Reference substances and reference spectra

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1. International Chemical Reference Substances

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 of 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.

1.2 Terminology

Chemical reference substance

The term chemical reference substance, as used in this text, refers to an authenticated, homogenous material that is intended for use in specified physical and chemical tests, in which one or more of 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.

1.3 Purpose of ICRS

The purpose of establishing ICRS is to provide users of *The International Pharmacopoeia* 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 attributes of a sample with those of the reference substance. ICRS serve as such reference substances 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*. [1] In cases of doubtful results or dispute, however, the tests performed using ICRS are the only authoritative ones.

> [1] *WHO Expert Committee on Specifications for Pharmaceutical Preparations. Forty- first report.* Geneva, World Health Organization, 2007, Annex 3 (WHO Technical Report Series, No. 943).

1.4 Production 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* [1] and International Organization for Standardization (ISO) Guide 34 – *General requirements for the competence of reference material producers* (including related guides) have pre-eminence.

Manufacture

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

Candidate material for the establishment of ICRS may be synthesized and purified for this purpose or may be selected from the pharmaceutical production 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 process the reference substance (e.g. by freeze drying) or 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.

The candidate should comply with the relevant test(s) of the corresponding monograph as published in *The International*

Pharmacopoeia, where applicable.

Reference substances are dispensed into suitable containers under appropriate filling and closure conditions, to ensure the integrity of the substance. 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 by the user to assure their suitability.

Analytical characterization

The candidate 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 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, qNMR) 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 depend on the intended use of the reference substance to be established. If required, assay standards are characterized in interlaboratory studies to increase the accuracy of the assigned value.

A thorough purity investigation of the candidate material is performed to verify the presence of all relevant components (i.e. main component, organic and inorganic impurities, water and residual solvents) and to quantify them, if relevant. For standards used for quantitative purposes, the cumulative percentage of all components should yield 100% (mass balance approach).

The purity of a candidate material is 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 mass of sample taken the “as is” content can be calculated as follows:

Purity = 100 – organic impurities [%] – inorganic impurities [%] – water [%] – residual solvents [%]

Formula 1. Formula to calculate the purity of ICRS on an “as is” basis.

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 formula 1, can be calculated as follows:

Organic impurities = chromatographic result x (100 [%] – water [%] – residual solvents [%] – inorganic impurities [%]) / 100

Formula 2. Formula to calculate the percentage of organic impurities, determined by a chromatographic method, on an “as is” basis.

The content assigned to a quantitative ICRS depends on the purity of the candidate material and is specific to the method for which the substance will serve as a reference. If the reference substance is intended to be used with a method that has the same selectivity as 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 detector 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 substance, 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 high-performance liquid chromatography (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 the necessary information 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 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 continuous fitness for purpose.

The fitness-for-purpose of ICRS is monitored by regular re-examinations. Their frequency and extent is based on:

-the stability of the ICRS;
-the container and closure systems;
-the storage conditions;
-the hygroscopicity;
-the physical form;
-the intended use.

The analytical methods employed to verify the stability are chosen among those used 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.

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. The analytical specifications and test methods in these documents are regularly revised to stay abreast of advances in analytical science and regulatory requirements. In addition, the intended use of a reference substance may change. For example, an ICRS previously used only for identification shall also be employed in a quantitative test. Information on the actually established intended uses of an ICRS can be found in the leaflet enclosed with the substance when distributed or accessible via the ICRS online database (see http://www.edqm.eu). The information found in the current leaflets is applicable to all standards of the respective batch number.

If used for other purposes the responsibility of assessing the suitability rests with the user or the authority that prescribes or authorizes this use. If reference substances other than ICRS or other than those listed under Annex 1 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 apply 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 units 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 website 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 not considered to be deleterious to the reference substance. Reference substances stored at -20 °C are packed on ice or dry ice and dispatched by courier. Reference substances stored at -80 °C or stored under liquid nitrogen are packed on dry ice and dispatched by courier.

