

Reference materials as crucial tools for quality assurance and control in food analysis*

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Abstract: The role of reference materials (RMs) in analytical quality assurance (QA) is outlined with special emphasis on trace element analysis of foodstuff. Crucial aspects for the development of such food RMs are illustrated by a recent example of trace elements in rice flour. Major influences on the uncertainty of certified values are discussed and possibilities to enhance the availability and fitness-for-purpose of RMs as well as their proper use are indicated.

Keywords: food analysis; reference materials; trace elements; quality assurance; uncertainty.

INTRODUCTION

The analysis of food samples with respect to chemical constituents suitable for the characterization of food quality and food safety is still attracting increasing interest. Corresponding analytical results have to be reliable and fit-for-purpose because of the potential importance under commercial and health aspects, but also because of the public attention on those topics. From an analytical point of view, food samples constitute often complex and difficult matrices with potentially important chemical species ranging from ultra-trace to major components. Therefore, a large variety of analytical methods have been developed and many more will be required to characterize food samples.

Any analysis always includes a series of operations, usually from sampling via measurements to evaluations leading to the analytical result. This so-called “total analytical process” has to be selected and designed in dependence on the problem to be solved. Consequently, the quality of the final analytical result is also influenced by many factors. However, the objective of the analytical process is always to provide a result that is fit for its intended purpose, i.e., that has a certain “quality”. Therefore, appropriate methods, procedures, and practices have to be designed and applied to ensure that the end result will meet “fitness-for-purpose” requirements.

In dependence on the specific quality requirements of the analytical task, the appropriate levels of quality assurance (QA) and more specifically for quality control (QC) have to be defined. Internal QC measures typically include the analysis of reference materials (RMs) and measurement standards, the analysis of blanks and blind samples, replicate analysis, and the establishment of control charts. External QC is commonly performed by proficiency testing (PT) of the laboratory performance. The level of QC adopted must be demonstrably sufficient to ensure the validity of the results. Moreover, third-party assessment, namely in form of accreditation, is increasingly recognized as a structured way of demonstrating competence.

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Without underestimating the role and importance of the other QC tools, the following focuses on aspects for the development and application of appropriate RMs for QA/QC in food analysis. In line with the topic of the TEF-2 Symposium, specific examples have been chosen with respect to trace element analysis in foodstuff. But it should be kept in mind that most of the QA/QC aspects are generic and independent of the specific chemical composition of the analyte of interest.

ROLE OF REFERENCE MATERIALS

The total analytical process mentioned above can be described in a general flow chart (Fig. 1). Two types of RMs are indicated in this figure. RMs for calibration (often called also “measurement standards” or “calibrants”) are needed for the quantification step, such as the instrumental measurement of Pb in a digestion solution by using inductively coupled plasma–mass spectrometry (ICP–MS). RMs that are more adapted to the nature of the original sample to be analyzed (such as pork meat RMs, milk powder RMs, etc.) are called “matrix RMs”. They can also be used for method validation and QC of a larger part of the total analytical process, including analytical sample preparation. This is of particular importance for QA/QC of such complex samples as foodstuff, and matrix RMs play therefore a key role in well-designed quality systems for food analysis.

