2 hours at a temperature 5° to 10° below the melting temperature, then dry at the specified temperature.

Where the sample under test is Capsules, use a portion of the mixed contents of not less than 4 capsules.

Where the sample under test is Tablets, use powder from not less than 4 tablets ground to a fine powder.

Where the individual monograph directs that loss on drying be determined by thermogravimetric analysis, a sensitive electrobalance is to be used.

Where drying in vacuum over a desiccant is directed in the individual monograph, a vacuum desiccator or a vacuum drying pistol, or other suitable vacuum drying apparatus, is to be used. Where drying in a desiccator is specified, exercise particular care to ensure that the desiccant is kept fully effective by frequent replacement.

Where drying in a capillary-stoppered bottle in vacuum is directed in the individual monograph, use a bottle or tube fitted with a stopper having a 225±25 µm diameter capillary, and maintain the heating chamber at a pressure not exceeding 0.7 kPa (about 5 Torr). At the end of the heating period, admit dry air to the heating chamber, remove the bottle, and with the capillary stopper still in place allow it to cool in a desiccator before weighing.

APPENDIX 5 CHEMICAL TESTS

5.2 LIMIT TESTS
HEAVY METALS IN HERBAL DRUGS AND HERBAL DRUG PREPARATIONS

> ***Caution*** When using closed high-pressure digestion vessels and microwave laboratory equipment, be familiar with the safety and operating instructions given by the manufacturer.

Apparatus

The apparatus typically consists of the following:

(a) a digestion flasks, polytetrafluoroethylene, perfluoroalkoxy polymer, quartz or glass flasks with a volume of 20 to 150 mL, fitted with a tightly closed closure, a valve to adjust the pressure inside the container and a polytetrafluoroethylene tube to allow release of gas;

(b) a programmable microwave oven (e.g., with a magnetron frequency of 2450 MHz, with a selectable output from 0 to 1500 ± 70 W in 1 per cent increments);

(c) an atomic spectrometer, an inductively coupled plasma-atomic emission spectrometer, or an inductively coupled plasma-mass spectrometer.

Method

Carry out the test as described in the "Atomic Spectrometry: Emission and Absorption" (AAS) (Appendix 2.3), "Inductively Coupled Plasma-Atomic Emission Spectrometry" (ICP-AES) (Appendix 2.10), or "Inductively Coupled Plasma-Mass Spectrometry" (ICP-MS) (Appendix 2.10).

Deviations from the experimental parameters of the sample preparation procedure and the method description below are acceptable provided that the validation requirements are met and the system suitability test is fulfilled on the day of the analysis.

SAMPLE PREPARATION

(**Note** Clean all the glassware and laboratory equipment with a 1 per cent w/v solution of *nitric acid* before use. All liquid samples should be weighed.)

Use either of the following methods.

Method I Wet Digestion

FOR ARSENIC, CADMIUM AND LEAD

Test solution Transfer about 250 mg of the herbal drug, coarsely powdered and accurately weighed, to a 100-mL glass beaker (in case of liquid samples, use 5 g). Add 2.5 mL of a 50 per cent v/v solution of *heavy metal-free nitric acid*, mix well, cover, and heat on a water-bath for 15 minutes. Allow to cool and add 5 mL of *heavy metal-free nitric acid*. Reheat on a water-bath for 60 minutes or until completely digested. Transfer the solution to a 50-mL volumetric flask and dilute to volume with water. Filter or centrifuge at 3421 × *g* (3000 rpm) for 10 minutes, if necessary. Prepare simultaneously the blank solution by carrying out the digestion in the same manner as for the test solution.

FOR MERCURY

Proceed as directed under the determination for arsenic, cadmium and lead except using a 100-mL volumetric flask in place of a 100-mL beaker and heat in a water-bath. The temperature of a water-bath is 60°.

Method II Closed Vessel Digestion or Microwave Digestion

TEST SOLUTION In a digestion flask, place the prescribed quantity of the substance to be examined (about 500 mg of the herbal drug, in *No. 1400 powder*). Add 4 mL of *heavy metal-free hydrochloric acid* and 6 mL of *heavy metal-free nitric acid*. Close the flask tightly.

Place the digestion flasks in the microwave oven. Carry out the digestion in 3 steps according to the following programme, used for 7 flasks each containing the test solution: 80 per cent power for 15 minutes, 100 per cent power for 5 minutes, 80 per cent power for 20 minutes.

At the end of the cycle, allow the flasks to cool in air or water. After cooling, open each digestion flask and introduce the clear, colourless solution obtained into a 50-mL volumetric flask. Rinse each digestion flask with two 15-mL portions, of *heavy metal-free dilute nitric acid*, collect the rinsings in the volumetric flask and dilute to 50.0 mL with *water*. Modifiers (e.g., in the case of AAS with electrothermal atomization, 1.0 mL of a 1 per cent w/v solution of *magnesium nitrate* and 1.0 mL of a 10 per cent w/v solution of *ammonium dihydrogen phosphate*) and stabilizing agents may be used, if necessary.

BLANK SOLUTION Mix 4 mL of *heavy metal-free hydrochloric acid* and 6 mL of *heavy metal-free nitric acid* in a digestion flask. Carry out the digestion in the same manner as for the test solution.

Determination of Arsenic, Cadmium and Lead Using AAS with Electrothermal Atomization

Carry out the test as described by the "Atomic Spectrometry: Emission and Absorption" (Appendix 2.3) and measure the content of arsenic, cadmium and lead by the "Method of Direct Calibration" (Method I, Appendix 2.3) or by the "Method of

Standard Additions" (Method II, Appendix 2.3), using standard solutions of each heavy metal and the instrumental parameters recommended in Table 1.

The absorbance value of the blank solution is subtracted from the value obtained with the test solution.

Table 1 Instrumental Parameters for AAS with Electrothermal Atomization

		As	Cd	Pb
Wavelength	nm	193.7	228.8	283.5
Slit width	nm	0.5	0.5	0.5
Lamp current	mA	10	6	5
Ignition temperature	°C	1400	800	800
Atomization temperature	°C	2600	1800	2200
Gas flow rate	L/min	3	3	3

Determination of Arsenic, Cadmium and Lead Using AAS with Cold-Vapour or Hydride Atomization

Carry out the test as described by the "Atomic Spectrometry: Emission and Absorption" (Appendix 2.3) and measure the content of arsenic and mercury by the "Method of Direct Calibration" (Method I, Appendix 2.3) or by the "Method of Standard Additions" (Method II, Appendix 2.3), using standard solutions of arsenic or mercury and an automated continuous-flow hydride vapour generation system.

The absorbance value of the blank solution is subtracted from the value obtained with the test solution.

ARSENIC

Sample solution To 19.0 mL of the test solution or of the blank solution as prescribed above, add 1 mL of a 20 per cent w/v solution of *potassium iodide*. Allow the test solution to stand at room temperature for about 50 minutes or at 70° for about 4 minutes.

Acid reagent Use *Heavy metal-free hydrochloric acid*.

Reducing reagent Prepare a 0.6 per cent w/v solution of *sodium tetrahydroborate* in a 0.5 per cent w/v solution of *sodium hydroxide*.

The recommended instrumental parameters in Table 2 may be used.

MERCURY

Sample solution Use *Test solution* or *Blank solution*, as prescribed above.

Acid reagent Prepare a 51.5 per cent w/v solution of *heavy metal-free hydrochloric acid*.

Reducing reagent Prepare a 1.0 per cent w/v solution of *stannous chloride* in *heavy metal-free dilute hydrochloric acid*.

The recommended instrumental parameters in Table 2 may be used.

Table 2 Instrumental Parameters for AAS with Cold-Vapour or Hydride Atomization

		As	Hg
Wavelength	nm	193.7	253.7
Slit width	nm	0.2	0.5
Lamp current	mA	10	4
Acid reagent flow rate	mL/min	1.0	1.0
Reducing reagent flow rate	mL/min	1.0	1.0
Sample solution flow rate	mL/min	7.0	7.0
Absorption cell		Quartz (heated)	Quartz (unheated)
Nitrogen flow rate	L/min	0.1	0.1

Determination of Arsenic, Cadmium, Mercury, and Lead Using ICP-AES

Carry out the test as described by the "ICP-AES" (Appendix 2.10) and measure the content of arsenic, cadmium, mercury, and lead by the "Method of Direct Calibration" (Method I, Appendix 2.3), using standard solutions of each heavy metal or a mixture of all measured metals, and the instrumental parameters recommended in Table 3.

The emission value of the blank solution is subtracted from the value obtained with the test solution.

Table 3 Instrumental Parameters for ICP-AES

		As	Cd	Hg	Pb
Wavelength	nm	193.696/ 197.197/ 189.042	214.438/ 226.502/ 228.802	184.950/ 253.652/ 435.835	220.351/ 283.306/ 168.215
Ar, Monitorline	nm	430.010	430.010	430.010	430.010
Plasma energy	W	1200	1200	1200	1200
Peak algorithm with background correction		yes	yes	yes	yes

Determination of Arsenic, Cadmium, Mercury, and Lead Using ICP-MS

Carry out the test as described by the "ICP-MS" (Appendix 2.10) and measure the content of arsenic, cadmium, mercury, and lead by the "Method of Direct Calibration" (Method I, Appendix 2.3) using standard solutions of each heavy metal and the analytical isotopes and additional masses recommended in Table 4.

The signal intensity of the blank solution is subtracted from the value obtained with the test solution.

System Suitability

A system suitability test must be carried out on the day of the analysis to ensure that the sample preparation and measurement system are appropriate.

Acceptance criterion for preparation of sample solution A clear solution is obtained.

Acceptance criterion for measurement system The measured concentration of a standard solution of the metal at a concentration within the range of the used calibration curve does not differ from the actual concentration by more than 20 per cent.

Table 4 Recommended Analytical Isotopes and Additional Masses for ICP-MS

Isotope	Element of Interest
75	Arsenic
106, 108, 111, 114	Cadmium
202	Mercury
206, 207, 208	Lead

Validation Requirements

The analytical procedures used must be validated in accordance with the relevant general methods as described in the "Atomic Spectrometry: Emission and Absorption" (Appendix 2.3), "ICP-AES" (Appendix 2.10) and "ICP-MS" (Appendix 2.10). Additionally, the following criteria must be fulfilled.

Specificity

Specificity is the ability to ensure that the analytical procedures for sample preparation and measurement allow a reliable determination of the metal(s) in the presence of components (e.g., carrier gas, impurities, matrix) that may be expected to be present.

Acceptance criteria The procedure must be able to assess unequivocally each heavy metal to be determined with this procedure in the presence of components that may be expected to be present, including other heavy metals, matrix components and other sources of interference; specificity is demonstrated by complying with the accuracy requirement for the metal(s) to be determined.

Range

The calibration range of each metal is within the linear range of the method; test solutions containing residues at a level outside the calibration range may be diluted to concentrations within the calibration range.

Acceptance criterion Range is demonstrated by complying with the recovery requirement.

