Consultation Document

The International Pharmacopoeia

4. Reference Substances and Reference Spectra

Draft proposal for the Supplementary Information section of The International Pharmacopoeia (January 2013). Please address any comments to Quality Assurance and Safety: Medicines, World Health Organization, 1211 Geneva 27, Switzerland or e-mail to schmidth@who.int. Working documents are available for comment at http://www.who.int/medicines.

[Note from the Secretariat: The Supplementary Information section of The International Pharmacopoeia provides the user with texts for guidance and information and will not constitute part of the standards.]

4.1 International Chemical Reference Substances

4.1.1 Introduction

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

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

This chapter is not applicable to WHO International Biological Reference Preparations.

4.1.2 Terminology

Chemical reference substance

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

Primary chemical reference substance

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

4.1.3 Purpose of ICRS

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

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

4.1.4 Establishment of ICRS

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

Production

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

Compliance with the relevant tests of the corresponding monograph as published in *The International Pharmacopeia* is required where applicable.

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

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

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

A thorough purity investigation is usually performed with the aim to identify and quantify all components of the candidate material (i.e., main component, organic and inorganic impurities, water and residual solvents). The elucidation of the composition of the candidate material is usually considered as accomplished when all components have been identified and quantified, if relevant. The cumulative percentage of all components should yield 100% (mass balance approach).

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

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

$$purity = 100 - \text{organic impurities\%} - \text{inorganic impurities\%} - \text{water\%} - \text{residual solvent\%}$$

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

$$\text{organic impurities\%} = \text{chromatographic result} \times \left(100 - \text{water\%} - \text{residual solvent\%} - \text{inorganic impurities\%}\right)/100$$

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

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

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

**Release and adoption**
ICRS are established and released under the authority of the WHO Expert Committee on Specifications for Pharmaceutical Preparations. The Committee adopts new ICRS and new lots as being suitable for use as described in *The International Pharmacopoeia* or in other WHO quality assurance documents.

**Stability monitoring and distribution**
At the WHO custodian centre for ICRS the established reference substances are stored and distributed under conditions suitable to ensure their stability.

The stability of ICRS is monitored by regular re-examinations. Their frequency and extent is based on the:

- liability of the ICRS to degradation
- container and closure system
- storage conditions
- hygroscopicity
- physical form
- intended use

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

**4.1.5 Use and storage of ICRS by the user**

The letters RS after the name of a substance in a test or assay described in *The International Pharmacopoeia* or in other WHO quality assurance documents adopted by the WHO Expert Committee on Specifications for Pharmaceutical Preparations indicate the use of the respective ICRS.
ICRS are suitable for the analytical purpose described in *The International Pharmacopoeia* or other WHO quality assurance documents adopted by the WHO Expert Committee on Specifications for Pharmaceutical Preparations. Directions for use of ICRS are also given in the leaflet enclosed with the substance when distributed. When used for other purposes the responsibility for assessing the suitability rests with the user or the authority that prescribes or authorizes this use. If reference standards other than ICRS are used for purposes described in *The International Pharmacopoeia* or in other WHO quality assurance documents adopted by the WHO Expert Committee on Specifications for Pharmaceutical Preparations the suitability of these substances has to be demonstrated by the user.

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

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

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

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

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

### 4.1.6 Ordering information

Since April 2010 the European Directorate for the Quality of Medicines and Health-Care (EDQM), Council of Europe, is responsible for the establishment, preparation, storage and distribution of ICRS for *The International Pharmacopoeia*. A list of ICRS currently available can be found on their web site (see http://www.edqm.eu).

Orders for International Chemical Reference Substances should be sent to:

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

The current price for ICRS is 70 Euros per package. Extra charges will be added for the delivery of the reference substances. For details see the above-mentioned web site.
The WHO International Standard for endotoxin is available from the National Institute for Biological Standards and Control (NIBSC).

National Institute for Biological Standards and Control  
Blanche Lane  
South Mimms  
Potters Bar  
GB-Hertfordshire EN6 3QG  
United Kingdom of Great Britain

4.2 International Infrared Reference Spectra

International Infrared Reference Spectra (IIRS) are provided for use in identification tests as described in monographs of The International Pharmacopoeia or other WHO quality assurance documents adopted by the WHO Expert Committee on Specifications for Pharmaceutical Preparations.

The reference spectra are produced from authenticated material using an appropriate sample preparation technique. They are recorded with a Fourier transform infrared spectrophotometer (FTIR). Instructions for the preparation of spectra are given in 1.7 Spectrophotometry in the infrared region; Identification by reference spectrum. A spectrum of the test substance is considered to be concordant with a reference spectrum if the transmission minima (absorption maxima) of the principal bands in the test spectrum correspond in position, relative intensities and shape to those in the reference spectrum.