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Good practice in reference material preparation

Bonne pratique pour la préparation des matériaux de référence

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Contents

Page

Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO document should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 334, *Reference materials*.

This first edition of ISO/TR 33402 cancels and replaces ISO Guide 80:2014, which has been technically revised.

The main changes are as follows:

— this document provides guidance for the preparation of reference materials and does not include information about characterization or the assessment of homogeneity and stability;

— the scope of this document has been broadened to include all types of matrix reference materials and not only reference materials used for statistical quality control.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Reference materials (RMs) are widely used in measurement laboratories for a variety of purposes, and it is important to ensure that the material most appropriate for a particular application is used. Certified reference materials (CRMs), i.e. those which have at least one certified value with associated uncertainty assigned by a metrologically valid procedure, are primarily used for method validation and calibrations providing metrological traceability.

While many RMs do not require characterization by metrologically valid procedures, they can be prepared to meet specific measurement requirements, including quality control. The key requirements for these RMs are sufficient homogeneity and stability, with respect to specific properties, for the intended use. Proper preparation processes can ensure the material's homogeneity and stability.

This document provides general information on key steps in material preparation of candidate matrix RMs. It is intended for laboratory staff involved in preparing and using matrix materials for specific applications. Reference material producers (RMPs) can also use it as an information source for the preparation steps of RM production.

The document includes case studies highlighting key considerations in RM preparation. Most of the case studies describe the production of matrix RMs used for statistical quality control and include information about the preparation of the materials as well as additional information about the characterization of the property values and the assessment of homogeneity and stability, as applicable.

The general requirements for the competence of reference material producers (RMPs) are outlined in ISO 17034, specifying necessary sample preparation steps. ISO 33405 covers guidance for assessing homogeneity and stability, characterization, and value assignment of property values. ISO 33403 provides guidance for the correct use of RMs. The requirements and guidance in these documents rely on the competent preparation of the candidate RM. However, preparation steps, especially for candidate matrix RMs, are intricate, and there is a lack of guidance focusing on these steps.

Good practice in reference material preparation

1 Scope

This document gives general information on the key steps for the preparation of candidate matrix reference materials (RMs) including the material specification, sourcing and selection of bulk material, and the processing of the material, which are important steps for the production of matrix RMs.

The document provides information on the preparation of candidate RMs for laboratory staff who prepare and use matrix materials for their specific applications. This document can also be used by reference material producers (RMPs) as an information source for the preparation of the RMs that they produce.

This document also offers examples of specific case studies covering the preparation of matrix RMs in different fields of application (see Annexes A to F). These are not complete "production manuals" but highlight key considerations for the preparation steps of RMs.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO Guide 30, *Reference materials — Selected terms and definitions*

ISO/IEC Guide 99, *International vocabulary of metrology — Basic and general concepts and associated terms (VIM)*

ISO 3534-1, *Statistics — Vocabulary and symbols — Part 1: General statistical terms and terms used in probability*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO Guide 30, ISO/IEC Guide 99 and ISO 3534-1 apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

4 Overview of preparation of candidate reference materials (RMs)

Many RMs and CRMs are produced by RMPs and are commercially available. However, laboratories conducting routine tests frequently encounter difficulties in acquiring matched matrix RMs that possess a comparable matrix and analyte content level, or even just one of these aspects. In cases where matched matrix RMs are challenging to obtain from the market, the capability to prepare samples closely matched to those used in routine tests becomes crucial.

For such RM users, the preparation of homogeneous and stable materials prior to conducting assessment of homogeneity and stability is crucial. If the preparation steps are inadequate to ensure a sufficient level of homogeneity and stability, the material will not be suitable for the intended use. Therefore, the preparation of any candidate RM requires a level of technical and organizational competence. It is acknowledged that in

many cases the candidate RMs are prepared by technically competent staff that are knowledgeable about the materials and processes being used.

This document focusses on the steps involved with the preparation of RMs. The key steps involved in the production of a matrix RM are summarized in the flow chart in Figure 1 and are described in more detail in References [12] and [13].

The following clauses offer detailed information on material specification (Clause 5), sourcing and selection of bulk material (Clause 6) and material processing (Clause 7). More guidance on the rest of the production steps, which includes the characterization, as well as the assessment of homogeneity and stability is covered in ISO 33405. Any requirements for the production of RMs can be found in ISO 17034.

Materials can be sourced from, and processed by third parties, where they have specialized equipment and/or expertise. Materials can even be products that are commercially available and meet the user's specifications (e.g., food products available in appropriately sized units from a single production batch).

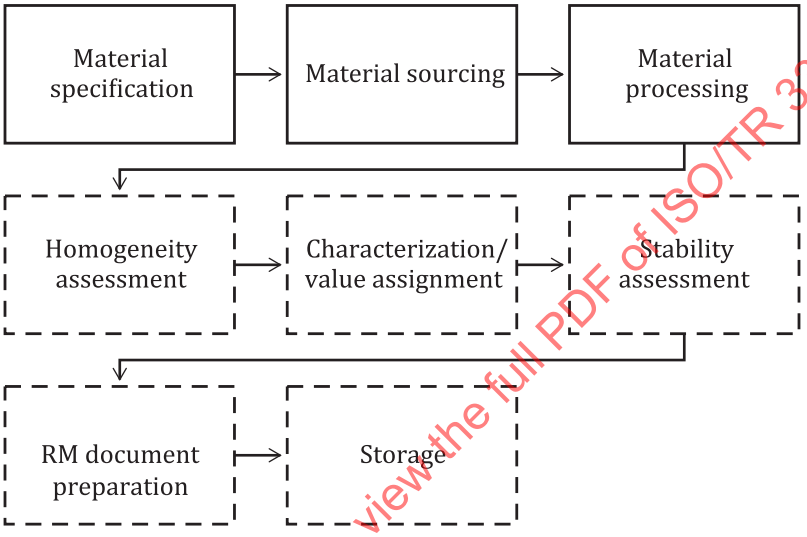


Figure 1 — Key steps in the production of RMs. This document focusses on the steps for the preparation of an RM (blocks with a solid black outline).

5 Material specification

5.1 General

The specification of a candidate matrix RM is a crucial consideration. The intended use, including the acceptable level of uncertainty for the measurement or test results, is an important consideration for both the production and use of all RMs. Key criteria include similarity to real samples and availability in appropriate quantities. In practice, the impetus for the preparation of such candidate RMs can often be the fact that adequate matrix RMs are not available. Therefore, the specific matrix and property combination for the test sample in question are likely to be used, and matching is not an issue for laboratories.

5.2 Matrix type and matching

In general terms, the uncertainties associated with a measurement result from a homogeneous sample arise from the two main stages of the measurement procedure:

— the preparation of a sample comprising digestion, extraction, clean-up, etc.;

— the measurement of the property in the prepared sample by a suitable technique.

The key criteria in the specification and selection of an RM are therefore for the material to be as similar as possible to real samples and available in appropriate quantities.

The matrix of the RM needs to be the same or as similar as possible to the matrix of the routine test samples, so that a satisfactory result for the RM is genuinely indicative of satisfactory results for the test samples. This matrix matching requires some knowledge of the analytical procedure used on the routine samples, so that a judgment can be made as to the degree of variation of the physical/chemical properties of the sample and test matrices that can cause them to respond differently to a particular measurement procedure. For example, a freeze-dried food matrix can behave differently during analysis to a similar foodstuff with higher moisture content.

Commutability has particular significance in clinical chemistry and has been described elsewhere[14].

5.3 Properties and property values

For all RMs, the properties of a candidate matrix RM are crucial for its intended use in measuring routine test samples.

When the material is employed to verify quantitative measurement results, having a property value close to the mean value of typical test samples or values near the decision limit for the application becomes crucial. This can be verified through preliminary screening measurements on several candidate source materials to ensure the selection of the most appropriate one.

In situations where an RM is used for the statistical control of a measurement method using a quality control chart, the important characteristic of the RM is that its matrix is closely resembling that of the test sample.

For drift monitoring, the important characteristics of the RM include stability and the ability to provide a measured signal that minimizes counting statistical uncertainty. An optimal drift monitor material can have a higher concentration of the measurand compared to the test sample.

5.4 Unit size

Unit size is the amount of material that comprises a single unit of the RM. When preparing a candidate RM, the size of individual units is based on the likely use, i.e., the amount of material needed for the measurements concerned and whether the units are to contain sufficient material for a single analysis or multiple measurements.

5.5 Total bulk amount of material

An estimate is needed of the total bulk amount of candidate matrix RM that needs to be sourced. In principle, this can be estimated by considering:

— the expected number of units needed for the lifetime of the material;

— the expected number of units needed for homogeneity and stability testing and characterization (as applicable);

— the unit size;

— the preparation yield;

— the quantity of material that can readily be homogenized;

— the assumed stability of the material;

— the type and size of the storage facility.

6 Sourcing and selection of bulk material

Sourcing of bulk materials for RM production can at first seem difficult, especially in those cases where large quantities of material are needed. However, there are several options that are available including:

— leftover sample material from testing activities;

— accurate gravimetric formulation.

Processing the bulk material can have significant cost implications for the production of RMs and simple, straightforward processing methods need to be used to ensure cost-effective RM production. The sourcing of the material usually considers the difficulty and cost implications of the processing of the material. The exact preparation procedures to be followed for a particular RM will depend on the nature of the matrix and the properties of interest.