Accuracy

Verify the accuracy using a certified reference material or by performing a test for recovery.

RECOVERY Recovery may be determined on a sample of the substance to be examined, spiked with a known quantity of a reference standard of the metal (three concentration levels in the range of 50 to 150 per cent of the intended specification limit, even if the original concentration of the reference standard is at the specified value), in triplicate.

Acceptance criterion Spike recovery is within 70 per cent and 150 per cent for the mean of three replicates at each concentration.

Repeatability

TEST SAMPLES Either six independent samples of the substance to be examined spiked with a suitable reference standard at the specified concentration level, or three concentration levels prepared in triplicate.

Acceptance criterion The relative standard deviation is in both cases not greater than the value indicated in Table 5.

Intermediate Precision

The effect of random events (intra-laboratory variations) on the analytical precision of the method must be established. Acceptable experiments for establishing intermediate precision include performing the repeatability analysis on different days, or with different instrumentation, or with different analysts. Only one of the three experiments is required to demonstrate intermediate precision.

Acceptance criterion The relative standard deviation is not greater than the value indicated in Table 5.

Limit of Quantification

Determine the lowest concentration meeting the acceptance criterion. Use the results from the accuracy study.

Acceptance criterion The limit of quantification is below the specification limit.

Table 5

Concentration Range of the Metal (mg/kg)	Repeatability (RSD) (Per Cent)	Intermediate Precision (RSD) (Per Cent)
0.01 - 1	20	32
> 1	10	16

Limit of Detection (only Applicable to Limit Tests)

Determine the lowest concentration giving a signal clearly distinct from that obtained with a blank solution.

Acceptance criterion The limit of detection is not more than 0.1 times the concentration of the specification limit.

APPENDIX 7 CRUDE DRUG

7.22H PESTICIDE RESIDUES

Herbal drugs are liable to contain pesticide residues which accumulate from agricultural practices, such as spraying, treatment of soils during cultivation and administration of fumigants during storage. Many herbal drug preparations of plant origin are taken over long periods of time; limits for residues should therefore be established.

Definition For the purposes of the Pharmacopoeia, a pesticide is any substance or mixture of substances intended for preventing, destroying or controlling any pest, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of herbal drugs. The item includes substances intended for use as growth-regulators, defoliants or desiccants and any substance applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport.

Limits Unless otherwise indicated in the monograph, the herbal drug being examined at least complies with the limits indicated in Table 1. (The calculation is based on 60 kg of body weight.) Limits for pesticides that are not listed in Table 1 are calculated using the following formula:

$$\frac{ADI \times M}{MDD \times 100}$$

where *ADI* = acceptable daily intake, as published by FAO-WHO in milligrams per kilogram of body weight,

M = body weight in kilograms (60 kg), and

MDD = daily dose of the plant material in kilograms.

If the plant herbal drug is intended for the preparation of extracts, tinctures or other pharmaceutical forms the preparation method of which modifies the content of pesticides in the finished product, the limits are calculated using the following formula:

$$\frac{ADI \times M \times E}{MDD \times 100}$$

where *E* = extraction factor of the method of preparation, determined experimentally.

Higher limits can also be authorized, in exceptional cases, especially when a plant requires a particular cultivation method or has a metabolism or a structure that gives rise to a higher than normal content of pesticides.

The competent authority may grant total or partial exemption of the test when the complete history (nature and quantity of the pesticides used, date of each treatment during cultivation and after the harvest) of the treatment of the batch is known and can be checked precisely.

Sampling

METHOD For containers up to 1 kg, take one sample from the total content, thoroughly mixed, sufficient for the tests. For containers between 1 and 5 kg, take three samples, equal in volume, from the upper, middle and lower parts of the container, each being sufficient to carry out the tests. Thoroughly mix the samples and take from the mixture an amount sufficient to carry out the tests. For containers of more than 5 kg, take three samples, each of at least 250 g from the upper, middle and lower parts of the container. Thoroughly mix the samples and take from the mixture an amount sufficient to carry out the tests.

SIZE OF SAMPLING If the number (n) of containers is three or fewer, take samples from each container as indicated above under Method. If the number of containers is more than three, take samples as indicated under Method, from $\sqrt{n} + 1$ containers, rounding up to the nearest unit if necessary.

The samples are to be analyzed immediately to avoid possible degradation of the residues. If this is not possible, the fresh samples should be stored in the refrigerator, but typically no longer than 5 days. Dried samples may be stored at room temperature, but if storage time is expected to exceed two weeks, they should be sub-sampled and stored in the freezer (–10°).

Table 1

Substance	Limit (mg/kg)
Acephate	0.1
Alachlor	0.05
Aldrin and dieldrin (sum of)	0.05
Azinphos-ethyl	0.1
Azinphos-methyl	1.0
Bromide, inorganic (calculated as bromide ion)	125.0
Bromophos-ethyl	0.05
Bromophos-methyl	0.05
Bromopropylate	3.0
Chlordane (sum of *cis*-, *trans*-, and oxychlordane)	0.05
Chlorfenvinphos	0.5
Chlorpyriphos-ethyl	0.2
Chlorpyriphos-methyl	0.1
Chlorthal-dimethyl	0.01
Cyfluthrin and isomers (sum of)	0.1
λ-Cyhalothrin	1.0
Cypermethrin and isomers (sum of)	1.0
DDT (sum of *o,p′*-DDE, *p,p′*-DDE, *o,p′*-DDT, *p,p′*-DDT, *o,p′*-TDE, and *p,p′*-TDE)	1.0
Deltamethrin	0.5
Diazinon	0.5
Dichlofluanid	0.1
Dichlorvos	1.0
Dicofol	0.5
Dimethoate and omethoate (sum of)	0.1
Dithiocarbamates (expressed as CS_2)	2.0
Endosulfan (sum of isomers and endosulfan sulfate)	3.0
Endrin	0.05
Ethion	2.0
Etrimphos	0.05
Fenchlorophos (sum of fenchlorophos and fenchlorophos-oxon)	0.1
Fenitrothion	0.5
Fenpropathrin	0.03
Fensulfothion (sum of fensulfothion, fensulfothion-oxon, fensulfothion-oxon sulfone, and fensulfothion sulfone)	0.05
Fenthion (sum of fenthion, fenthion-oxon, fenthion-oxon sulfone, fenthion-oxon sulfoxide, fenthion sulfone, and fenthion-sulfoxide)	0.05

Table 1 (Continued)

Substance	Limit (mg/kg)
Fenvalerate	1.5
Flucythrinate	0.05
τ-Fluvalinate	0.05
Fonophos	0.05
Heptachlor (sum of heptachlor, *cis*-heptachlorepoxide, and *trans*-heptachlorepoxide)	0.05
Hexachlorbenzene	0.1
Hexachlorocyclohexane (sum of isomers α-, β-, δ-, and ε-)	0.3
Lindan (γ-hexachlorocyclohexane)	0.6
Malathion and malaoxon (sum of)	1.0
Mecarbam	0.05
Methacriphos	0.05
Methamidophos	0.05
Methidathion	0.2
Methoxychlor	0.05
Mirex	0.01
Monocrotophos	0.1
Parathion-ethyl and paraoxon-ethyl (sum of)	0.5
Parathion-methyl and paraoxon-methyl (sum of)	0.2
Pendimethalin	0.1
Pentachloranisole	0.01
Permethrin and isomers (sum of)	1.0
Phosalone	0.1
Phosmet	0.05
Piperonyl butoxide	3.0
Pirimiphos-ethyl	0.05
Pirimiphos-methyl (sum of pirimiphos-methyl and *N*-desethyl-pirimiphos-methyl)	4.0
Procymidone	0.1
Profenophos	0.1
Prothiophos	0.05
Pyrethrum (sum of cinerin I, cinerin II, jasmolin I, jasmolin II, pyrethrin I, and pyrethrin II)	3.0
Quinalphos	0.05
Quintozene (sum of quintozene, pentachloraniline, and methyl pentachlorphenyl sulfide)	1.0
S-421	0.02

Table 1 (Continued)

Substance	Limit (mg/kg)
Tecnazene	0.05
Tetradifon	0.3
Vinclozolin	0.4

Qualitative and quantitative analysis of pesticide residues

Use either of the following analytical procedures:

— Analytical procedures issued by National Authorities or International Standard Organizations, or those published in internationally recognized manuals or publications;

— Analytical procedures with appropriate and reliable performance characteristics validated by a collaborative study or single-laboratory study according to the internationally recognized protocols. The validated analytical procedures shall be assessed in compliance with the latest version of ISO/IEC 17025.

The analytical procedures stated above shall be capable of providing the reliable results of the specified maximum pesticide residue level.

In addition, they shall satisfy the following criteria:

— The chosen method, especially with respect to its purification steps, is suitable for the combination of pesticide residue and substance to be examined, and is not susceptible to interference from co-extractives;

— The limit of quantification for each pesticide matrix combination to be analyzed is not more than the corresponding tolerance limit; the method is shown to recover between 70 and 120 per cent of each pesticide with a repeatability not less than 20 per cent relative standard deviation. (**Note** The lower recoveries may be acceptable in certain cases.) The concentration of the test and standard solutions and the setting of the apparatus are such that a linear response is obtained from the analytical detector.

APPENDIX 10 MICROBIOLOGICAL TEST

10.2 MICROBIAL LIMIT TESTS

Introduction

The hazard of microbiological contamination in non-sterile pharmaceuticals has been well realized, especially in those products of vegetable, animal and mineral origins and in those which lack good manufacturing practices (GMP). Microbiological attributes of non-sterile pharmaceutical products are described in Appendix 10.4.

This appendix, therefore, comprises two parts of tests. Part I allows quantitative enumeration of mesophilic bacteria and fungi that may grow under aerobic condition and Part II allows determination of the absence or limited occurrence of specified micro-organisms that may be detected under the conditions described.

The tests are designed primarily to determine whether a substance or preparation complies with an established specification for microbiological quality. When used for such purposes, follow the instructions given below, including the number of samples to be taken, and interpret the results as stated below.

The methods are not applicable to products containing viable micro-organisms as active ingredients.

Alternative microbiological procedures, including automated methods, may be used, provided that their equivalence to the Pharmacopoeial method has been demonstrated.

Acceptance criteria for microbiological quality of non-sterile pharmaceuticals are given in the table under "Limits for Microbial Contamination" (Appendix 10.5).

In the following, the term "micro-organisms" is covering bacteria and fungi only; the term "pharmaceuticals" means pharmaceutical products of any kind, from raw materials to the finished forms; the term "growth" is used to designate the presence and presumed proliferation of micro-organisms.

Part I Microbial Enumeration Tests

PROCEDURE

In preparing for and in applying the tests, precautions are taken so as to avoid the accidental microbial contamination of the product to be examined, as well as the inadvertent suppression of the growth of any micro-organisms that are to be revealed in the test.

If the product to be examined has antimicrobial activity, this is insofar as possible removed or neutralized. If inactivators are used for this purpose, their efficacy and their absence of toxicity for micro-organisms must be demonstrated.

