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# WHO GENERAL GUIDELINE FOR THE ESTABLISHMENT, MAINTENANCE, AND DISTRIBUTION OF CHEMICAL REFERENCE SUBSTANCES

The initial guideline published in 1975 was revised in the light of developments in analytical chemistry and international collaboration during an informal consultation held in April 1996. This guideline also takes into account established practices, modified as appropriate for specific application to chemical reference substances for pharmacopoeial purposes. It is intended for all issuing bodies of chemical reference substances giving advice on the establishment of both primary and secondary chemical reference substances. The general use of a primary or secondary chemical reference substance is an integral part of a compliance-oriented, compendial monograph of test procedure to demonstrate the strength, quality and purity of pharmaceutical substances and preparations.

It is intended to present this revised guideline, together with any changes or additions, if necessary, to the forthcoming WHO Expert Committee on Specifications for Pharmaceutical Preparations.

We would appreciate your views and comments to be mailed to Quality Assurance, Division of Drug Management & Policies, World Health Organization, 1211 Geneva 27, Switzerland, Fax: (0041 22) 791 07 46, or e-mail: schmidm@who.ch or koppkubels@who.ch, by the end of February 1997 at the latest.

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## INTRODUCTION

In 1975, the WHO Expert Committee on Specifications for Pharmaceutical Preparations recommended "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 who are responsible for collections of chemical reference substances. This concept is still relevant. The guidelines were initially drawn-up for use in particular by the WHO Collaborating Centre for Chemical Reference Substances in Sweden who provide 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 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. In addition this Collaborating Centre was not in a position to meet all demands. Another difficulty was to ship the substances in a timely manner. Since the meticulous work carried out at the WHO Collaborating Centre to establish the international collection would have to be duplicated in local or regional laboratories, guidelines would be necessary to ensure the integrity of national or regional collections. In order to clarify the need for national and regional collections, the guidelines as prepared in 1975 were reviewed and modified in 1982.[3] In view of refinements in pharmaceutical and analytical methods since then, the present revision was considered essential.

The purpose of chemical reference substances is to achieve accuracy and reproducibility of analytical results required in the context of pharmacopoeial testing and pharmaceutical control in general. These substances are normally prepared and issued by the Regional/National Pharmacopoeial Commission or the Regional/National Quality Control Laboratory on behalf of the Drug Regulatory Authority. In the context of this guideline, the general use of a chemical reference substance should be considered an integral part of a compliance-oriented, compendial monograph or test procedure to demonstrate the identity, purity and content of pharmaceutical substances and preparations.

The establishment of chemical reference substances should be based on reports in which results of analytical testing have been evaluated. These reports should be subsequently approved and adopted by a certifying body, normally the relevant pharmacopoeial committee or the Drug Regulatory Authority. Such establishment can be on an international, national or regional basis. Each substance is generally established for a specific analytical purpose as defined by the issuing body. Use for any other purpose becomes the responsibility of the user and a suitable caution is included in the information sheet accompanying a reference substance. The guideline is concerned with both primary and secondary chemical reference substances as defined below.

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1 *WHO Expert Committee on Specifications for Pharmaceutical Preparations, Twenty-fifth Report.* World Health Organization, Geneva, 1975 (WHO Technical Report Series, No. 567), Annex 3, p.98.
The term *chemical reference substances*, as used here, refers to an authenticated uniform material that is intended for use in specified chemical and physical tests, in which its properties are compared with the properties of a product under examination, and which possesses a degree of purity adequate for its intended use.

2 *The International Pharmacopoeia, Vol. 1. General methods of analysis.* Geneva, World Health Organization, 1979.
*Vol. 2. Quality specifications.* Geneva, World Health Organization, 1981.
*Vol. 3. Quality specifications.* Geneva, World Health Organization, 1988.
*Vol. 4. Tests, methods, and general requirements. Quality specifications for pharmaceutical substances, excipients, and dosage forms.* Geneva, World Health Organization, 1994.

3 *WHO Expert Committee on Specifications for Pharmaceutical Preparations, Twenty-eighth Report.* World Health Organization, Geneva, 1982 (WHO Technical Report Series, No. 681), Annex 1, p.19.

The preparation of a chemical reference substance should comply with requirements for quality assurance systems, including principles of good manufacturing practices and good laboratory practices.[4]

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, however, to the availability of resources.

### Primary chemical reference substance

A designated primary chemical reference substance is widely acknowledged as having appropriate qualities within a specified context, and whose value is accepted without reliance on comparison to 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 extensive than for a primary chemical reference substance. This definition may apply *inter alia* to some substances termed "working standards".

## PART A: PRIMARY CHEMICAL REFERENCE SUBSTANCES

## 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 of great importance to determine in a critical way whether a need for a given substance exists. Requests for new chemical reference substances usually arise when a certain approach to the development of a specification for a new substance or product has been adopted. Methods may have been proposed in a specification that requires 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 procedure could be adopted that does not require a comparative standard and is considered to be equally satisfactory.

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

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4 *Good laboratory practices in governmental drug control laboratories. WHO Expert Committee on Specifications for Pharmaceutical Preparations, Thirtieth Report.* World Health Organization, Geneva, 1987 (WHO Technical Report Series, No. 748), Annex 1, p.20.
*Good manufacturing practices for pharmaceutical products. WHO Expert Committee on Specifications for Pharmaceutical Preparations, Thirty-second Report.* World Health Organization, Geneva, 1992 (WHO Technical Report Series, No. 823), Annex 1, p.14.
*Good manufacturing practices for biological products. WHO Expert Committee on Specifications for Pharmaceutical Preparations, Thirty-third Report.* World Health Organization, Geneva, 1993 (WHO Technical Report Series, No. 834), Annex 3, p.20.