In general, liquid matrix RMs are much easier to produce than their solid counterparts. The main reason for this is that homogeneous liquids can easily be achieved even with rudimentary equipment (e.g., large mixing containers equipped with paddles or magnetic stirrers). A liquid is easily spiked, filtered, or mixed with additives and stabilizers. The corresponding processes for solid materials, milling, grinding, mixing, and sieving are much more difficult to accomplish homogeneously, especially for quantities greater than 20 kg. These techniques require a significant investment in major capital equipment when large-scale preparation is envisaged.

When sourcing biological materials for example, for control of measurement procedures for medical laboratories, the following specific issues need to be considered:

— ethics of the retention and use of residual patients' samples for the production of RMs;

— legal liabilities of retention and use of residual patients' samples purchased for the production of RMs;

— medical laboratories creating RMs need to have a high degree of confidence in the identity of the material selected, to avoid use of misidentified organisms;

— materials sourced for RM production are screened for potential risks including health hazards, especially if the processing includes the use of contaminated sharps or has the potential for aerosol formation.

7 Material processing

7.1 General

Once the bulk material has been sourced for the candidate matrix RM, there are several processing stages that need to be carried out to ensure the candidate matrix RM has the appropriate homogeneity and stability for its intended use. Take care to ensure consistency in processing across multiple days. Some of the more common processes are described in 7.2 to 7.10.

7.2 Avoidance of contamination

For all candidate matrix RMs, it is important to prevent contamination by substances which can potentially interfere with the intended measurement process (e.g., a similar material or contamination of a blank material). Hence, all containers are carefully cleaned and dried before filling to remove possible contaminants.

In addition, consideration needs to be given to the possible interaction of bulk material with processing equipment and/or leaching of contaminants/impurities from the processing equipment parts, or the container, into the bulk material.

7.3 Drying

Removal of water makes candidate matrix RMs far easier to handle and improves both their transportation and long-term stability. Drying of soils and similar matrices is carried out at ambient or elevated temperatures, depending on the properties of interest, since the more volatile components could be partly lost at higher temperatures. Water removal also reduces the likelihood of microbial growth formation, which is a particular problem with biological materials. Freeze-drying is a technique which is useful with temperature sensitive properties or matrices.

7.4 Milling and grinding

For solids, some form of crushing, milling, grinding and particle size reduction is often necessary to ensure uniform particle size and to improve homogeneity of the candidate matrix RM. For large quantities, these processes are slow and can take several days to complete. Take care not to introduce contamination from the apparatus during the grinding process. The health and safety aspects of grinding large quantities of particulate matter, which could have toxic components, needs to also be considered. Cryogenic grinding at –78 °C (solid CO_2) or –196 °C (liquid N_2) could be necessary for polymers, biological, oily/fatty, and thermally labile materials.

Specialised equipment can allow producing a material with a smaller particle size than laboratory samples, which can lead to changed extraction or digestion behaviours. This can result in the reference material not being representative of the real sample anymore. It is therefore important to ensure that also the particle size of the RM is representative for real samples.

7.5 Sieving

Sieving is often carried out after milling and grinding to improve the homogeneity of the candidate matrix RM. Particulate materials such as soils, ores, ashes, and ground biological materials are passed through a standard sieve to remove large particles that are above a prescribed size.

Sieving, however, changes the matrix composition. If a large fraction is removed by sieving, the analyte concentration can change, and the matrix can no longer reflect the composition of regular test samples.

7.6 Mixing and blending

When the candidate matrix RM is in solid form, it is homogenized by thorough mixing, using for example a roll-mixer, shaker, or end-over-end mixer. Such mixing is carried out after milling, grinding, and sieving.

Blending of two or more materials with sufficiently similar matrix compositions and differing property values can enable the production of RMs with a desired property value, a set of similar RMs covering a range of property values, or the production of RMs from an existing RM.

To obtain homogeneous mixtures, the materials to be mixed need to have similar densities and particle size distributions.

7.7 Filtration

Filtration of solutions before bottling removes any particulate and fibrous solids that would compromise the homogeneity of the bulk candidate matrix RM. However, some liquids cannot be filtered due to:

a) viscosity;

b) potential loss of active ingredients by adsorption to the filter;

c) the introduction of contamination. Qualification of the filter is critical to avoiding loss of active ingredients.

Typically, liquids such as waters and leachates, are filtered through a 0,45 µm filter prior to bottling or ampouling.

7.8 Stabilization

Certain analytes in the candidate matrix RM are unstable and therefore need to be stabilized at the bulk stage of the preparation procedure. Metals, for example, can precipitate out of neutral or alkaline solutions because of hydrolysis or oxidation, and adjustment of the pH of the solution to below 2 counteracts this problem. Copper at a concentration of 1 $mg \cdot l^{-1}$ has been used to counteract algal growth in aqueous solutions. Different materials can require other approaches such as addition of antioxidants, preservatives, texture stabilizers, etc.

7.9 Sterilization

Prepared soils, sewage sludges, and biological materials can contain persistent pathogens that are potentially harmful to humans. They can also contain spores that cause fungal moulds to develop on storage, which could initiate changes in either the composition of the bulk material or the individual units. Such organisms need to be destroyed before the final units are prepared and packaged.

Before sterilizing any candidate matrix RMs, it is important to consider the impact of the proposed sterilization process on the properties of interest and/or the matrix, particularly those which degrade at elevated temperatures.

Autoclaving is an inexpensive and convenient means of sterilization that can be used for materials that are temperature resistant, for example, metals in sediments. Autoclaving can be done on the bulk material prior to final homogenization and unit preparation or on the final samples. However, it is important to ensure that the core of the material reaches 121 °C.

Irradiation can be used on the final packaged units (e.g., ampoules, bottles, or pouches). Gamma irradiation is a convenient means of sterilization at ambient temperature so changes in matrix composition are less likely than with autoclaving. Dose values need to be determined such that they are effective in removing pathogens but do not adversely affect the material by, for example, raising the temperature to unacceptable levels (e.g., chocolate). However, gamma irradiation is beyond the means of most laboratories, requiring specialist subcontractors.

Sterilization is performed after subdivision and packaging has been completed, and the material is in its final packaged form, otherwise the material will not be sterile.

7.10 Subdivision and packaging

7.10.1 General

The last steps of the material processing are subdivision and packaging. Subclauses 7.2 and 7.3 describe some of the key considerations for the subdivision process and choice of containers to ensure the RMs are sufficiently homogeneous and stable for their intended use.

Some candidate matrix RMs are used as bulk materials and do not need to be packaged into individual units. In-house reference materials are often not distributed and are therefore not always packaged into units, but subsamples are taken from the bulk prepared material as and when needed.

7.10.2 Choice of containers

For RMs to be prepared cost-effectively, one aspect that needs careful consideration is the choice of appropriate containers for the individual units. If unsuitable containers are used, the material could quickly degrade. The type of container used depends on the inherent stability of the material and the length of time it is expected to remain stable. For particularly susceptible materials, two layers of containment (e.g., a vial within a polyethylene bag) can provide additional protection against degradation and contamination.

The following examples serve to illustrate the need for careful consideration of the container and its closure.

— Materials can either lose or pick up moisture if the container is not securely closed. Glass containers with screwcaps fitted with “polycone” inserts[1)] are preferable to simple screw caps. Sealed cans, foil pouches, or septum-lined crimp-top vials offer more security.

— Oxygen sensitive materials need to be prepared and sub-sampled under an inert gas atmosphere (nitrogen or argon).

— For aqueous samples containing low concentrations of metals (e.g., mg/kg or below), glass containers are not recommended because of possible adsorption of the metals onto the walls over time. High-density polyethylene (HDPE) bottles with screwcaps are more suitable for this application but have the potential

1) Polycone liners are cone-shaped polyethylene cap liners that provide a better seal than simple wadded cap closure.

problem of loss of water by evaporation through the bottle walls. This can be minimized by storage in a refrigerator (rather than at ambient temperature) or using fluorine-treated polyethylene bottles.

— The possibility of contamination of the RM by the leaching of impurities from the container also needs to be considered. For example, the iron content of canned foodstuff RMs could be subject to unpredictable increases on a can-by-can basis, as iron leaches from the can wall into the food matrix. Bottles (whether glass or HDPE) containing aqueous acid solutions could also give rise to leaching problems. As a rule, containers that can interact with the RM need to be carefully evaluated before use by suitable leaching trials.

— For relatively inert matrices, such as soils and other dried environmental or biological materials, screw-cap glass jars are usually satisfactory. Amber glass gives additional protection against degradation induced by light.

— RMs comprising relatively volatile components susceptible to evaporation, such as some organic solvents, will normally require a septum-lined crimp-top, glass vial, or flame-sealed glass ampoules. It is preferable for vials and ampoules to be amber to reduce the impact of light.

Some preliminary experimental work, including blank and stability studies, can be necessary to identify the most suitable container type to use for a particular RM.

If the unit size is larger than the sample size taken for a measurement, then consideration must be given to the stability of the material for reuse according to the requirements of ISO 17034. The effect of repeated opening and closing of the sample containers is also assessed if repeated use of the material is anticipated. If a material is unlikely to be stable once opened, then it is preferable to restrict the unit size to a single use portion.

Tamper evident closures need to be considered if the unit is only intended to be used once.

7.11 Subdivision procedures

Once a homogeneous bulk candidate matrix RM has been produced, the essential requirement of any subdivision process is that the homogeneity of the material is maintained. Ensure that the sub-division process itself, or the time taken to complete the subdivision of bulk material, does not re-introduce heterogeneity into the material. This could conceivably occur in several ways.

Matrices comprised of mixtures of liquids of differing volatilities (e.g., ethanol in water) can undergo selective evaporation of one component during a prolonged subdivision run, causing a rising or falling trend in property value from the first to the last units produced. Effects of this sort can be minimized by protecting the bulk material from evaporation and by completing the subdivision in as short a time as is consistent with accurate dispensing.

