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Guidance for the in-house preparation of quality control materials (QCMs)

Lignes directrices pour la préparation interne des matériaux de référence utilisés pour le contrôle qualité

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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 documents 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 on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/REMCO, *Committee on reference materials* (which has the task to prepare guidance documents for the preparation, characterization, certification and use of reference materials (RMs) and the competence assessment of reference material producers.

Introduction

Reference materials (RMs) are widely used in measurement laboratories for a variety of purposes and it is important to recognize that the material most appropriate for a particular application should be used. Certified reference materials (CRMs), i.e. those which have property values and associated uncertainties assigned by metrologically valid procedures are primarily used for method validation and calibrations providing metrological traceability.

The preparation of reference materials for metrological quality control (i.e. control of the quality of measurements not products) is an important activity which provides materials suitable for the day-to-day demonstration that a particular (part of a) measurement system is under statistical control. Such materials do not require characterization by metrologically valid procedures, and can be prepared "in-house", i.e. by laboratory staff familiar with their behaviour, to fulfil specific quality control requirements.

Reference materials which are sufficiently homogeneous and stable are necessary for metrological quality control purposes, such as demonstrating a measurement system is under statistical control, performs as expected and provides reliable results; where the trueness of the measurement result is not critical. Different industries use various terminologies to describe such materials (e.g. in-house reference materials, quality control materials, check samples, etc.). For the purposes of this Guide, the term "Quality Control Materials" (QCMs) will be used to simplify repeated citation.

While CRMs are produced by established reference material producers and are commercially available, QCMs are often prepared by a laboratory for its own internal use. Frequently, QCMs are characterized only for a limited scope (a limited number of property values) and for specific laboratory applications.

The rationale for preparing quality control materials can be one or a combination of the following factors:

— to have an RM representing as closely as possible routine samples, suitable for quality control;

— to have a suitable day-to-day RM to complement a commercially available CRM;

— no suitable CRM exists;

— the application does not require a material having the full characteristics of a CRM (e.g. traceability and uncertainty for specified property values).

QCMs are RMs and as such the requirements of ISO Guide 34[1] for the production of RMs apply. However, if the material is only used in-house by the preparing laboratory, some requirements (e.g. for transport stability) can be relaxed. The preparation of a QCM is related to that of a CRM and those preparing QCMs may wish to consult ISO Guides 34[1] and 35[2] for further guidance. Where appropriate, this Guide will refer to relevant parts of these Guides.

It is recognized that the aim of many laboratories requiring QCMs is to minimize the time and effort needed to prepare the materials. To this end, many laboratories use samples of real products for which there is a body of analytical data available. A number of case studies are included as annexes of this guidance document to provide examples of how such data may be processed to confirm fitness for purpose of the materials.

Guidance for the in-house preparation of quality control materials (QCMs)

1 Scope

This Guide outlines the essential characteristics of reference materials for quality control (QC) purposes, and describes the processes by which they can be prepared by competent staff within the facility in which they will be used (i.e. where instability due to transportation conditions is avoided). The content of this Guide also applies to inherently stable materials, which can be transported to other locations without risk of any significant change in the property values of interest.

The primary audience for this Guide is laboratory staff who are required to prepare and use materials for specific in-house quality control applications. Preparation of QCMs, where transportation is a necessary component of the supply chain, such as laboratory sites at different locations or for proficiency testing schemes, should conform to the relevant requirements of ISO Guides 34[1] and 35.[2]

The description of the production of reference materials (RMs) as detailed in ISO Guide 34[1] and ISO Guide 35[2] is also applicable to the preparation of quality control materials (QCMs). However, the requirements for "in-house" QCMs are less demanding than those for a certified reference material (CRM). The preparation of QCMs should involve homogeneity and stability assessments, and a limited characterization of the material to provide an indication of its relevant property values and their variation, prior to use. This document provides the quality criteria that a material should fulfil to be considered fit-for-purpose for demonstrating a measurement system is under statistical control. Guidance on uses of such materials, for example setting up a QC chart, is adequately covered elsewhere [3],[4],[5],[6] and is not included in this Guide.

The layout and structure of this Guide provides general information on the preparation of QCMs in the main chapters, with specific case studies covering a range of sectors in the annexes. The case studies are not complete "process manuals" but are included to highlight some of the key considerations when preparing QCMs. The case studies vary in complexity and detail, including sector specific terminology, but provide a range of information for laboratory staff to draw from.

It is expected that those involved in QCM preparation will have some knowledge of the type of material to be prepared and be aware of any potential problems due to matrix effects, contamination, etc.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. 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 in ISO Guide 30[7] ISO/IEC Guide 99[8] and ISO 3534-1[9] and the following apply.

3.1
indicative value
value of a quantity or property, included in the certificate of a CRM or otherwise supplied, which is provided for information only (i.e. is not certified by the producer or the certifying body)

Note 1 to entry: Values assigned to quality control materials (QCMs) can only be indicative in that they have no metrological traceability. ISO Guide 30:1992[7] uses the term "uncertified value" to describe a value of a quantity provided for information only.

4 Quality control materials (QCMs)

The term "quality control material" or "QCM" has been devised for the purposes of this Guide solely to simplify repeated reference to materials used routinely to assess the precision of test procedures. It is not intended to define a new class of reference materials. Such materials are variously referred to in the open literature as "in-house reference materials", "quality control samples", "check samples", "set up samples", etc.

Where no suitable CRM exists, laboratories may use QCMs to provide an assessment of the repeatability / intermediate precision / reproducibility of a measurement result. QCMs cannot be used to establish metrological traceability or trueness of a measurement result.

QCMs should always comply with the basic requirements of any reference material, i.e. they should be sufficiently homogeneous and stable with respect to the properties of interest. The level of heterogeneity should be less than the expected standard deviation of the measurement process or an established criterion value against which the assessment of laboratory performance or the "normalization" of results is acceptable. The QCM should be stable for a period of time that is at least as long as that during which it is intended to be used.

5 Applications of quality control materials (QCMs)

The principal function of QCMs is to provide laboratories with an economical means of checking their routine test procedures for precision on a regular basis (e.g. daily, weekly or monthly).

While CRMs can in all cases replace QCMs; QCMs are not replacements for CRMs; they are complementary to them having a specific, limited purpose in the measurement process. CRMs produced according to the principles of ISO Guide 34[1] are essential to establish the concept of metrological traceability in a meaningful manner, and provide the highest standard with respect to reference materials. There is no requirement for QCMs to have metrologically traceable assigned values; consequently, QCMs cannot be used to establish metrological traceability or to estimate uncertainty. For method validation and uncertainty estimation, QCMs may be used to a limited extent (e.g. for establishment of a precision estimate as part of the total measurement uncertainty).

Uses of QCMs include (but are not limited to):

— preparation of QC charts – to demonstrate control of a measurement process within a laboratory or to confirm the effectiveness of a laboratory's quality control process or to demonstrate control of a measurement process over a period of time;

— comparison of results (e.g. from two or more series of related samples either in a short period of time or over an extended period of time when a measurement process is known to vary);

— method development – to establish consistency (for validation a certified reference material should be used);

— instrument performance checks;

— repeatability and reproducibility studies – repeated use over an extended period of time, instruments, operators, etc., to estimate long-term reproducibility or robustness of a measurement process or laboratory;

— as check samples – for example, to confirm the degree of equivalence of measurement results from two or more laboratories (e.g. provider and user), where the materials are inherently stable;

— operator variability;

— impact of any changes to the environmental conditions (e.g. temperature, humidity).

