Best practices for the use of reference materials in the testing of organic and elemental analytes in foods and environmental matrices

Reference Material Use in Trace Analysis

Edition Two

by the NACRW Reference Materials Working Group

NACRW
Reference Materials
Working Group

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NACRW Reference Materials Working Group, July 2023
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1 INTRODUCTION

1.1 Introduction

The NACRW Reference Material Working Group is pleased to present this "best practices" manual, with a special emphasis on analyses of organic and elemental chemicals at concentrations less than 100 µg/g (trace) in foods and environmental matrices.

1.1.1 Trace Analysis: A test measurement of a chemical analyte at a concentration less than 100 µg/g in a material.[1]

1.2 Revisions in Edition 2

Revisions include multiple additions and rewording to include discussions of elemental analysis, clarify terminology, reorder content into more relevant chapters and add information to address reviewer suggestions.

Take special note of:

- Chapter 8: *Contamination in Elemental Analysis* and Chapter 9: *Mitigating Elemental Interferences*;
- revisions to Chapter 2: *Accreditation and ISO Standards*, to emphasize ISO 17034:2016 standard which has now been adopted by the majority of reference material producers;
- revisions to Chapter 12: *Measurement Uncertainty*, to emphasize assessment calculations; and
- addition of Appendix 1: *Challenges of Adding New Compounds into a Multi-residue Method,* which presents a study of analyte interactions in multi-analyte mixes.

1.3 Reference Materials (RMs)

RMs play an essential role in ensuring that analytical results are accurate, precise, verifiable, and legally defensible. An analytical chemistry RM defines a common standard of reference, similar to those used in metrology, by providing a material with a reliable and reproducible composition. The analysis of contaminants and residues in human and animal foods presents special challenges due to the large numbers of analytes with varied chemical properties being analyzed at low concentrations in a single method. In addition, a single multi-analyte method can be utilized to screen a wide variety of complex food, dietary supplement, and environmental matrices for compliance with strict regulatory requirements. Recognizing these challenges, the Reference Materials Working Group of the North American Chemical Residue Workshop (NACRW) developed a 'Best Practices Manual' to facilitate the understanding and effective use of RMs.

As an introduction to the terminology used in this field, the complex nature of the term 'Reference Material' will be described.

1.3.1 Reference Material (RM): A material, sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process.[2]

Some types of RMs include:

1.3.2 Certified RM (CRM): A RM characterized by a metrologically valid procedure for one or more specified properties, accompanied by a RM Certificate (RMC) that provides the value of the specified property, its associated uncertainty, and a statement of metrological traceability.[2]

Also defined as a reference material accompanied by documentation issued by an authoritative body and providing one or more specified property values with associated uncertainties and traceabilities using valid procedures.[3]

1.3.3 Proficiency Testing (PT) Material: Upon completion of proficiency testing, some PT materials are characterized as RMs or CRMs. A PT material is a quality control material (QCM) distributed to a laboratory as an unknown test (analytical) sample to allow an external assessment of the ability of the laboratory to generate acceptable results. PT providers can be accredited to ISO Guide 17043.[4]

1.4 CRMs

A CRM provides metrological traceability and must also fulfill the criteria of an RM as being sufficiently homogeneous and stable. RMs that are *certified* for a specific property will be accompanied by a RMC issued by an authoritative body that describes the certified amount of the specified property and the expanded uncertainty of that value. The total combined uncertainty includes contributions from the characterization, homogeneity, transportation, and long-term stability uncertainties.

Metrological traceability must be stated on the RMC indicating that the property is traceable to the international system of units (SI) or to some other common standard or method. Metrological traceability provides the basis for comparability of results. The International Organization for Standardization (ISO) Guide 17034 provides the basis for accreditation of CRM producers used by multiple accreditation bodies.[2]

1.5 Calibrants and Quality Control Materials

Besides PT materials and CRMs, calibrants and quality control materials also belong to the RM family as described by Emons[5] and illustrated in FIGURE 1.

1.5.1 Reference Standard: A substance of known identity and purity, generally with a certificate of quality from an authoritative body and used to prepare calibration standards and/or for the calibration of other measurement standards.

1.5.2 Calibrant (CAL), such as an analytical standard or a calibration standard, is used to quantify instrument response during measurement. A CAL should have a metrologically traceable property value with an uncertainty suitable for the intended calibration.

1.5.3. Quality Control Material (QCM), such as a non-certified RM or an in-house RM, is characterized as sufficiently homogeneous and stable so as to be fit for the intended use. In-house RMs are typically materials prepared in-house for use as an internal or daily RM and validated for accuracy, homogeneity and stability for the period of time they are expected to be used. They support many internal or external quality control measures. QCMs are not characterized sufficiently to be used for method calibration or to provide metrological traceability of a measurement result.[6]