All liquids and solutions need to be stirred continuously while individual aliquots are being dispensed. Solutions need to be filtered before dispensing commences if particulates are likely to be present to an extent that could affect the properties of interest.

Take care with solid particulate matrices such as soils, sediments, industrial products, etc. to ensure that segregation of finer particles does not occur during subdivision. Take special care when sampling bulk material from a large drum, to ensure that there is no vertical segregation. Riffling is a process for representatively subdividing free-flowing powdered materials so that each aliquot receives similar particulate fractions. When operated effectively, riffling minimizes flow segregation and produces units with a low between-unit variation. Commercial riffling devices can be used to subdivide such materials without introducing heterogeneity. Sampling and subdivision of particulate materials are described in more detail in ISO 14488[15].

In food matrices with a high fat content (e.g., mackerel paste), there could be a tendency for the fat to separate as a discrete phase. If such effects occur, the matrix needs to be stirred continuously during dispensing and/or additives included in the matrix to slow down the separation process.

As a general principle, subdivision of a bulk material is completed as quickly as possible to minimize the opportunities for the matrix to revert to heterogeneity. Where appropriate, take steps to maintain a homogeneous bulk material during the subdivision process. It could be necessary to discard the first and/or

last portions dispensed from the bulk material, especially of complex matrices that are especially prone to segregation effects.

In the case of RMs intended for trace analysis, take special care not to introduce additional impurities (e.g., from the air, apparatus, laboratory vessels, etc.) during subdivision of the material as this could change the property value being measured.

Annex A
(informative)

Case study 1 — Production of a quality control material (QCM) from coal[2)]

A.1 Objective

A coal testing laboratory uses a quality control material (QCM) for daily quality control for proximate and ultimate analysis in accordance with the applicable ISO standards. One can of 1 L, holding approximately 1 kg coal, is sufficient for checking the analysis results for a week. The laboratory would like to use the material for one year and calculates that it needs 100 kg of starting material. The starting material, as delivered, is 50 mm in top size.

The laboratory is interested in a QCM that represents blended coal of the type used in power plants.

A.2 Sampling

The samples are mechanically removed from a conveyor belt, crushed, and sieved to a top size of 10 mm and split into 6 portions of 10 kg per sample. In total, 12 samples are taken from the blend. The laboratory receives 12 plastic bags, each containing 10 kg of blended coal.

A.3 Checking the suitability of the material

The laboratory takes a sample from one of the bags and prepares it for analysis. It determines the volatile matter, and ash contents, gross calorific value (proximate analysis), as well as the contents of carbon, hydrogen, nitrogen, and sulfur (elemental analysis). These results confirm the suitability of the material with respect to the content levels and calorific value.

A.4 Sample preparation

The coal is dried in air at ambient temperature to remove the excess water, sieved, split into 10 portions, and subdivided.

For the subdivision, a laboratory riffler is used with 10 tubes. To eliminate possible differences between the bags, the subdivision scheme shown in Table A.1 is used.

2) This case study was provided by Adriaan M.H. van der Veen, NMi Van Swinden Laboratorium (VSL) B.V., Thijsseweg 11, 2629 JA Delft, The Netherlands. Subclauses A.1 to A.4 focus on the preparation of the RM.

Table A.1 — Subdivision scheme

01	02	03	04	05	06	07	08	09	10		
↓	↓	↓	↓	↓	↓	↓	↓	↓	↓		
01.01	02.02	03.03	04.04	05.05	06.06	07.07	08.08	09.09	10.10	→	A
01.02	02.03	03.04	04.05	05.06	06.07	07.08	08.09	09.10	10.01	→	B
01.03	02.04	03.05	04.06	05.07	06.08	07.09	08.10	09.01	10.02	→	C
01.04	02.05	03.06	04.07	05.08	06.09	07.10	08.01	09.02	10.03	→	D
01.05	02.06	03.07	04.08	05.09	06.10	07.01	08.02	09.03	10.04	→	E
01.06	02.07	03.08	04.09	05.10	06.01	07.02	08.03	09.04	10.05	→	F
01.07	02.08	03.09	04.10	05.01	06.02	07.03	08.04	09.05	10.06	→	G
01.08	02.09	03.10	04.01	05.02	06.03	07.04	08.05	09.06	10.07	→	H
01.09	02.10	03.01	04.02	05.03	06.04	07.05	08.06	09.07	10.08	→	I
01.10	02.01	03.02	04.03	05.04	06.05	07.06	08.07	09.08	10.09	→	J

Starting with the 10 bags (top row), 100 subsamples are made by dynamic riffling. The numbering of the subsamples reveals the sample from which it is subdivided (first pair of digits) and from which tube of the riffler it stems (second pair of digits). The subsamples are combined in such a fashion, that each composite sample A through J contains one subsample from each bag and one subsample from each tube of the dynamic riffler.

In a second step, the 10 composite samples A to J are riffled again to give 100 samples. The samples are put into small plastic bags in cans. From 10 cans, chosen at random, 2 subsamples are taken for a homogeneity test. The samples for the between-bottle homogeneity study are analysed for moisture and ash content, and gross calorific value.

The cans containing the 100 samples are closed and labelled with the date of blending, and the sequence number obtained from the second sub-sampling. Composite sample A delivered cans 1 to 10, and so on. The laboratory considers preservation of the history from the sample production essential to support a root cause analysis if needed later.

A.5 Between-bottle homogeneity study

The between-bottle homogeneity study is carried out with two replicates on 10 cans from the batch of 100. One-way analysis of variance is used to determine the between-bottle standard deviation[22]. Previous experience has shown that for the selected parameters (ash content and gross calorific value) the between-bottle standard deviation needs to be smaller than the repeatability standard deviation of the tests. For both parameters, this objective is achieved in the homogeneity study.

A.6 Characterization

The laboratory monitors its quality using a Shewhart chart. The standard deviation is taken from a previous chart from a similar blend. The mean value is obtained from 10 measurements from one can, taken over 10 consecutive days. On days 1 and 10, a CRM was analysed as well to confirm the laboratory results. The QCM was used with the mean from these 10 measurements, after carefully scrutinizing the data. The data analysis indicated no irregularities.

Annex B
(informative)

Case study 2 — Production of geological or metallurgical quality control materials (QCMs)[3)]

B.1 General

The materials produced include various geological or metallurgical particulate materials sourced from customers of the analytical facility (matrix matched), typically in the order of 600 kg each. This includes, but is not restricted to, ores, concentrates, feeds, tails, slags, and un-mineralized rock, soils, or sediments.

B.2 Project initiation

The need for RM production generally stems from the difficulty in sourcing a suitable commercial RM for the analysis of a material of a unique sample matrix.

B.3 Sourcing of material

The following factors are considered.

— The matrix of the material to be as close as practically possible to the samples for which it will be used as a quality control. By mixing mineralized ore with barren material/ lower grade ore of similar overall composition, materials of different grades and a predetermined matrix can be manufactured. Once the material is of the requisite composition it can be prepared into one homogenous bulk RM.

— Materials are stored and prepared in separate facilities according to their grade to prevent cross contamination. Precious metal concentrates can require additional safekeeping procedures.

— The quantity of material needs to be sufficient to last for the duration of an analytical campaign or at least long enough that it can be replaced in good time, allowing for some overlap period during which subsequent materials are used in conjunction.

— The value or toxicity of the material could be such that it would not be safe to handle large quantities, nor feasible to obtain large quantities.

B.4 Processing of RM

The processing encompasses the crushing, pulverization, screening, splitting, and packaging of solid geological/metallurgical materials into “homogenous” pulps. The term “homogenous” is used with caution. These materials normally consist of 100 % sub 75 µm or 43 µm particles below which the material consists of discrete mineralogical grains. The term “homogeneity” naturally has a mass constraint and is often assessed purely upon a “fit for purpose” basis.

Equipment used:

a) jaw crusher capable of crushing to 100 % passing 5 mm;

b) closed circuit mill capable of producing 50 % 43 µm or greater;

3) This case study is based upon information provided by Vicky Anderson of Anglo Research, 8 Schonland Street Theta Johannesburg, P O Box 106, Crown Mines, 2025 Republic of South Africa. Subclauses B.1 to B.6 and B.8 focus on the preparation of the RM.

c) ultrasonic sieve shaker with 75 µm or 43 µm screen;

d) large 10 cup rotary splitter;

e) 100 L plastic drums;

f) V-Blender or equivalent;

g) 50 L plastic drums;

h) large balance (scale);

i) packing machine;

j) assorted marking pens, polythene bags, labels, and packing tape.

B.5 Crushing, blending and milling source material

Prior to sampling, the component materials for grade determination purposes, the material is be crushed to 5 mm or finer to minimize the sampling error. Crushing is usually accomplished using a laboratory jaw crusher, but other crushers can be used to expedite the process.

Sampling stockpiles is inherently difficult and prone to sampling error due to segregation. The following procedure has been adopted to obtain an approximate grade.

— Sample 30 samples of about 500 g each at random intervals from the stockpile at random heights. Try to sample the inside of the stockpile as best as possible.

— Composite the sample and riffle split it down to about 5 kg.

— Mill the riffled composite and analyse in the usual manner.

The planned final grade of a RM is calculated from the grade of its components. The accuracy of the grade calculation of the components, and thus the mixture, is predominately determined by the sampling error. The composition of the mixture is simply determined as follows. The concentration c of element i,

$$c_i = \sum c_{ij} w_j$$

where

c_{ij} is the concentration of element i in component j;

w_j is the mass fraction of component j.