When confirming that a measurement process is under statistical control,[3],[4],[5],[6] the acceptability of laboratory performance is generally assessed by comparing either the standard deviation or the range of the individual results for the QCM against a pre-established criterion. If a lack of control of the measurement process is identified, the laboratory needs to take action. In the simplest case, this may require repeating the "suspect" measurements, perhaps following a re-calibration of instruments.

A more in-depth discussion of the uses of quality control materials can be found in ISO Guide 33.[10]

Regardless of the intended use, it is necessary to assess homogeneity and stability of a QCM.[11]

6 Steps in the in-house preparation of quality control materials (QCMs)

The fundamental purpose of QCMs is to detect change. In general, more pragmatic and less rigorous protocols can be used for stability and homogeneity steps to strike a balance between material development costs on the one hand and the intended use of the material on the other.

The production of any reference material requires a level of technical and organizational competence. It is acknowledged that in many cases "in-house" QCMs will be prepared by technically competent staff that is knowledgeable about the materials/processes being used.

The key steps involved in the in-house preparation of a typical QCM are summarized in the flow chart in Figure 1 and are described in more detail in References [12] and [13]. Materials can be sourced from, processed, sub-divided and packaged by third parties, where they have specialized equipment and/or expertise. Materials may even be products which are commercially available and meet the user's specification (e.g. food products available in appropriately sized units from a single production batch).

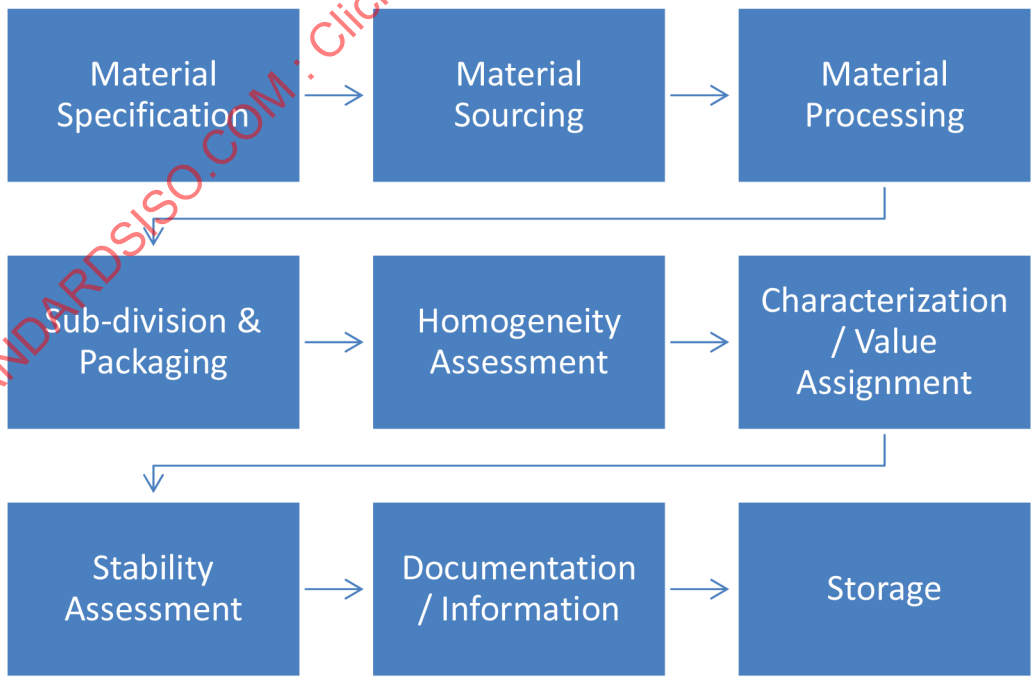

Figure 1 — Key steps in the preparation of a typical QCM

NOTE Any of these steps may be subcontracted to a technically competent subcontractor.

7 Material specification

The key criteria in the specification and selection of a QCM are for the material to be as close as possible to real samples and available in appropriate quantities.

7.1 Matrix type, matching and commutability

In general terms, the uncertainties associated with a measurement result 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 scope and applicability of a matrix reference material is an important consideration for both the production and use of all reference materials.

The matrix of the QCM should be the same or as similar as possible to the matrix of the routine test samples, so that a satisfactory result for the QCM 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 may cause them to respond differently to a particular measurement procedure. For example, a freeze-dried food matrix may behave differently during analysis to a similar foodstuff with higher moisture content.

Generally, QCMs are prepared for specific purposes and the materials' properties can be closely matched to the samples under analysis.

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

In practice, the impetus for the preparation of a QCM may often be the fact that adequate matrix CRMs are not available and therefore the QCM producer is likely to use the specific matrix/property combination in question and matching is not an issue.

7.2 Properties and property values

As for any reference material, the QCM should be characterized for those properties that are of particular importance in the measurement of the routine test samples. The properties of the QCM should be as similar as possible to those expected in the test samples. This may require some preliminary screening measurements to be carried out on a number of candidate materials, to enable the most appropriate to be selected.

7.3 Unit size

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

7.4 Total bulk amount of material

An estimate is required of the total bulk amount of candidate material that should be sourced. In principle, this may be estimated by considering

— the number of units per year required by the laboratory,

— the unit size,

— the preparation yield,

— the quantity of material that can readily be homogenized,

— the length of time the supply is to be maintained and the assumed stability of the material,

— the type and size of the required storage facility.

8 Preparation of quality control materials (QCMs)

8.1 Sourcing of bulk material

Sourcing and processing of bulk materials for QCM preparation may at first seem difficult especially in those cases where large quantities of material are required. However, there are a number of options that may be available including:

— excess sample material;

— accurate gravimetric formulation.

Processing the bulk material can have significant cost implications for the preparation of QCMs and simple, straightforward processing methods should be used to ensure cost-effective QCM preparation. The exact preparation procedures required for a particular QCM will depend on the nature of the matrix and the properties of interest.

In general, liquid matrix QCMs are much easier to produce than their solid counterparts. The main reason for this is that homogeneous liquids can easily be achieved even with fairly rudimentary equipment (e.g. large mixing containers equipped with paddle 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.

During preparation of both liquid and solid materials 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 bottles, vials or flasks to be used for final containment must be carefully cleaned and dried before filling to remove possible contaminants.

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 preparation of QCMs;

— legal liabilities of retention and use of residual patients' samples purchased for the preparation of QCMs;

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

— materials sourced for QCM preparation should be screened for potential risks for health hazards, especially if the preparation includes the use of contaminated sharps or has the potential for aerosol formation.

8.2 Material processing

8.2.1 General

Once the bulk material has been sourced there are a number of processing stages which may need to be carried out to ensure the material has the appropriate homogeneity and stability for its intended purpose. Some of the more common processes are described in the following sub clauses.

8.2.2 Drying

Removal of water makes matrix materials far easier to handle and also improves both their short and long term stability. Drying of soils and similar matrices may be carried out at ambient or elevated temperature, depending on the properties of interest, since the more volatile components may 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.

8.2.3 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. For large bulk quantities, these processes are slow and may take several days to complete. Care should be taken not to introduce contamination from the apparatus during the grinding process. The health and safety aspects of grinding large quantities of particulate matter, which may have toxic components, should also be considered. Cryogenic grinding at −78 °C (solid CO_2) or −196 °C (liquid N_2) may be necessary for polymers, biological, oily/fatty and thermally labile materials.

8.2.4 Sieving

Sieving is often carried out after milling and grinding to improve material homogeneity. 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 may change and the matrix may no longer reflect the composition of regular test samples.

8.2.5 Mixing and blending

Bulk solid material should be 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 may enable the preparation of QCMs with a desired property values, a set of similar QCMs covering a range of property values, or the preparation of QCMs from an existing reference material.