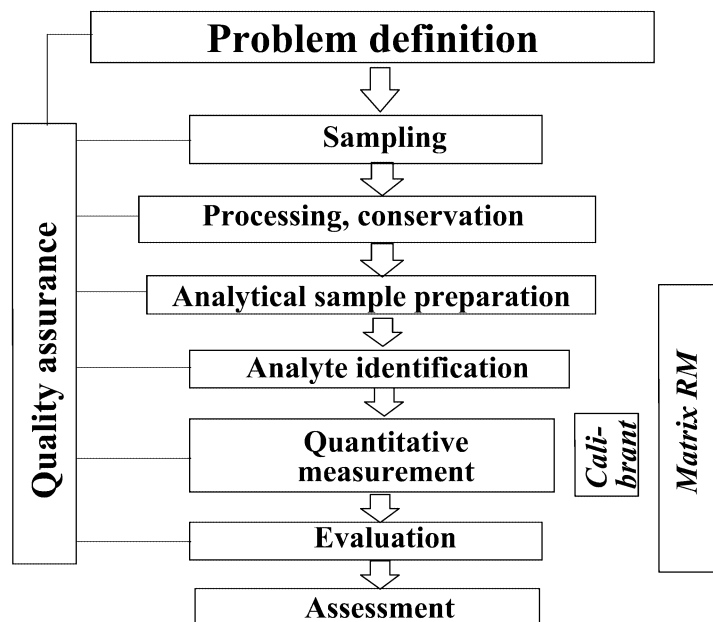


Fig. 1 Flow diagram of general steps of the total analytical process.

According to the definitions in ISO Guide 30 [1] and VIM [2], an RM is a “material or substance one or more of whose property values are sufficiently homogeneous and well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials”, whereas a certified reference material (CRM) is a “reference material accompanied by a certificate, one or more of whose property values are certified by a procedure which establishes its traceability to an accurate realization of the unit in which the property values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence.” According to these definitions, CRMs form a subgroup of RMs, namely, those RMs which possess additional characteristics—a certificate and traceable assigned values with an uncertainty statement. But there is still

considerable confusion about terminology for various types of RMs. A systematic classification based on a strict separation between RM characteristics and uses has been recently proposed [3].

RMs are mainly used for internal QC purposes in the analytical laboratory. During the development and validation of a new analytical method, CRMs allow the evaluation if correct results can be obtained and the reliable estimation of their uncertainties. Moreover, the formal qualification of analytical equipment used and the regular proof of method performance via control charts can be realized with the help of RMs. Another important field of application consists in the establishment of traceable results of the laboratory. Here, the unknown sample and the CRM are measured with the same analytical method, and thus a link is provided for the analytical result of the unknown to an internationally accepted reference point. It should not be forgotten that RMs are also required for the calibration of various analytical measuring instruments, mostly in the form of pure substances or corresponding solutions. But element analysis with the help of solid sample-techniques, such as XRF (X-ray fluorescence) or solid sampling-AAS (atomic absorption spectrometry), needs even the calibration with appropriate matrix CRMs.

External measures of analytical QC are focused nowadays on PT. For the verification of laboratories' competences via such interlaboratory exercises, sufficiently homogeneous and at least short-term stable materials are needed. Therefore, RMs are widely applied PT materials. They can also serve as analytical samples in corresponding training activities.

CRMs could be used for all the applications mentioned above and would provide an added value because they are well characterized and offer traceability. But various tasks such as PT or establishment of control charts can also be performed with noncertified RMs (increasingly called "quality control materials", QCMs [3]).

DEVELOPMENT OF FOOD CERTIFIED REFERENCE MATERIALS

The major limiting factor for the general use of RMs in food analysis laboratories is their restricted availability considering the huge range of possible analyte/concentration level/matrix combinations of the real samples to be analyzed. A recent search in the COMAR database [4] showed 94 food-related RMs with certified values for elements. Among them are 19 CRMs available from IRMM [5], such as bovine liver/muscle, bread, cod muscle, haricots verts, mussel tissue, pig kidney, pork muscle, rye flour, skim milk powder, white cabbage, tuna fish, and wheat flour.

The further development and production of new RMs according to modern internationally accepted guidelines, namely, ISO Guides 34 [6] and 35 [7] require a significant number of studies, processing operations, measurements, and evaluations as well as considerable management efforts. The major steps of such a CRM project are: selection of raw material, feasibility studies for processing and characterizing the material, preparation of the candidate RM, homogeneity study, short- and long-term stability studies, characterization of the candidate RM with respect to the targeted properties (often concentrations of certain constituents), assignment of the certified values and their uncertainties, drafting of certificate and certification report, and finally, the reviewing of the project results and approval of the certification documents. Several crucial aspects of this process will be further outlined by using a recent project for the certification of trace elements in rice flour as example.