For example, if a low-grade material contains 0,50 g/t Cu and a feed 4,00 g/t Cu, a grade calculation for a mixture of 100 kg blank and 400 kg feed will be as follows:

c_i = 0,50 * 100/(100+400) + 4,00* 400/(100+400) = 3,30 g/t Cu

Prior to milling the material, the crusher products are blended in a mixer or V blender adding the final proportions of the different crusher products. If the amount of material is more than the mixer can accommodate, several batches are made up comprising approximately the same proportions of the components.

Closed circuit mills are preferred for their blending properties. Dry milling is preferred to avoid leaching or altering the oxidation state of the source material. Mill pots are thoroughly cleaned prior to milling a new RM but not between milling cycles of the same material. Once approximately 100 kg of milled material has accumulated, screening commences while the milling is completed. Materials are screened at the expected top size since more malleable metal particles can tend to resist size reduction unlike the associated silicate or sulfide gangue. Oversized particles are recycled to the mill. The material passing the screen is

used for manufacturing RMs. Before using the large ultrasonic screen, it is inspected for holes exceeding the specification and for damage at the edge of the screen. The screen is thoroughly cleaned before use. The process is continued until less than 1 kg of oversize material remains. This is then discarded in an environmentally responsible manner.

B.6 Homogenizing milled material

The final screened fraction is divided into several equal mass portions such that each portion can be accommodated in the V-Blender. These portions are designated Drum 1, Drum 2, Drum 3, etc. Note: if these do not fit into the blender then they are split further. The blender is not filled to maximum capacity; otherwise, it will not function adequately.

Prior to commencing the blending step, the material is dried to ensure that it does not contain excess moisture that would cause it to form clumps that do not disaggregate in the V blender. A sub-sample is tested for moisture by drying a portion at 60 °C overnight. If the sample contains > 2 % moisture, the entire batch is dried at 60 °C before further processing. The moisture content is recorded. Samples are not dried at a higher temperature lest minerals such as pyrrhotite or gypsum alter into other species.

Each of the original drums Drum 1, Drum 2, Drum 3, etc. (which are of equal mass) is be homogenized for at least four hours in the V blender that is not filled beyond the mark. For a 600 kg RM, there needs to be four drums of about 150 kg each for blending in a V-Blender of 100 L capacity (50 L to the mark). The material in each of the homogenized drums is split using a cleaned, large 10 cup rotary splitter. The feed rate and rotation rate of the rotary splitter are adjusted so that each cup receives a minimum of 30, but preferably 35 increments. Each split is weighed. If the percent relative standard deviations (%RSD) of these weights exceed 2 %, the splits are recombined, blended, and split again. Each acceptable split from each drum is designated A, B, C….to J. The A's, B's, C's … J's are combined into 10 new composites. V-blend each of the new composites (A to J) for 4 h. Rotary split each homogenized drum using the large 10 cup rotary splitter again checking the %RSD of splitting. Each split from each drum (A, B, C, etc.) is designated 1 through to 10. Splits 1 to 10 from drums A to J are combined into 10 new composites (repeat of earlier procedure). These 10 new composites relabelled, for example, D1 to D10, are each blended in the V-Blender for 4 hours (see Figure B.1).

Homogeneity tests are performed on the 10 composites. If homogeneity testing fails and blending is implicated, repeat the rotary splitting and recombination of each drum and blend for 4 h (another repeat of the earlier procedure). Repeat the homogeneity test again.

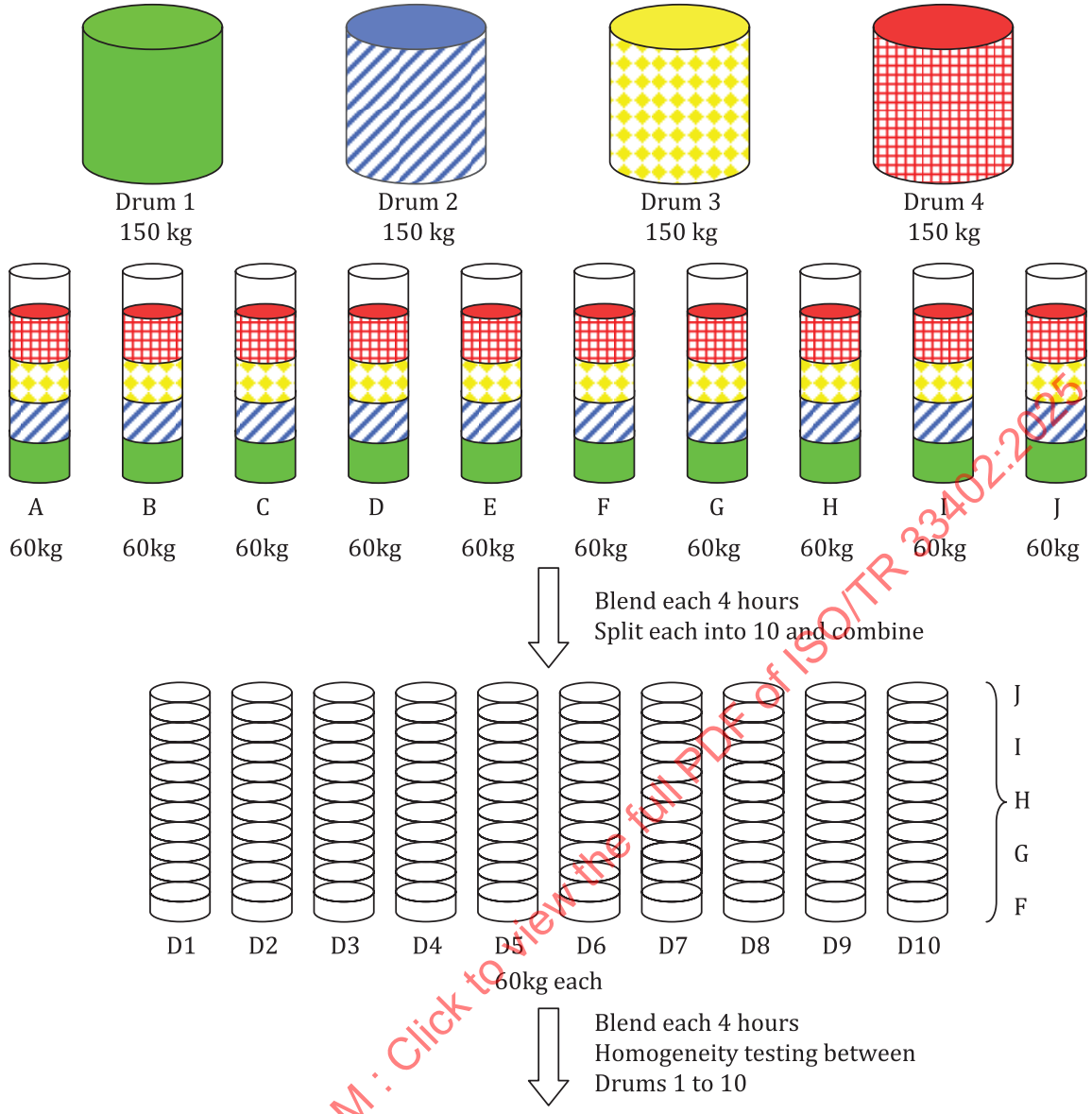


Figure B.1 — Flow diagram of splitting and blending

B.7 Homogeneity testing

From each of the ±60 kg splits (D1 to D10), a minimum of five random samples are submitted to the laboratory, preferably for analysis by the method that will ultimately be the routine method of choice for the material. The samples are taken at different points across the surface of the sample and at different depths using a clean auger sampler. A random systematic or stratified random technique is acceptable.

When these samples are submitted to the laboratory, the order is stratified on the worksheets. In other words, the first sample from each split/drum is analysed in sequence, followed by the second sample from each split/drum, etc. If the samples from the splits (1 to 10) were analysed sequentially, bias, and instrumental drift could produce statistical differences that are not a function of the composition of the samples, but rather a result of the analytical process. The samples are submitted in the predetermined sequence.

The data received from the laboratory are transferred to an Excel[4] spreadsheet for manipulation and statistical analysis. Outliers for the full array of data are first identified using Chauvenet's principle (see Table B.1).

Table B.1 — Use of Excel to determine Chauvenet outliers

Objective	Excel Formulae
Mean	=AVERAGE(array)
Standard deviation	=STDEV(array)
Count	=COUNT(array)
Rejection probability (RP)	=1/(2*count)
Range	=-NORMSINV(0,5*RP)
Rejection limit (RL)	=range*stdev
Chauvenet outlier	=if(ABS(value-mean)>RL, fail, pass)

Outliers are hence selected based on the standard deviation of the data. By removing outliers from a data set, the standard deviation is altered, and a second set of outliers is identified and subsequently a third. The successive sets of outliers are only removed with caution, as at this stage the objective is to determine the spread of the data and not to assign an accurate mean. As a rough guide, if it is necessary to reject > 10 % of the data to obtain an acceptable % RSD, repeat analyses are requested before a final decision is made (rejected data are those suspected of poor analyses).

If the material shows unacceptable overall precision (the overall variance is higher than expected for the material, the grade, and the laboratory/method requirements), the production process is stopped, and the cause of the poor precision determined. Material inhomogeneity can be the result of insufficient particle size reduction or poor mixing. In the former case, further mixing of the material will not result in an improvement of the overall error. Poor overall precision could, for example, be attributed to poor screening, insufficient grinding/crushing, excessive grinding/crushing or poor quality assays. Further progress in preparing the material depends on the cause of the poor precision. A simple skewness test is used to indicate if the distribution formed by the pooled data is positively skewed or not. Positively skewed data arising from underlying Poisson or log-normal distributions can complicate the value assignment process and could indicate insufficient grinding/crushing. Limits for significantly skewed tables are determined at a 90 % confidence limit.