In order to obtain homogeneous mixtures, the materials to be mixed should have similar densities and particle size distributions.

8.2.6 Filtration

Filtration of solutions before bottling removes any particulate and fibrous solids that would compromise the homogeneity of the bulk material. However, some liquids cannot be filtered due to i) viscosity, ii) potential loss of active ingredients by adsorption to the filter or iii) the introduction of contamination. Qualification of the filter is critical to avoiding loss of active ingredients.

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

8.2.7 Stabilization

Certain analytes are unstable in solution and as a consequence 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 may require other approaches such as addition of antioxidants, preservatives, texture stabilizers, etc.

8.2.8 Sterilization

Prepared soils, sewage sludges and biological materials may contain persistent pathogens that are potentially harmful to humans. They may 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 QCM, it is important to consider the impact of the proposed sterilization process on the material, 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 sub-contractors.

8.3 Sub-division and packaging

8.3.1 General

Once the bulk material has been processed it will need to be sub-divided and packaged. The following sub clauses describe some of the key considerations for the sub-division process and choice of containers to ensure the QCM is sufficiently homogeneous and stable for its intended purpose.

8.3.2 Choice of containers

For QCMs to be produced cost-effectively one aspect that needs careful consideration is the choice of appropriate containers for the individual units. If unsuitable containers are used, a material may quickly degrade to the extent that time-consuming and expensive sourcing and preparation work on the bulk material may have to be repeated. The type of container used depends on the inherent stability of the material and the length of time it is required to remain stable. For particularly susceptible materials, two forms 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.

— Organic materials can either lose or pick up ***moisture*** if the container is not securely closed. Glass containers with screw-caps 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*** should be prepared and sub sampled under an inert gas atmosphere (nitrogen or argon).

— For water 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 screw-caps are more suitable for this application, but they themselves have the potential 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 by the use of fluorine-treated polyethylene bottles.

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

— The possibility of contamination of the QCM by the ***leaching of impurities*** from the container should also be considered. For example, the iron content of canned foodstuff QCMs may 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 may also give rise to leaching problems. As a general rule, containers that might interact with the QCM should 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.

— QCMs 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. Vials and ampoules should preferably be amber to reduce the impact of light.

Some preliminary experimental work, including blank studies, may be required to identify the most suitable container type to use for a particular QCM.

The effect of repeated opening and closing of the sample containers may also be assessed if repeated use of the material is anticipated.

Tamper evident closures should be considered if the unit should only be used once.

8.3.3 Sub-division procedures

Once a homogeneous bulk material has been produced, the essential requirement of any sub-division process is that the homogeneity of the material is maintained. That is, the sub-division process itself, or the time taken to complete the sub-division of a bulk material, should not re-introduce heterogeneity into the material. This may conceivably occur in a number of ways.

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

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

Care should be taken with solid particulate matrices such as soils, sediments, industrial products, etc. to ensure that segregation of finer particles does not occur during sub-division. Special care should be taken 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 low between-unit variation. Commercial riffling devices can be used to sub-divide such materials without introducing heterogeneity. Sampling and sub-division of particulate materials are described in more detail in ISO 14488:2007.[15]

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

As a general principle, sub-division of a bulk material should be completed as quickly as possible to minimize the opportunities for the matrix to revert to heterogeneity. Where appropriate, steps should be taken to maintain a homogeneous bulk material during the sub-division process. It may 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 QCMs intended for trace analysis, special care must be taken 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.

9 Homogeneity

9.1 Overview

Homogeneity is a relative concept. The required level of homogeneity of a QCM is dependent on an understanding of the expected variation of the amount of sample used in the measurement process under investigation (see 7.3). In all cases, the level of inhomogeneity should result in a smaller effect on the measurement result than the expected variation of the measurement process or should be below an established criterion value.

Once a candidate QCM has been sub-divided into individual aliquots it is important to establish whether there are any variations in its property values between aliquots. For certain QCM matrices, such as true solutions which have been prepared by procedures such as filtration (to remove particulates) and thorough mixing, formal homogeneity testing is, in principle, not necessary. Such materials may be formally regarded as being inherently homogeneous. Nevertheless, because of the risk of contamination (e.g. introduced due to packaging) or imperfect subdivision, it is recommended to carry out a simple homogeneity study.

For more complex matrices such as foodstuffs, soils and solid matrices that are inherently heterogeneous, a formal experimental investigation of homogeneity is required. A sufficient number of units, representative of the entire batch of the QCM should be chosen and analysed for selected properties. [1] In certain instances, one property can be chosen to represent and quantify the homogeneity of several properties of a similar general type. This should be based on scientific evidence or on previous experience that certain properties exhibit similar behaviour [13] or are known to have a strong tendency to homogeneous distribution in the sample (e.g. some metals in alloys).

A statistical evaluation of the data and a test for sufficient homogeneity are carried out, which can be readily achieved using spreadsheet software (see 9.3). ISO Guide 35[2] details the requirements for the assessment of homogeneity and gives full details and examples of recommended approaches for the design of homogeneity studies and the statistical treatment of homogeneity data.

Homogeneity has two aspects, between-unit homogeneity and within-unit homogeneity. Between-unit homogeneity reflects the variation in the measurement results in each unit of the material. Within-unit homogeneity is reflected in the minimum size of subsample that is representative for the whole unit. It should be confirmed that sample sizes typically used in the day-to-day analysis are larger or at least equal to this size.

9.2 Analytical approach

A validated analytical method having a sufficient degree of repeatability should be selected for evaluation of the homogeneity. The selected units should be representative of the entire batch and the number of units is dictated by the total number of units produced.

Sampling guidelines [16] for the homogeneity testing of multi-unit batches recommend that for a stock comprising "*n*" individual units of material the number of units to be analysed for homogeneity should be three times the cubed root of "*n*". For a stock of 600 to 1 000 units this equates to between 27 and 30 units to be analysed in duplicate. This represents a considerable analytical effort which will be time-consuming and expensive. A study of the impact of reducing the number of units selected for homogeneity assessment of a QCM [2] concluded that in certain circumstances 10 units analysed in duplicate were sufficient. For the preparation of a QCM therefore, there may be scope for cost savings by reducing the number of units selected for homogeneity testing, although this should be assessed on a case by case basis.

ISO Guide 35[2] acknowledges the fact that, in some circumstances, it may not be feasible technically or economically to determine the homogeneity of all properties of interest. Where only selected properties are assessed for homogeneity, these must be representative of the other properties of interest (e.g. on the basis of established physical or chemical relationships).

The following represents a suitable approach for homogeneity assessment.

— Select a number g of the samples in their final packaged form at random, where $g \geq 10$.

— Prepare two test portions from each sample using techniques appropriate to the test material to minimize between-test-portion differences.

— Taking the $2g$ test portions in a random order, obtain a measurement result on each, completing the whole series of measurements under repeatability conditions.

— Calculate the arithmetic mean $\bar{x}$, within-sample standard deviation S_w, and between-sample standard deviation S_s, as shown in 9.3.

The measurements should be carried out such that measurement drift can be distinguished from any trend in the batch of samples. This can be achieved by measuring the replicates in randomized order or by reversing the order of the replicates.

9.3 Statistical treatment of homogeneity data

The principles of ISO Guide 35[2] should be applied with respect to the statistical evaluation of the results for homogeneity.