The "product" CRM consists of the RM itself, and the certificate containing information about this material as defined in ISO Guide 31 [8]. The potential user will usually inspect before purchase if a CRM fulfils the requirements for his QC procedure. Therefore, the user laboratory should check during CRM selection in addition to the above-mentioned criteria matrix and analyte and concentration level, if the stated uncertainty of the certified value is suitable for the demands of this specific purpose. The reliable determination of such uncertainties is still a very demanding task, despite various practical guides developed in recent years [9,10]. A number of factors that have to be taken into account are illustrated in Fig. 2. The total uncertainty of the stated value for a CRM property, such as the concentration of Cd in a fine powder of freeze-dried pig kidney, depends on intrinsic material characteristics

(microhomogeneity, kinetics of degradation), characteristics of the analytical methods used for studying the CRM (sample intake, calibrants used, uncertainties), environmental conditions (temperature, radiation), and statistical parameters (number of replicates, laboratories, methods, etc.). It should be noted that Fig. 2 contains only major influences and may be much more complex for certain materials.

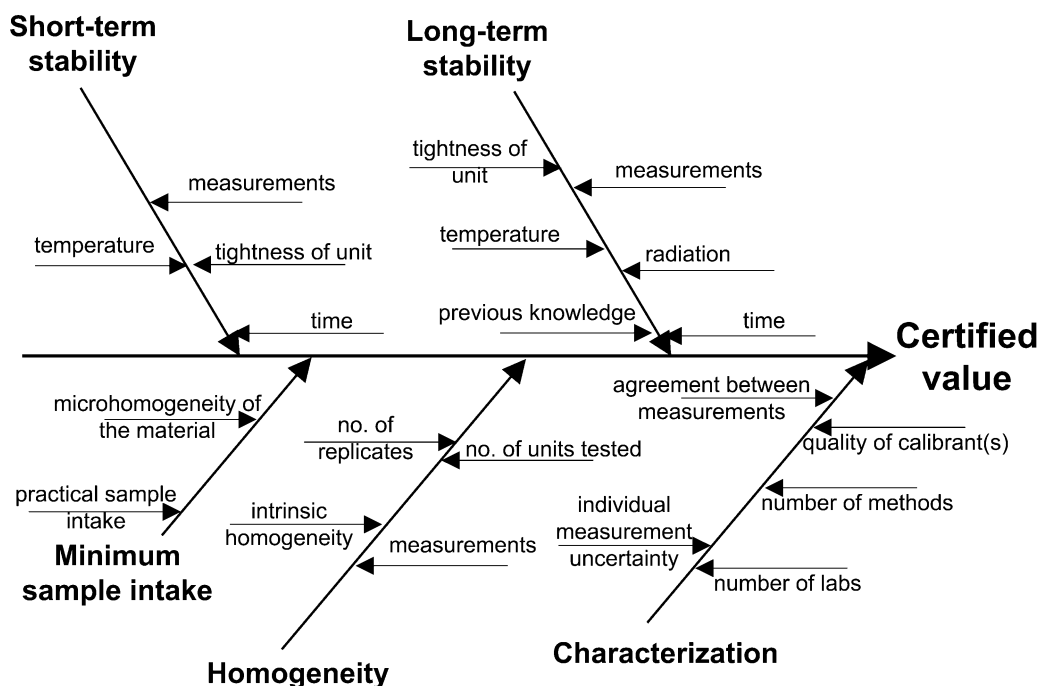


Fig. 2 Major influences on the uncertainty of a certified value of a CRM. The figure does not try to be exhaustive.