A single factor ANOVA is used for testing the hypothesis that means from two or more samples (in the statistical sense) are equal, i.e., the samples are drawn from populations with the same mean. At a 95 % confidence limit, difference between the batches will be indicated, by chance alone, 5 % of the time. If the ANOVA indicates that there is a difference in the population means between the drums, the sample is re-homogenized (see Figure B.2).

4) Excel is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

Drum 1	Drum 2	Drum 3	Drum 4	Drum 5	Drum 6	Drum 7	Drum 8	Drum 9	Drum 10
0,245	0,245	0,237	0,251	0,265	0,228		0,250	0,244	0,242
0,254	0,234	0,252	0,240	0,240	0,248	0,248	0,255	0,249	0,255
0,276	0,245	0,249	0,255	0,250	0,253	0,265	0,243	0,245	0,258
0,261	0,245	0,268	0,254	0,259	0,244	0,250	0,262		0,275
0,254	0,269	0,249	0,236	0,257	0,257	0,237	0,256	0,269	0,253

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Drum 1	5	1,29	0,258	0,000 134
Drum 2	5	1,238	0,247 6	0,000 166
Drum 3	5	1,255	0,251	0,000 124
Drum 4	5	1,236	0,247 2	7,47E-05
Drum 5	5	1,271	0,254 2	9,17E-05
Drum 6	5	1,23	0,246	0,000 126
Drum 7	4	1	0,25	0,000 133
Drum 8	5	1,266	0,253 2	5,07E-05
Drum 9	4	1,007	0,251 75	0,000 137
Drum 10	5	1,283	0,256 6	0,000 142

ANOVA

Source of variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between groups	0,000 722	9	8,02E-05	0,686 767	0,716 084	2,137 528
Within groups	0,004 44	38	0,000 117			
Total	0,005 162	47				

Comment: F<Fcrit (Between Drum Homogeneity Acceptable)

Figure B.2 — Microsoft Excel single factor ANOVA to determine homogeneity

If the material passes all the homogeneity criteria detailed above, it is important to note that the material is only considered sufficiently homogenous, at the sample mass used for analysis, to be fit for use for the method used to determine its homogeneity (as the %RSD determined will be unique to the testing method). For further guidance on how to compute the between-bottle heterogeneity, two scenarios are possible using ANOVA. If MS between groups is larger than the MS within groups, the between bottle variance is computed by subtracting MS between groups from MS within groups and dividing by the number of replicates made per unit (in this case 5). The between-bottle standard deviation is then the square root of this variance. If MS between groups is smaller than MS within groups, the between-bottle heterogeneity is computed as given for case study 4. Further explanation can be found in ISO 33405[2].

B.8 Packaging

Once initial homogeneity tests have been completed, the material is ready for packaging. Generally, for internal laboratory use, each drum is split into 10 kg buckets using the rotary splitter. If the material requires long term storage, the material can be packaged according to method requirements which could include sealed and nitrogen-purged foil packets of 150 g each or sealed glass bottles depending upon sample matrix. For final packaging (as needed), a minimum of 10 random packets/bottles are selected for analysis to verify that the packaging procedure has not resulted in segregation or a loss of fines. The %RSD between analyses (packets) is compared to the method %RSD and that obtained during initial homogeneity testing.

For a proven processing procedure for a regularly handled (consistent) matrix, the %RSD of analyses of randomly selected final packets can be used as stand-alone evidence that the material is sufficiently homogeneous for its intended use.

B.9 Assigning accepted performance limits

For a material intended to monitor consistency/repeatability of a method only, it could be considered sufficient to assign values based upon traceability to other CRMs, or even other RMs and long-term method repeatability. In such a case, the material would be used in conjunction with a simple, one-page description of determined mean and performance gates based upon two standard deviations in either direction, signed off by the Laboratory Quality Manager.

Annex C
(informative)

Case study 3 — Production of a wheat flour fortified with folic acid quality control material (QCM)[5)]

C.1 Introduction

Folic acid fortification of wheat flour for bread making became mandatory in Australia in September 2009. The specified level of fortification is between 2 mg and 3 mg of folic acid per kilogram of wheat flour. The analytical methods used by laboratories measuring folic acid need to detect and accurately quantify added folic acid near the regulated levels. The results from laboratories need to be comparable to enable a consistent assessment of industry compliance with the Food Standards Code. The material described here was prepared as a proficiency test (PT) study sample and has subsequently been used as a QC material. It has been used for analytical method development purposes and to ensure comparability of results between analytical batches. The purity of the commercial folic acid used to prepare the sample and the adsorption of folic acid onto the walls of the mixing containers were two important issues that were identified in the processing of this material.

In this example, development of an analytical method for the determination of folic acid in flour commenced at the same time as the processing of this material. Consequently, there was no reliable method to determine homogeneity of folic acid in the material and an alternative approach was used.

Barium carbonate was used to demonstrate (validate) that the mixing protocol ensures a homogeneous mix at the expected fortification level. Barium carbonate was mixed into a separate flour sample in similar concentrations to the anticipated folic acid concentration, the idea being to demonstrate that a solid i.e., barium carbonate mimicking folic acid can be evenly distributed within the flour matrix. Subsamples of the flour were analysed for barium by inductively coupled plasma mass spectrometry (ICPMS) and found to be homogeneously distributed throughout the flour with the concentration of the barium in the subsamples at the expected concentration. It was therefore assumed that the mixing protocol produced homogeneous materials at the expected concentration, i.e. it was validated.

The folic acid analytical method when developed was used to analyse other similar folic acid/flour PT study samples produced using the described procedure. However, it was found that although the folic acid was homogeneously distributed in the flour it was not present at the expected concentration. Eventually, after a full mass balance experiment it was determined that the folic acid was sticking to the sides of the stainless-steel mixing vessels, and this was why the concentration of the folic acid in the study samples was less than the expected fortification level. The fact that folic acid is pale yellow made this easier to determine. Further experiments determined that the stainless-steel vessels were probably the least suitable for mixing and polypropylene the best option.

C.2 Material description and specification

C.2.1 **White wheat flour fortified with folic acid** at 1,86 mg/kg.

5) This case study was provided by Meg Croft and Stephen Davies, National Measurement Institute Australia. PO Box 138, North Ryde NSW 1670, Australia. Subclauses C.1 to C.4 focus on the preparation of the RM.

C.3 Preparation

C.3.1 General

The steps in the preparation of this QC material are shown in Figure C.1

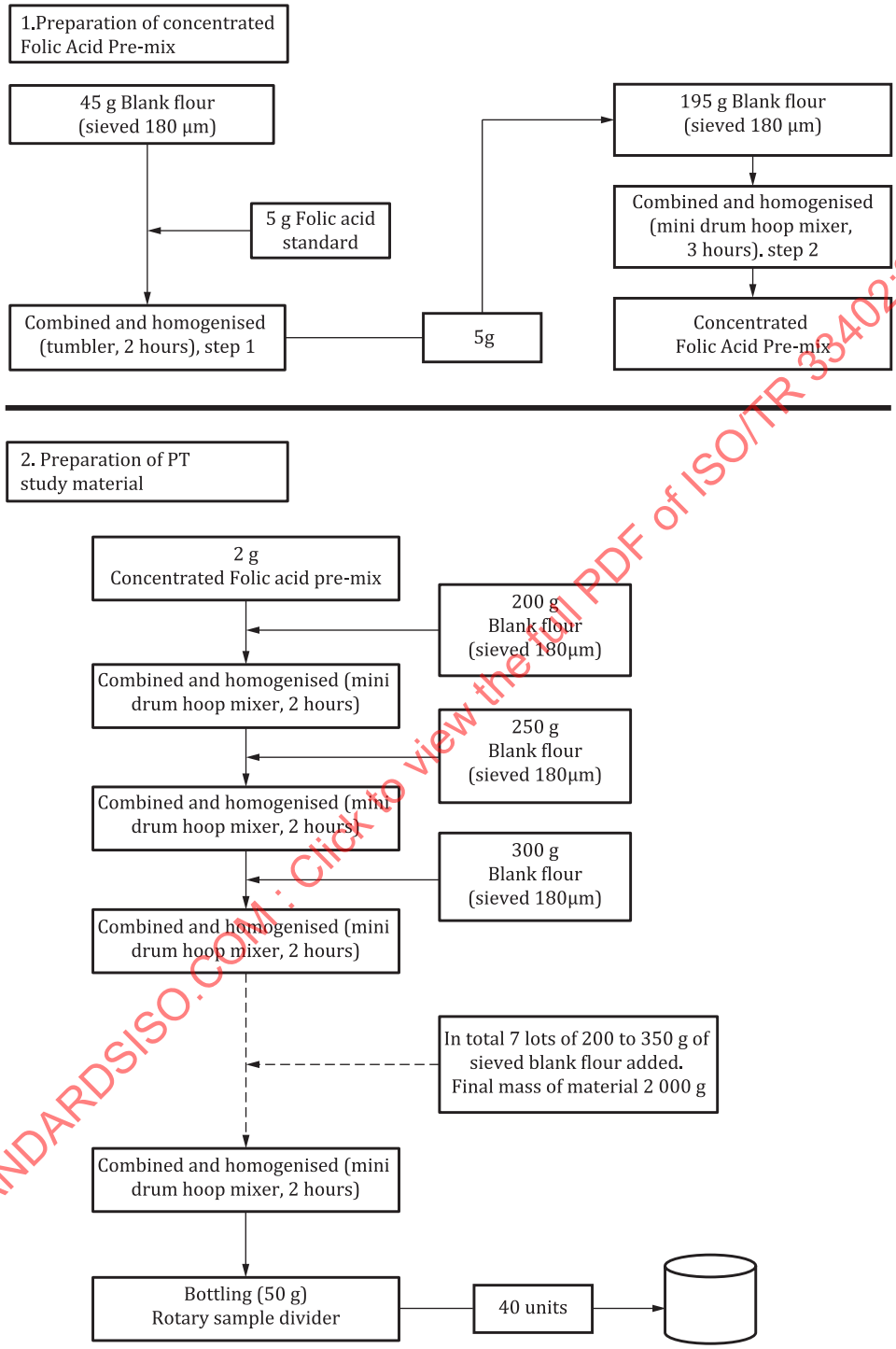


Figure C.1 — Preparation steps of a wheat flour QCM fortified with folic acid

C.3.2 Issues

The following issues were considered:

— Purity determination of the commercial folic acid used to fortify the flour;

— adsorption of folic acid onto the surface of mixing containers;

— degradation of folic acid due to light sensitivity (literature);

— possible risk of deterioration of the matrix (flour) in storage by insects (e.g., weevils);

— fortification of a solid (powder) material with another solid (powder);

— uniformity of particle size; and

— sub-sampling.