If surface-active substances are used for sample preparation, their absence of toxicity for micro-organisms and their compatibility with inactivators used must be demonstrated.

ENUMERATION METHODS

Use the membrane filtration method or the plate-count method, as prescribed. The most probable number (MPN) method is generally the least accurate method for microbial counts; however, for certain product groups with a very low bioburden, it may be the most appropriate method. The choice of method is based on factors such as the nature of the product and the required limit of micro-organisms. The chosen method must allow testing of a sufficient sample size to judge compliance with the specification. The suitability of the method chosen must be established.

GROWTH PROMOTION TEST, SUITABILITY OF THE COUNTING METHOD AND NEGATIVE CONTROLS

The ability of the test to detect micro-organisms in the presence of product to be tested must be established. Suitability must be confirmed if a change in testing performance, or the product, which may affect the outcome of the test is introduced.

Preparation of test strains

Use standardized stable suspensions of test strains or prepare them as stated below. Seed lot culture maintenance techniques (seed-lot systems) are used so that the viable micro-organisms used for inoculation are not more than five passages removed from the original master seed-lot. Grow each of the bacterial and fungal test strains separately as described in Table 1.

Use Buffered sodium chloride-peptone solution pH 7.0 or Phosphate buffer pH 7.2 to make test suspensions; to suspend *Aspergillus niger* spores, 0.05 per cent of *polysorbate 80* may be added to the buffer. Use the suspensions within 2 hours or within 24 hours if stored at 2° to 8°. As an alternative to preparing and then diluting a fresh suspension of vegetative cells of *A. niger* or *Bacillus subtilis*, a stable spore suspension is prepared and then an appropriate volume of the spore suspension is used for test inoculation. The stable spore suspension may be maintained at 2° to 8° for a validated period of time.

Negative control

To verify testing conditions, a negative control is performed using the chosen diluent in place of the test preparation. There must be no growth of micro-organisms. A negative control is also performed when testing the products as described in Testing of Products. A failed negative control requires an investigation.

Table 1 Preparation and Use of Test Micro-organisms

Micro-organism*	Preparation of Test Strain	Growth Promotion		Suitability of Counting Method in the Presence of the Product	
		Total Aerobic Microbial Count (TAMC)	Total Yeasts and Moulds Count (TYMC)	Total Aerobic Microbial Count (TAMC)	Total Yeasts and Moulds Count (TYMC)
Staphylococcus aureus such as: ATCC 6538 DMST 8013 NCIMB 9518 C.I.P. 4.83 NBRC 13276	Soybean-caseins digest agar or Soybean-casein digest broth 30° to 35° 18 to 24 hours	Soybean-casein digest agar or Soybean-casein digest broth ≤100 CFU 30° to 35° ≤3 days		Soybean-casein digest agar/MPN Soybean-casein digest broth ≤100 CFU 30° to 35° ≤3 days	
Pseudomonas aeruginosa such as: ATCC 9027 DMST 15501 NCIMB 8626 C.I.P. 82.118 NBRC 13275	Soybean-casein digest agar or Soybean-casein digest broth 30° to 35° 18 to 24 hours	Soybean-casein digest agar or Soybean-casein digest broth ≤100 CFU 30° to 35° ≤3 days		Soybean-casein digest agar/MPN Soybean-casein digest broth ≤100 CFU 30° to 35° ≤3 days	
Bacillus subtilis such as: ATCC 6633 DMST 15896 NCIMB 8054 C.I.P. 52.62 NBRC 3134	Soybean-casein digest agar or Soybean-casein digest broth 30° to 35° 18 to 24 hours	Soybean-casein digest agar or Soybean-casein digest broth ≤100 CFU 30° to 35° ≤3 days		Soybean-casein digest agar /MPN Soybean-casein digest broth ≤100 CFU 30° to 35° ≤3 days	

Table 1 (Continued)

Micro-organism*	Preparation of Test Strain	Growth Promotion		Suitability of Counting Method in the Presence of the Product	
		Total Aerobic Microbial Count (TAMC)	Total Yeasts and Moulds Count (TYMC)	Total Aerobic Microbial Count (TAMC)	Total Yeasts and Moulds Count (TYMC)
Candida albicans such as: ATCC 10231 DMST 5815 NCPF 3179 I.P. 48.72 NBRC 1594	Sabouraud dextrose agar or Sabouraud dextrose broth 20° to 25° 2 to 3 days	Soybean-casein digest agar ≤100 CFU 30° to 35° ≤5 days	Sabouraud dextrose agar ≤100 CFU 20° to 25° ≤5 days	Soybean-casein digest agar ≤100 CFU 30° to 35° ≤5 days MPN: not applicable	Sabouraud dextrose agar ≤100 CFU 20° to 25° ≤5 days
Aspergillus niger such as: ATCC 16404 DMST 15538 IMI 149007 I.P. 1431.83 NBRC 9455	Sabouraud dextrose agar or Potato dextrose agar 20° to 25° 5 to 7 days, or until good sporulation is achieved	Soybean-casein digest agar ≤100 CFU 30° to 35° ≤5 days	Sabouraud dextrose agar ≤100 CFU 20° to 25° ≤5 days	Soybean-casein digest agar ≤100 CFU 30° to 35° ≤5 days MPN: not applicable	Sabouraud dextrose agar ≤100 CFU 20° to 25° ≤5 days

*ATCC = American Type Culture Collection, USA; DMST = Department of Medical Sciences, Thailand; NCIMB = National Collection of Industrial and Marine Bacteria Ltd., Great Britain; C.I.P. = Collection de Bactéries de l' Institut Pasteur, France; NBRC = Biological Resource Center, Japan; NCPF = National Collection of Pathogenic Fungi, London School of Hygiene and Tropical Medicine, Great Britain; I.P. = Collection Nationale de Culture de Micro-organismes (C.N.C.M.) Institut Pasteur, France; IMI = International Mycological Institute, Great Britain

Growth promotion of the media

Test each batch of ready-prepared medium and each batch of medium, prepared either from dehydrated medium or from the ingredients described.

Inoculate portions/plates of Soybean-casein digest broth and Soybean-casein digest agar with a small number (not more than 100 CFU) of the micro-organisms indicated in Table 1, using a separate portion/plate of medium for each. Inoculate plates of Sabouraud dextrose agar with a small number (not more than 100 CFU) of the micro-organisms indicated in Table 1, using a separate plate of medium for each. Incubate in the conditions described in Table 1.

For solid media, growth obtained must not differ by a factor greater than 2 from the calculated value for a standardized inoculum. For a freshly prepared inoculum, growth of the micro-organisms comparable to that previously obtained with a previously tested and approved batch of medium occurs. Liquid media are suitable if clearly visible growth of the micro-organisms comparable to that previously obtained with a previously tested and approved batch of medium occurs.

Suitability of the counting method in the presence of product

PREPARATION OF THE SAMPLE The method for sample preparation depends upon the physical characteristics of the product to be tested. If none of the procedures described below can be demonstrated to be satisfactory, an alternative procedure must be developed.

Water-soluble products Dissolve or dilute (usually a 1 in 10 dilution is prepared) the product to be examined in Buffered sodium chloride-peptone solution pH 7.0, Phosphate buffer solution pH 7.2 or Soybean-casein digest broth. If necessary, adjust to pH 6 to 8. Further dilutions, where necessary, are prepared with the same diluent.

Non-fatty products insoluble in water Suspend the product to be examined (usually a 1 in 10 dilution is prepared) in Buffered sodium chloride-peptone solution pH 7.0, Phosphate buffer solution pH 7.2 or Soybean-casein digest broth. A surface-active agent such as 0.1 per cent w/v of *polysorbate 80* may be added to assist the suspension of poorly wettable substances. If necessary, adjust to pH 6 to 8. Further dilutions, where necessary, are prepared with the same diluent.

Fatty products Dissolve in *isopropyl myristate*, sterilized by filtration or mix the product to be examined with the minimum necessary quantity of sterile polysorbate 80 or another non-inhibitory sterile surface-active agent, heated if necessary to not more than 40°, or in exceptional cases to not more than 45°. Mix carefully and if necessary maintain the temperature in a water-bath. Add sufficient of the pre-warmed chosen diluent to make a 1 in 10 dilution of the original sample. Mix carefully whilst maintaining the temperature for the shortest time necessary for the formation of an emulsion. Further serial ten-fold dilutions may be prepared using the chosen diluent containing a suitable concentration of sterile *polysorbate 80* or another non-inhibitory sterile surface-active agent.

Fluids or solids in aerosol form Chill the container(s) for approximately 1 hour, cut open the container(s), and allow to reach room temperature, permitting the propellant to escape, or warming to drive off the propellant if feasible. Aseptically transfer the product into a membrane filter apparatus or a sterile container for further sampling. Use either the total contents or a defined number of metered doses from each of the containers tested.

Transdermal patches Remove the protective cover sheets ("release liners") of the transdermal patches and place them, adhesive side upwards, on sterile glass or plastic trays. Cover the adhesive surface with a sterile porous material, for example sterile gauze, to prevent the patches from sticking together, and transfer the patches to a suitable volume of the chosen diluent containing inactivators such as polysorbate 80 and/or lecithin. Shake the preparation vigorously for at least 30 minutes.

INOCULATION AND DILUTION Add to the sample prepared as described in Preparation of the Sample and to a control (with no test material included) a sufficient volume of the microbial suspension to obtain an inoculum of not more than 100 CFU. The volume of the suspension of the inoculum should not exceed 1 per cent of the volume of diluted product.

To demonstrate acceptable microbial recovery from the product, the lowest possible dilution factor of the prepared sample must be used for the test. Where this is not possible due to antimicrobial activity or poor solubility, further appropriate protocols must be developed. If inhibition of growth by the sample cannot otherwise be avoided, the aliquot of the microbial suspension may be added after neutralization, dilution or filtration.

NEUTRALIZATION/REMOVAL OF ANTIMICROBIAL ACTIVITY The number of micro-organisms recovered from the prepared sample diluted as described in Inoculation and Dilution and incubated following the procedure described in Recovery of Micro-organism in the Presence of Product, is compared to the number of micro-organisms recovered from the control preparation.

If growth is inhibited (reduction by a factor greater than 2), then modify the procedure for the particular enumeration test to ensure the validity of the results. Modification of the procedure may include, for example, (1) an increase in the volume of the diluent or culture medium, (2) incorporation of specific or general neutralizing agents into the diluent such as Casein digest-soy lecithin polysorbate 20 broth, (3) membrane filtration, or (4) a combination of the above measures.

Neutralizing agents Neutralizing agents may be used to neutralize the activity of antimicrobial agents (Table 2). They may be added to the chosen diluent or the medium preferably before sterilization. If used, their efficacy and their absence of toxicity for micro-organisms must be demonstrated by carrying out a blank with the neutralizer and without the product.