Adapting example B.3 from ISO Guide 35:2006[2] (chromium in soil) to a homogeneity study of 10 units in duplicate, the data become:

Table 1 — Homogeneity study of 10 units

Unit number	Result 1 mg·kg^{-1}	Result 2 mg·kg^{-1}	Mean mg·kg^{-1}	Variance mg·kg^{-1}
1	121,3	128,74	125,02	27,68
2	120,87	121,32	121,10	0,10
3	122,4	122,96	122,68	0,16
4	117,60	119,66	118,63	2,12
5	110,65	112,34	111,50	1,43
6	117,29	120,79	119,04	6,12
7	115,27	121,45	118,36	19,10
8	118,96	123,78	121,37	11,62
9	118,67	116,67	117,67	2,00
10	126,24	123,51	124,88	3,73

with an overall mean of 120,02 mg·kg^{-1}.

This data need to be assessed to determine whether the material is sufficiently homogeneous for use as a QCM. To be of use as a QCM, the between–bottle standard deviation should be no greater than one third of the within–laboratory reproducibility standard deviation (which can be obtained from existing control chart data, where available, or from existing reproducibility and repeatability data for the method). This is analogous to the approach recommended for the homogeneity acceptance criteria for proficiency testing materials.[17]

When reviewing this data, consideration should be given to the nature of the material (in this example - a soil) and whether such variation is within acceptable limits.

A useful first step is to review the data graphically. This enables any discordant features (such as outlying samples, trends or other systematic effects) to be readily identified.

The data from Table 1 are plotted in Figure 2 and while there are no obvious trends, there is variability of the within unit duplicate results and the data from unit 5 requires investigation.

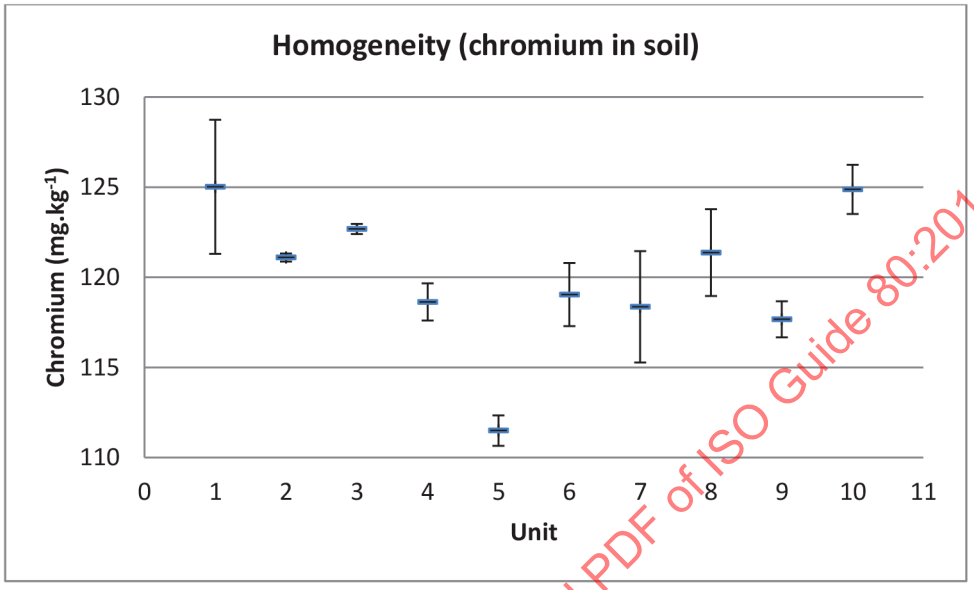

Figure 2 — Data plot

This graphical analysis of the data are complemented by statistical analysis to determine the within unit and between-unit standard deviations. Single factor analysis of variance using spreadsheet software gives the following values in Table 2.

Table 2 — Results of single factor analysis of variance

Source of variation	Sum of squares (SS)	Degrees of freedom	Mean squares (MS)
Between-units	284,94	9	31,67
Within-unit	74,05	10	7,41
Total	358,99	19	

The between-unit variance is estimated using:

$$s_A^2 = \frac{\mathrm{MS}_{\text{between}} - \mathrm{MS}_{\text{within}}}{n_0} = \frac{31{,}67 - 7{,}41}{2} = 12{,}13\,\mathrm{mg}^2 \cdot \mathrm{kg}^{-2}$$

The between-unit standard deviation is the square root of this variance:

$$s_{bb} = \sqrt{12{,}13} = 3{,}48\,\mathrm{mg} \cdot \mathrm{kg}^{-1}$$

The repeatability standard deviation can be computed as:

$$s_r = \sqrt{\mathrm{MS}_{\text{within}}} = \sqrt{7{,}41} = 2{,}72\,\mathrm{mg} \cdot \mathrm{kg}^{-1}$$

The result shows a rather high between-bottle heterogeneity which is mainly caused by unit 5. If the heterogeneity is above pre-set limits and if it makes the QCM unsuitable for use, further manipulation of

the material may be necessary in order to improve homogeneity. In this case, it would also be advisable to perform an in-depth analysis of the data for point 5 to determine if there are any technical reasons to question the validity of this data which could lead to its exclusion from the data set.

Other examples for assessing the homogeneity of QCMs are described in the case studies in Annexes A through F.

10 Characterization and value assignment

The purpose of QCMs is to monitor measurement processes for change. In order to achieve this effectively an indication of the property values of the QCM used to monitor the process is needed. It is also necessary to have an indication of the likely variation in values due to heterogeneity between different aliquots.

An effective way of determining an indicative property value is to use the overall mean derived from the homogeneity study. The range within which the property values may reasonably be expected to lie can be estimated by the deviation from this overall mean value. This deviation from the mean can be used to establish control chart warning limits. Conventionally, warning and action limits (also described as lower and upper action limits) are established at two and three times the standard deviation respectively.

11 Stability

11.1 Overview

Different materials will exhibit different types of (in)stability, some of which may be excluded from consideration given long-term historic data and knowledge; some may be undetectable but cannot be excluded from consideration; some can be detected and should be assessed and some follow certain, and well-established, chemical or physical principles.

In principle, the approaches to stability testing described in ISO Guides 34[1] and 35[2] apply. However, if the material is not to be transported beyond the confines in which it was prepared, no short-term tests for degradation need to be performed. For stability during storage, stability assessment of all properties of the material can be both costly and time-consuming and may not be necessary if sufficient checks are in place to distinguish between an out of specification result due to a faulty test sample, measurement instrument drift or QCM degradation. However, those responsible for QCM preparation have to be aware of the trade-off between costs for stability testing and the costs incurred by investigating a method drift, which is in reality caused by degradation of the material.

It should be pointed out that as some QCMs are made for repeated use, investigation of the stability of opened units may be particularly useful.

Adequate stability of QCMs is required; so that users can be confident they will not undergo any significant change affecting the property value(s) during the period of its intended use, which in some cases could be a number of years.

Careful choice of the container used for the material can address some intrinsic stability issues (e.g. sensitivity to oxygen, light, moisture; susceptibility to evaporation of components).

Some materials (e.g. metallurgical materials) are inherently stable, while for others storage at a precautionary low temperature may lengthen the period of stability.

Failures in preparation or unexpected contamination/impurities may impair the stability significantly.

11.2 Assessing stability

Full stability assessment for any reference material is a costly, time consuming and involved process, and is inappropriate for QCMs as described in this Guide, which are prepared in the laboratory where they are intended to be used. It is likely that the laboratory will have previous experience of the stability

of the types of matrix and property values it is preparing as QCMs, or there may be well established background information from similar materials.