An example for assessing the between-bottle homogeneity is shown in Fig. 3 for the Pb content of the candidate RM IRMM-804, rice flour. The Pb concentration has been measured on three different subsamples from 12 different bottles. This allows separating the variability due to the measurement method from the between-bottle variability using ANOVA [11]. In this case, no significant between-bottle inhomogeneity could be found. The maximum inhomogeneity that could be hidden by the method variability was estimated to be 1.1 %. This value is further used as an estimate for the uncertainty of between-bottle homogeneity u_{bb} of Pb.

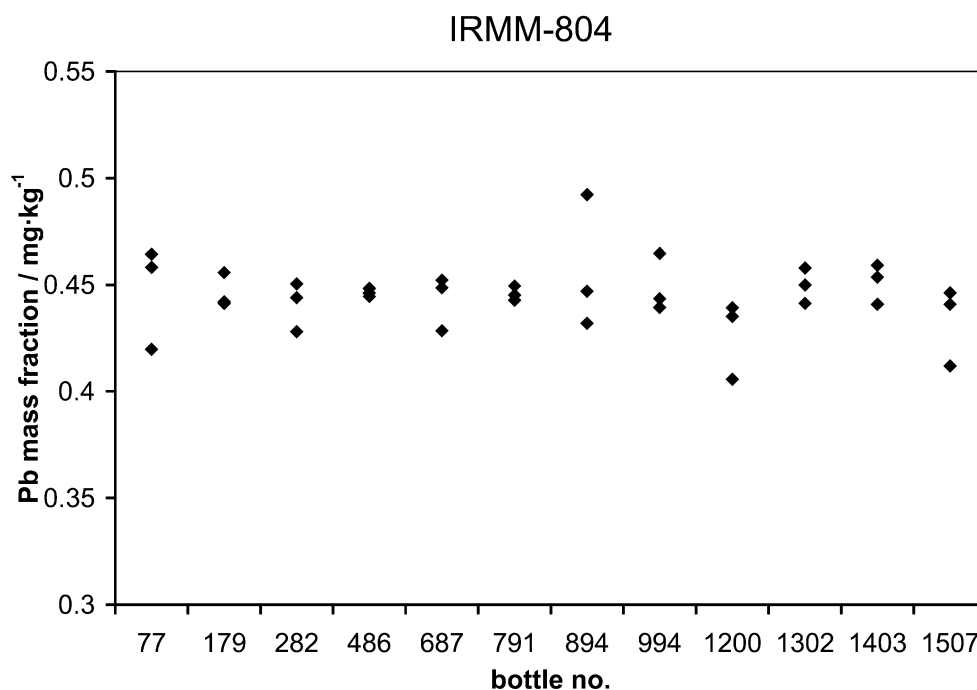


Fig. 3 Characterization of the between-bottle homogeneity of the candidate RM IRMM-804 “rice flour” with respect to Pb. Absolute numbers as depicted here are not relevant.

The long-term stability is the crucial parameter for estimating the shelf-life of a CRM stored under certain environmental conditions. For example, Zn measurements of samples of IRMM-804 were performed in an isochronous scheme for 24 months [12]. The results (Fig. 4) demonstrate that storing this material at 4 °C does not significantly influence the Zn content of the material within a predefined uncertainty (in this case, the uncertainty of long-term stability u_{lt} is 4 % for a shelf-life of 48 months).

There are different strategies for the so-called characterization (i.e., the measurement of property values intended to be certified) of the RM during the certification process. It should be kept in mind that in the case of trace elements in food, any analysis is directed to determine the value of the quantity of interest (e.g., Pb concentration) in a material for well-defined chemical constituents. Such quantity values should be SI-traceable and independent of the analytical method used. Therefore, the one very suitable approach for characterizing food matrix RMs is the application of different analytical methods in a specifically designed interlaboratory comparison involving expert laboratories. As an example, results for the determination of Pb in the rice flour material are shown in Fig. 5. Nine laboratories, using ICP–MS with “conventional calibration”, ICP–MS with isotope dilution, or AAS with a graphite furnace have measured the Pb content. From the resulting data, an uncertainty contribution of the characterization u_{char} of 3.3 % can be derived.