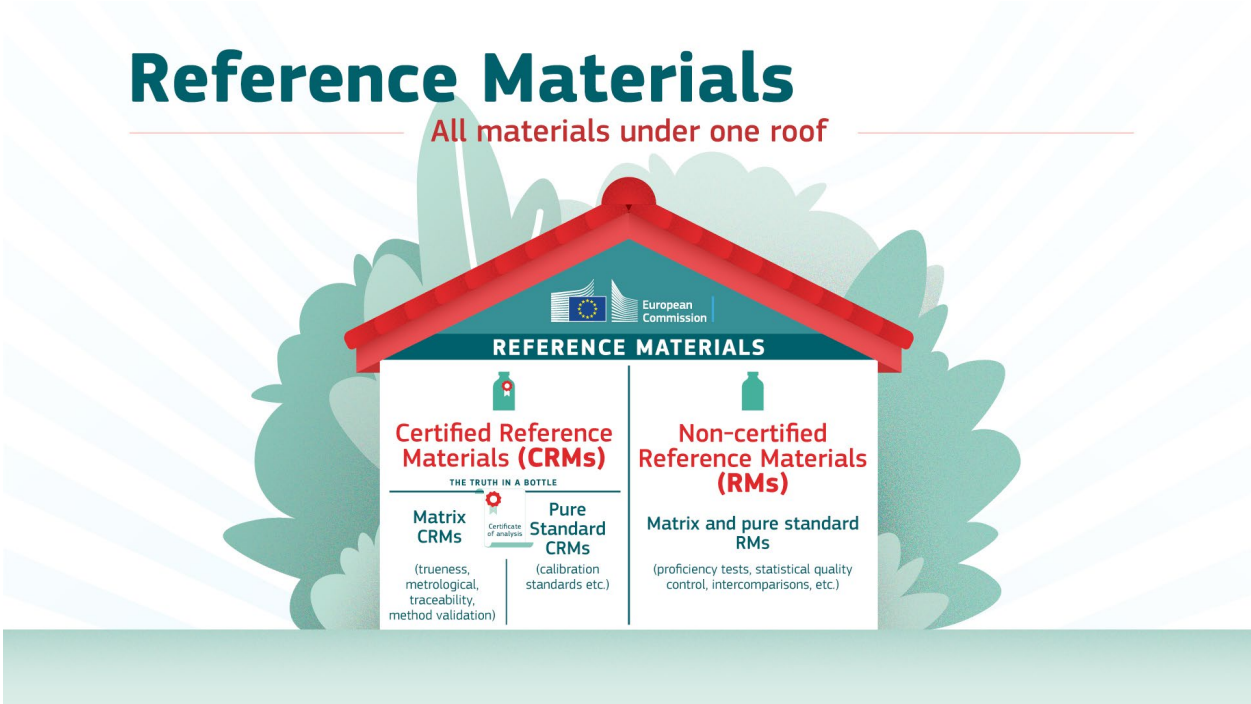

FIGURE 1: Reference Materials under One Roof
Image provided by the European Commission's Joint Research Centre, Geel, Belgium.

1.6 Non-certified RMs

Non-certified RMs, in the form of pure chemicals, stable multi-analyte solutions, and well characterized matrix materials, are needed to support the determination of trace level contaminants and residues in food, animal feed, and environmental materials. Target analytes may include pesticides, veterinary drugs, natural toxins, toxic elements, metals/metalloids, environmental contaminants, processing contaminants, packaging migrants, unapproved additives, adulterants, and others. A variety of spectrometric analytical

techniques including, but not limited to, liquid and gas chromatography mass spectrometry (LC-MS, GC-MS), are used to provide simultaneous identification and quantitation of organic compounds. Spectroscopic methods including inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma optical emission spectrometry (ICP-OES) enable the identification and quantitation of multiple elements, where the signals are captured either sequentially or simultaneously, depending upon the instrument design. These analytical determinations can be challenging due to the lack of information around stability, analyte interactions affecting solubility, and the presence of spectroscopic interferences in multi-analyte mixtures. In organic analytical techniques, the complexity and cost of analyses has increased due to routine use of isotopically labeled compounds as internal standards and ^{14}C radiolabeled compounds for metabolic studies. This is also true for trace elemental analytical techniques such as ICP-MS, where enriched isotopes of the element of interest can be used in isotope dilution methods. These standards are expensive and, in some cases, difficult to acquire. While many RMs are available commercially to support these methods, more are needed.

1.6.1 In a laboratory setting, complex calibration, validation and working standards are prepared either in-house, or purchased from reference material producers (RMP) who manufacture these RMs and offer them to laboratories “ready-to-use". While research and some industry laboratories may only use RMs, most regulatory laboratories use a combination of CRMs, CALs and QCMs. In the case of organic studies and elemental speciation techniques, research and manufacturing laboratories can synthesize new compounds, for which there are no RMs available. The extent to which manufacturer-provided CALs are characterized for purity and stability is often not adequately documented, leaving the user to determine how suitable these RMs are for use. Moreover, extensive characterization of neat chemicals is often required before RMPs can certify a CRM.

1.6.2 Regulatory laboratories and their contracted partners conduct both monitoring and enforcement testing. The purity and stability of RMs is especially important when monitoring over long periods at low levels, as monitoring data is often used as the basis for establishing new regulatory limits. When testing for enforcement and compliance with regulations, CRMs may be required to produce results that will be defensible in a court of law. Validation of new methods, especially when used for regulatory enforcement, requires the use of CRMs to demonstrate that test results are traceable to a metrologically valid SI unit. CRMs may also be used as a “check” to identify and correct for method bias. The laboratory should know the specific requirements that may be applicable to the testing purpose, such as requirements for regulatory enforcement testing or method validation testing. For example, The Code of Federal Regulations (CFR) Title 21, Chapter 58[7] prescribes Good Laboratory Practices (GLP) for conducting non-clinical laboratory studies related to products regulated by the U.S. Food and Drug Administration (FDA). The U.S. Environmental Protection Agency (US EPA) also has GLP practices to safeguard the quality and integrity of data submitted to the EPA.[8]

1.6.3 This ”Best Practices Manual” is a collection of information intended to provide analysts with practices that provide reliable, effective, and efficient use of precious RMs, whether purchased from a RMP or prepared in the user’s laboratory. Information provided includes the proper use and handling of RMs; recommendations to prevent analyte degradation, losses, or metabolite creation; and the

identification of challenges in obtaining suitable RMs. A glossary of RM terminology is included (see Chapter 13) to reduce ambiguity and clearly define important terms and concepts.

1.6.4 This manual is not intended to be a mandatory guide. Information is intended to assist the RM user and provide recommendations. The use of the words "shall" and "must" have been avoided, except when referring to an established standard or government guideline requirement.

1.6.5 In developing these best practices, our authors included many valuable references. The authors intend to continue building on the content of these best practices to meet the needs of the trace level analysis community. Users' suggestions and contributions are welcomed.

Chapter 1: References

1. IUPAC. Compendium of Chemical Terminology, 2nd ed. (the "Gold Book"). Compiled by A. D. McNaught and A. Wilkinson. Blackwell Scientific Publications, Oxford (1997). *Based on definition for trace element*. Online version (2019-) created by S. J. Chalk. ISBN 0-9678550-9-8. https://goldbook.iupac.org/terms/search (accessed 1/15/2023)
2. ISO 17034:2016, General requirements for the competence and consistent operation of reference material producers, https://www.iso.org/standard/29357.html (accessed 9-10-2020)
3. International Vocabulary of Metrology (VIM): Basic and general concepts and associated terms, 3rd Ed., 2008 version with minor corrections. Bureau International des Poids et Mesures (BIPM) (2012) https://www.bipm.org/en/publications/guides/vim.html (accessed 1/16/2023)
4. ISO 17043:2010, Conformity assessment — General requirements for proficiency testing, https://www.iso.org/standard/29366.html (accessed 1/16/2023)
5. Emons, H. The 'RM family'—Identification of all of its members., *Accred Qual Assur* (2006) 10:690–691. https://doi.org/10.1007/s00769-005-0046-z

Definitions from ISO 17034 have been adopted for:

- **Shall or Must:** **indicates a requirement**
- **Should:** **indicates a recommendation**
- **May:** **indicates a permission**
- **Can:** **indicates a possibility or a capability**

6. ISO Guide 80: Guidance for In-house Preparation of Quality Control Materials (QCMs) (2014) https://www.iso.org/standard/44313.html (accessed 1/16/2023)
7. Code of Federal Regulations, Title 21, Part 58 – Good Laboratory Practice for Nonclinical Laboratory studies. https://www.ecfr.gov (accessed 9/11/2020)
8. USEPA Good Laboratory Practices Standards (GLPS), https://www.epa.gov/compliance/good-laboratory-practices-standards-compliance-monitoring-program (accessed 9/8/2020).