However, the financial implications of using QCMs whose property values have changed significantly can be large (e.g. release of out-of-specification products, or non-release of in-specification products) and laboratories using QCMs should have procedures in place which describe the actions to be taken in the event of a QCM giving an unexpected result. Possible actions include use of freshly prepared calibrants, comparison with certified reference materials and frequent sensitivity checks to confirm any deviations or trends in the QCM results. More information can be found in ISO standards on control charts (e.g. ISO 7870-1[3] and ISO/IEC 17025[18]).

Should a formal assessment of stability be deemed necessary, the principles of ISO Guide 35[2] should be followed. An example of this approach is given in Annex F (Case study 6).

11.3 Assigning an expiry date to a QCM

The quality systems of many laboratories require standard materials and reagents to be labelled with expiry dates.[19] The assignment of a formal expiry date is one of the most problematic tasks in reference material production. It involves making a prediction of the future behaviour of a material based on an extrapolation of its past behaviour. Any stated expiry date for a QCM should be based upon previous experience of the stability of the types of matrix and property values and any background information. The stated expiry date should not be regarded as absolute and the analyst using the QCM should follow laboratory procedures in the event of a QCM giving an unexpected result (see 11.2).

Materials which are considered to be inherently stable do not need an expiry date, but a statement justifying the basis for the assumption of inherent stability is required. A typical statement for a metallurgical material might read – "*this is a metallurgical material used as a QC material in the analysis of elements in ore and is considered inherently stable provided it is stored and handled under the recommended conditions*".

12 Transportation

In general, this guidance relates to materials which are prepared and used in the same locality (i.e. not for wider distribution). However, it is recognized that some materials are inherently stable and their property values will not be adversely impacted by transportation (controlled or otherwise).

Should there be a requirement to transport the material and there is any concern about its stability, a stability assessment following the principles of ISO Guide 35[2] should be carried out.

13 Documentation for quality control materials (QCMs)

13.1 General

As with laboratory reagents, QCMs should be appropriately labelled and have instructions for their safe and effective use. ISO Guide 31[20] provides details of the requirements of the contents of certificates and labels for reference materials.

13.2 Information to be available with quality control materials (QCMs)

The following information should be readily available to the QCM user:[10],[20]

— name and description of the material;

— reference number and/or batch number;

— date of preparation;

— intended use of the material and any special instructions for its use (e.g. specific drying procedures to follow or instruction on dry mass correction should be given, if applicable);

— indicative value(s), if applicable;

— the minimum amount of material required to achieve consistent results (this will depend upon the quantities used for the homogeneity study), i.e. minimum sample intake;

— storage instructions;

— information on expected shelf-life;

— any special safety precautions to be applied by users.

13.3 Labelling of QCM units

Each unit of the QCM should be clearly labelled, in a manner that enables it to be unambiguously linked to the information for the material. The label should therefore contain the following essential information:

— name and description of the material;

— reference number and/or batch number;

— hazard and safety labels, where appropriate;

— storage area environmental conditions including temperature and humidity;

— preparation date;

— expected expiry date.

It may be useful to provide the following additional information on the label:

— unit size (e.g. 20 g);

— individual unit number (this may help to identify any bottling trends in the event of an unexpected result being obtained);

13.4 Useful information to be retained

Information relating to the preparation of the QCM will be required if a query arises regarding the material during use, or if a new batch of the material has to be produced. It is good practice to retain/archive data relating to the following aspects of the preparation of the QCM in an accessible form:

— relevant specifications;

— source and preparation of the bulk material;

— types of containers used for the individual units;

— sub-division procedures used;

— any special treatments used to stabilize or sterilize the material;

— supplementary data on the material (e.g. particle size, moisture content, etc.);

— full details of all methods used to produce the material;

— all data from methods used to produce the material;

— specific experience useful to avoid pitfalls and costly errors during preparation of new batches or related QCMs.

14 Storage

14.1 General

Completed batches of QCMs should be stored under conditions that will ensure they remain unchanged. Generally, this entails ensuring that the individual containers are securely closed and are stored away from extremes of heat, light and humidity.

Storage in dark conditions at the appropriate temperature (e.g. room temperature, 4 °C or −18 °C) is usually the key aspect of ensuring long-term stability. For QCMs comprised of matrices where stability is/might be considered questionable, such as food matrices and aqueous solutions, it is recommended that long-term storage is carried out at sub-ambient temperatures. For matrix/property types where stability is more ensured, such as soils containing metals or PCBs, long-term storage may usually be carried out at ambient temperature.

14.2 Monitoring of storage conditions

The relevant storage conditions should be monitored at regular intervals, to ensure that, for example, the appropriate storage temperature is being maintained. A recommended procedure is to check the temperatures of refrigerators/freezers, etc. on a daily basis, so that if a failure does occur, the user can take appropriate action (e.g. transfer the material to another storage location, if available, or carry out an assessment of the continued fitness for purpose of the material). A record should be kept of the temperature readings of refrigerators/freezers, etc.

15 Using quality control materials (QCMs)

15.1 General

All reference materials, whether from commercial suppliers or in-house producers, should have instructions for their use. These instructions should include details on how the material should be stored and handled, including specific instructions for sub-sampling and dry-mass correction.

15.2 Minimum sample size

All reference materials (with the exception of homogeneous solutions and gases) are inherently heterogeneous but for practical purposes taking sufficiently large samples reduces the heterogeneity to an acceptable value.

The minimum sample size to be taken is the smallest sub-sample that is still representative of the whole unit for the target property value. Frequently, this is estimated as the minimum sample size used in the assessment of the homogeneity of the material. The minimum sample size stated to be used is therefore a conservative estimate and not the absolute minimum.

15.3 Mixing procedure

Prolonged storage of multi-component mixtures and matrix materials may cause settling and separation of the material. It is therefore important that aliquots are adequately mixed before a new sub-sample is withdrawn. This can often be achieved by simple shaking of the units. Where stirring is required to re-mix the material sufficiently for use, care should be taken not to introduce any impurity that will interfere with the measurement process.

Where possible, simple visual inspection of the material before sub-sampling for use, can often identify heterogeneity, such as agglomeration.

15.4 Dry mass correction

Many property values are presented as *content per dry mass of sample*. This has the advantage that potential moisture uptake does not change the value, but has the disadvantage that the property value is then dependent on the method for the determination of the dry mass. Studies[21] have shown that results from different methods (e.g. drying oven, Karl Fischer titration, vacuum drying oven) differ significantly – not least because they measure different things.

For moisture sensitive materials, appropriate packaging would be the preferred method of controlling moisture content. Where this is not possible and the moisture content of the material may change over time, a consistent method for dry-mass correction should be applied each time the material is used.

15.5 Storing opened containers of QCMs

Repeated opening and closing of reference material containers increases the risk of contamination. In the process of preparing the QCM the effect of opening and closing of the container and repeated freezing/thawing of units in terms of stability and homogeneity should be considered[2].

Where possible the unit size should be chosen such that it reflects the amount of sample needed for the type of analysis for which it is designed. In this way, once opened each unit is entirely used as soon as possible after opening to avoid potential degradation. Preparing QCMs as a number of small, single use units may be advantageous in this case.

If opened containers of QCMs are to be stored, storage conditions should be selected under which the material has been demonstrated to be stable. These conditions should protect the material from accidental contamination and be included in the user instructions.

2) This is not always easy as illustrated by the reference material BCR-522 (haemoglobincyanide in bovine blood lysate), which does not re-dissolve properly after it has been frozen.

Annex A
(informative)

Case study 1 — Preparation of a QCM from coal[3)]

A.1 Objective

A coal testing laboratory uses a QCM for daily quality control for proximate and ultimate analysis in accordance with the applicable ISO standards. One can of 1 l, holding approximately one 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 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 blended coal.

