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HANDLING AND USE OF REFERENCE STANDARDS IN THE OMCL NETWORK

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Handling and use of reference standards in the OMCL Network

1. Introduction

The ISO Standard ISO/IEC 17025 describes the requirements for the establishment, use and handling of reference standards and reference materials in chapter 5.6.3 “Reference standards and reference materials”.

“The laboratory shall have a program and a procedure for the calibration of its reference standards” (5.6.3.1). Furthermore, it is stated that “checks needed to maintain confidence in the calibration status of reference, primary, transfer and working standards and reference materials shall be carried out according to defined procedures and schedules” (5.6.3.3). ISO/IEC 17025 also requires the elaboration of procedures for safe handling, transport, storage and use of reference standards and reference materials (5.6.3.4).

Experience shows that the handling of compendial (*i.e.* pharmacopoeial) reference standards, reference materials or use of in-house reference standards is not harmonised amongst the different OMCLs. However, the correct use, handling and value assignment of reference standards has a direct influence on the final results of analytical tests.

Therefore, the intention of this document is to give guidance on how to deal with the aforementioned requirements in order to guarantee a harmonised interpretation and application of reference standard usage within the OMCL Network.

This document should be considered as a guide to OMCLs, providing advice for the correct handling of reference standards. It does not, however, replace an SOP nor should it be taken as an exhaustive list of compulsory tests for the establishment or control of a reference standard.

2. Definitions and Guidelines

In the first instance it might be necessary to explain some important terms that are occasionally confused and not always correctly interpreted, such as reference standard, reference material, certified reference material (CRM), primary or secondary standard, working standard and others.

Some useful definitions are given in the European Pharmacopoeia, General Chapter 5.12, Reference standards:

2.1 European Pharmacopoeia chemical reference substance:

A substance or mixture of substances intended for use as stated in a monograph or general chapter of the European Pharmacopoeia. European Pharmacopoeia chemical reference substances are primary standards, except for those (notably antibiotics) that are calibrated in International Units. The latter are secondary standards traceable to the international standard.

2.2 European Pharmacopoeia biological reference preparation:

A substance or mixture of substances intended for use as stated in a monograph or general chapter of the European Pharmacopoeia. European Pharmacopoeia biological reference preparations are either secondary standards calibrated in International Units or primary standards, which may be used to define a European Pharmacopoeia Unit. Other assigned values may also be used, for example, virus titre or number of bacteria.

2.3 Primary standard:

A standard shown to have suitable properties for the intended use; the demonstration of suitability being made without comparison to an existing standard.

2.4 Secondary standard:

A standard established by comparison with a primary standard.

Note: In the European Pharmacopoeia, another type of reference standard is used in the testing of herbal drugs and preparations; namely herbal reference standards (HRS), which are dry extracts, tinctures or dried drugs. They are mainly employed in chromatographic tests or assays, where they may be used for system suitability tests, peak identification or as assay standards.

The ISO Guides 30 to 35 provide definitions of the terms “reference material” (RM), “certified reference material” (CRM), but also of primary and secondary standards as follows:

2.5 Reference material (RM):

Material, sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process.

2.6 Certified reference material (CRM)

Reference material characterised by a metrologically valid procedure for one or more specified properties, accompanied by a certificate that provides the value of the specified property, its associated uncertainty, and a statement of metrological traceability.

2.7 Primary standard

Standard that is designated or widely acknowledged as having the highest metrological qualities and whose value is accepted without reference to other standards of the same quantity, within a specified context.

2.8 Secondary standard

Standard whose value is assigned by comparison with a primary standard of the same quantity.

Similar definitions of primary and secondary standards can be found in the WHO guidelines. From these definitions it becomes clear that pharmacopoeial standards mainly represent primary standards because the values of their properties (*e.g.* assigned contents) are usually not established based on comparisons to other reference standards. However, they should not be confused with certified reference materials as defined by ISO. The latter are often benchmark standards that are employed as comparator substances, which are analysed at the same time as the test sample. These CRMs are used to ensure the suitability of the analytical procedure by demonstrating the final result to be within the uncertainty range. CRMs are also often used for the calibration of instruments.

As pharmacopoeial standards are usually primary standards, they can be directly used in testing procedures, but it is also acceptable to establish secondary standards against these primary standards. Even though there is usually no uncertainty declared for pharmacopoeial standards (except for certain USP standards), consideration should be given to the uncertainty budgets that are added when such secondary standards are established by calibration against primary standards. The establishment and use of tertiary standards, derived from secondary standards, should therefore be avoided.

For the purpose of this guideline, a non-compendial, working or in-house standard can be defined as a standard that is not an official pharmacopoeial standard or an International Standard. It may have been derived as a secondary standard from a primary official standard or a standard supplied by an MAH or be any other substance where the suitability for the intended purpose has been demonstrated by the laboratory that uses the standard.