Table 2 Common Neutralizing Agents for Interfering Substances

Interfering Substance	Potential Neutralizing Method
Glutaraldehyde, mercurials	Sodium hydrogensulfite (sodium bisulfite)
Phenolics, ethanol, aldehydes, sorbate	Dilution
Aldehydes	Glycine
Quaternary Ammonium Compound (QACs), parahydroxy benzoates (parabens), bisbiguanides	Lecithin
QACs, iodine, parabens	Polysorbate
Mercurials	Thioglycolate
Mercurials, halogens, aldehydes	Thiosulfate
EDTA (edetate)	Mg^{2+} or Ca^{2+} ions

If no suitable neutralizing method can be found, it can be assumed that the failure to isolate the inoculated organism is attributable to the microbicidal activity of the product. This information serves to indicate that the product is not likely to be contaminated with the given species of the micro-organism. However, it is possible that the product only inhibits some of the micro-organisms specified herein, but does not inhibit others not included amongst the test strains or for which the latter are not representative. Then, perform the test with the highest dilution factor compatible with microbial growth and the specific acceptance criterion.

RECOVERY OF MICRO-ORGANISMS IN THE PRESENCE OF PRODUCT For each of the micro-organisms listed, separate tests are performed. Only micro-organisms of the added test strain are counted.

Membrane filtration Use membrane filters having a nominal pore size not greater than 0.45 µm. The type of filter material is chosen such that the bacteria-retaining efficiency is not affected by the components of the sample to be investigated. For each of the micro-organisms listed, one membrane filter is used.

Transfer a suitable amount of the sample prepared as described under Preparation of the Sample, under Inoculation and Dilution, and under Neutralization/Removal of Antimicrobial Activity (preferably representing 1 g of the sample, or less if large numbers of CFU are expected) to the membrane filter, filter immediately and rinse the membrane filter with an appropriate volume of diluent. For the determination of total aerobic microbial count (TAMC), transfer the membrane filter to the surface of Soybean-casein digest agar. For the determination of total combined yeasts and moulds count (TYMC), transfer the membrane to the surface of Sabouraud dextrose agar. Incubate the plates as indicated in Table 1. Perform the counting.

Plate-count methods Perform plate-count methods at least in duplicate for each medium and use the mean count of the result.

Pour-plate method For Petri dishes 9 cm in diameter, add to the dish 1 mL of the sample prepared as described under Preparation of the Sample, under Inoculation and Dilution, and under Neutralization/Removal of Antimicrobial Activity and 15 to 20 mL of Soybean-casein digest agar or Sabouraud dextrose agar, both media being at not more than 45°. If larger Petri dishes are used, the amount of agar medium is increased accordingly. For each of the micro-organisms listed in Table 1, at least two Petri dishes are used. Incubate the plates as indicated in Table 1. Take the arithmetic mean of the counts per medium and calculate the number of CFU in the original inoculum.

Surface-spread method For Petri dishes 9 cm in diameter, add 15 to 20 mL of Soybean-casein digest agar or Sabouraud dextrose agar at about 45° to each Petri dish and allow to solidify. If larger Petri dishes are used, the volume of the agar is increased accordingly. Dry the plates, for example in a laminar-air-flow cabinet or an incubator. For each of the micro-organisms listed in Table 1, at least two Petri dishes are used. Spread a measured volume of not less than 0.1 mL of the sample prepared as described under Preparation of the Sample, under Inoculation and Dilution, and under Neutralization/Removal of Antimicrobial Activity over the surface of the medium. Incubate and count as prescribed under Pour-plate Method.

Most probable number (MPN) method The precision and accuracy of the MPN method is less than that of the membrane filtration method or the plate-count method. Unreliable results are obtained particularly for the enumeration of moulds. For these reason the MPN method is reserved for the enumeration of TAMC in situations where no other method is available. If the use of the method is justified, proceed as follows.

Prepare a series of at least three serial ten-fold dilutions of the product as described under Preparation of the Sample, under Inoculation and Dilution, and under Neutralization/Removal of Antimicrobial Activity. From each level of dilution, three aliquots of 1 g or 1 mL are used to inoculate three tubes with 9 to 10 mL of Soybean-casein digest broth. If necessary, a surface-active agent such as polysorbate 80 or an inactivator of antimicrobial agents may be added to the medium. Thus, if three levels of dilution are prepared, nine tubes are inoculated.

Incubate all tubes at 30° to 35° for not more than 3 days. If reading of the results is difficult or uncertain owing to the nature of the product to be examined, subculture in the same broth, or in Soybean-casein digest agar, for 1 to 2 days at the same temperature and use these results. Determine the most probable number of micro-organisms per g or per mL of the product to be examined from Table 3.

Results and interpretation

When verifying the suitability of the membrane filtration method or the plate-count method, a mean count of any of the test organisms not differing by a factor greater than 2 from the value of the control defined in Inoculation and Dilution in the absence of the product must be obtained. When verifying the suitability of the MPN method the calculated value from the inoculum must be within 95 per cent confidence limits of the results obtained with the control.

If the above criteria cannot be met for one or more of the organisms tested with any of the described methods, the method and test conditions that come closest to the criteria are used to test the product.

TESTING OF PRODUCTS

Amount used for the test

Unless otherwise prescribed, use 10 g or 10 mL of the product to be examined taken with the precautions referred to above. For fluids or solids in aerosol form, sample 10 containers. For transdermal patches, sample 10 patches.

The amount to be tested may be reduced for active substances that will be formulated in the following conditions: the amount per dosage unit (e.g., tablet, capsule, injection) is less than or equal to 1 mg or the amount per g or per mL (for preparations not presented in dosage units) is less than 1 mg. In these cases, the amount to be tested is not less than the amount present in 10 dosage units or 10 g or 10 mL of the product.

For materials used as active substances where sample quantity is limited or batch size is extremely small (i.e., less than 1000 mL or 1000 g), the amount tested shall be 1 per cent of the batch unless a lesser amount is prescribed or justified and authorized.

For products where the total number of entities in a batch is less than 200 (e.g., samples used in clinical trials), the sample size may be reduced to 2 units, or 1 unit if the size is less than 100.

Select the sample(s) at random from the bulk material or from the available containers of the preparation. To obtain the required quantity, mix the contents of a sufficient number of containers to provide the sample.

Examination of the product

MEMBRANE FILTRATION Use a filtration apparatus designed to allow the transfer of the filter to the medium. Prepare the sample using a method that has been shown suitable as described in Growth Promotion Test, Suitability of the Counting Method and Negative Controls. Transfer the appropriate amount to each of two membrane filters and filter immediately. Wash each filter following the procedure shown to be suitable.

For the determination of TAMC, transfer one of the membrane filters to the surface of Soybean-casein digest agar. For the determination of TYMC, transfer the other membrane to the surface of Sabouraud dextrose agar. Incubate the plate of Soybean-casein digest agar at 30° to 35° for 3 to 5 days and the plate of Sabouraud dextrose agar at 20° to 25° for 5 to 7 days. Calculate the number or CFU per g or per mL of product.

When examining transdermal patches, filter 10 per cent of the volume of the preparation described under Preparation of the Sample separately through each of two sterile filter membranes. Transfer one membrane to Soybean-casein digest agar for TAMC and the other membrane to Sabouraud dextrose agar for TYMC.

PLATE-COUNT METHODS

Pour-plate method Prepare the sample using a method that has been shown to be suitable as described in Growth Promotion Test, Suitability of the Counting Method and Negative Controls. Prepare for each medium at least two Petri dishes for each level of dilution. Incubate the plates of Soybean-casein digest agar at 30° to 35° for 3 to 5 days and the plates of Sabouraud dextrose agar at 20° to 25° for 5 to 7 days. Select the plates

Table 3 Most Probable Number (MPN) Values of Micro-organisms

Observed Combinations of Numbers of Tubes Showing Growth in Each Set			MPN per g or per mL of Product	95 Per Cent Confidence Limits
Number of Grams or Millilitres of Product per Tube				
0.1	0.01	0.001		
0	0	0	<3	0–9.4
0	0	1	3	0.1–9.5
0	1	0	3	0.1–10
0	1	1	6.1	1.2–17
0	2	0	6.2	1.2–17
0	3	0	9.4	3.5–35
1	0	0	3.6	0.2–17
1	0	1	7.2	1.2–17
1	0	2	11	4–35
1	1	0	7.4	1.3–20
1	1	1	11	4–35
1	2	0	11	4–35
1	2	1	15	5–38
1	3	0	16	5–38
2	0	0	9.2	1.5–35
2	0	1	14	4–35
2	0	2	20	5–38
2	1	0	15	4–38
2	1	1	20	5–38
2	1	2	27	9–94
2	2	0	21	5–40
2	2	1	28	9–94
2	2	2	35	9–94
2	3	0	29	9–94
2	3	1	36	9–94
3	0	0	23	5–94
3	0	1	38	9–104
3	0	2	64	16–181
3	1	0	43	9–181
3	1	1	75	17–199
3	1	2	120	30–360
3	1	3	160	30–380
3	2	0	93	18–360
3	2	1	150	30–380
3	2	2	210	30–400
3	2	3	290	90–990
3	3	0	240	40–990
3	3	1	460	90–1980
3	3	2	1100	200–4000
3	3	3	>1100	

corresponding to a given dilution and showing the highest number of colonies less than 250 for TAMC and 50 for TYMC. Take the arithmetic mean per culture medium of the counts and calculate the number of CFU per g or per mL of product.

Surface-spread method Prepare the sample using a method that has been shown to be suitable as described in Growth Promotion Test, Suitability of the Counting Method and Negative Controls. Prepare at least two Petri dishes for each medium and each level of dilution. For incubation and calculation of the number of CFU proceed as described for the pour-plate method.

MOST PROBABLE NUMBER METHOD

Prepare and dilute the sample using a method that has been shown to be suitable as described in Growth Promotion Test, Suitability of the Counting Method and Negative Controls. Incubate all tube at 30° to 35° for 3 to 5 days. Subculture if necessary, using the procedure shown to be suitable. Record for each level of dilution the number of tubes showing microbial growth. Determine the most probable number of micro-organisms per g or per mL of the product to be examined from Table 3.

Interpretation of the results

The total aerobic microbial count (TAMC) is considered to be equal to the number of CFU found using Soybean-casein digest agar; if colonies of fungi are detected on this medium, they are counted as part of the TAMC. The total combined yeasts and mould count (TYMC) is considered to be equal to the number of CFU found using Sabouraud dextrose agar; if colonies of bacteria are detected on this medium, they are counted as part of the TYMC. When the TYMC is expected to exceed the acceptance criterion due to the bacterial growth, Sabouraud dextrose agar with antibiotics may be used. If the count is carried out by the MPN method the calculated value is the TAMC.

The recommended solutions and media are described in Part II.

The limits prescribed in the Limits for Microbial Contamination (Appendix 10.5) are the maximum acceptable limits.

Part II Test for Specified Micro-organisms

PROCEDURE

The preparation of the samples is carried out as described in Part I.

If the product to be examined has antimicrobial activity, this is insofar as possible removed or neutralized as described in Part I.