A.3 Checking of 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 of water, sieved, split into 10 portions and subdivided.

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

3) This case study was provided by Adriaan M.H. van der Veen, NMi Van Swinden Laboratorium B.V., Thijsseweg 11, 2629 JA Delft, The Netherlands.

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), a 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 a 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 preparation essential to support a root cause analysis, if required 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 should not be greater 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 put to use with the mean from these 10 measurements, after carefully scrutinizing the data. The data analysis indicated no irregularities.

Annex B
(informative)

Case study 2 — Preparation of geological or metallurgical quality control materials (QCMs)[4)]

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 in-house reference material preparation generally stems from the difficulty in sourcing a suitable commercial reference material for the analysis of a material of a unique sample matrix.

B.3 Sourcing of material

The following factors are taken into account.

— The matrix of the material must 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 reference material.

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

— The quantity of material should 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 may be such that it would not be safe to handle large quantities, nor feasible to obtain large quantities.

B.4 Preparation of reference material

The preparation 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;

4) 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.

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, packing tape.

B.5 Crushing, blending and milling source material

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

$$c_i = \Sigma\, c_{ij}\, w_j$$

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:

$$c_i = 0{,}50 \times 100/(100+400) + 4{,}00^* 400/(100+400) = 3{,}30 \text{g/t Cu}$$

Prior to milling the material, the crusher products are blended in a mixer or V blender adding approximately the required final proportions of the different crusher products. If more material is required to be mixed than the mixer can accommodate, a number of 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 reference material 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 required top size since more malleable metal particles may 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 reference materials. 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 a number of ***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 must be split further. The blender must not be full, otherwise, it will not function adequately.

Prior to commencing the blending step, the material must 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 preparation. The moisture content is recorded. Samples are not dried at a higher temperature lest minerals such as pyrhotite or gypsum alter into other species.

Each of the original drums Drum 1, Drum 2, Drum 3, etc. (which are of equal mass) must be homogenized for at least four hours in the V blender that is not filled beyond the mark. For a 600 kg RM, there should 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 per cent relative standard deviations (%RSD) of these weights exceed 2 %, the splits must be 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.

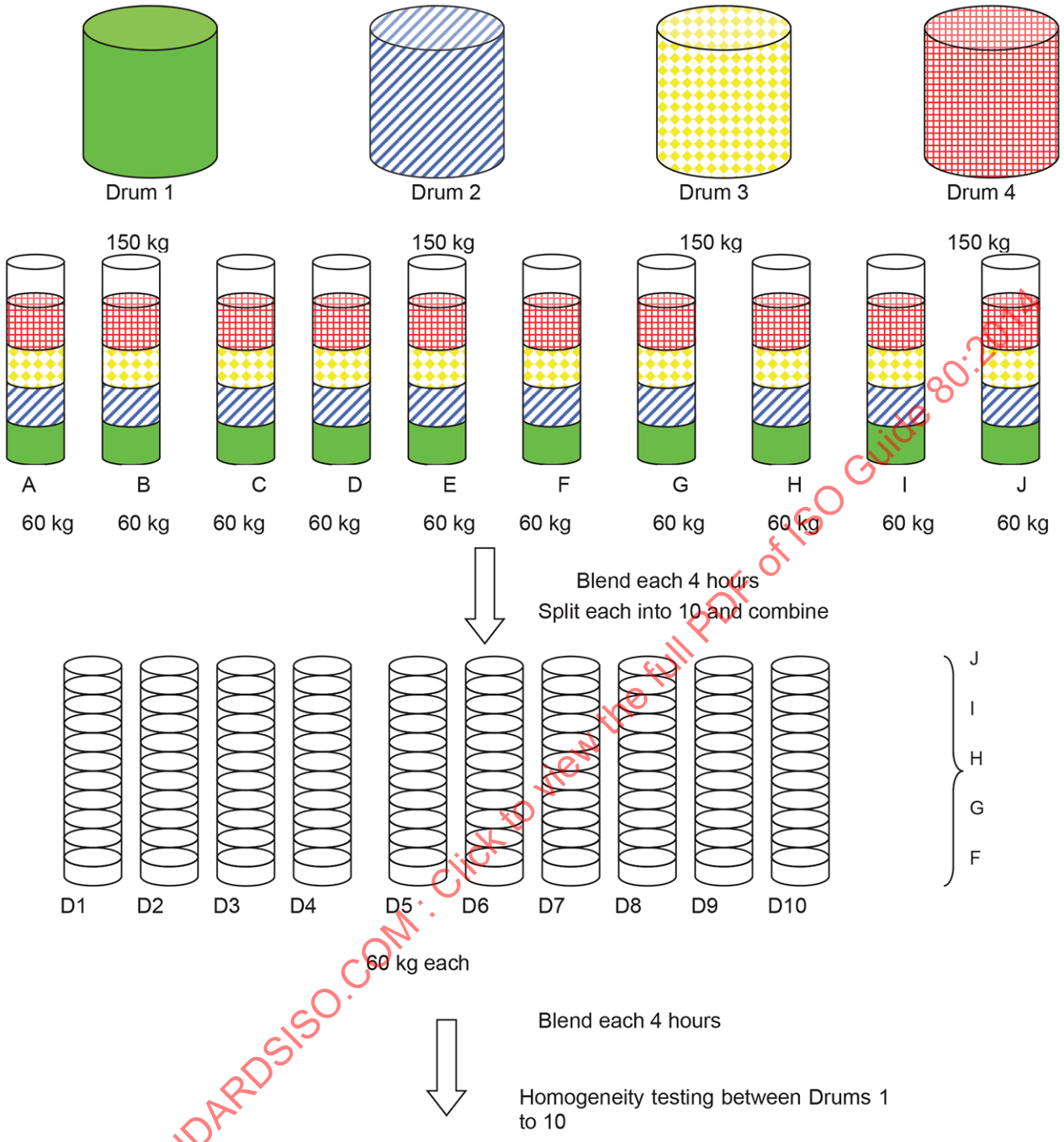

Figure B.1 — Flow diagram of splitting and blending

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, rather a result of the analytical process. The samples are submitted in the required sequence.

The data received from the laboratory are transferred to an Excel[5)] 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 upon 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 in order 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 preparation process is stopped and the cause of the poor precision determined. Material in-homogeneity may 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 may, 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 are positively skewed or not. Positively skewed data arising from underlying Poisson or log-normal distributions can complicate the certification process and may indicate insufficient grinding/crushing. Limits for significantly skewed table are determined at a 90 % confidence limit.

A single factor ANOVA is used to 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).

5) 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	0.000134
Drum 2	5	1.238	0.2476	0.000166
Drum 3	5	1.255	0.251	0.000124
Drum 4	5	1.236	0.2472	7.47E-05
Drum 5	5	1.271	0.2542	9.17E-05
Drum 6	5	1.23	0.246	0.000126
Drum 7	4	1	0.25	0.000133
Drum 8	5	1.266	0.2532	5.07E-05
Drum 9	4	1.007	0.25175	0.000137
Drum 10	5	1.283	0.2566	0.000142

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.000722	9	8.02E-05	0.686767	0.716084	2.137528
Within Groups	0.00444	38	0.000117			
Total	0.005162	47				

Comment: F<Fcrit (Between Drum Homogeneity Acceptable)

Figure B.2 — Microsoft Excel ANOVA to determine homogeneity

Should the material pass all of the required 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 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 Guide 35.[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. Should the material require long term storage, the material may 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. Should final packaging be required, 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 required method %RSD and that obtained during initial homogeneity testing.