2 ACCREDITATION AND ISO STANDARDS

2.1 Introduction to Accreditation

2.1.1 Analytical Laboratories around the world process and analyze hundreds of thousands of samples each day. The results they produce are used by a variety of interested parties to make critical decisions that impact health and safety throughout the world. For instance, regulatory authorities use analytical results to monitor compliance or to set limits on certain priority pollutants. These results need to be reliable to enable the regulatory authorities to properly apply the regulations.

2.1.2 The reliability of results obtained by laboratories depends on the technical competency of the analyst to perform the method. A mechanism must therefore be in place to assure the generation of reliable results from the laboratory analysts. This assurance is accomplished through accreditation.

2.2 Laboratory Accreditation

2.2.1 Laboratory Accreditation is a verification process performed by an accreditation body to determine the impartiality and technical competency of a laboratory to carry out specific tasks or methods. A recognized standard is used as the basis for this evaluation. Laboratories that perform testing and calibration use ISO/IEC 17025[1] as the basis for their competence in performing either of these tasks. Those that manufacture reference materials pursue accreditation to ISO 17034[2]. Finally, those laboratories that provide and administer PTs seek accreditation to ISO 17043[3].

2.2.2 Accreditation bodies operate in accordance with *ISO/IEC 17011 Conformity assessment – Requirements for accreditation bodies accrediting assessment bodies*[4]. This standard "specifies the requirements for the competence, consistent operation, and impartiality of accreditation bodies assessing and accrediting" laboratories.

2.2.3 Candidate accreditation bodies are evaluated and mutually accepted by member signatories to the International Laboratory Accreditation Cooperation Mutual Recognition Arrangement (ILAC MRA).[5] These admitted bodies sign this ILAC MRA to demonstrate their competence.

2.2.4 ILAC[6] is an international organization of accreditation bodies that develops and harmonizes accreditation practices for testing laboratories, PT providers, RMPs, and inspection bodies through an ILAC MRA. It achieves this through working collaboratively with regional co-operation bodies involved in accreditation. These bodies include European Accreditation (EA) in Europe, Asia Pacific Accreditation Cooperation (APAC) in Asia-Pacific, Inter-American Accreditation Cooperation (IAAC) in the Americas, African Accreditation Cooperation (AFRAC) in Africa, Southern African Development Community Cooperation in Accreditation (SADCA) in Southern Africa, and Arab Accreditation Cooperation (ARAC) in the Arab region. An updated list of accreditation bodies is maintained an the ILAC MRA Signatory Search application.[6]

2.2.5 ILAC partners with ISO through its participation in various committees and working groups involved in ISO standards development.

2.2.6 The accreditation process for a laboratory begins with the laboratory identifying the need for accreditation. This involves identifying the type and scope of accreditation needed. A detailed description of 'scope of accreditation' is provided later in this chapter. The laboratory then runs a search for a potential accrediting body on the ILAC MRA Signatory Search. Once an accrediting body is selected, the laboratory contacts the accrediting body and follows whatever process the body uses. This usually begins with drafting a contract that is signed by both parties. Next, the laboratory sends quality documentation for screening before scheduling an onsite assessment. Once this process is completed, including resolution of any non-conformances, an onsite assessment is scheduled and conducted. This results in a report containing the accreditation recommendation and any non-conformances identified. A laboratory is usually given up to 30 days to correct any non-conformances before the final accreditation decision is made. Once complete, if successful, the laboratory receives an accreditation certificate and other accompanying documentation.

2.2.7 Laboratory accreditation offers benefits to various users of laboratory data. These include the following:

- **Benefits to Regulatory Authorities:** Regulators are confident that the results they receive from accredited laboratories can be relied upon to make decisions about compliance and to set regulatory limits. This reliance eliminates the need for government agencies to retest the samples thereby avoiding the cost of analysis.
- **Benefits to the Laboratory:** A laboratory that is accredited to these ISO standards gains international recognition of its competence to test, manufacture RMs, or administer PT programs. The company can leverage this achievement to enter new global markets. Another benefit is that the burden of independent assessment by clients is substantially reduced because most clients now rely on these accreditations. Most government agencies and conformity assessment certification bodies include applicable ISO accreditation as a requirement to bid on their work. Therefore, accreditation creates opportunities for the laboratories to bid on these requests for quotes/proposals.
- **Benefits to Customers:** Customers have a peace of mind when they know that the products and services provided to them come from an accredited laboratory. This is very critical for products that may affect the health and safety of individuals. For example, customers need to be assured that toys and baby food products are free of toxic metals.

2.2.8 Most organizations follow some form of Quality Management System (QMS) outlined by standards established under an authoritative body such as a government regulatory entity or recognized scientific group. The systems and practices can vary and serve specific purposes for their unique needs. These include: the Good Laboratory Practice (GLP) standards[7], the Good Manufacturing Practice (GMP) standards[8], the National Institute of Standards and Technology (NIST)[9] standards and the ISO standards and guidelines. A QMS includes a complete program of organized structures, methods, techniques,

policies, documents, and training which enables adopting companies to meet or exceed expectations. These systems include objectives, procedures, improvements, quality assurance, and quality control for the products and services. Quality Assurance (QA) is the ongoing process responsible for retention and improvement of quality services and products. The QA process is usually established and/or regulated by an external organization such as a government entity, an accrediting body, or a certifying agency. Quality Control (QC) is the process or method by which products or services are examined for adherence to methods, standard operating procedures, or quality manuals established by the QA infrastructure. QC and QA are both tasked with the identification of deviations in products or services and deciding whether the product or service meets the criteria set in the established standards (either voluntary or mandated). In the event a product or service fails to meet the expected criteria, a QMS system has procedures for customer notification, root cause investigation, and process improvement to prevent the deficiency from being repeated.