IRMM-804; Zn

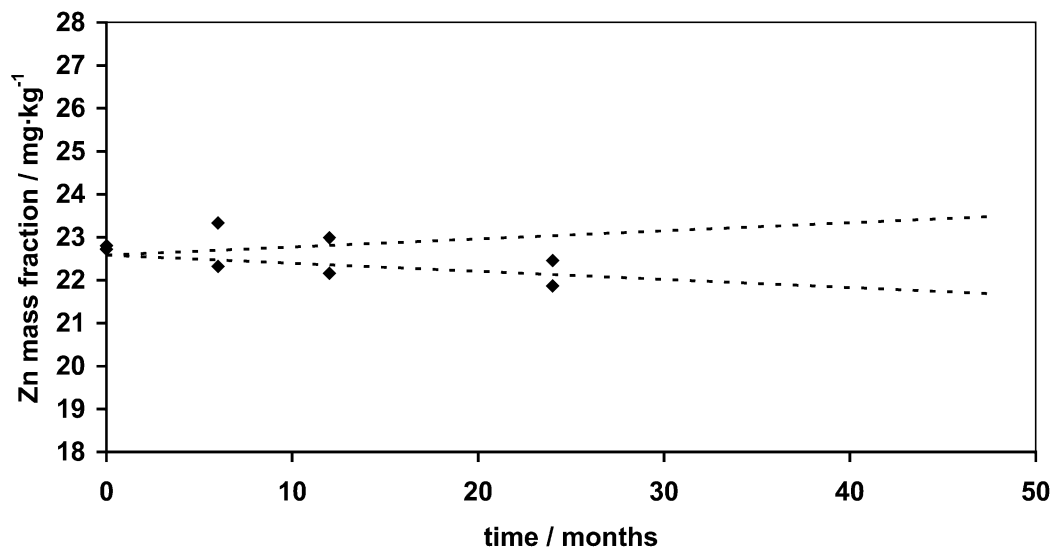


Fig. 4 Evaluation of the long-term stability of IRMM-804 with respect to its Zn content. Dotted lines represent the increase of u_{ts} with an increase of shelf-life.