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

B.9 Assigning accepted performance limits

For a material intended to monitor consistency / repeatability of a method only, it may be considered sufficient to assign values based upon traceability to other CRMs, or even other reference materials 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 — Preparation of a wheat flour fortified with folic acid quality control material (QCM)[6)]

C.1 Introduction

Folic acid fortification of wheat flour for bread making became mandatory in Australia in September 2009. The level of fortification required 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 an in-house QC reference 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 preparation 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 preparation 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 actually 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.

C.3 Preparation

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

6) This case study was provided by Meg Croft and Stephen Davies, National Measurement Institute Australia. PO Box 138, North Ryde NSW 1670, Australia.

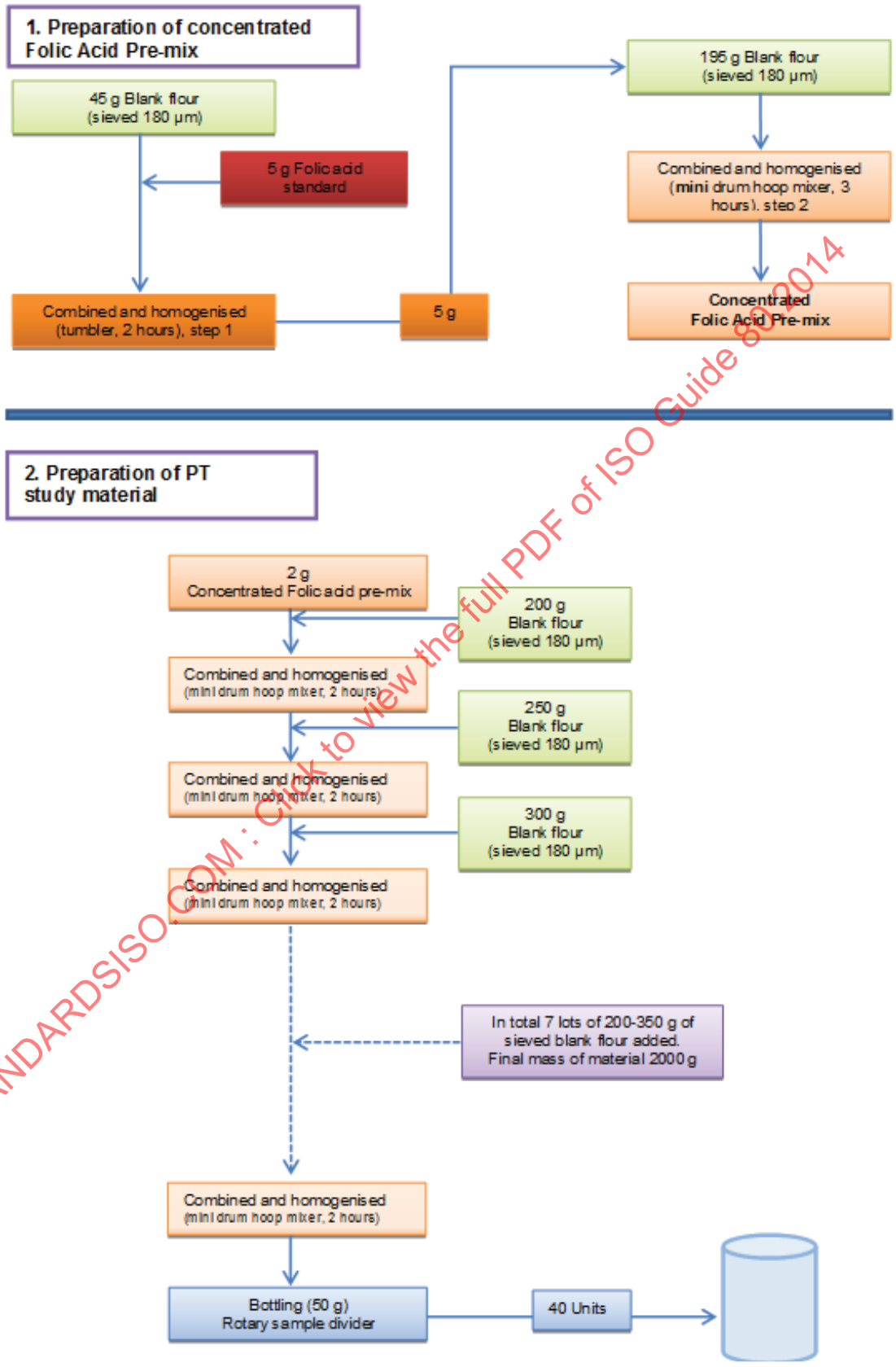

Figure C.1 — Production steps

C.3.1 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.3.2 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 mole 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 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 preparation 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 may 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 preparation of folic acid fortified flour samples confirmed the adsorption of folic acid onto the surfaces of the various mixing vessels employed in the preparation process. While various different 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[7)].

7) A Retsch PT 100 Rotary Sample Divider is an example of a suitable product available commercially. This

C.4 Sub-division and packaging (e.g. 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 preparation procedure produced homogeneous samples containing the expected (gravimetric) concentration of barium.

Testing the methodology using barium carbonate made the assumption that folic acid and barium carbonate would behave similarly during the preparation process. 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 within (analytical) and between bottle variance.[17]

C.5.2 Examples of data and data treatment

The in-house 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.

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	2,3785	0,00042
Row 2	2	4,685	2,3425	0,003784
Row 3	2	4,723	2,3615	0,000265
Row 4	2	4,741	2,3705	0,013285
Row 5	2	4,728	2,364	3,2E-05
Row 6	2	4,584	2,292	0,001152
Row 7	2	4,78	2,39	0,001352

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0,012476	6	0,0020793	0,717365	0,6492	3,865968853
Within Groups	0,02029	7	0,0028986			
Total	0,032766	13				

Prepared by _____

Checked by _____

Figure C.2 — Production steps

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 Guide 35.[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 of time – 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 upon 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)[8)]

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[9)], reactive silica[10)] 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 Preparation

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 reference material.

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 in order to safeguard against contamination.

D.3.3 Packaging requirements

Packaging requirements are:

— plastic or glass bottles with screw cap;

— units of the reference material clearly labelled.

8) This example was provided by Dr Maria Alice de Goes, CETEM, Rio de Janeiro - RJ – Brazil, 21941-908.

9) Amount of alumina that is digested in a caustic solution (150 ºC) at similar conditions of Bayer Process.

10) Amount of silica that reacts with sodium hydroxide (150 ºC) 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 preparation to reduce the batch inhomogeneity.

To assess homogeneity, a subset of the batch of units of the reference material 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. In order 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.