3. Use of reference standards in an OMCL

3.1 General considerations:

In general, reference standards are established in a particular manner that takes account of the intended use. Standards that are employed in a quantitative test therefore usually require more intensive characterisation than those that are only qualitatively used. Therefore, one of the most important principles to consider is that reference standards must always be used for the purposes for which they are intended. This means that, for instance, pharmacopoeial monographs require the use of pharmacopoeial reference standards that have been established for this particular purpose or secondary standards that have been derived from these primary standards. Therefore, when compendial standards are not available, other non-official reference standards, *e. g.* from marketing authorisation holders, can be used, provided that they have been established for the purpose for which they will be used and that sufficient documentation proving their suitability is available. It is important to consider the expiry or validity dates given to reference standards, given that different suppliers may have different policies. Ph. Eur. standards, for instance, are accompanied (on the website) by a batch validity statement (BVS) and other compendia have similar systems. Non-valid standards must not be used. Vials of pharmacopoeial standards should be used immediately after opening and subsequent storage is strongly discouraged.

3.2 Pharmacopoeial reference standards:

Pharmacopoeial standards do not need any further characterisation by the OMCL as long as they are used for their intended purpose. Detailed information about the establishment and use of Ph. Eur. reference standards can be found in General Chapter 5.12 of the European Pharmacopoeia. For their correct use it is important to know that values assigned to Ph. Eur. assay standards used in physico-chemical assays (CRS) are given on an “as is” basis, *i.e.* after subtraction of impurities, water and solvents from 100 per cent of the total mass. This is not necessarily the case for other Pharmacopoeias.

As mentioned above, pharmacopoeial standards should be used as described in the corresponding monograph. Therefore, a standard that is employed for an identification test and/or qualitatively in a test for related substances does not carry an assigned content and can therefore not be used in an assay. It is not admissible to assume a purity of 100 % for such a standard and then to use it as an assay standard. It should be noted that Ph. Eur. reference standards have a legally-binding status. In the General Notices of the European Pharmacopoeia it is stated that: “The European Pharmacopoeia Commission establishes the official reference standards, which are alone authoritative in case of arbitration”. Similar statements can be found in the United States Pharmacopoeia (USP) and the British Pharmacopoeia (BP). As a

consequence, it is necessary to use a Ph. Eur. reference standard when a European Pharmacopoeia monograph is being applied in testing, USP standards where USP monographs are employed and BP standards for those BP monographs for which there is no available Ph. Eur. standard, *i.e.* often for finished products. This is necessary because the monographs of the different compendia are usually not interchangeable, except if they have undergone a harmonisation process. Alternatively, secondary standards can be established against a primary pharmacopoeial standard and used instead for testing in conjunction with the corresponding Pharmacopoeia.

In exceptional cases such as screening tests, if the OMCL uses non-pharmacopoeial standards for compendial testing, it is the responsibility of the OMCL to demonstrate their suitability for use.

3.3 Non-compendial reference standards:

In certain cases, reference standards may be obtained from marketing authorisation holders (MAH). These are useful, particularly in such cases where the testing is done according to a manufacturer's method and is not covered by a pharmacopoeial monograph. However, before use it must be ensured that the standard has been established for the intended purpose by the MAH and the accompanying documentation must be complete and self-explanatory. The assigned value, if there is any, must be based on the analytical data provided. MAH standards do not represent official standards. For this reason, particularly in case of doubtful results, it could be useful to perform a minimum of testing of the standard, *e.g.* identification and/or purity determination using the chromatographic method in which the standard will be used. Should such a reference standard be used in a method that differs from the MAH method, it is the responsibility of the OMCL to demonstrate its suitability for use. This could be done by additional testing of the standard or adequate system suitability tests. The same is true when non-official reference standards are obtained from certified reference standard producers (ISO 34 certified); their suitability for the intended purpose must be demonstrated by the OMCL.

3.4 Certified reference materials

In an analytical laboratory, a certified reference material (CRM) is mainly used for the calibration of equipment. For this purpose it must be accompanied by a certificate of analysis specifying the assigned value and the attributed uncertainty. It must fulfill the requirements of ISO Guide 35 and be traceable to SI-units by an unbroken chain of calibrations.

4. Non-availability of official reference standards (establishment of in-house standards)

If neither suitable compendial or International Standards nor reference standards from the MAH or from certified producers of reference standards are available, in-house reference standards may be established. It is generally unacceptable for a commercial reagent to be used as a reference standard without further verification of its suitability. In exceptional cases, *e.g.* solvents of high purity used in gas chromatography, it may be acceptable to use a commercial reagent instead of a reference standard, provided the OMCL demonstrates its suitability for the intended purpose.