C.3.3 Approaches

The approaches taken for each of these issues are listed below.

— Purity of the commercial folic acid used to fortify the flour*:* A commercially sourced bottle of folic acid had a label stating the folic acid content as being "approx. 98 %" from which it was not clear if this referred to mass fraction or amount fraction. The manufacturer's "certificate of analysis" stated the presence of 8 % moisture, which suggested that the statement "approx. 98 %" refers to the purity of the organic component (i.e., folic acid and impurities of similar structure) and not the total purity of folic acid in the so-called pure material. In this case, an in-house assessment of purity was conducted (quantitative NMR, thermogravimetric analysis (TGA), and Karl Fischer titration) to confirm the total purity of the folic acid. A standard comparison was also performed using folic acid sourced from alternate commercial suppliers.

— Light sensitivity: Sample production was performed in foil-covered containers or inside other containers that blocked exposure to light.

— Minimize risk of deterioration of the matrix (flour) by insects: The final product was stored overnight at −80 °C to destroy any insect larvae that could have been present.

— Fortification of a solid (powder) material with another solid (powder): A concentrated pre-mix was gravimetrically prepared by combining and thoroughly mixing sieved folic acid and sieved white flour. The final material was prepared by dilution of an appropriate sub-sample of this pre-mix with unfortified sieved flour. To ensure homogeneity, the mixing process was done in stages. The concentrated flour pre-mix was added to approximately 200 g unfortified flour and mixed thoroughly by tumbling in a mini drum hoop mixer. Every 2 h approximately 200 g of unfortified flour was added to the mix and the process continued until the desired concentration was achieved.

— Adsorption onto surface of mixing containers: The concentration of the folic acid in the fortified flour as determined by replicate analysis was less than the expected gravimetric fortification level. A rigorous mass balance approach involving analysis of solutions used to wash the empty container walls at each stage during the production of folic acid fortified flour samples confirmed the adsorption of folic acid onto the surfaces of the various mixing vessels employed during processing. While various containers (Perspex, metal, polypropylene) were tested to minimize sample adsorption, this problem could not be completely eliminated. Accordingly, the gravimetric fortification level was not used to assign the mass fraction of folic acid in the flour.

— Uniformity of particle size: Both the unspiked flour and folic acid were passed through a 180 µm sieve before mixing.

— Sub-sampling: The folic acid fortified material was divided into 50 g portions using a Retsch PT 100 Rotary Sample Divider[6].

C.4 Subdivision and packaging (any contamination issues, preferential evaporation, special sealing requirements)

C.4.1 Issues

The following issues were considered:

— adsorption on containers;

— light sensitivity.

C.4.2 Approaches

The issues were addressed by:

— use of polypropylene screw cap containers to minimize adsorption and provide ease of access to the material;

— recommending storage in the dark at room temperature.

C.5 Homogeneity

C.5.1 Achieving and confirming homogeneity

Homogeneity was achieved through serial dilution and thorough mixing of a concentrated pre-mix (see C.3.2).

Statistical analysis determined the processing procedure produced homogeneous samples containing the expected (gravimetric) concentration of barium.

Testing the methodology using barium carbonate assumed that folic acid and barium carbonate would behave similarly during the processing stages. This was not found to be the case as folic acid was found to adsorb onto the walls of the mixing containers. (See discussion above.)

The homogeneity of samples produced using the procedure has since been tested multiple times for subsequent folic acid in flour PT studies. In these studies, the homogeneity of samples was confirmed by analysis of 7 × 1 g subsamples in duplicate for folic acid. The folic acid mass fraction was determined using exact-matching isotope dilution with liquid chromatography tandem mass spectrometry (LC-MS/MS) in Selected Reaction Mode for detection.

ANOVA was used to determine the within (analytical) and between-bottle variance[17].

C.5.2 Examples of data and data treatment

The QC material was produced before an analytical method for folic acid was available (see previous discussion re validation of the homogeneity using barium carbonate). The data provided below were obtained for a similar PT study material fortified at a similar concentration, confirming the homogeneity of the folic acid fortified wheat flour QC materials produced using the described procedure. See Figure C.2.

6) A Retsch PT 100 Rotary Sample Divider is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

Study S3 Folic acid

Bottle No.	A	B
301	2,39	2,36
306	2,39	2,30
307	2,35	2,37
314	2,29	2,45
317	2,37	2,36
323	2,32	2,27
325	2,36	2,42

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Row 1	2	4,757	2,378 5	0,000 42
Row 2	2	4,685	2,342 5	0,003 784
Row 3	2	4,723	2,361 5	0,000 265
Row 4	2	4,741	2,370 5	0,013 285
Row 5	2	4,728	2,364	3,2E-05
Row 6	2	4,584	2,292	0,001 152
Row 7	2	4,78	2,39	0,001 352

ANOVA

Source of variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between groups	0,012 476	6	0,002 079 3	0,717 365	0,6492	3,865 968 853
Within groups	0,020 29	7	0,002 898 6			
Total	0,032 766	13				

Prepared by _____

Checked by _____

Figure C.2 — Data for the homogeneity study performed on a similar PT material at a similar fortified concentration as the QC material

For further guidance on how to compute the between-bottle heterogeneity, two scenarios are possible using ANOVA. If MS between groups is larger than the MS within groups, the between bottle variance is computed by subtracting MS between groups from MS within groups and dividing by the number of replicates made per unit (in this case 2). The between-bottle standard deviation is then the square root of this variance as also given under 9.3. If MS between groups is smaller than MS within groups, the between-bottle heterogeneity is computed as given for case study 4. Further explanation can be found in ISO 33405[2].

C.5.3 Achieving and confirming stability

Initially, the stability of folic acid was assumed based on literature precedents.

The stability of the flour was also a concern, and this was addressed by treatment at −80 °C of the final product.

The stability of the material was demonstrated by analysis of the material with batches of samples over a period – no trends were observed.

C.6 Storage and handling

Store in a dry environment, away from light at room temperature. The decision to store at room temperature was made based on literature precedents and confirmed over time.

The minimum sample size requirement was 1 g based on homogeneity testing.

Safety information: Non-hazardous. For laboratory use only. Do not consume.

Annex D
(informative)

Case study 4 — Bauxite quality control material (QCM)[7)]

D.1 Introduction

Aluminium industry laboratories use bauxite quality control materials to monitor day-to-day and batch-to-batch analytical performance. The stability of the analytical process is checked, and the magnitude of the time-and-operator-different intermediate precision standard deviation is evaluated by applying the control chart method [23] to the analysed values of available alumina[8)], reactive silica[9)] and major oxides content in a bauxite quality material.

D.2 Material description and specification

D.2.1 Washed bauxite sample with high content of gibbsite.

D.3 Processing

D.3.1 Issues

The following issues are addressed:

— material in sufficient quantity, as to be available for analysis over a given period of time, but also adequate to be handled in a chemical laboratory facility;

— particle size less than 150 µm;

— avoid material contamination;

— minimize heterogeneity between units of RM.

D.3.2 Approaches

The approaches taken are listed below.

— A batch of 5 kg of washed bauxite is oven-dried, crushed, ground, and sieved to pass a 150 µm screen. The product is mixed and then bottled in 200 g units using a rotary sample divider.

— Use inert equipment and handle the material to safeguard against contamination.

D.3.3 Packaging requirements

Packaging requirements are:

— plastic or glass bottles with screw caps;

— units of the RM clearly labelled.

7) This example was provided by Dr Maria Alice de Goes, CETEM, Rio de Janeiro - RJ – Brazil, 21941-908. Subclauses D.1 to D.3 focus on the preparation of the RM.

8) Amount of alumina that is digested in a caustic solution (150 °C) at similar conditions of Bayer Process.

9) Amount of silica that reacts with sodium hydroxide (150 °C) at similar conditions of Bayer Process.

D.4 Homogeneity

D.4.1 Achieve and confirming homogeneity

Materials such as ores are heterogeneous in composition by nature. Much depends on the options available during processing to reduce the batch inhomogeneity.

To assess homogeneity, a subset of the batch of units of the RM is selected by a stratified random sampling scheme. For each selected unit, measurements for available alumina, reactive silica, and major oxides are carried out in triplicate, under repeatability conditions.

A one-way analysis of variance approach [22] is performed on the data to compute the within and the between-unit standard deviations. The uncertainty component due to sampling expressed as a percentage of the grand mean, is evaluated.