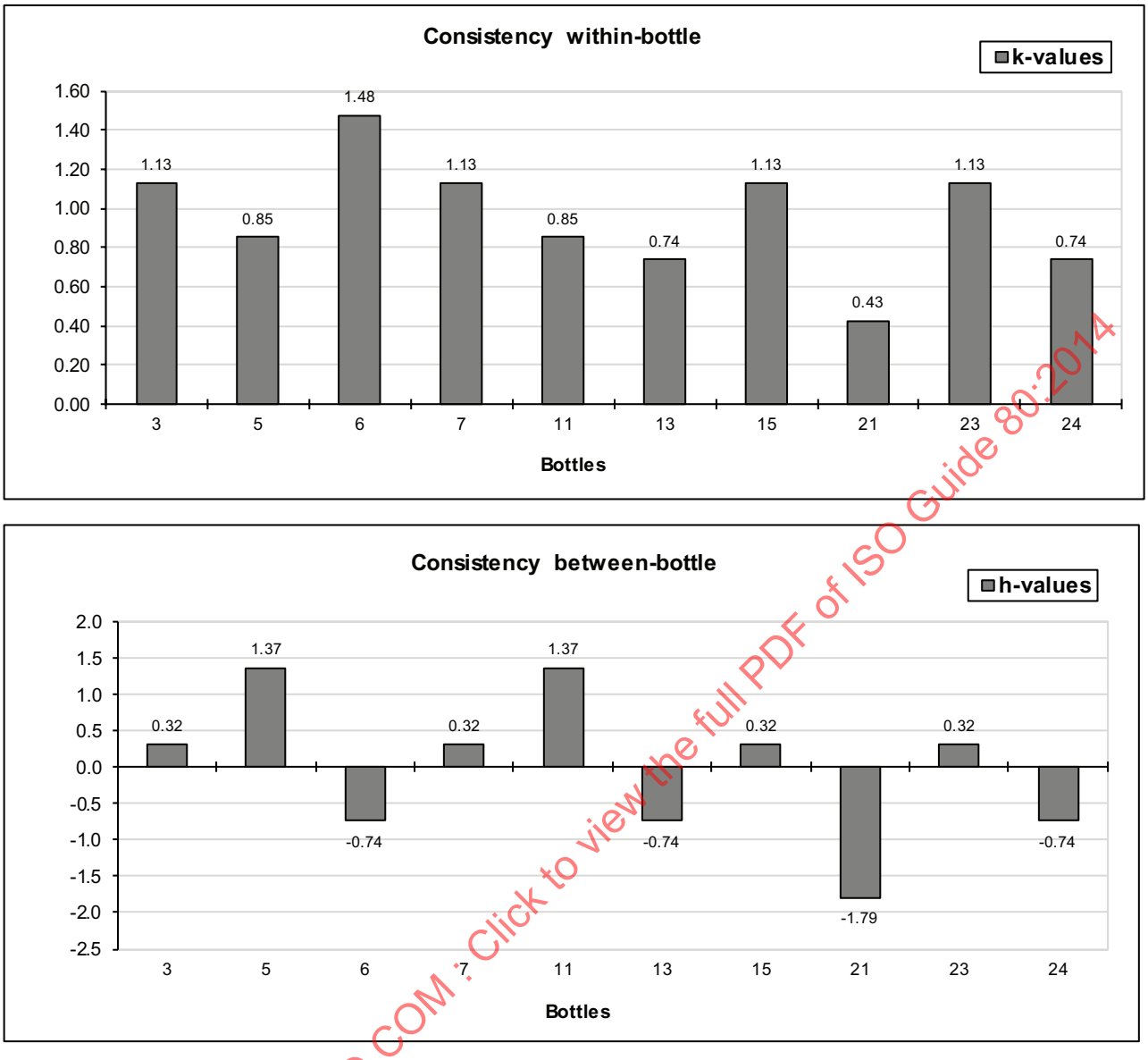

Figure D.1 — Plots of consistency within- and between -bottle

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				

$s_{an} = 0{,}018$

$s_{sam} = 0$

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 Guide 35.[2]

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

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

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

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 reference material 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 reference material should be stored at ambient temperature in a dry place.

D.6.2 Minimum sample size requirements

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

D.6.3 Safety information

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

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

Annex E
(informative)

Case study 5 — Pharmaceutical reference standards[11)]

E.1 Introduction

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

Reference standards are needed almost at every stage in the development of a drug candidate in order 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 preparation of bulk materials for reference material production can at first seem a difficult and daunting task, and large quantities may sometimes be required. High quality reference materials (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, should this be necessary, this case study provide guidance for non-specialist laboratories to prepare their own reference materials for quality control. Some of the key issues that need to be considered are: selection of materials (appropriateness, native material versus spikes, material preparation, etc.), traceability, testing, preparation 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 in 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 a number of processing stages which may need to be carried out to ensure the material has the appropriate level of homogeneity and stability for its intended purpose.

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 reference materials only.

11) This case study is based on information provided by Iffaaz Salahudeen, PhD, and Frank Hu, PhD, Bristol-Myers Squibb Pharmaceuticals, New Brunswick, NJ, USA.

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

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

E.2.2 Specification of material

A reference standard should 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 should 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 should be controlled throughout the manufacturing process. Impurities that are process-related should be kept to a minimum to avoid degradation and unwanted pharmacological effects. Compounds that are susceptible to hydrolysis, for example, should be thoroughly dried to remove moisture and then stored in a desiccator. Reference standards that contain a high percentage of organic volatile impurities may experience purity changes over time as the solvents evaporate.

If the reference standard is in a salt form, the amount of salt present must be determined so that the purity can be corrected for content. Applying the molecular weight to the correction will not account for residual salt that may 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 must be characterized. Both the reference standards and drug substance may be synthesized initially using the same process. The reference standard should be of the highest purity possible; the drug substance may require further purification to become a reference standard (additional purification steps used for a drug substance should 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 has a tendency to form lumps, a delumping procedure can be employed. Large particles can be reduced by grinding. In order 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 may be necessary in order to make sure the mixture is homogeneous. An old or API lot also can be used after fresh testing as long as its properties are within the acceptable range.

E.2.5 Qualification

For the initial lot, an example requalification period may 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 should 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 is required to 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 must be identified and quantified. The synthesis

of the reference standard should be evaluated to predict and identify potential impurities from raw materials. Potential degradation product also can occur as a result of storage. Short-term (forced degradation) and long-term (evaluation under accelerated conditions) stress testing, therefore, should 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 per cent or from the relative response of the standard being used. The technique used to obtain this data will depend on the amount 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 per cent versus relative response factor, the following scenario may 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 may be insufficient to affect overall purity results. If there was 1 % impurity based on area per cent 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 should be considered. The relative-response factor approach requires additional development because the component needs to be isolated and the relative response factor must be determined. In addition, as the reference standard ages, new unknown impurities may be detected. The relative-response factor of these new impurities must be determined, and the method updated if the new unknown is significant enough to alter the purity. Much of this information may be ascertained during the development of the drug substance.

Impurities that arise from raw materials, synthesis, purification, and storage require careful consideration because they may not produce detector responses that are related to the reference-standard material. Quantification by area per cent would not be appropriate in such cases. Rather, the impurities must be 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 is required for the cation, and a separate analytical method for quantification may 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 further analysis is not required.

Residual solvents. The potential for residual solvents should 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, may be specific to the manufacturing process and require a specific test procedure. An additional specific test procedure may be required 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 required at subsequent intervals. If the amount of residual solvents present affects the purity, however, they should be evaluated at each requalification interval.

E.2.6 Sub-division and packaging

Reference materials 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. Vials should preferably be amber to reduce the light impact. The amount per vial depends on its application. Repeated opening and closing of reference material containers increases the risk of contamination. Stability may also be affected by repeated

freeze/thaw cycle. Therefore, sufficient quantity of material needed for analysis should be vialed separately, instead of using bulk containers for repeated use.

Light sensitive materials are sub-divided 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 may 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 may be necessary in order to making sure the mixture is homogeneous. These mixtures need to be assessed for homogeneity.

E.2.8 Documentation requirements

Ideally, a document complying with ISO Guide 31[20] and a report covering the characterization and value assignment will be produced.

For quantitative standards, purity values should be assigned, and based on past history and stability, a re-test date should also be provided. However, for qualitative standards (e.g. retention time markers or impurity mixtures), assigned purity values are not needed, as long as 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-certification 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) also should be 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 should be 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 should be 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 standard. 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 should be considered as a guide instead of general requirement for selecting a reference standard.