For the establishment of in-house reference standards the OMCL should have a procedure describing the necessary tests (ISO/IEC 17025 5.6.3) to be performed for its characterisation. The extent of testing required should be appropriate for the intended use of the standard and the quantity of material available. Guidance can be found in the General Chapter "Reference Standards", 5.12. of the European Pharmacopoeia.

Typically, the establishment of a standard that is only qualitatively used, *e.g.* for identification, system suitability tests or peak identification in HPLC and GC, requires less testing than a standard that shall be used quantitatively. In any case, it is the responsibility of the analyst to decide upon the extent of analytical testing, based on their scientific judgment and the data already available in the CoA. The examples given in 4.1 and 4.2 are particularly valid for standards used in physico-chemical tests. If biological products are being established as in-house standards, specific tests that are particularly relevant for those standards and their intended uses may need to be carried out.

4.1 Qualitatively used standards: the identity of the reference standard must be unequivocally confirmed; suitable methods are, for instance, IR-spectrophotometry, mass spectrometry, NMR and CHN-analysis. It is not necessary to carry out all of these tests, but the analyst must make sure that the reference standard is clearly identified before use. In particular, should the substance be used for identification by IR-spectrophotometry, it is recommended to carry out additional chromatographic purity testing and determination of water/LOD, as an insufficient purity may endanger the correct interpretation of the spectrum.

4.2 Quantitatively used standards: more testing is required when a standard will be used quantitatively. In such cases, it is essential that the identity tests are complemented by purity tests, *e.g.* chromatographic, water determination/LOD and possibly determination of residual solvents. The value assigned to a standard used in a physico-chemical assay should be based on the chromatographic method for which

it is intended. The selective methods should be complemented by an absolute method to confirm the mass balance, *e.g.* a non-aqueous titration or other suitable techniques, such as CHN-analysis. If the total mass is not confirmed by an absolute method, inorganic impurities might be present and should be investigated. As content will be assigned to such a reference standard, it is recommended that a second series of tests is performed by a different operator. It is recommended that content be assigned on an “as is” basis using the following calculation:

Assigned value = $(100 - \text{LOD/water/solvents/inorganics}) \times \text{chromatographic purity (\%)} / 100$.

These examples are particularly valid for reference standards used in physico-chemical tests.

With regard to biological testing, usually International Standards, biological reference preparations (BRPs) or manufacturers’ standards are used. The latter two may be secondary standards that have been established against the current, primary WHO standard, or in some cases, primary standards.

5. Filling and storage of standards

In general, compendial standards should be used as soon as possible after receipt, *i.e.* it is recommended to order them when required for a test and not with the intention to store them. If the latter is unavoidable they should only be stored as long as the batch is valid and under the recommended storage conditions. The content of vials once opened should immediately be used. Subsequent storage of such vials bears the risk of moisture-uptake, particularly when they are stored in the refrigerator. As content values are assigned on an “as is” basis, moisture-uptake might therefore modify the content and, as a consequence, erroneous test results may be obtained.

Reference standards obtained from an MAH or a certified reference standard producer should be handled and stored according to the recommendations of the producer. Expiry or re-testing dates have to be defined by the OMCL if they are not given by the producer.

If OMCLs establish in-house standards, either primary or secondary standards, it is recommended to fill/dispense the material in single-use containers in order to avoid moisture-uptake of the bulk material caused by repeated opening and closing of containers. The conditions of filling/dispensing should be defined depending on the characteristics of the substance. Thus, hygroscopic substances, for instance, should be aliquoted in a glove box under controlled humidity. Freeze-drying is a suitable

alternative for producing high-stability standards of water-soluble substances, provided the required equipment is available.

The containers must be clearly labeled with the name and batch number so that traceability can be guaranteed. Also, an expiry date should be defined. Often it may be preferable to define a re-test date that allows for an extension to the validity period, provided that the re-test results confirm suitability of the batch.

In order to guarantee controlled handling of the reference substances, access to the standards should be restricted and a responsible person should be nominated.

6. Monitoring or re-testing of standards

For all non-compendial reference standards, a procedure for re-testing should be in place (ISO/IEC 5.6.3.3 Intermediate checks) in order to guarantee their continued "fitness for use". For this purpose it is not necessary to re-establish the standard, but properties that are subject to change, *e.g.* chromatographic purity and water content, should be checked. As is the case during establishment of a standard, the extent of testing depends on the intended use of the reference standard and is, ultimately, left to the discretion of the analyst.

The testing intervals should be based on the known or predicted stability, the physico-chemical characteristics and also the intended use of the standard. An assay standard with an assigned content, for instance, might require more frequent re-testing because even a minor degradation or other modification of its characteristics may change the value and therefore compromise the results obtained in assays in which the standard is used.

No monitoring is needed for pharmacopoeial standards as long as they are stored as recommended and used within their period of validity. After that date they should be discarded.