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

## 2. PROCUREMENT OF SOURCE MATERIAL

Source material of satisfactory quality can be selected from a batch[5] of the substance originating from the normal production process if the purity is acceptable. Further purification techniques may need to be employed 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 notable 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% (on the anhydrous or volatiles-free basis) or higher is desirable for such chemical reference substances. However, in cases 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 impair the usefulness of a chemical reference substance, whereas even traces of impurities with significantly different properties may render a substance unsuitable as a chemical reference substance.

When procuring a source material with a view to its use as a chemical reference substance from a supplier, the following should be supplied with the material:

- Certificate of analysis with complete information as to test methods employed, values found and number of replicates used, where applicable, and relevant spectra and/or chromatograms.

- Information on optimal storage conditions required for stability (temperature and humidity considerations).

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5 Also referred to as "lot".

- Results of any hygroscopicity study and/or statement of the hygroscopicity of the source material.
- Results of any accelerated stability studies.
- Identification of detected impurities (by preference), and/or specific information as to the relative response factor in compendial methods concerning the principal component, and/or the mass in per cent of the impurity.
- Updated Material Safety Data Sheet (MSDS) outlining any health hazards associated with the material.

For new drug substances, manufacturers should be aware that elaboration of pharmacopoeial monographs will be needed and a batch of the new substance should be set aside to be used as the possible chemical reference substance . In this respect, it is desirable for issuing bodies of chemical reference substances to provide each other with a sample of the same batch of material, even if the substance will be employed for different test methods. This will require interchange of information concerning the establishment process, supplier(s), availability and conditions of supply.

## 3. EVALUATION OF CHEMICAL REFERENCE SUBSTANCES

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

A. Use in identification tests, by infrared spectrophotometry and/or chromatographic methods;

B. Use in purity tests (usually chromatographic) for specific impurities;

C. Use in assays: colorimetry, liquid or gas chromatography and ultraviolet spectrophotometry;

D. Use for calibration of an instrument.

A. A batch of material of good quality, selected from the normal production process is satisfactory if it is of acceptable purity. Additional purification may be necessary by the supplier. The most important control is the application of the test(s) for which the substance is intended. It is usual for at least one laboratory to apply all tests of the monograph.

B. The characterization of a chemical reference substance to be employed to determine an impurity is more extensive especially when used in a limit test. If the technique employed is TLC, an acceptable minimum purity is recommended (normally at least 90%) but purer material may be required for liquid or gas chromatographic methods (HPLC or GC). If the proposed reference substance is prepared or isolated for the first time, appropriate chemical and physicochemical tests must be applied to characterize it, e.g. NMR, mass spectrometry (MS), elemental analysis, etc.

C. If the chemical reference substance is to be employed in an assay method, the extent of testing is very much greater. It is desirable that several (a minimum of three) laboratories collaborate in the testing of 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 in a proposed substance must be checked when a non-specific assay method is employed, e.g. by colorimetry or UV spectrophotometry. When a selective assay method is employed, it is particularly important to determine and express the quantity of the impurities. In such a case, it is best to examine the proposed substance by as many methods as practicable, including, where possible, absolute methods. For proposed 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 (PSA) and differential scanning calorimetry (DSC) may also be employed in certain cases.

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

D. In cases 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. It is desirable that several laboratories collaborate in the testing of the proposed substance using a variety of techniques to ascertain that its purity is adequate. An appropriate number of collaborating laboratories should also participate in the study, after the reference substance has been deemed suitable, to establish a value by measurement of the essential property of the substance using an appropriate instrument.

## 4. CHEMICAL AND PHYSICAL METHODS USED IN EVALUATING CHEMICAL REFERENCE SUBSTANCES

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

The methods used to establish such a substance fall into two broad groups: those intended primarily to identify the substance and those 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. In such instances, the quoted purity is an estimate based upon the data obtained by use of the various analytical methods.

### 4.1 Methods useful for verifying the identity of chemical reference substances

Where a proposed substance consists of a compound whose structure has been satisfactorily defined, its identity may be confirmed by matching the IR spectra of the substance to that of an authentic compound. Particular care should be taken when polymorphism exists.[6] Other highly specific techniques, such as NMR spectroscopy, MS, or X-ray diffraction crystallography, may also be used for such comparisons. The identity of 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 infrared (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 the identity of the substance by applying several analytical techniques currently used to characterize new compounds. Such analytical methods may include elemental analyses, crystallographic studies, MS, NMR spectroscopy, functional group analyses, IR spectrophotometry, and UV spectrophotometry, as well as other supplementary tests as are necessary and sufficient to establish that the proposed substance is characterized.

### 4.2 Methods used in determining the purity of chemical reference substances

The analytical methods to be employed in examining a proposed substance should be considered in relation to its intended use. These analytical methods may be divided into three broad categories, those that require comparison with an external chemical reference substance (e.g. chromatographic or spectrophotometric methods), those that depend solely on an intrinsic dynamic property (e.g. phase solubility analysis and differential scanning calorimetry), and other methods.

*4.2.1 Separation techniques*. The methods used for the determination of purity should be established and validated with system suitability requirements as appropriate for the techniques described below.

4.2.1.1 Chromatographic methods. Methods of analysis based on chromatographic separation are especially useful for detecting and determining impurities in chemical reference substances. HPLC is the most widely used chromatographic method for purity determinations in chemical reference substances, 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 the first two

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