D.4.2 Examples of data and data treatment

Measurements for available alumina content (%*m*/*m*) were carried out under repeatability conditions. To separate a trend in the measurements from a trend in the batch of bottles, the replicates were measured in a randomized order. See Table D.1.

Table D.1 — Measurements for available alumina content

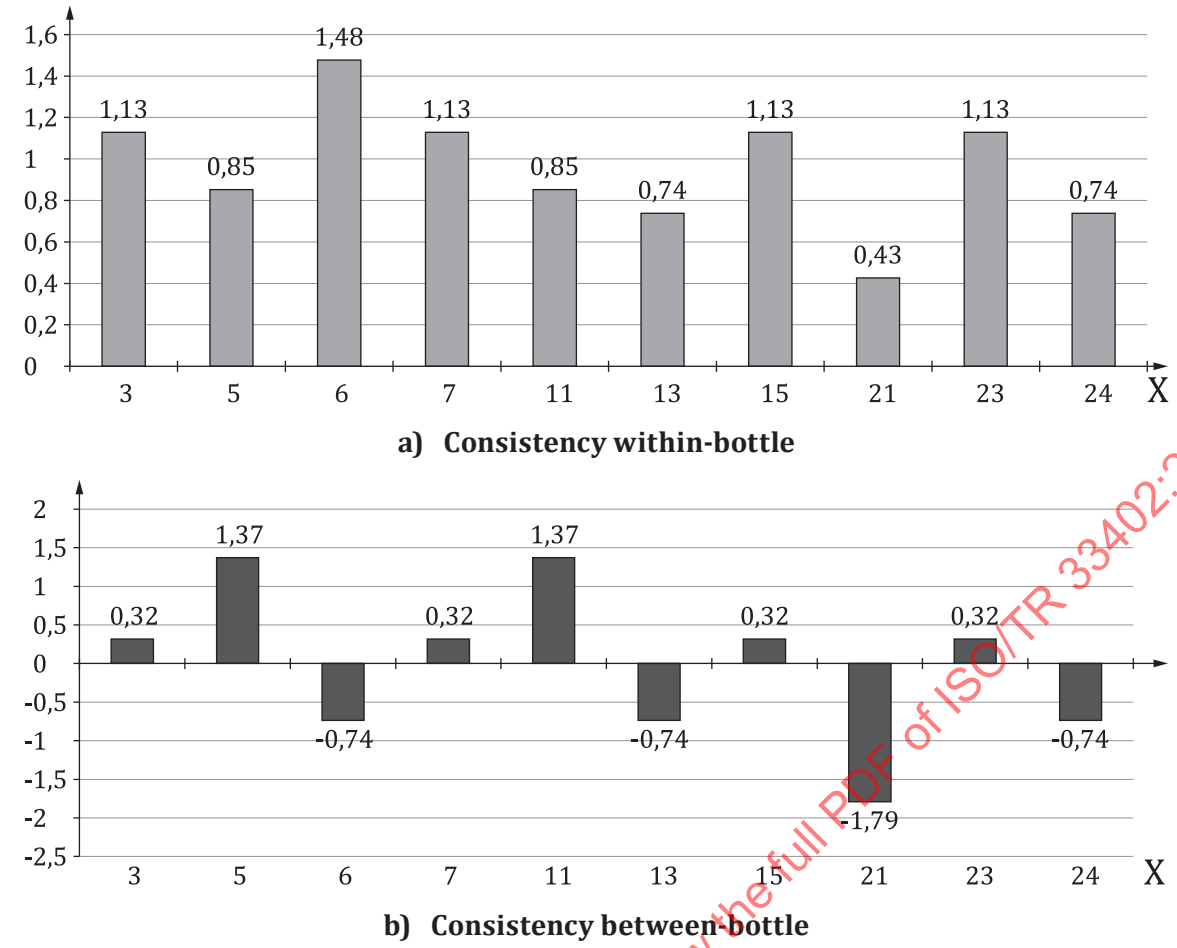
Bottle no.	Available alumina %(*m*/*m*)		
3	50,2	50,0	49,9
5	50,0	50,2	50,0
6	50,0	50,2	49,8
7	50,0	50,2	49,9
11	50,0	50,2	50,0
13	50,0	50,1	49,9
15	50,0	50,2	49,9
21	50,0	49,9	50,0
23	50,0	50,2	49,9
24	50,1	50,0	49,9

Analytical method:

— Alkali digestion [NaOH (80 g/L); digestor 150 °C + addition ($C_6H_{11}O_7Na$) + KF]/Titrimetry (HCl);

— Sample size – 0,65 g.

The data was evaluated for consistency using *h* and *k* statistics[22]. The *h* and *k* plots in Figure D.1 indicate that no specific bottle exhibit patterns of results that are markedly different from others in the homogeneity study.



a) Consistency within-bottle

b) Consistency between-bottle

Key

X	bottles
	k-values
	h-values

Figure D.1 — Plots of consistency within- and between -bottle

A one-way analysis of variance approach[22] was performed on the data to compute the within and the between-unit standard deviations which are, respectively, estimations of the analytical standard deviation (s_{an}) and the sampling standard deviation (s_{sam}). See Tables D.2 and D.3.

Table D.2 — ANOVA: Single factor

SUMMARY				
Group	**Count**	**Sum**	**Average**	**Variance**
bottle 3	3	150,1	50,033 33	0,023 333
bottle 5	3	150,2	50,066 67	0,013 3 33
bottle 6	3	150	50	0,04
bottle 7	3	150,1	50,033 33	0,023 333
bottle 11	3	150,2	50,066 67	0,013 333
bottle 13	3	150	50	0,01
bottle 15	3	150,1	50,033 33	0,023 333
bottle 21	3	149,9	49,966 67	0,003 333
bottle 23	3	150,1	50,033 33	0,023 333
bottle 24	3	150	50	0,01

Table D.3 — ANOVA: Source of variation

ANOVA						
Source of variation	**SS**	***df***	**MS**	*F*	*P*-value	*F* crit
Between groups	0,027	9	0,003	0,163 636	0,995 792	2,392 814
Within groups	0,366 666 7	20	0,018 333			
Total	0,393 666 7	29				

$s^2_{an} = 0{,}018$

$s^2_{sam} = 0$

For further guidance on how to compute the between-bottle heterogeneity, two scenarios are possible using ANOVA. If MS between groups is larger than the MS within groups, the between bottle variance is computed by subtracting MS between groups from MS within groups and dividing by the number of replicates made per unit (in this case 3). The between-bottle standard deviation is then the square root of this variance as also given under 9.3. If MS between groups is smaller than MS within groups, the between-bottle heterogeneity is computed as given below. Further explanation can be found in ISO 33405[2].

The uncertainty component due to sampling estimate that accounts for insufficient repeatability of the analytical method was calculated using the following formula[24]:

$$u_{sam} = \sqrt{s_{sam}^2 + \frac{MS_{within}}{n}} \sqrt{\frac{2}{v_{MS_{within}}}}$$

$$u_{sam} = 0,044$$

where

MS_{within} is the within mean square;

$v_{MS_{within}}$ is the respective degrees of freedom;

The uncertainty component due to sampling, expressed as a percentage of the grand mean, is less than 0,1 %. Therefore, the RM could be considered sufficient homogeneous.

D.5 Achieving and confirming stability

Based on the nature of the material, deterioration is not anticipated provided the material is properly handled and stored.

D.6 Storage and handling

D.6.1 Temperature and other environmental conditions for storage

Units of the RM needs to be stored at ambient temperature in a dry place.

D.6.2 Minimum sample size requirements

The minimum sample size that was used for the homogeneity assessment, i.e., 0,65 g.

D.6.3 Safety information

Avoid dispersion of, or exposure to dust by inhalation, eye contact or skin contact.

Dispose residual material in accordance with regulations pertaining to inorganic chemical and mineralogical waste.

Annex E
(informative)

Case study 5 — Pharmaceutical reference standards[10)]

E.1 Introduction

Establishment of reference standards during the drug development and commercialization process is very complex and needs a thorough understanding. From a very early stage in the process, pharmaceutical industries need to consider establishing reference standards to evaluate the raw materials, process impurities, intermediates, metabolites, degradation products, and active pharmaceutical ingredients (APIs)[25].

NOTE Reference standard is used as a synonym for reference material in the context of pharmaceuticals.

Reference standards are needed almost at every stage in the development of a drug candidate to make sure the final drug product is of the highest quality. In the absence of available official reference standards, manufacturers often establish in-house reference standards.

Sourcing and processing of bulk materials for RM production can at first seem a difficult and daunting task, especially if large quantities of the material is needed. High quality RMs are demanding and costly to produce and if materials are available from other sources, it is not normally cost effective for laboratories to make their own. However, if this is necessary, this case study provides guidance for non-specialist laboratories to prepare their own RMs for quality control. Some of the key issues that need to be considered are: selection of materials (appropriateness, native material versus spikes, material processing, etc.), traceability, testing, processing and packaging (homogeneity, contamination, etc.), stability testing, value assignment exercises, uncertainty estimation, documentation, mechanism for the approval of the assigned value, storage, and distribution.

Note that biologics (large molecules) are beyond the scope of this guidance.

E.2 General selection criteria used in establishing a reference standard by a pharmaceutical company

E.2.1 General

The criteria used in selecting a material to use as a reference standard is complex and depends on many factors. There is no magic bullet, and every situation is different. The following is a general guideline for selecting a reference standard followed by a specific example of a standard selected by a pharmaceutical industry.

Once a bulk material has been sourced there are usually several processing stages which need to be carried out to ensure the material has the appropriate level of homogeneity and stability for its intended use.