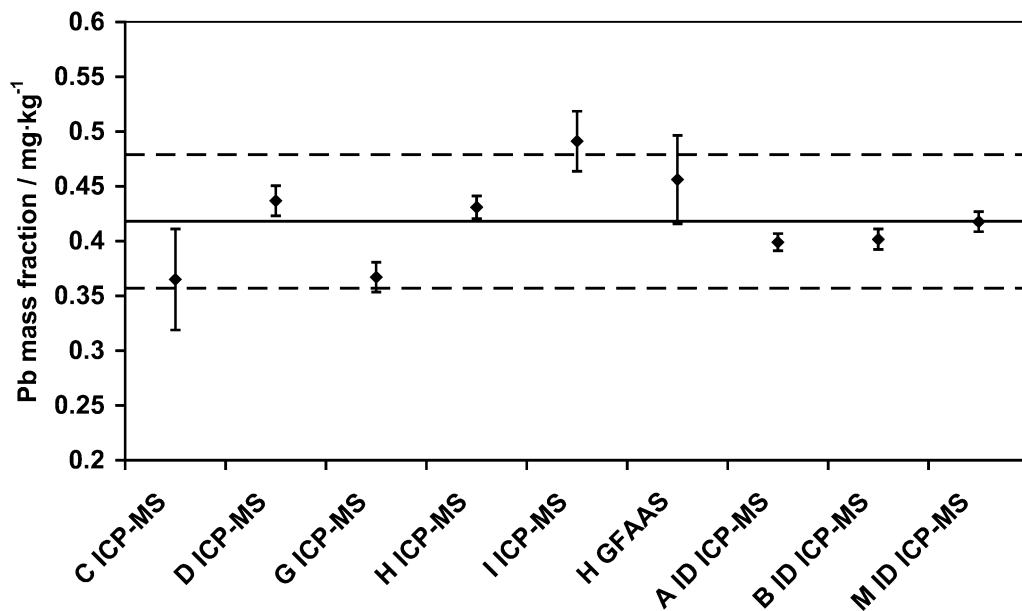


Fig. 5 Measurements of Pb in IRMM-804 with different methods and in various laboratories. The first letter of the x-axis labels identifies the laboratory, followed by the method acronym; ICP-MS, ID ICP-MS (ICP-MS with isotope dilution technique), GFAAS (graphite furnace atomic absorption spectrometry). Uncertainty bars represent one standard deviation of 6 independent replicate analyses performed by each laboratory. The solid line represents the proposed certified value, the dotted lines give the range of the expanded uncertainty of the certified value U_{CRM} .

Such systematic studies of a candidate RM are providing the required overview about the contributions of homogeneity, stability, and analytical characterization to the total uncertainty of the certified property value of the material. The various contributions can differ in their relative effect on this total uncertainty, as depicted in Fig. 6 for Cd, Pb, Mn, and Zn in IRMM-804. The height of the pie charts represents the relative uncertainty of the property value, while the sections represent the various contributions to this uncertainty. The differences in the relative effects of the various contributions can be attributed to different reasons. For Cd and Zn, u_{char} (and consequently the uncertainty of the property value) is particularly small, because the characterization data were derived from a CCQM (Consultative Committee for Amount of Substance: metrology in chemistry) key comparison K24 [13] and pilot study P29 [14] that used the same material. It is not the main goal of these CCQM intercomparisons to provide data for RM certifications. Nevertheless, as the participants are usually national metrology institutes, the resulting data frequently shows better agreement (and consequently lower u_{char}) than can be obtained by an interlaboratory comparison using other expert laboratories. In this case, the CCQM participants were asked for permission to use the data for this purpose.