If surface-active substances are used for sample preparation, their absence of toxicity for micro-organisms and their compatibility with inactivators used must be demonstrated as described in Part I.

GROWTH-PROMOTING AND INHIBITORY PROPERTIES OF THE MEDIA, SUITABILITY OF THE TEST AND NEGATIVE CONTROLS

The ability of the test to detect micro-organisms in the presence of the product to be tested must be established. Suitability must be confirmed if a change in testing performance, or the product, which may affect the outcome of the test is introduced.

Preparation of test strains

Use standardized stable suspensions of test strains or prepare them as stated below. Seed lot culture maintenance techniques (seed-lot systems) are used so that the viable micro-organisms used for inoculation are not more than five passages removed from the original master seed-lot.

AEROBIC MICRO-ORGANISMS Grow each of the bacterial test strains separately in Soybean-casein digest broth or on Soybean-casein digest agar at 30° to 35° for 18 to 24 hours. Grow the test strain for *Candida albicans* separately on Sabouraud dextrose agar or in Sabouraud dextrose broth at 20° to 25° for 2 to 3 days.

— *Staphylococcus aureus* such as ATCC 6538, DMST 8013, NCIMB 9518, C.I.P. 4.83 or NBRC 13276;

— *Pseudomonas aeruginosa* such as ATCC 9027, DMST 15501, NCIMB 8626, C.I.P. 82.118 or NBRC 13275;

— *Escherichia coli* such as ATCC 8739, DMST 15537, NCIMB 8545, C.I.P. 53.126 or NBRC 3972;

— *Salmonella enterica* subsp. *enterica* serovar Typhimurium, such as ATCC 14028, DMST 13311, or, as an alternative, *Salmonella enterica* subsp. *enterica* serovar Abony such as NCTC 6017, DMST 21863, C.I.P. 80.39, or NBRC 100797;

— *Candida albicans* such as ATCC 10231, DMST 5815, NCPF 3179, I.P. 48.72 or NBRC 1594.

Use Buffered sodium chloride-peptone solution pH 7.0 or Phosphate buffer pH 7.2 to make test suspensions. Use the suspensions within 2 hours or within 24 hours if stored at 2° to 8°.

CLOSTRIDIUM SPP. Use *Clostridium sporogenes* such as ATCC 11437 (DMST 15536, NCIMB 12343, C.I.P. 100651, NBRC 14293) or ATCC 19404 (DMST 15282, NCTC 532, C.I.P. 79.03). Grow the clostridial test strain under anaerobic conditions in Reinforced medium for clostridia at 30° to 35° for 24 to 48 hours.

As an alternative to preparing and then diluting down a fresh suspension of vegetative cells of *Cl. sporogenes*, a stable spore suspension is used for test inoculation. The stable spore suspension may be maintained at 2° to 8° for a validated period.

Negative control

To verify testing conditions, a negative control is performed using the chosen diluent in place of the test preparation. There must be no growth of micro-organisms. A negative control is also performed when testing the products as described in Testing of Products. A failed negative control requires an investigation.

Growth promotion and inhibitory properties of the media

Test each batch of ready-prepared medium and each batch of medium prepared either from dehydrated medium or from ingredients.

Verify suitable properties of relevant media as described in Table 4.

TEST FOR GROWTH PROMOTING PROPERTIES, LIQUID MEDIA: inoculate a portion of the appropriate medium with a small number (not more than 100 CFU) of the appropriate micro-organism. Incubate at the specified temperature for not more than the shortest period of time specified in the test. Clearly visible growth of the micro-organism comparable to that previously obtained with a previously tested and approved batch of medium occurs.

TEST FOR GROWTH PROMOTING PROPERTIES, SOLID MEDIA: perform the surface-spread method, inoculating each plate with a small number (not more than 100 CFU) of the appropriate micro-organism. Incubate at the specified temperature for not more than the shortest period of time specified in the test. Growth of the micro-organism comparable to that previously obtained with a previously tested and approved batch of medium occurs.

TEST FOR INHIBITORY PROPERTIES, LIQUID OR SOLID MEDIA: inoculate the appropriate medium with at least 100 CFU of the appropriate micro-organism. Incubate at the specified temperature for not less than the longest period of time specified in the test. No growth of the test micro-organism occurs.

TEST FOR INDICATIVE PROPERTIES: perform the surface-spread method, inoculating each plate with a small number (not more than 100 CFU) of the appropriate micro-organism. Incubate at the specified temperature for a period of time within the range specified in the test. Colonies are comparable in appearance and indication reactions to those previously obtained with a previously tested and approved batch of medium.

Suitability of the test method

For each product to be tested, perform the sample preparation as described in the following paragraph in Testing of Products. Add each test strain at the time of mixing, in the prescribed growth medium. Inoculate the test strains individually. Use a number of micro-organisms equivalent to not more than 100 CFU in the inoculated test preparation. Perform the test as described in the following paragraph in Testing of Products using the shortest incubation period prescribed.

The specified micro-organisms must be detected with the indication reactions as described in Testing of Products.

Any antimicrobial activity of the sample necessitates a modification of the test procedure described in Neutralization/Removal of Antimicrobial Activity under Part I.

If for a given product the antimicrobial activity with respect to a micro-organism for which testing is prescribed cannot be neutralized, then it is to be assumed that the inhibited micro-organism will not be present in the product.

TESTING OF PRODUCTS

Bile-tolerant gram-negative bacteria

SAMPLE PREPARATION AND PRE-INCUBATION Prepare a sample using a 1 in 10 dilution of not less than 1 g of the product to be examined as described in Part I, but using Soybean-casein digest broth as the chosen diluent, mix and incubate at 20° to 25° for a time sufficient to resuscitate the bacteria but not sufficient to encourage multiplication of the organisms (usually 2 hours but not more than 5 hours).

TEST FOR ABSENCE Use the volume corresponding to 1 g of the product, as prepared in Sample Preparation and Pre-incubation, to inoculate Enterobacteria enrichment broth-Mossel. Incubate at 30° to 35° for 24 to 48 hours. Subculture on plates of Violet red bile dextrose agar. Incubate at 30° to 35° for 18 to 24 hours. The product passes the test if there is no growth of colonies of Gram-negative bacteria on any plate.

Table 4 Growth Promoting, Inhibitory and Indicative Properties of Media

Test	Medium	Property	Test Strains
Test for bile-tolerant gram-negative bacteria	Enterobacteria enrichment broth-Mossel	Growth promoting Inhibitory	*E. coli* *P. aeruginosa* *S. aureus*
	Violet red bile dextrose agar	Growth promoting + indicative	*E. coli* *P. aeruginosa*
Test for *Escherichia coli*	MacConkey broth	Growth promoting Inhibitory	*E. coli* *S. aureus*
	MacConkey agar	Growth promoting + indicative	*E. coli*
Test for *Pseudomonas aeruginosa*	Cetrimide agar Pseudomonas for detection fluorescin Pseudomonas for detection pyocyanin	Growth promoting Inhibitory	*P. aeruginosa* *E. coli*
Test for *Salmonella*	Rappaport-Vassiliadis broth Tetrathionate bile brilliant green broth	Growth promoting Inhibitory	*Salmonella enterica* subsp. *enterica* serovar Typhimurium or *Salmonella enterica* subsp. *enterica* serovar Abony *S. aureus*
	Xylose-lysine-deoxycholate agar Brilliant green agar Bismuth sulfite agar	Growth promoting + indicative	*Salmonella enterica* subsp. *enterica* serovar Typhimurium or *Salmonella enterica* subsp. *enterica* serovar Abony
Test for *Staphylococcus aureus*	Mannitol salt agar Baird-Parker agar Vogel-Johnson agar	Growth promoting + indicative Inhibitory	*S. aureus* *E. coli*
Test for *Clostridium* spp.	Reinforced medium for Clostridia	Growth promoting	*Cl. sporogenes*
	Columbia agar Defibrinated sheep blood agar	Growth promoting	*Cl. sporogenes*
Test for *Candida albicans*	Sabouraud dextrose broth	Growth promoting	*C. albicans*
	Sabouraud dextrose agar	Growth promoting + indicative	*C. albicans*

QUANTITATIVE TEST

Selection and subculture Inoculate suitable quantities of Enterobacteria enrichment broth-Mossel with the preparation as described under Sample Preparation and Pre-incubation and/or dilutions of it containing respectively 0.1 g (or 0.1 mL), 0.01 g (or 0.01 mL), and g (or 0.001 mL) of the sample to be examined. Incubate at 30° to 35° for 24 to 48 hours. Subculture each of the cultures on a plate of Violet red bile dextrose agar to obtain selective isolation. Incubate at 30° to 35° for 18 to 24 hours.

Interpretation Growth of colonies constitutes a positive result. Note the smallest quantity of the product that gives a positive result and the largest quantity that gives a negative result. Determine from Table 5 the probable number of bacteria.

Table 5 Probable Number of Bacteria

Results for Each Quantity of Product			Probable Number of Bacteria per g or per mL of Product
0.1 g (or 0.1 mL)	0.01 g (or 0.01 mL)	0.001 g (or 0.001 mL)	
+	+	+	More than 10^3
+	+	–	Less than 10^3 and more than 10^2
+	–	–	Less than 10^2 and more than 10
–	–	–	Less than 10

***Salmonella* species**

SAMPLE PREPARATION AND PRE-INCUBATION

Prepare the product to be examined as described in Part I, and use the portion corresponding to not less than 10 g or 10 mL to inoculate a suitable amount (determined as described under Suitability of the Test Method) of Soybean-casein digest broth, mix and incubate at 30° to 35° for 18 to 24 hours.

SELECTION AND SUBCULTURE Separately transfer 0.1 mL and 1 mL of the enrichment culture to 10 mL of Rappaport-Vassiliadis broth and Tetrathionate bile brilliant green broth, respectively, mix and incubate at 30° to 35° for 18 to 24 hours. Subculture on plates of Xylose-lysine-deoxycholate agar, Brilliant green agar, and Bismuth sulfite agar. Cover and invert the dishes, and incubate at 30° to 35° for 18 to 48 hours.

INTERPRETATION Upon examination, if none of the colonies conforms to the description given in Table 6, the product meets the requirements of the test for absence of the genus *Salmonella*. If colonies of Gram-negative rods matching the description in Table 6 are found, proceed with further identification.

Table 6 Morphology Characteristics of *Salmonella* Species on Selective Agar Media

Selective Medium	Characteristic Colonial Morphology
Xylose-lysine-deoxycholate agar	Red, with or without black centres
Brilliant green agar	Small, transparent, colourless or pink to white opaque (frequently surrounded by pink to red zone)
Bismuth sulfite agar	Black or green

IDENTIFICATION Transfer representative suspect colonies individually, by means of an inoculating wire, to a butt-slant tube of Triple sugar-iron agar by first streaking the surface of the slant and then stabbing the wire well beneath the surface, and incubate. If the examination discloses no evidence of tubes having alkaline (red) slants and acid (yellow) butts (with or without concomitant blackening of the butt from hydrogen sulfide production), the product meets the requirements of the test for absence of the genus *Salmonella*. The presence of *Salmonella* may be confirmed by other suitable cultural or biochemical and serological tests, if necessary.