1.6 Rational to limit the number of ICRS in *The International Pharmacopoeia*

Specifications and test procedures of *The International Pharmacopoeia* are intended to be applicable in all WHO Member States wishing to implement them. Procuring reference substances may, however, be difficult in certain areas of the world due to delays in their delivery and the cost of purchase. *The International Pharmacopoeia* therefore endeavours to reduce the number of reference substances required to perform the included tests and assays. For this purpose the following strategies and practices may be applied during the elaboration of monographs:

-in situ preparation of impurities for identification of related substances/impurities;

-quantification of impurities by comparing their detector responses with the response of the parent compound in a diluted sample solution along with the establishment of correction factors to compensate for differences in the responses of the impurity and the parent compound;

-provision of International Infrared Reference Spectra (IIRS) for use in identification tests;

-provision of assay methods not requiring reference substances, like titrations and ultraviolet spectrophotometry using absorptivity values. These methods shall be provided as alternatives in particular to chromatographic assays in monographs for pharmaceutical substances.

These strategies, however, shall only be applied when, during the elaboration of the methods, evidence has been obtained demonstrating that the intended measures do not compromise the quality of the analytical results or the ability of the tests to conclusively demonstrate conformance to the applicable standards.

1.7 Analytical information provided in the leaflet of the ICRS

The leaflets of the ICRS may provide analytical information, including, but not limited to:

-the IR spectrum of the substance (together with information on the sample preparation);

-additional analytical information generated at the time of establishment;

-the assigned content.

The section “Additional analytical information generated at the time of establishment” provides data about the purity of the reference substance and the methods used for its determination. The information was valid at the time of the establishment of the standard and will not be monitored or adjusted. The information may help the user to understand the calculation of the content that has been assigned to a standard for quantification. It may further be of value to assess risks associated with an unintended use of an ICRS. This information, however, is not given to authorize such an unintended use. As laid down under section 1.5, ICRS are adopted by the WHO Expert Committee on Specifications for Pharmaceutical Preparations for their intended uses only; the responsibility for an unintended use of an ICRS rests with the user or the authority that prescribes or authorizes this use.

1.8 Ordering information

The European Directorate for the Quality of Medicines & HealthCare (EDQM), Council of Europe, is responsible for the establishment, preparation, storage and distribution of ICRS for *The International Pharmacopoeia*. A list of currently available ICRS can be found on its website (see http://www.edqm.eu).

Orders for ICRS 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
Email: orders@edqm.eu

The current price for ICRS per package, as well as the cost for the delivery is available on the above-mentioned website.

2. International Infrared Reference Spectra

International infrared reference spectra 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 sample preparation 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.

3. Biological reference substances

3.1 Reference substances for the microbiological assay of antibiotics

*[**Note from the Secretariat.** It is intended to add a chapter dealing with the biological, in particular microbiological reference substances referred to in* The International Pharmacopoeia.*]*

4. Use of reference substances of other pharmacopoeias

In order to foster harmonization of pharmacopoeial standards and to exploit possibilities for work sharing between pharmacopoeias the WHO Expert Committee on Specifications for Pharmaceutical Preparations may prescribe reference substances that have been established by other pharmacopoeias for use according to *The International Pharmacopoeia*.

The suitability of these reference substances for the additional intended use is assessed during the elaboration or revision of monographs or other tests in which they are mentioned. With the adoption of these texts the WHO Expert Committee on Specifications on Pharmaceutical Preparations authorizes the use of the respective reference substances for the purposes described in *The International Pharmacopoeia*.

To order these reference substances contact the issuing pharmacopoeia. Any question related to the use of such reference substance according to a monograph of *The International Pharmacopoeia* shall be addressed to the Secretariat of *The International Pharmacopoeia* (empinfo@who.int).

The list of reference substances found suitable for use according to *The International Pharmacopoeia* is published on the website of *The International Pharmacopoeia* (http://www.who.int/medicines/areas/quality_safety/quality_assurance/qas_icrs/en/).