World Health Organization

DRAFT WORKING DOCUMENT FOR COMMENTS:

WHO General guidelines for the establishment, maintenance and distribution of chemical reference substances

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For any technical questions, you may contact **Dr Herbert Schmidt**, Technical Officer, Norms and Standards for Pharmaceuticals, Technical Standards and Specifications (schmidth@who.int), with a copy to **Ms Sinéad Jones** (jonessi@who.int, nsp@who.int).
Comments should be submitted through the online platform on or by **07 May 2025**. Please note that only comments received by this deadline will be considered for the preparation of this document.

SCHEDULE FOR DRAFT WORKING DOCUMENT QAS/25.972

WHO General guidelines for the establishment, maintenance and distribution of chemical reference substances

Description of Activity	Date
Public consultation to evaluate whether the current guideline still reflects the status of technology and if not, which sections would need to be updated or amended.	February – May 2025
Review of feedback received and preparation of the first draft working document with an informal drafting group.	July – December 2025
Public consultation of the first draft working document inviting comments and posting of the working document on the WHO website.	February – March 2026
Discussion of the feedback received in a virtual meeting with an informal drafting group. Preparation of a revised draft working document.	April 2026
Public consultation of the revised draft working document inviting comments and posting of the working document on the WHO website.	May – June 2026
Review of feedback received and preparation of the draft working document for submission to the ECSPP with an informal drafting group.	August 2026
Presentation of the draft working document to the Sixtieth meeting of the ECSPP.	October 2025
Any other follow-up action as required.	

Note from the Secretariat. Access to suitable chemical reference substances remains a challenge for many laboratories in low- and middle-income countries (LMICs). These challenges arise not only from the lack of reference standards for certain active ingredients but also from the high costs associated with their procurement and supply.

While official primary reference substances are essential for conclusively demonstrating compliance with pharmacopoeial standards, manufacturers, national quality control laboratories and other stakeholders often rely on secondary reference standards for routine testing.

However, the use of secondary reference substances, whether produced in-house or sourced from national or regional collections, has several limitations. Secondary reference substances should only be used for the same purposes as the primary reference standards to which they are calibrated. The uncertainty of their assigned values is typically higher than that of primary reference substances, which may render them unsuitable for certain applications. Moreover, secondary standards lack authoritative status in cases of doubt or dispute, often requiring repeated testing with primary reference substances. Additionally, multiple collections of secondary standards may lead to divergent test results, potentially causing inconsistencies in medicine dosing regimens across regions.

The World Health Organization (WHO) is considering revising the *General guidelines for the establishment, maintenance and distribution of chemical reference substances*. To ensure the guideline reflects current scientific and technological advancements and improves global access to suitable reference substances, WHO invites feedback on the current version of the document below. The Secretariat will review the feedback received and invite selected experts, based on their contributions, to discuss and incorporate necessary updates or amendments to address the identified gaps and challenges.

WHO General guidelines for the establishment, maintenance and distribution of chemical reference substances

For any further information or request, please send an email to the Norms and Standards for Pharmaceuticals (NSP) Team at WHO, at email nsp@who.int.

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Introduction

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

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

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

The purpose of establishing chemical reference substances is to achieve accuracy and reproducibility of the analytical results required by pharmacopoeial testing and pharmaceutical control in general. These substances are normally prepared and issued by the regional or national pharmacopoeia commission or the regional or national quality control laboratory on behalf of the drug regulatory

authority. In the context of these guidelines, the general use of a chemical reference substance should be considered an integral part of a compliance-oriented monograph or test procedure used to demonstrate the identity, purity and content of pharmaceutical substances and preparations.

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

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

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

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

Glossary

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

meanings in other contexts.

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

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

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

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

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

Part A. Primary chemical reference substances

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

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

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

- infrared (IR) spectrophotometry, whether for identification or quantitative purposes;
- quantitative methods based on ultraviolet (UV) absorption spectrophotometry;
- quantitative methods based on the development of a colour and the measurement of its intensity, whether by instrumental or visual comparison;
- methods based on chromatographic separation for identification or quantitative purposes;
- quantitative methods (including automated methods) based on other separation techniques that depend on partition of the substance to be determined between solvent phases, where

the precise efficiency of the extraction procedure might depend upon ambient conditions that occasionally vary and from laboratory to laboratory;

- quantitative methods, often titrimetric but sometimes gravimetric, that are based on non-stoichiometric relationships;
- assay methods based on measurement of optical rotation; and
- methods that might require a chemical reference substance consisting of a fixed ratio of known components (for example, *cis/trans* isomers, spiked samples).