Escherichia coli

SAMPLE PREPARATION AND PRE-INCUBATION Prepare a sample using a 1 in 10 dilution of not less than 1 g of the product to be examined as described in Part I, and use 10 mL or the portion corresponding to 1 g or 1 mL to inoculate a suitable amount (determined as described under Suitability of the Test Method) of Soybean-casein digest broth, mix and incubate at 30° to 35° for 18 to 24 hours.

SELECTION AND SUBCULTURE Transfer 1 mL of the enrichment culture to 100 mL of MacConkey broth and incubate at 42° to 44° for 24 to 48 hours. Subculture on plates of MacConkey agar and incubate at 30° to 35° for 18 to 72 hours.

INTERPRETATION Upon examination, if none of the colonies conforms to the description given in Table 7, the product meets the requirements of the test for absence of *Escherichia coli*. If colonies matching the description in Table 7 are found, proceed with further identification.

Table 7 Morphology Characteristics of *Escherichia coli* on MacConkey Agar

Gram Stain	Characteristic Colonial Morphology
Negative rods (cocco-bacilli)	Brick-red; may have surrounding zone of precipitated bile

IDENTIFICATION Transfer the suspect colonies individually, making subculture the suspect colonies individually on plates of Levine eosin-methylene blue agar, and incubate at 30° to 35° for 18 to 24 hours.

Upon examination, if none of the colonies exhibits both a characteristic metallic sheen under reflected light and a blue-black appearance under transmitted light, the product meets the requirements of the test for absence of *Escherichia coli*. The presence of *Escherichia coli* may be confirmed by suitable cultural and, if necessary, biochemical tests. Further serological test may be performed.

Staphylococcus aureus* and *Pseudomonas aeruginosa

SAMPLE PREPARATION AND PRE-INCUBATION Prepare a sample using a 1 in 10 dilution of not less than 1 g of the product to be examined as described in Part I, and use 10 mL or the portion corresponding to 1 g or 1 mL to inoculate a suitable amount (determined as described under Suitability of the Test Method) of Soybean-casein digest broth and mix. When testing transdermal patches, filter the volume of sample corresponding to one patch of the preparation described under Preparation of the Sample in Part I through a sterile filter membrane and place in 100 mL of Soybean-casein digest broth. Incubate at 30° to 35° for 18 to 24 hours.

SELECTION AND SUBCULTURE If growth is present, use an inoculating loop to streak a portion of the culture medium on the surface of Mannitol-salt agar, or Baird-Parker agar, or Vogel-Johnson agar and of Cetrimide agar, and incubate at 30° to 35° for 18 to 72 hours.

INTERPRETATION Upon examination, if none of the plates contains colonies having the characteristics listed in Tables 8 and 9 for the media used, the product meets the requirements for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa.* If colonies matching the description in Table 8 and 9 are found, proceed with further identification.

IDENTIFICATION

Coagulase test (for *Staphylococcus aureus*) With the aid of an inoculating loop, transfer representative suspect colonies from the agar surfaces of the Mannitol-salt agar (or Baird-Parker agar or Vogel-Johnson agar) to individual tubes, each containing 0.5 mL of mammalian, preferably rabbit or horse, plasma with or without suitable additives. Incubate in a water-bath at 37°, examining the tubes at 3 hours and subsequently at suitable intervals

up to 24 hours. Test positive and negative controls simultaneously with the unknown products. If no coagulation in any degree is observed, the product meets the requirements of the test for absence of *Staphylococcus aureus*.

Oxidase and pigment tests (for *Pseudomonas aeruginosa*) With the aid of an inoculating loop, streak representative suspect colonies from the agar surfaces of Cetrimide agar on the agar surface of Pseudomonas agar for detection of fluorescin and Pseudomonas agar for detection of pyocyanin contained in Petri dishes. Cover and invert the inoculated media, and incubate at 30° to 35° for not less than 3 days. Examine the streaked surfaces under UV light. Examine the plates to determine whether colonies having the characteristics listed in Table 9 are present.

Table 8 Morphology Characteristics of *Staphylococcus aureus* on Selective Agar Media

Selective Medium	Characteristic Colonial Morphology	Gram Stain
Mannitol-salt agar	Yellow colonies surrounded by yellow zone	Positive cocci (in clusters)
Baird-Parker agar	Black, shiny colonies surrounded by clear zones of 2 to 5 mm	Positive cocci (in clusters)
Vogel-Johnson agar	Black surrounded by yellow zones	Positive cocci (in clusters)

Table 9 Morphology and Diagnostic Characteristics of *Pseudomonas aeruginosa* on Selective Agar Media

Selective Medium	Characteristic Colonial Morphology	Fluorescence in UV Light	Oxidase Test	Gram Stain
Cetrimide agar	Generally greenish	Greenish	Positive	Negative rods
Pseudomonas agar for detection of fluorescin	Generally colourless to yellowish	Yellowish	Positive	Negative rods
Pseudomonas agar for detection of pyocyanin	Generally greenish	Blue	Positive	Negative rods

Confirm any suspect colonial growth on one or more of the media as *Pseudomonas aeruginosa* by means of the oxidase test. Upon the colonial growth, place or transfer colonies to strips or discs of filter paper that previously has been impregnated with *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride. If there is no development of a pink colour, changing to purple, the product meets the requirements of the test for the absence of *Pseudomonas aeruginosa*. The presence of *Pseudomonas aeruginosa* may be confirmed by suitable cultural and, if necessary, biochemical tests.

Candida albicans

SAMPLE PREPARATION AND PRE-INCUBATION Prepare the product to be examined as described under Preparation of the Sample and use 10 mL or the portion corresponding to 1 g or 1 mL to inoculate a suitable amount (determined as described under Suitability of the Test Method) of Sabouraud dextrose broth and mix. Incubate at 30° to 35° for 3 to 5 days.

SELECTION AND SUBCULTURE Subculture on a plate of Sabouraud dextrose agar and incubate at 30° to 35° for 24 to 48 hours.

INTERPRETATION When growth of white colonies may indicate the presence of *Candida albicans* occurs, proceed with further identification.

IDENTIFICATION Transfer the suspect colonies individually, making subculture the suspect colonies individually on plates of a suitable selective medium[1].

Upon examination, the product passes the test if there is no growth of colonies of *Candida albicans* on any plate.

***Clostridium* spp.**

SAMPLE PREPARATION AND HEAT TREATMENT Prepare the product to be examined as described under Preparation of the Sample in Part I. Use two 10-mL portions each corresponding to 1 g or 1 mL of the product to be examined to inoculate a suitable amount (determined as described under Suitability of the Test Method) of Reinforced medium for clostridia. Heat one portion at 80° for 10 minutes and cool rapidly. Do not heat the other portion. Incubate both containers under anaerobic conditions at 30° to 35° for 48 hours.

SELECTION AND SUBCULTURE After incubation, make subcultures from each container on plates of Columbia agar to which gentamicin has been added and incubate under anaerobic conditions at 30° to 35° for 48 to 72 hours.

INTERPRETATION If no growth occurs, the product passes the test for absence of Clostridium spp. When growth of rods (with or without endospores) giving a negative catalase reaction occurs, subculture each distinct colony from on plates of Columbia agar, without gentamicin, and incubate at 30° to 35° for 48 to 72 hours, one plate anaerobically and the other aerobically, to check that the organism will not grow under aerobic condition.

Examine the appearance of only anaerobic growth of Gram-positive bacilli giving a negative catalase reaction together with the extent of hemolysis, by making subculture on a plate of Defibrinated sheep blood agar, and also examine microscopically for spore formation, using Gram stain or spore stain technique and confirmed by further suitable biochemical and biological tests. The description in Table 10 gives the characteristics of some *Clostridium* species on Defibrinated sheep blood agar.

Table 10 Characteristics of *Clostridium* Species on Defibrinated Sheep Blood Agar

Selective Species	Colonies	Hemolysis	Spores (Staining)
Clostridium botulinum	Irregular, translucent with a granular surface and indefinited fimbriated spreading edge	+	Oval, central, subterminal distend bacilli
Clostridium perfringens	Large, circular, convex, semitranslucent, smooth with an entire edge	Double zone	Oval and subterminal (very rare)
Clostridium tetani	Transparent with long feathery spreading projections	+	Spherical and terminal (drumstick)

Buffer Solution and Media

Culture media may be prepared as follows, or dehydrated culture media may be used if they have similar or comparable nutritive and selective properties for the micro-organisms to be tested for.

In preparing the media according to the formulae set forth herein, dissolve the soluble solids in the water, using heat, if necessary, to effect complete solution, and add other ingredients. Add, if necessary, a solution of hydrochloric acid or sodium hydroxide in quantities sufficient to yield the desired pH in the medium when it is ready for use. Determine the pH at 25°±2°.

Where agar is called for in a formula, use agar that has a moisture content of not more than 15 per cent.

Unless otherwise indicated, the buffer solution and media should be dispensed and sterilized by

[1]Biggy agar, CHROMagar Candida, or Candida isolation agar is recommended.

heating in an autoclave at 121°±2° for not less than 15 minutes, depending on the volume to be sterilized. Store under refrigeration.

BUFFER SOLUTION

Stock buffer solution

Place 34 g of *potassium dihydrogenphosphate* in a 1000-mL volumetric flask, dissolve in 500 mL of *water*, adjust to pH 7.2±0.2 with *sodium hydroxide*, dilute to 1000.0 mL with *water* and mix. Dispense into containers and sterilize. Store at 2° to 8°.

Phosphate buffer pH 7.2

Prepare a mixture of 1 volume of stock buffer solution and 800 volumes of *water* and sterilize.

Buffered sodium chloride-peptone solution pH 7.0

Potassium dihydrogenphosphate	3.56	g
Disodium hydrogenphosphate heptahydrate	10.89	g
Sodium chloride	4.30	g
Peptone, dried	1.0	g
Water	1000	mL

Polysorbate 20 or *80* may be added to obtain a 0.1 to 1.0 per cent w/v solution.

pH after sterilization: 7.0±0.1.

MEDIA

Baird-Parker agar

Pancreatic digest of casein	10.0	g
Beef extract	5.0	g
Yeas t extract	1.0	g
Lithium chloride	5.0	g
Agar	20.0	g
Glycine	12.0	g
Sodium pyruvate	10.0	g
Water	950	mL

Heat with frequent agitation, and boil for 1 minute. Sterilize, cool to between 45° and 50°, and add 10 mL of a sterile, 1 per cent w/v solution of *potassium tellurate(IV)* and 50 mL of egg-yolk emulsion. Mix intimately but gently, and pour into plates.

pH after sterilization: 6.8±0.2.