2.3 International Organization for Standardization (ISO)

2.3.1 Since the 1940s, ISO has become one of the world's largest developers of voluntary international standards for all manner of manufactured, agricultural, and technological products and services. In the 1990s, ISO began creating standards for laboratories to harmonize procedures and provide competency and accuracy. Through the years, laboratories and RMPs have pursued ISO accreditation for their facilities as a mark of quality and reliability.

2.3.2 A laboratory or company can request to become accredited to a particular ISO standard by applying to an external accreditation body. The laboratory or company enters into an agreement with the third-party accreditation body to perform the necessary evaluations of their competency, which involves a technical review of their procedures and periodic on-site audits. In addition, measurement using CRMs and participation in PT programs or interlaboratory comparisons are normal requirements to demonstrate competency. An assessment report is created by the auditors listing any deficiencies or deviations to the standards that were noted and these deficiencies or non-conformances should be corrected before the company can receive accreditation to a particular ISO standard. To retain ISO certification, laboratory proficiency should be periodically re-certified.

Accreditation is confirmation of the competence of an organization by an unbiased independent third-party to an ISO or other international standard.

2.4 ISO/IEC 17025: General Requirements for the Competence of Testing and Calibration Laboratories

2.4.1 ISO/IEC 17025 is the standard used by testing and calibration laboratories worldwide to demonstrate their technical competence.[1] The standard was originally issued in 1999 and was followed by a revision in 2005. The 2005 version contained five elements including Scope, Normative References, Terms and Definitions, and two main sections covering Management Requirements and Technical

Requirements. The Management Requirements section describes the documentation needed to establish the QMS of the laboratory. The Technical Requirements section outlines criteria for adequate laboratory performance including trueness & precision (accuracy), and uncertainty of the analyses and calibrations performed in the laboratory.

2.4.2 The standard was revised to ISO/IEC 17025:2017. Both the earlier ISO/IEC 17025 standards, and the newly implemented 2017 version address the issues of documenting, estimating, and verifying accuracy (trueness and precision). Many of the changes between the 2005 and the 2017 versions of ISO/IEC 17025 close verbal loopholes in the standard which allowed for different interpretations of the requirements. The changes were recommended and reviewed by industry experts through web-based user surveys, support and guidance notes, suggestions for new quality concepts and the examination of common terminology and structure of other standards.

2.4.3 Some key points in ISO/IEC 17025:2017 include:

- Emphasis on impartiality and confidentiality.
- Changes to document range and scope of laboratory activities.
- Increased periodic review measures and control of environmental conditions (e.g., laboratory access, contamination, etc.).
- Expanded definition of equipment to include instruments, software, data, standards, RMs, reagents, consumables, and other apparatus.
- Documentation and definitions of competency for staff.
- Use of statistical methods such as control charts, stability charts and uncertainty estimations.
- Records for supervising and monitoring staff and personnel.
- Management review of risks (i.e., changed to risk-based analysis and impartiality).
- Requirements for use of CRM and traceability.
- Metrological traceability addressed in more detail with reference to relevant international agreements.
- Focus on competency of personnel and removal of deputy role for key positions.
- Strict requirements set about participation in proficiency testing.
- Stronger focus on information technologies and electronic documents.
- Alignment with the other existing ISO/IEC conformity assessment standards.
- Revised scope to cover all laboratory activities, including testing, calibration, and the sampling associated with subsequent calibration and testing.

2.5 ISO 17034 General Requirements for the Competence of Reference Material Producers

2.5.1 ISO Guide 34, *"General requirements for the competence of RM producers"* was originally authored by ISO/REMCO in 1991 and published in 1996. An update was published in 2009 and in 2016 was changed from a guide to become an international standard, ISO 17034.

2.5.2 ISO 17034[2] was developed to enable the comparison of results between testing, analytical, and measurement laboratories by using CRMs produced by accredited manufacturers. These materials would be used for the calibration of measurement equipment, method verification, and evaluation or validation of measurement procedures. For the CRM producers, ISO 17034 requires demonstration of scientific and technical competence, which is demonstrated by additional ISO/IEC 17025 accreditation. ISO 17034 accreditation confirms competence of an RMP for a specific scope of RM and CRM production. The standard describes a set of stringent requirements that make certain all aspects of the production of RMs can be carried out according to established and relevant procedures. The comprehensive requirements of the standard cover production planning, material selection, assignment of certified values, uncertainty, traceability, homogeneity, and stability, as well as packaging and documentation. Thus, any accredited ISO/IEC 17025 and ISO 17034 facility producing RMs should estimate and report uncertainty measurements for all values that are certified. The guide also requires that certified values and supplementary information be provided for RMs, including traceability statements, uncertainty, homogeneity, stability, preparation, and methods of measurement.

2.5.3 Traceability: According to ISO guidelines, traceability is the ability to identify and trace the history, distribution, location, and application of products, parts, and materials.[10]

2.5.4 Metrological traceability is the property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty.[11]

2.5.5 RMPs should establish that the certified property values of a CRM can be traced back to a primary standard, one of the highest obtainable metrological values that is accepted without reference to another standard since a direct traceability to the SI unit by primary methods has been realized in its characterization. Secondary standards are standards for which a value is assigned by comparison of the same quantity to a primary standard. All property values of RMs should, where possible, be traceable to SI units of measurement, or to CRMs. Koeber et.al.[12] and ERM Application Note 3[13] provide more explanations on the concept of metrological traceability.

2.5.6 Many changes from ISO Guide 34 are updates to wording or terminology to either harmonize with other standards or clear up previous ambiguity. Additions to the standard have been made to improve impartiality, confidentiality, and security. A number of changes and additions have also been made to the standard to improve the accuracy and stability of RMs.

2.5.7 Major points in ISO Guide 17034 that affect RM users, require RMPs to:

- Verify the identity of the RM.
- Provide necessary advice on the storage and intended use of the material in order to maintain stability.
- Record secondary parameters (such as temperature, humidity etc.) that can influence a CRM's certified value (or it's uncertainty) for traceability.

- Assess the effect of repeated use or sampling of a RM (under the instructions for use) for stability of the material and provide guidance for maintaining material stability.
- Identify uncertainty contributions for a RM property value which are included in the combined uncertainty of assigned values.

2.5.8 ISO 17034 includes references to several other documents including ISO/IEC 17025 (previously discussed), ISO Guide 35: *Reference Materials – Guidance for characterization and assessment of homogeneity and stability*, and ISO/TR 16476: *Establishing and expressing metrological traceability of quantity values assigned to reference materials*.