A.2 Obtaining source material

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

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

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

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

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

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

A.3 Evaluation of chemical references substances

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

A.3.1 Use in identification tests

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

A.3.2 Use in purity tests

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

A.3.3 Use in assays

If the chemical reference substance is to be used in an assay (colorimetry, LC, GC or UV spectrophotometry), the extent of testing is much greater. Several (a minimum of three) laboratories should collaborate in testing the proposed substance, using a variety of established and validated techniques, including the method used in the pharmacopoeial specification. The relative reactivity or relative absorbance of the impurities present must be checked when a nonspecific assay method is employed, for example, by colorimetry or UV spectrophotometry. When a selective assay method is employed, it is particularly important to determine the quantity of impurities. In such a case, it is

best to examine the proposed reference substance by as many methods as practicable including, where possible, absolute methods. For substances that are acidic or basic a titration with alkali or acid is simple, but other reactions which are known to be stoichiometric may be used. Phase solubility analysis and differential scanning calorimetry may also be employed in certain cases.

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

A.3.4 Use in the calibration of an instrument

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

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

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

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

A.4.1 Methods used to verify the identity of chemical reference substances

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

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

A.4.2 Methods used to determine the purity of chemical reference substances

The analytical methods to be employed in examining a substance should be considered in relation to its intended use. These analytical methods may be divided into three broad categories:

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

A.4.2.1 Separation techniques

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

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

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

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

TLC uses apparatus that is simple and inexpensive; the technique is easy to carry out and is readily applicable even in the microgram range. It can separate closely related compounds, such as geometric isomers and the members of a homologous series. All the constituents of a substance subjected to chromatography appear somewhere on the chromatogram. However, some

constituents may remain on the starting line, some may move with the solvent front, some may migrate at the same rate as the main component, and some may remain undetected. For this reason, the usefulness of the method may be greatly enhanced by performing two- dimensional chromatography and by using a number of different solvent systems and a variety of detection methods. In some cases, the method may be used quantitatively with acceptable accuracy by using a densitometer.

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

A.4.2.2 Methods based on intrinsic thermodynamic properties

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

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

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

A.4.2.3 Other methods

Spectrophotometric methods. UV spectrophotometry is occasionally used to determine purity. Since it depends upon the presence of a characteristic chromophore, it can detect impurities that

contribute excessively to the absorbance value and may indicate the presence of impurities that have a negligible or distinctive absorbance.

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

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

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

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

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

A.5 Assignment of content

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

A.6 Handling and distribution of chemical reference substances

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

A.6.1 Packaging operations

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

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

are opened.

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

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

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

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

A.6.2 Storage

Information about suitable storage conditions can often be obtained from the manufacturer of the source material and should be requested routinely when a new chemical reference substance is established. Theoretically, the stability of the substances should be enhanced by keeping them at low temperatures but, for substances that contain water, storage below 0 °C may impair the stability. It should also be remembered that the relative humidity in normal refrigerators or cold rooms may be high and, unless ampoules or other tightly closed containers are used, the improvement in stability may be more than offset by degradation due to the absorption of moisture. Storage at about 5 °C, with precautions to prevent such absorption, has proved satisfactory for most chemical

reference substances. Vials should, however, not be opened until they have attained room temperature to prevent ingress of moisture by condensation.

A.6.3 Stability

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

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

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

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

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

A.6.4 Information to be supplied with chemical reference substances

The labels on chemical reference substances should give the following information:

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

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

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

– health hazard information or warnings in conformity with national and regional regulations or international agreements.

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

A.6.5 Distribution and supply

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

A.6.6 Period of use

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

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

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

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

Part B. Secondary chemical reference substances

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

B.1 Assessment of need

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

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

documented.

B.2 Obtaining source material

B.2.1 Selection of candidate substance

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

B.2.2 Documentation to be supplied with the candidate reference substance

The supplier of the candidate reference substance is requested to supply the same documentation as required for a candidate primary reference substance (see section 2, Part A).

B.2.3 Initial testing for compliance with the requirements of the monograph

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

B.3 Packaging

See section 6.1 in Part A of these guidelines.

B.4 Interlaboratory testing to establish the assigned content

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

B.4.1 Competence of the participating laboratories

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

B.4.2 Dispatch of the candidate materials

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

The following documents should be supplied with the material:

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

B.4.3 Test protocol

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

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

B.4.4 Evaluation of test results

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

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

B.4.5 Traceability

The term for “traceability”, for the purposes of this document, is defined as the property of a result of measurement which can be related to the appropriate standards, generally international or

national standards, through an unbroken chain of comparison. In other words, when the result of a measurement is described as traceable, it is essential to specify to what (value of) "appropriate standards" traceability has been established.

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

B.5 Adoption of the secondary reference substance

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

B.6 Retesting programme

See also section 6.3 in Part A of these guidelines.

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

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

The frequency and extent of re-testing reference substances depends on a number of factors

including stability, container and closure system, storage conditions, hygroscopicity, physical form and intended use. The frequency of testing and the testing methods to be employed for each reference substance must be documented.

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

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

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

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

B.8 Period of use

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

B.9 Distribution and supply

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

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

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