Some of the more common processes are drying to remove water or solvents so that the material is easier to handle, and its stability improves. Water removal also reduces the likelihood of microbial growth formation, which is a particular problem with biological materials, and for such materials, freeze drying is employed to remove water. Please note that our scope in this paper is restricted to smaller organic RMs only.

Sieving is an additional process carried out after milling and grinding to improve material homogeneity. Bulk solid materials are be homogenized by thorough mixing.

10) This case study is based on information provided by Iffaaz Salahudeen, PhD, and Frank Hu, PhD, Bristol-Myers Squibb Pharmaceuticals, New Brunswick, NJ, USA. Subclauses E.2.1 to E.2.6 as well as E.3.1 to E.3.5 focus on the preparation of the RM.

Certain analytes can be unstable and appropriate salt would be selected to stabilize the material.

E.2.2 Specification of material

A reference standard needs to be made using a best-known synthetic process yielding the highest purity with minimum amount of other external components such as residual solvents and heavy metals. The physical properties of the material are also important. It needs to be less hygroscopic, with free-flowing crystalline structure with minimum aggregation or lumping.

Impurities classified as organic (process and drug related), inorganic, or residual solvents can be introduced during the manufacturing process for the drug substance, drug product, or excipient and/or through storage of the material. Impurities needs to be controlled throughout the manufacturing process. Impurities that are process-related needs to be kept to a minimum to avoid degradation and unwanted pharmacological effects. Compounds that are susceptible to hydrolysis, for example, needs to be thoroughly dried to remove moisture and then stored in a desiccator. Reference standards that contain a high percentage of organic volatile impurities could experience purity changes over time as the solvents evaporate.

If the reference standard is in a salt form, the amount of salt present is determined so that the purity can be corrected for content. Applying the molecular weight to the correction will not account for residual salt that could be produced during synthesis.

E.2.3 Sourcing of material

Reference-standard materials that are synthesized by the user or supplied by a contract manufacturer or secondary company are characterized. Both the reference standards and drug substance can be synthesized initially using the same process. The reference standard needs to be of the highest purity possible; the drug substance can require further purification to become a reference standard (additional purification steps used for a drug substance needs to be fully described and included in any regulatory filing).

E.2.4 Processing

If needed, the material selected can be dried, solvent removed, re-crystallized, or purified. If the material tends to form lumps, a delumping procedure can be employed. Large particles can be reduced by grinding. To increase its stability, a different salt can be selected, or certain additives can be added to the material. For materials with < 95 % purity, or mixtures, thorough mixing or homogenization is necessary to make sure the mixture is homogeneous. An old or API lot also can be used after fresh testing if its properties are within the acceptable range.

E.2.5 Qualification

For the initial lot, an example requalification period could be 3, 6, or 12 months for the first year and annually thereafter. In this scenario, it is recommended that during development, the reference standard be assessed after 3 months at the intended storage condition and at an accelerated storage condition. Validation of the analytical method for organic impurities needs to occur after the full accelerated storage condition has been evaluated. The total length of the requalification program will depend on the intended life of the reference standard and the length of the stability and clinical programs. If the initial lot is proven to be stable for at least one year, then subsequent lots will require annual requalification only. In all study scenarios, a protocol will outline the reference-standard material, lot, storage conditions, frequency of test, analytical procedures, acceptance criteria, and reporting criteria[26].

— Organic impurities. Determination of organic impurities is the most challenging aspect of developing a suitable analytical method because these impurities are unique to the parent compound and because various degradation pathways can lead to various impurities. Actual and potential organic impurities that arise during synthesis, purification, and storage are identified and quantified. The synthesis of the reference standard needs to be evaluated to predict and identify potential impurities from raw materials. Potential degradation of products also can occur because of storage. Short-term (forced degradation)

and long-term (evaluation under accelerated conditions) stress testing, therefore, needs to be evaluated during development. The design of the long-term stress test depends on the intended storage condition.

The quantity of organic impurities present can be determined with high-performance liquid chromatography (HPLC) and ultraviolet (UV) detection. Degradation products and compounds related to the product can be evaluated by the area percent or from the relative response of the standard being used. The technique used to obtain this data will depend on the number of impurities and related compounds present and the decomposition pathway of the reference-standard material.

To consider the impact on the purity evaluation using area percent versus relative response factor, the following scenario could be considered. If analysis shows an impurity at 0,05 % and the relative response factor of the impurity is half of the standard (i.e., the amount of impurity present shows a 50 % detector response compared with the equivalent amount of standard), then there could be 0,1 % of actual impurity. This level could be insufficient to affect overall purity results. If there was 1 % impurity based on area percent present, however, then there would be 2 % of actual impurity that could affect overall purity.

The approach to determining the relative-response factor for each impurity is a more accurate process, but potential pitfalls need to be considered. The relative-response factor approach requires additional development because the component needs to be isolated, and the relative response factor is determined. In addition, as the reference standard ages, new unknown impurities can be detected. The relative-response factor of these new impurities is determined, and the method updated if the new unknown is significant enough to alter the purity. Much of this information can be ascertained during the development of the drug substance.

Impurities that arise from raw materials, synthesis, purification, and storage require careful consideration because they might not produce detector responses that are related to the reference-standard material. Quantification by area percent would not be appropriate in such cases. Rather, the impurities are isolated and identified so that an appropriate reference standard can be used, or a relative response factor determined. For example, if the reference-standard material is a salt, then the cation response would not be equivalent to the reference standard. In such instances, a specific reference standard will be used for the cation, and a separate analytical method for quantification could be needed.

— Inorganic impurities. Inorganic impurities such as metals and non-combustible materials are typically evaluated using compendial procedures. If inorganic impurities are proven to be less than the reporting threshold at initial characterization, then no further analysis is needed.

— Residual solvents. The potential for residual solvents needs to be evaluated during development of the drug substance and can be estimated by reviewing the synthesis pathway. USP General Chapter Residual Solvents[1] details a generic procedure for this evaluation. Residual solvents, however, could be specific to the manufacturing process and require a specific test procedure. An additional specific test procedure could be necessary if the USP procedure is not suitable for the reference standard being evaluated, or if the solvents used during synthesis are not included in USP. If residual solvents (previously referred to as organic volatile impurities, or OVIs, by USP) are proven to be less than the reporting threshold at initial characterization, further analysis is generally not needed at subsequent intervals. If the amount of residual solvents present affects the purity, however, they need to be evaluated at each requalification interval.

E.2.6 Subdivision and packaging

RMs are subdivided from the bulk material by either manually or by automated means. Many materials can either lose or pick up moisture if the container is not securely closed. Therefore, septum lined crimp-top vials are more suitable. It is preferable for vials to be amber to reduce the light impact. The amount per vial depends on its application. Repeated opening and closing of RM containers increases the risk of contamination. Stability could also be affected by repeated freeze/thaw cycles. Take care to vial enough material for analysis separately, instead of using bulk containers for repeated use.

Light sensitive materials are subdivided into amber vials, and heat sensitive materials are stored at lower temperatures. Any material with a significant amount of solvent needs to be stored carefully.

Prolonged storage of inherently heterogeneous matrix materials can cause settling and separation of the material. It is therefore important the units are adequately mixed before a new sub-sample is withdrawn. This can often be achieved by simple shaking of the units (bottles).

For moisture-sensitive materials, appropriate packaging would be the preferred method of controlling moisture content.

E.2.7 Homogeneity assessment

For materials with < 95 % purity, or mixtures, thorough mixing or homogenization is usually necessary to make sure the mixture is homogeneous. These mixtures need to be assessed for homogeneity.

E.2.8 RM document requirements

Ideally, a document complying with ISO 33401[20] and a report covering the characterization and value assignment will be produced.

For quantitative standards, purity values are assigned, and based on history and stability, a re-test date is also be provided. However, for qualitative standards (e.g., retention time markers or impurity mixtures), assigned purity values are not needed, if a chromatographic verification is performed to prove that the impurities are detected (above the detection limits).

Upon testing a Certificate of Analysis (COA) will be issued. The COA will have all the necessary information including the chemical name, catalogue #, Lot #, test date, re-test date (most reference standards can be used upon re-evaluation of the assigned value and therefore, they do not need any expiry date, but a re-test date). Any special handling instruction such as hazardous category, light, or heat sensitivity) is also provided in the COA.

E.2.9 Storage requirements

Reference-standard materials are often expensive to manufacture and are generally of limited supply. It is important, therefore, to consider how the material will be stored, distributed, and controlled. Once the storage conditions are ascertained, the reference-standard material is monitored continually using a suitable environmental monitoring system. It is advisable to store the material in at least two different locations in case there is a prolonged excursion from the storage condition. The material is stored in a secure environment with controlled access and distribution. Even if the material is proven to be stable at room temperature, most of the solid or powdered materials can be refrigerated or frozen so that their shelf lives can be extended after re-testing.

E.2.10 Quantity

In the early phase of product development (prior to Phase 3), batches of 10 g to 100 g quantity would be qualified as reference standards. After the program moves into the Phase 3, a larger quantity (500 g to 1 000 g) from one pilot-scale batch would be qualified as reference standard.

E.3 Specific example of the selection criteria used in establishing a reference standard

E.3.1 General

The criteria described in the example below are appropriate for the specific standard under discussion and is considered a guide instead of a general requirement for selecting a reference standard.

E.3.2 Specification of material

Compound X is a monohydrate and mono propylene glycol solvate. A representative batch of Compound X prepared with the best-known synthetic process available at the time was selected. The batch has a high purity (>99,0 %) and contains < 0,1 % of residual solvent. Compound X is a free-flowing crystalline material with minimum aggregates or lumps.