2.6 ISO Guide 35: Guidance for Characterization and Assessment of Homogeneity and Stability

2.6.1 ISO Guide 35[14] supports the implementation of ISO 17034 by outlining principles for characterization of a RM and assessment of RM homogeneity and stability which are needed to estimate the uncertainty of a certified value. For an RM to be considered certified (i.e., a certified RM or CRM), statistical data should be incorporated into its validation and verification. Statistical data should be collected during the manufacturing processes, development of the product, and final testing. These data include measurements of homogeneity, reproducibility, accuracy, stability, and metrological data (balances, volumetrics, pipettes etc.). All measurements should be traceable directly to the SI unit through suitable measurement standards. Standard uncertainty is the term used for the uncertainty components before multiplying them with a coverage factor.

2.6.2 Measurement Uncertainty is a non-negative parameter characterizing the dispersion of the quantity values being attributed to a measurand, based on the information used.[15] It is the estimate attached to an assigned value which characterizes the range of values within which the 'true or consensus value' lies within a stated coverage probability. Also refer to Chapter 11: Measurement Uncertainty.

2.6.3 Measurement uncertainty of normal analytical laboratory method performance is part of method validation and is an integral part of ISO/IEC 17025 accreditation, analytical reporting, and decision rules. The uncertainty contributions should encompass the impact of random effects such as changes in temperature, humidity, extraction efficiency, clean-up, instrumental drift corrections and variability in performance of an instrument or analyst. There is also a systematic part of the uncertainty estimation, which takes into account the uncertainty for trueness. Uncertainty, however, does not cover analyst errors or mistakes. For estimation of method-related uncertainties, two different approaches exist, top-down and bottom-up as outlined in Chapter 11.

2.6.4 References on estimation of measurement uncertainty include two guides from Eurachem, Quantifying Uncertainty in Analytical Measurement[16] and Terminology in Analytical Measurements[17]. In addition, a technical report from Nordtest provides practical advice on top-down approaches which are more easily realized in normal analytical laboratories.[18]

2.6.5 Standard uncertainty is the term used for the uncertainty components, expressed as a standard deviation, before multiplying them with the coverage factor, which only takes place after combining all uncertainty components as listed above. ISO Guide 35 provides comprehensive guidance in how to assess uncertainty of certified values.[14]

2.6.6 Expanded uncertainty is the estimate attached to an assigned value which characterizes the range of values within which the 'true value' lies within a stated coverage probability (typically multiplied with a coverage factor of k = 2 for the 95% coverage probability). It is the most used measurement uncertainty. The expanded uncertainty of a certified value typically includes contributions from between-bottle homogeneity, contributions from minor instability due to transport (short-term stability) and the uncertainty contribution from storage (long-term stability) to cover the stated shelf-life of the RM guaranteed by the RMP. Contribution to the uncertainty of the certified value from the characterization exercise is also part of the combined standard uncertainty of the certified value.

2.7 Scope of Accreditation and Certification of a Reference Material Producer

2.7.1 The scope of accreditation for a RMP or laboratory is the detailed statement of all the activities, tests, analyses, compound classes or compounds, instruments, equipment, etc., for which the laboratory or company has demonstrated compliance with the accreditation standard. The accreditation body certifies that the laboratory or RMP has the competence to provide the products or services defined within the scope. The accrediting body has the authority to certify the performance of methods in the laboratory whereas the accredited laboratory obtains the authority to issue certificates of analysis. The scope of accreditation for products or materials with numerical values includes the capabilities to perform calibration, measurement, and assignment of uncertainty of an organization, laboratory, or manufacturer. These values (expressed as either a number or formula) are assessed by the accreditation body of the laboratory or manufacturer taking into consideration their personnel, equipment, and processes.

2.7.2 The accreditation scope usually contains tables of information and ranges or values, which are often divided up by parameters. For example, for RMPs, the table may contain a list of uncertainty sources with their corresponding estimations.

2.7.3 RMPs accredited to ISO/IEC 17025 and ISO 17034 provide certificates using combined and expanded uncertainties within a normal distribution containing stated values, an associated uncertainty for each value, and an outline of contributions to those uncertainties. Refer to the chapter on method uncertainty for more details.

2.8 Impact of Changes on Laboratories

2.8.1 Laboratories operating under ISO 17025:2017 now must provide much more documentation regarding risk analysis and security. In addition to documentation requirements, laboratories are now tasked with proving their accuracy (trueness & precision) and competency (e.g., through successful participation in interlaboratory comparisons, use of second source standards, etc.). Whenever available and applicable, laboratories should be using CRMs provided by accredited RMPs for measurements under the ISO/IEC 17025 scope for their measurements to be considered traceable. If an RMP is not accredited to ISO 17034, signatories of the Bureau International des Poids et Mesures (in French) Mutual Recognition Agreement (CIPM/MRA)[19] are equally acceptable providers of RMP accreditation.

The scope of accreditation for a RMP or laboratory is a detailed statement of all the activities, tests, analyses, compound classes or compounds, instruments, equipment, etc., for which the laboratory or company has demonstrated compliance with the accreditation standard.

2.8.2 RMPs will now have to provide more detailed information regarding use, storage, and stability in addition to instructions on use, storage, and storage after opening to maintain the assigned values of the standards during normal use within the declared product lifetime (expiry date or re-assay date). Any special handling or normal use conditions should be noted. Some reference materials are sensitive to light, heat, or moisture. Warnings, such as the Globally Harmonized System of Classification and Labeling of Chemical (GHS) labels, should be included on all paperwork, as well as on the reference material container itself.

Chapter 2: References

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3 APPLICATION AND USE OF RMS

RMs used in the organic and elemental trace analysis include pure substances (neat materials), standard solutions prepared from pure substances and matrix RMs. Use of CRMs is preferred but not always available for all analytes and especially not for all analyte-matrix combinations.

3.1 RM Types

3.1.1 Neat materials should be characterized and certified for their identity and purity in order to serve as RMs for calibration and other purposes. Laboratories may obtain neat materials from various sources as discussed in Chapter 10, *RMs Prepared In-House*. In addition to neat materials for analytes, suitable substances serving as internal standards are also used in the analysis of chemical residues and contaminants and their selection and application are discussed below.

Reference materials serve a variety of purposes in organic and elemental trace analysis. Understanding the advantages and limitations of each will enable successful implementation in the laboratory.