Preparation of the egg-yolk emulsion: Disinfect the surface of whole shell eggs, aseptically crack the eggs, and separate out intact yolks into a sterile graduated cylinder. Add *saline TS* to obtain a 3 to 7 ratio of egg-yolk to saline. Add to a sterile blender cup, and mix at high speed for 5 seconds.

Bismuth sulfite agar

Beef extract	5.0	g
Pancreatic digest of casein	5.0	g
Peptic digest of animal tissue	5.0	g
Dextrose monohydrate	5.0	g
Disodium hydrogenphosphate heptahydrate	4.0	g
Iron(II) sulfate	0.3	g
Bismuth sulfite indicator	8.0	g
Agar	20.0	g
Brilliant green	25.0	mg
Water	1000	mL

Heat the mixture of solids and *water*, with swirling, just to the boiling point. *Do not overheat or sterilize*. Transfer at once to a water-bath maintained at about 50°, and pour into plates as soon as the medium has cooled.

Final pH: 7.6±0.2.

Brilliant green agar

Yeast extract	3.0	g
Peptic digest of animal tissue	5.0	g
Pancreatic digest of casein	5.0	g
Lactose	10.0	g
Sodium chloride	5.0	g
Sucrose	10.0	g
Phenol red	80.0	mg
Agar	20.0	g
Brilliant green	12.5	mg
Water	1000	mL

Boil the solution of solids for 1 minute. Sterilize just prior to use. Melt the medium, pour into Petri dishes, and allow to cool.

pH after sterilization: 6.9±0.2.

Casein digest-soy lecithin polysorbate 20 broth

Pancreatic digest of casein	20.0	g
Soy lecithin	5.0	g
Polysorbate 20	40	mL
Water	960	mL

Dissolve pancreatic digest of casein and soy lecithin in 960 mL of *water*, heating in a water-bath at 48° to 50° for about 30 minutes to effect solution. Add 40 mL of *polysorbate 20*. Mix and dispense as desired.

pH after sterilization: 7.3±0.2.

Cetrimide Agar

Pancreatic digest of casein	20.0	g
Magnesium chloride	1.4	g
Potassium sulfate	10.0	g
Agar	13.6	g

Cetrimide	0.3	g
Glycerol	10.0	mL
Water	1000	mL

Dissolve all solid components in *water*, and add *glycerol*. Heat, with frequent agitation, and boil for 1 minute to effect solution.

pH after sterilization: 7.2±0.2.

Columbia agar

Pancreatic digest of casein	10.0	g
Peptic digest of animal tissue	5.0	g
Heart pancreatic digest	3.0	g
Yeast extract	5.0	g
Maize starch	1.0	g
Sodium chloride	5.0	g
Agar, according to gelling power	10.0 to 15.0	g
Water	1000	mL

Hydrate the agar, and dissolve by heating to boiling with continuous stirring. Sterilize, cool to between 45° and 50° and add, where necessary, gentamicin sulfate corresponding to 20 mg of gentamicin base. Pour into Petri dishes.

pH after sterilization: 7.3±0.2.

Defibrinated sheep blood agar (Blood agar)

Heat Soybean casein digest agar and cool to 45° to 50° in a water-bath. Add sufficient amount of defibrinated sheep blood to make 5 per cent and mix.

Enterobacteria enrichment broth-Mossel

Pancreatic digest of gelatin	10.0	g
Dextrose monohydrate	5.0	g
Dehydrated ox bile	20.0	g
Potassium dihydrogenphosphate	3.0	g
Disodium hydrogenphosphate dihydrate	8.0	g
Brilliant green	15.0	mg
Water	1000	mL

Mix and heat at 100° for 30 minutes to sterilize and cool immediately. *Do not autoclave*.

Final pH: 7.2±0.2.

Lactose broth

Beef extract	3.0	g
Pancreatic digest of gelatin	5.0	g
Lactose	5.0	g
Water	1000	mL

Cool as quickly as possible after sterilization.

pH after sterilization: 6.9±0.2.

Levine eosin-methylene blue agar

Pancreatic digest of gelatin	10.0	g
Dipotassium hydrogenphosphate	2.0	g
Agar	15.0	g
Lactose	10.0	g
Eosin Y	0.4	g
Methylene blue	65.0	mg
Water	1000	mL

Dissolve pancreatic digest of gelatin, *dipotassium hydrogenphosphate* and *agar* in *water*, with warming, and allow to cool. Just prior to use, liquefy the gelled agar solution, add the remaining ingredients, as solutions, in the following amounts, and mix: for each 100 mL of the liquefied agar solution 5 mL of a 20 per cent w/v solution of *lactose*, 2 mL of a 2 per cent w/v solution of *eosin Y*, and 2 mL of a 0.33 per cent w/v solution of *methylene blue.* The finished medium may not be clear.

pH after sterilization: 7.1±0.2.

MacConkey agar

Pancreatic digest of gelatin	17.0	g
Pancreatic digest of casein	1.5	g
Peptic digest of animal tissue	1.5	g
Lactose	10.0	g
Bile salts mixture	1.5	g
Sodium chloride	5.0	g
Agar	13.5	g
Neutral red	30.0	mg
Crystal violet	1.0	mg
Water	1000	mL

Boil the mixture of solids and water for 1 minute to effect solution.

pH after sterilization: 7.1±0.2.

MacConkey broth

Pancreatic digest of gelatin	20.0	g
Lactose	10.0	g
Dehydrated ox bile	5.0	g
Bromocresol purple	10.0	mg
Water	1000	mL

Prepare as directed under Buffer Solution and Media.

pH after sterilization: 7.3±0.2.

Mannitol-salt agar

Pancreatic digest of casein	5.0	g
Papaic digest of animal tissue	5.0	g
Beef extract	1.0	g
Mannitol	10.0	g
Sodium chloride	75.0	g
Agar	15.0	g

Phenol red	25.0	mg
Water	1000	mL

Mix, then heat with frequent agitation, and boil for 1 minute to effect solution.

pH after sterilization: 7.4±0.2.

Potato dextrose agar

Cook 300 g of peeled and diced potatoes in 500 mL of *water* prepared by distillation, filter through cheesecloth, add *water* prepared by distillation to make 1000 mL, and add the following:

Agar	15.0	g
Dextrose monohydrate	20.0	g

Dissolve by heating and sterilize.

pH after sterilization: 5.6±0.2.

For use, just prior to pouring the plates, adjust the melted and cooled to 45° medium with a sterile 10 per cent w/v solution of *tartaric acid* to a pH of 3.5±0.1. *Do not reheat the pH 3.5 medium.*

Pseudomonas agar for detection of fluorescin

Pancreatic digest of casein	10.0	g
Peptic digest of animal tissue	10.0	g
Dipotassium hydrogenphosphate	1.5	g
Magnesium sulfate	1.5	g
Agar	15.0	g
Glycerol	10.0	mL
Water	1000	mL

Dissolve the solid components in *water* before adding *glycerol*. Heat, with frequent agitation, and boil for 1 minute to effect solution.

pH after sterilization: 7.2±0.2.

Pseudomonas agar for detection of pyocyanin

Pancreatic digest of gelatin	20.0	g
Magnesium chloride	3.0	g
Potassium sulfate	10.0	g
Agar	15.0	g
Glycerol	10.0	mL
Water	1000	mL

Dissolve the solid components in *water* before adding glycerol. Heat, with frequent agitation, and boil for 1 minute to effect solution.

pH after sterilization: 7.2±0.2.

Rappaport-Vassiliadis broth

Soya peptone	4.5	g
Sodium chloride	8.0	g
Dipotassium phosphate	0.4	g
Potassium dihydrogenphosphate	0.6	g
Magnesium chloride	29.0	g
Malachite green	36.0	mg
Water	1000	mL

Mix and heat to effect solution.

pH after sterilization: 5.±0.2.

Reinforced medium for clostridia

Beef extract	10.0	g
Peptone	10.0	g
Yeast extract	3.0	g
Soluble starch	1.0	g
Dextrose monohydrate	5.0	g
Cysteine hydrochloride	0.5	g
Sodium chloride	5.0	g
Sodium acetate	3.0	g
Agar	0.5	g
Water	1000	mL

Hydrate the agar, and dissolve by heating to boiling with continuous stirring.

pH after sterilization: 6.8±0.2.

Sabouraud dextrose agar

Dextrose monohydrate	40.0	g
Mixture of equal parts of Peptic digest of animal tissue and Pancreatic digest of casein	10.0	g
Agar	15.0	g
Water	1000	mL

Mix and boil to effect solution.

pH after sterilization: 5.6±0.2.

Sabouraud dextrose agar with antibiotics

Dextrose monohydrate	40.0	g
Mixture of equal parts of Peptic digest of animal tissue and pancreatic digest of casein	10.0	g
Agar	15.0	g
Water	1000	mL

Mix and boil to effect solution. Immediately before use, add 0.10 g of *benzylpenicillin sodium* and 0.10 g of *tetracycline* per litre of medium as sterile solutions or alternatively, add 50 mg of *chloramphenicol* per litre of medium before sterilization.

pH after sterilization: 5.6±0.2.

(**Note** Other antibiotics can all be used, individually or in combination.)

Sabouraud dextrose broth

Dextrose monohydrate	20.0	g
Mixture of equal parts of Peptic digest of animal tissue and Pancreatic digest of casein	10.0	g
Water	1000	mL

Prepare as directed under *Buffer Solution and Media*.

pH after sterilization: 5.6±0.2.

Soybean-casein digest agar

Pancreatic digest of casein	15.0	g
Papaic digest of soybean meal	5.0	g
Sodium chloride	5.0	g
Agar	15.0	g
Water	1000	mL

Prepare as directed under *Buffer Solution and Media.*

pH after sterilization: 7.3±0.2.

Soybean-casein digest broth

Pancreatic digest of casein	17.0	g
Papaic digest of soybean meal	3.0	g
Sodium chloride	5.0	g
Dipotassium hydrogenphosphate	2.5	g
Dextrose monohydrate	2.5	g
Water	1000	mL

Prepare as directed under *Buffer Solution and Media*.

pH after sterilization: 7.3±0.2.

Tetrathionate bile brilliant green broth

Peptone	8.6	g
Ox bile, dried	8.0	g
Sodium chloride	6.4	g
Calcium carbonate	20.0	g
Potassium tetrathionate	20.0	g
Brilliant green	70.0	mg
Water	1000	mL

Heat the solution of solids to boiling. *Do not reheat*.

Final pH: 7.0±0.2.

Triple sugar-iron-agar

Pancreatic digest of casein	10.0	g
Pancreatic digest of animal tissue	10.0	g
Lactose	10.0	g
Sucrose	10.0	g
Dextrose monohydrate	1.0	g
Ammonium iron(II) sulfate	0.2	g
Sodium chloride	5.0	g
Sodium thiosulfate	0.2	g
Agar	13.0	g
Phenol red	25.0	mg
Water	1000	mL

Prepare as directed under *Buffer Solution and Media.*

pH after sterilization: 7.3±0.2.