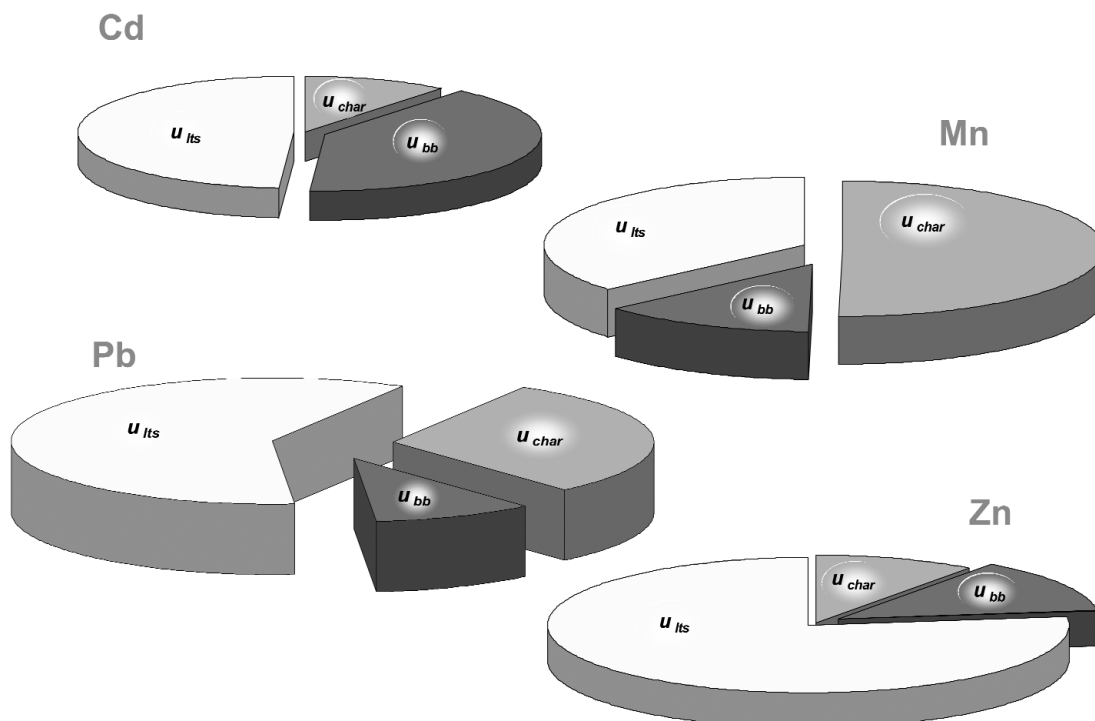


Fig. 6 Main uncertainty contributions for the certified values of Cd, Pb, Mn, and Zn in IRMM-804.

The estimates for u_{bb} and u_{its} are frequently limited by the quality of the analytical data on which they are based on. Only in few cases, they are caused by a significant between-bottle inhomogeneity or lack of long-term stability. Since these measurements are usually carried out by one single laboratory, this laboratory's method repeatability is quite decisive for the combined uncertainty of the property value.

Overall, the example illustrates the importance of well-designed and carefully executed studies of a candidate RM for obtaining reliable information about crucial characteristics of a food CRM certified for trace elements.

TOWARD BETTER FITNESS-FOR-PURPOSE

As already outlined, RMs are generally characterized by their high level of homogeneity and stability of both the analyte(s) and the matrix. Generally, it has been found easier to fulfil these criteria by providing matrix RMs in the form of dried samples. As a result, also most of the food RMs are dried (oven-, spray-, or freeze-drying) and finely ground powders (ball- or jet-milling or cyro-grinding). However, many RM users require the material to reflect the natural state of the samples which are routinely analyzed to provide a realistic picture regarding the validity of the applied analytical method.

Also, the particle size has a pronounced influence on the extractability of analytes from the matrix; finely ground, dry CRMs usually have a particle size distribution which differs from a real sample under investigation. Physical disruption of tissues during milling and the dehydration effects during freeze-drying can also contribute to an altered sorption/extraction behavior in comparison to wet or less rigorously processed samples. Alternatives to conventional powderous CRMs do exist, e.g., frozen homogenates and cryo-milled material, which behaves as a free-flowing powder at very low temperatures. Such materials will undoubtedly satisfy users requiring the matrix of the CRM as similar to the state of the samples, which are analyzed routinely. But logistics of delivering such CRMs, which need cooling at least to dry-ice temperatures, is complicated, and corresponding handling costs are relatively high. Distribution problems could be avoided for heat-stable analytes/matrices as they could be processed by canning and autoclaving. But autoclaving may also alter profoundly the texture of the material, thus impacting again on the sorption/extraction behavior of target analytes.

Information regarding selection and proper use of RMs for QA/QC purposes is available from a number of reputed sources. Nonetheless, some end users feel that CRM producers should offer even more supporting material with detailed instructions on how to make best use of CRMs. One reason for this perceived information gap might be the fact that ISO Guide 33 *Uses of Certified Reference Materials* is currently under revision and the major CRM producers are therefore reluctant to release guidance documents with the risk of not being fully compliant with the revised ISO Guide.

Checking the availability of CRMs on a global scale is fortunately straightforward. Major producers have joined forces and cooperatively submit their data (analyte, concentration level, matrix, etc.) to the COMAR database, which can be consulted free of charge [4].

New efforts to the concerted planning of RM development and preparation processes have been launched recently. A closer collaboration among the LGC, Ltd. (UK), the Federal Institute for Materials Research and Testing (BAM, Germany) and the European Commission's Joint Research Centre, Institute for Reference Materials and Measurements, led to the creation of the European Reference Materials (ERM[®]) initiative. It was rolled out during Analytica 2004 and has more than 150 CRMs in its portfolio. End users benefit from an additional peer review of the quality characteristics of CRMs before they are branded as ERM. The participating RM producers will guarantee an efficient market supply, and it is expected that this cooperation will also reduce the time-to-market need for new CRMs.

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