3.1.2 Standard solutions are prepared from neat materials gravimetrically either in-house or by RMPs. Typically, the first step is the preparation of an individual stock solution for one substance in a suitable solvent and at a suitable concentration, followed by dilutions to intermediate stock solutions and ultimately working solutions. Depending on the purpose of the analysis, the intermediate and working solutions can include a single compound (such as in the analysis of acrylamide or mercury) or multiple compounds (composite standard solutions), such as for multi-residue analysis of pesticides or veterinary drugs or in multi-contaminant analyses including the analysis of toxic elements, PCBs and dioxins, PAHs, mycotoxins, etc. Composite standard solutions may be prepared gravimetrically using multiple neat materials. Alternately, composite standard solutions may be prepared gravimetrically or volumetrically by mixing individual compound solutions. The former process is usually employed by RMPs whereas the latter process is typical for in-house preparation of composite (mixed) standard solutions. Reactivity and adsorption considerations of the solvent system and containment vessels should be evaluated for each test material. Some materials may require storage in silanized glassware, borosilicate glassware, high purity plastic, or PTFE containers. Special techniques are required for neat analytical standards that are in a gaseous form at room temperature.

3.1.3 Matrix RMs are RMs that have characteristics similar to the laboratory samples (i.e., similar commodity, processed food, soil type, etc.). Matrix CRMs are highly valuable and preferably used during method validation, but can be quite expensive because their production often involves extensive certification processes. Therefore, laboratories might not use matrix CRMs for routine quality control but reserve them for the estimation of bias during the method validation. Suitable matrix CRMs are often

unavailable for the many different analyte/matrix combinations. As a result, laboratories employ alternative options to matrix CRMs, such as the use of materials from PT programs, spiked test portions, laboratory samples with incurred residues, or other in-house produced QCMs as second-best options. Where possible, these QCMs should be characterized and traced to a CRM.

3.1.4 PT samples are primarily used for laboratory comparison during an actual testing round with a limited time duration. However, PT providers often sell unused PT materials, which have been previously characterized in a PT interlaboratory comparison, including the information of assigned value (mostly consensus mean value) and uncertainty. In contrast to a CRM, the PT samples are usually not characterized with metrological traceability or an evaluation of long-term stability. Therefore, laboratories must not use PT samples for trueness evaluations. The consensus mean of a set of PT data has value, but participating laboratories can submit inaccurate values and, consequently, the assigned value can contain an undisclosed element of error or uncertainty. Moreover, analyte/matrix combinations are also limited in PT programs, especially for the analysis of pesticide or veterinary drug residues. Therefore, the use of spiked (fortified) test portions is the most practical and cost-effective approach employed in method development, validation, and also routine quality control in analytical laboratories.

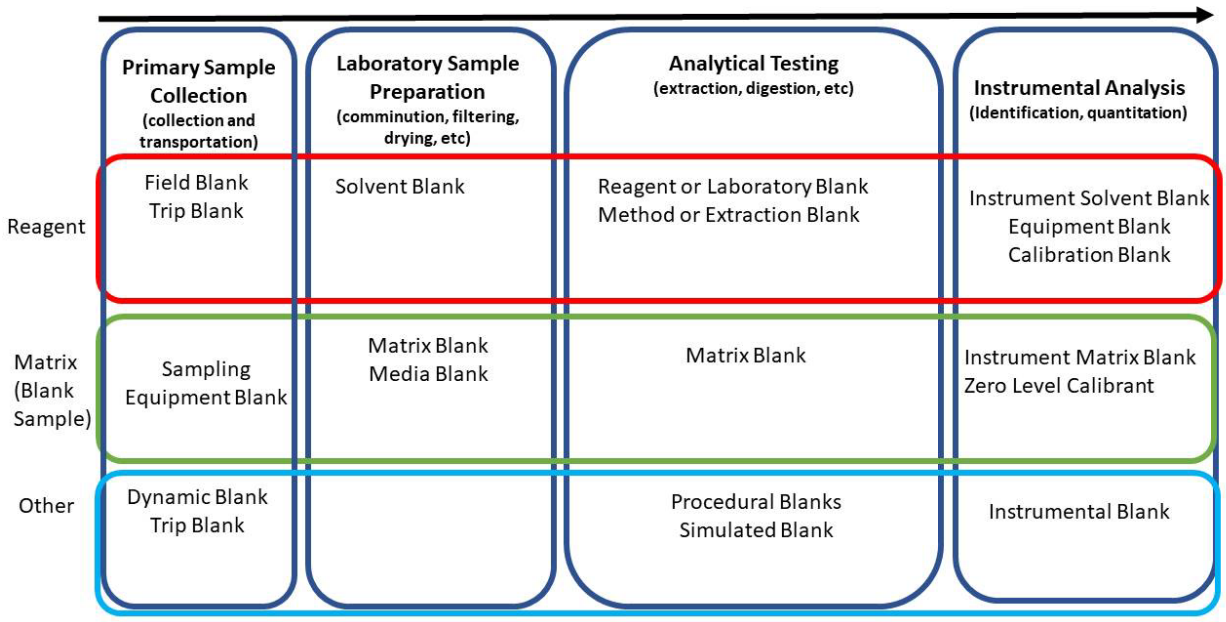


FIGURE 2: Quality Control Materials Used for Blanks

Refer to Chapter 13 for definitions.

3.1.5 FIGURE 2 illustrates many different types of blanks and where they are introduced in the analysis process. For example, field blanks may be introduced at the time of primary sample collection and treated

in the same manner as samples through the entire collection, shipment, preparation, analytical testing, and instrumental analysis steps.

3.1.6 A blank is a substance that is intended to not contain the analyte(s) of interest and is subjected to the usual measurement process. Blanks without analyte(s) of interest may not always be available, especially in elemental analysis where many elements are ubiquitous in the environment. Careful characterization of blanks is very important in these instances.

A few commonly used blanks are defined here while definitions for others may be found in the glossary (see Chapter 13). In several cases, there are multiple terms for the same blank material.

3.1.6.1 A field blank consists of additional sample collection media (e.g., bottles with preservative, sorbent tubes, reagents, filters) which are transported to the monitoring site, exposed briefly at the site when the samples are exposed, and transported back to the laboratory for analysis, similar to a field sample. A field blank is used to identify and estimate contamination immediately before and after sampling (evaluation of protocols), during sample shipment, and while awaiting measurement in the laboratory.

3.1.6.2 A matrix blank is a substance that closely matches the samples being analyzed with regard to matrix components. Ideally, the matrix blank does not contain the analyte(s) of interest but is subjected to all sample processing operations including all reagents used to analyze the test samples.

3.1.6.3 A method blank is a substance that does not contain the analyte(s) of interest but is subjected to all analytical testing operations including all reagents used to analyze the test samples.