Violet red bile dextrose agar

Yeast extract	3.0	g
Pancreatic digest of gelatin	7.0	g
Bile salts mixture	1.5	g
Lactose	10.0	g
Sodium chloride	5.0	g
Dextrose monohydrate	10.0	g
Agar	15.0	g
Neutral red	30.0	mg
Crystal violet	2.0	mg
Water	1000	mL

Mix and heat to boiling. *Do not overheat or sterilize.* Transfer at once to a water-bath maintained at about 50°, and pour into plates as soon as the medium has cooled.

Final pH: 7.4±0.2.

Vogel-Johnson Agar

Pancreatic digest of casein	10.0	g
Yeast extract	5.0	g
Mannitol	10.0	g
Dipotassium hydrogenphosphate	5.0	g
Lithium chloride	5.0	g
Glycine	10.0	g
Agar	16.0	g
Phenol red	25.0	mg
Water	1000	mL

Boil the solution of solids for 1 minute. Sterilize, cool to between 45° and 50°, and add 20 mL of a sterile 1 per cent w/v solution of *potassium tellurate(IV).*

pH after sterilization: 7.2±0.2.

Xylose-lysine-deoxycholate agar

Xylose	3.5	g
L-Lysine	5.0	g
Lactose	7.5	g
Sucrose	7.5	g
Sodium chloride	5.0	g
Yeast extract	3.0	g
Agar	13.5	g
Sodium desoxycholate	2.5	g
Sodium thiosulfate	6.8	g
Ammonium iron(III) citrate	0.8	g
Phenol red	80.0	mg
Water	1000	mL

Heat the mixture of solids and *water*, with swirling, just to the boiling point. *Do not overheat or sterilize.* Transfer at once to a water-bath maintained at about 50°, and pour into plates as soon as the medium has cooled.

Final pH: 7.4±0.2.

10.4 MICROBIOLOGICAL ATTRIBUTES OF NON-STERILE PHARMACEUTICAL PRODUCTS

Few raw materials used in making pharmaceutical products are sterile as received, and special treatment may be required to render them microbiologically acceptable for use. Strict adherence to effective environmental control and sanitation, equipment cleaning practices, and good personal hygiene practices in pharmaceutical manufacture is vital in minimizing both the type and the number of micro-organisms.

Monitoring, in the form of regular surveillance, should include an examination of the microbiological attributes of Pharmacopoeial articles and a determination of compliance with such microbiological standards as are set forth in the individual monographs. It maybe necessary also to monitor the early and intermediate stages of production, with emphasis being placed on raw materials, especially those of animal or botanical origin, or from natural mineral sources which may harbour objectionable micro-organisms not destroyed during subsequent processing. It is essential that ingredients and components be stored under conditions designed to deter microbial proliferation. Microbiological purity of the raw materials as well as manufacturing conditions, including water used for the production, should be such a degree that the microbiological purity requirements for the final product i.e., the pharmaceutical preparation, are observed to fulfill the requirements for good manufacturing practices (GMP).

The nature and frequency of testing vary according to the product. Monographs for some articles require freedom from one or more species of selected indicator microorganisms such as *Salmonella* spp., *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. For some articles, a specific limit on the total aerobic microbial count (TAMC) and/or the total combined yeasts and moulds count (TYMC) is set forth in the individual monograph. In these cases a requirement for freedom from specified indicator microorganisms is also included. The significance of micro-organisms in a non-sterile pharmaceutical product should be evaluated in terms of the use of the product, the nature of the product, and the potential hazard to the user. Also taken into account is the processing of the product in relation to an acceptable quality for pharmaceutical purposes. It is suggested that certain categories of products be tested routinely for total microbial count and for specified indicator microbial contaminants, e.g., natural plant, animal, and some mineral products for *Salmonella* species; oral solutions and suspensions for *E. coli;* articles applied topically for *P. aeruginosa* and *S. aureus*; and articles intended for rectal, urethral, or vaginal administration for yeasts and moulds.

Definitive microbial limits (stipulated micro-organisms and/or counts) are incorporated into specific monographs on the basis of a major criterion, i.e., the potential of the stipulated micro-organisms and/or counts, and of any others that they may reflect, to constitute a hazard in the end product. Such considerations also take into account the processing to which the product components are subjected, the current technology for testing, and the availability of desired quality material. Any of these may preclude the items from specific requirements in the "Microbial Limit Tests" (Appendix 10.2). Regardless of such preclusion, it remains essential to apply strict good manufacturing practices to assure a lowest possible load of micro-organisms. "Limits for Microbial Contamination" (Appendix 10.5) is also set forth to control microbial purity in non-sterile pharmaceutical products. For herbal remedies or herbal drug preparation described in categories Table 2, the same criteria are applicable to both crude drugs and their preparations.

The relevant tests for determining the total aerobic microbial count and the total combined yeasts and moulds count, and for detection and identification of designated species are given in the "Microbial Limit Tests" (Appendix 10.2). For reliable results, the personnel responsible for the conduct of the test should have specialized training in microbiology and in the interpretation of microbiological data.

10.5 LIMITS FOR MICROBIAL CONTAMINATION

In the manufacture, packaging, storage and distribution of pharmaceutical preparations, suitable means must be taken to ensure their microbiological quality. Unless otherwise specified in the individual monograph, the non-sterile pharmaceutical preparations should comply with the acceptance criteria given in Table 1 and the herbal drug preparations should comply with the acceptance criteria given in Table 2.

Table 1 Acceptance Criteria for Microbiological Quality of Non-sterile Pharmaceutical Preparations

Category	Types	Requirements*
1	Topical preparations for broken skins, abscess, lesions, and mucous total membranes excluding for vaginal and rectal routes.	- Total aerobic microbial count: not more than 2×10^1 CFU per g or per mL and combined yeasts and moulds count: not more than 2×10^1 CFU per g or per mL. - Absence of *Pseudomonas aeruginosa* and *Staphylococcus aureus* per g or per mL.
2	A. Preparations for inhalation use except where required to be sterile.	- Total aerobic microbial count: not more than 2×10^2 CFU per g or per mL and total combined yeasts and moulds count: not more than 2×10^1 CFU per g or per mL. - Absence of bile-tolerant gram-negative bacteria, *Pseudomonas aeruginosa* and *Staphylococcus aureus* per g or per mL.
	B. Preparation for vaginal use.	- Total aerobic microbial count: not more than 2×10^2 CFU per g or per mL and total combined yeasts and moulds count: not more than 2×10^1 CFU per g or per mL. - Absence of *Pseudomonas aeruginosa, Staphylococcus aureus* and *Candida albicans* per g or per mL.
	C. Preparation for rectal use.	- Total aerobic microbial count: not more than 2×10^3 CFU per g or per mL and total combined yeasts and moulds count: not more than 2×10^2 CFU per g or per mL.
3	Transdermal patchs and topical preparation for intact skin, e.g., creams, lotions, ointments, solutions, powders, etc.	- Total aerobic microbial count: not more than 2×10^2 CFU per g or per mL or per patch and total combined yeasts and moulds count: not more than 2×10^1 CFU per g or per mL or per patch. - Absence of *Pseudomonas aeruginosa* and *Staphylococcus aureus* per g or per mL or per patch.
4	A. Aqueous preparations for oral use.	- Total aerobic microbial count: not more than 2×10^2 CFU per g or per mL and total combined yeasts and moulds count: not more than 2×10^1 CFU per g or per mL. - Absence of *Escherichia coli* per g or per mL.
	B. Non-aqueous preparations for oral use.	- Total aerobic microbial count: not more than 2×10^3 CFU per g or per mL and total combined yeasts and moulds count: not more than 2×10^2 CFU per g or per mL. - Absence of *Escherichia coli* per g or per mL.

*Carry out the tests as described in the "Microbial Limit Tests" (Appendix 10.2).

Table 1 (Continued)

Category	Types	Requirements*
5	Preparations for oral administration containing raw materials of natural origin (animal, vegetable or mineral) which cannot be treated with a process for reduction of microbial count.**	- Total aerobic microbial count: not more than 2×10^4 CFU per g or per mL and total combined yeasts and moulds count: not more than 2×10^2 CFU per g or per mL. - Bile-tolerant gram-negative bacteria: not more than 10^2 probable number of bacteria per g or per mL. - Absence of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* per g or per mL. - Absence of *Salmonella* spp. per 10 g or per 10 mL.

*Carry out the tests as described in the "Microbial Limit Tests" (Appendix 10.2).

**Specified for raw materials of natural origin that are not obtainable with the required purity including those for manufacturing drugs where an antimicrobial treatment (e.g., with ethylene oxide or ionizing radiations) is not feasible or permissible. The examples are arabic gum, tragacanth, pancreas powder, pepsin, and trypsin.

Table 2 Acceptance Criteria for Microbiological Quality of Herbal Drug Preparations

Category	Types	Requirements*
1	Preparations from crude drug extracts which underwent a process for reduction of micro-organisms.	
	A. Preparations for oral use.	- Total aerobic microbial count: not more than 5×10^3 CFU per g or per mL and total combined yeasts and moulds count: not more than 5×10^2 CFU per g or per mL. - Absence of *Escherichia coli* per g or per mL. - Absence of *Salmonella* spp. per 10 g or per 10 mL.
	B. Topical preparations for intact skin.	- Total aerobic microbial count: not more than 5×10^2 CFU per g or per mL and total combined yeasts and moulds count: not more than 5×10^1 CFU per g or per mL. - Absence of *Pseudomonas aeruginosa* and *Staphylococcus aureus* per g or per mL.
2	Preparations of crude drugs and mixtures of crude drugs for internal use which will undergo a process for reduction of microbial count before use (e.g., by pouring boiling water over them).	- Total aerobic microbial count: not more than 5×10^7 CFU per g or per mL and total combined yeasts and moulds count: not more than 5×10^5 CFU per g or per mL. - Absence of *Escherichia coli* and *Clostridium* spp. per g or per mL. - Absence of *Salmonella* spp. per 10 g or per 10 mL.

*Carry out the tests as described in the "Microbial Limit Tests" (Appendix 10.2).

Table 1 (Continued)

Category	Types	Requirements*
3	Preparations of crude drugs and mixtures of crude drugs for external use.**	- Total aerobic microbial count: not more than 5×10^7 CFU per g or per mL and total combined yeasts and moulds count: not more than 5×10^5 CFU per g or per mL. - Absence of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Clostridium* spp. per g or per mL.
4	Other preparations for internal use containing whole or ground crude drugs.	- Total aerobic microbial count: not more than 5×10^5 CFU per g or per mL and total combined yeasts and moulds count: not more than 5×10^4 CFU per g or per mL. - Bile-tolerant gram-negative bacteria: not more than 10^3 probable number of bacteria per g or per mL. - Absence of *Escherichia coli* and *Clostridium* spp. per g or per mL. - Absence of *Salmonella* spp. per 10 g or per 10 mL.

*Carry out the tests as described in the "Microbial Limit Tests" (Appendix 10.2).

**The examples are LUKPRAKHOP (herbal compress), YAPHOK (herbal poultice).

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Thai Pharmacopoeia Application