3.1.6.4 A procedural blank is a test portion that does not contain the matrix, which is brought through the entire measurement procedure and analyzed in the same manner as a test sample. When preparing procedural blanks, water is often used in place of the matrix.

3.1.6.5 A reagent blank is a test portion consisting of reagent(s), without the target analyte or sample matrix, introduced into the analytical procedure at the appropriate point and carried through all subsequent steps to determine the contribution of the reagents and of the involved analytical steps to the error within the observed value.

3.1.6.6 An instrument blank is a blank test portion, processed through the instrumental steps of the measurement process and used to determine instrument-based contamination.

3.1.7 A matrix spike (laboratory fortified matrix) is a test portion prepared by adding a known amount of analyte(s) to a specified amount of matrix. A matrix spike is subjected to the entire analytical procedure to establish if the method is appropriate for the recovery and analysis of a specific analyte(s) in the presence of a particular matrix.

3.1.8 For trace organic analyses the matrix should be free of the target analyte(s) that are spiked. If that is not possible, the analyte(s) should be present at no more than 10% of the lowest reportable

concentration in test portions. The spike volume should be small enough that the spike solution can be easily absorbed by the test portion matrix but large enough to be measured with acceptable accuracy (e.g., spiking a 10 g test portion with 50-200 µL standard solution contributes acceptable uncertainty). The test portion should be mixed thoroughly after spiking and then allowed to stand for at least 15 min before adding the test extraction solvent in order to provide interaction of the added analytes with the matrix components. One drawback of this approach is that matrix spiking often does not reflect the situation of real laboratory samples, in which the analytes were incurred during various real-life processes, such as the plant uptake, animal metabolism, or food processing. This misrepresentation is especially true if the analytes occur in real laboratory samples in various forms (e.g., acids or esters), are conjugated or bound to matrix components, or if they are distributed in the matrix differently than what could be accomplished by spiking. As a result, spiking blank matrix test portions does not determine the extractability of the compounds of interest. Incurred residues should be used to evaluate extractability. Extraction efficiency of the pesticide compounds from a food crop, animal tissues, soil or sediment matrices is typically established during the early phase of the registration of a pesticide product and is determined by achieving a material balance for the analyte(s) recovered by the analytical method. Radiolabeled materials may be applied to a crop and residual radioactivity tested during the extraction process to determine if all the incurred residues and any metabolites are recovered and identified by the method.

3.1.9 A method blank spike (laboratory fortified method blank) is a test portion prepared by adding a known amount of analyte(s) to a specified amount of blank substance. A method blank spike is subjected to the entire analytical procedure to establish if the method is appropriate for the recovery and analysis of a specific analyte(s) in the absence of a sample matrix.

3.1.10 For trace elemental analyses, spiked test portions are also prepared by adding a known volume and concentration of a standard solution containing the analyte(s) of interest to a sample, matrix, or method blank test portion. In contrast to trace organic analysis, it is very difficult to find materials “free” of trace levels of elemental analytes because they are ubiquitous in the environment. For elemental analysis, deionized water with no detectable analytes (clean water) is often used to represent a blank matrix, and when spiked with the target analyte(s) is referred to as a “method blank spike”. When analyzing simple matrices, such as water, a method blank spike could be a fortification of water before filtration. The matrix spike is prepared, digested, diluted, and analyzed in the same manner as the test samples and can be used to assess the recovery of the spiked element through the entire preparation process. Additionally, a post-digestion spike (fortified analytical solution) of a test sample can be used to assess the impact of the sample matrix on the recovery of the spiked element(s).

The spiked analytes should be compatible with one another and with the acids being used for digestion. Avoid combinations which result in one analyte precipitating or becoming volatile. The concentration of analyte(s) in the spiked matrix is then compared against the expected spiked value(s) and a spike recovery determined. This allows the analyst to evaluate the impact of the sample preparation technique on the analyte recovery (loss during preparation) as well as evaluate matrix effects (suppression/enhancement).

3.1.11 Spike recovery is the fraction of analyte remaining in a fortified analytical test portion (spike) at the point of final determination. Spike recovery is typically expressed as a percentage. Spike recovery should be calculated for the method as written. For example, if the method prescribes using isotopically labeled internal standards or matrix-matched calibration standards, then the reported analyte recoveries should be calculated according to those procedures.

3.2 Method Development and Optimization

3.2.1 Method development for trace organic or elemental analysis usually begins with the selection of suitable neat materials or standard solutions for the target analytes and appropriate internal standards. These materials are first used for the development of the measurement (determination) method, such as GC-MS/MS, LC-MS/MS, ICP-MS, or HPLC-ICP-MS methods, by optimizing analyte-specific conditions for optimal sensitivity and selectivity. Ideally, single analyte solutions should be used when developing a completely new method to prevent potential compound misidentification and to assess behavior and stability of each individual compound, such as potential formation of degradation products (e.g., in the GC inlet or via species interconversions) or presence of impurities. Multi-analyte composite solutions are then employed in multi-analyte method development to optimize analyte separation and evaluate potential analyte interactions and matrix effects.

3.2.2 A CRM is utilized in method development and validation for the purpose of evaluating the efficiency of the extraction/digestion analytical procedures. It also assists in evaluation of any enhancement or suppression effects and ionization efficiency in the plasma in relation to the matrix or other ions. Spikes are used in the absence of a CRM or as an additional measure of quality control.

3.3 Matrix effects

3.3.1 For trace organic analysis or elemental analysis techniques signal suppression or enhancement during mass spectroscopy analyses, caused by coextracted/digested matrix components, should be taken into account by comparing the detector response of the analyte(s) of interest when injected in pure solvent to the response when injected in matrix. If organic mass spectrometry is used, a total ion chromatogram (TIC) is typically evaluated to determine the magnitude of the background from the specific matrix. Ion ratio criteria should be established between a primary ion and at least two secondary ions in order to establish a baseline for instrument suitability and stability during each analytical run. For multi-analyte evaluations, the mass ions chosen for detection and quantitation should be different for the various matrix components or, if this is not possible, the retention times should be established such that no overlap of signal is observed during routine test (analytical) sample analysis. For elemental mass spectrometry, various components in the analytical solution can cause enhancement or suppression and be evaluated by the use of internal standards.

3.4 Internal Standards (ISTDs)

The use of ISTDs is a well-established practice to control various steps in the analytical procedure.

3.4.1 In trace organic analysis, if mass spectrometry is employed for the analyte detection, then stable isotopically labeled compounds could be employed as ISTDs for all analytes but that is only practical for methods with one or a relatively small number of compounds. The availability and cost of stable isotopically labeled ISTDs limit their use in multiresidue analysis, where only a very small percentage of ISTDs (relative to the number of analytes) is used. Availability and cost are the main deciding factors when selecting ISTDs for a multiresidue method, followed by their suitability to serve as ISTDs considering their stability, recovery, chromatographic behavior, and matrix effects. Isotopically labeled or other ISTDs that are stable, have very good recoveries, and show minimum matrix effects may be suitable as ISTDs for a larger group of analytes (e.g., to control volumetric changes); whereas less stable or otherwise problematic analytes may benefit from the use of their own isotopically labeled version as an ISTD to compensate for potential losses during the analytical process (e.g., the use of stable isotopically labeled pesticides such as captan, folpet, or DDT in pesticide residue analysis).

Individual compound solutions should be used when developing a new method to prevent potential compound misidentification and to assess formation of degradation products or presence of impurities.

3.4.2 In elemental analysis an internal standard is an element which is similar in ionization potential to the analyte of interest and of a similar mass to the analyte but is not present in the sample, or is present in very low concentrations. Internal standards are used to track and account for signal enhancements or suppression for the analyte of interest as a function of the matrix. Here the internal standard is expected to behave the same way as the analyte in the plasma and therefore experience the same degree of enhancement or suppression.

3.4.3 For hyphenated elemental speciation applications using HPLC-ICP-MS, it is common to include an analyte of the same *m/z* and which appears at a different retention time, such as a separate species of the same element, or one injected after the analytical column at a controlled retention window. Additionally, an ISTD with the above criteria (ionization potential, *m/z*, etc.) could be introduced during the analysis (perhaps mixed with a mobile phase or introduced with the flow prior to entering the ICP-MS. Regardless of the form/type, the ISTDs for elemental speciation are used to correct analyte signal for instrument drift and differences in ionization.

3.5 Method Optimization (as needed)

3.5.1 Once the initial determination method is established, the development, optimization, and/or validation of the analytical method steps, such as extraction, derivatization (organic mass spectrometry and hyphenated elemental speciation techniques), digestion, or clean-up can start. Use of incurred matrix CRMs or at least well-characterized PT samples for the optimization of extraction parameters is ideal but is usually not possible in routine practice. Most methods are developed and optimized using spiked matrix test portions, which enable evaluation of all critical analyte/concentration/matrix combinations for analyte recovery and precision. However, as noted above, spiking may not reflect the situation in real-life

laboratory samples. Therefore, sufficiently homogeneous and stable analytical samples with incurred analytes should be used in the method development to optimize extraction parameters, such as the selection of the extraction solvent, solvent-to-test portion ratio, time, temperature, or mechanism. These incurred analytical samples do not need to be fully characterized because they serve as a relative comparison of the results obtained using different conditions.

3.5.2 Method development is an iterative process, so the conditions used initially, including the preparation of standard solutions, can change during the method optimization. For instance, the selection of a suitable solvent for the calibration solution is affected by the analyte solubility and stability but also by compatibility with the method conditions, such as suitability for the injection into GC, miscibility with the LC mobile phase, or the formation of new interferences in the plasma. It should be noted that even when using the same instrument model under the same conditions, the sensitivity and instrument performance can vary. The variance is mostly taken into account by establishing a specific calibration curve for each instrument and each analysis batch.

3.6 Method Validation

3.6.1 Method validation is performed to provide evidence that a method is fit for the intended purpose**.** Method validation requirements differ between qualitative (screening) and quantitative methods. For screening methods, the confidence of detection of an analyte at a certain concentration level in the representative matrices should be established along with evaluation of selectivity, robustness, and matrix effects. Validation of quantitative methods requires determination of the method accuracy (trueness and precision) and other important parameters, such as linearity, range, limit of quantitation (LOQ), specificity, robustness, and matrix effects.

3.6.2 To validate method trueness, matrix CRMs should be used if available.

3.6.2.1 For organic and hyphenated elemental speciation multi-analyte methods, there are only a few matrix CRMs available. Therefore, trueness for all analyte-matrix combinations cannot be evaluated with CRMs. Spikes using standard solutions prepared with CRMs serve as the second-best option for evaluation of accuracy (both spike recoveries and precision). The spike concentrations and number of replicates depend on the purpose of the analysis. For organic trace analysis, matrix selection is critical and should include typical matrices that will be analyzed for the specific analytes as matrices vary even within crop groups. Each validated method should cover the majority of relevant matrices and additional matrices may be evaluated concurrently by adding spikes to each analysis set. Similar considerations should be given for elemental speciation matrices, as only certain matrices can contain certain analytes.

3.6.2.2 For elemental analysis techniques, a similar matrix might be used as a substitute since the resulting digested solutions derived from various matrices can be quite similar (dilute acid solutions). For example, if the user is interested in citrus leaves but there is only a CRM for peach leaves, they could use peach leaves even though the type of leaf is different, is expected to have different concentrations of elements, and is a different pH. In elemental analysis you may have a different method per matrix, or you

may have a method that covers multiple matrices. The methods might be grouped based upon the elements present in the matrix and their concentrations as well as the total dissolved solids, acids used for digestion, and/or organic carbon content, etc. (e.g., all plants might be analyzed together; all freshwater samples analyzed together, etc.)

3.6.3 The SANTE 11312:2021 guidance document, *"Analytical quality control and method validation procedures for pesticide residue analysis in food and feed"* [1] requires a minimum of 5 replicates at the target LOQ (or reporting limit) and at least one other (typically 2 to 10-fold higher) level. If it is anticipated that the range of residue values detected often will exceed the 10-fold range, it is suggested that additional fortifications at the highest anticipated residue range be included to confirm the method suitability. Mean spike recoveries should be within the range of 70-120%, with an associated relative precision (repeatability) less than or equal to 20%. Mean recovery rates outside the range of 70-120% can be accepted if they are consistent (relative standard deviation (RSD) less than or equal to 20%) and the basis for this is well established (e.g., owing to analyte distribution in a partitioning step). In routine analysis, residue results do not have to be adjusted for method bias when the mean bias is less than 20% and the default expanded measurement uncertainty of 50% is not exceed.

3.6.4 The FDA's Guidelines for the "Validation of Chemical Methods for FDA FVM Programs, 3rd Edition"[2] provides requirements for various types of validations for quantitative and qualitative methods for four levels of validation (ranging from single laboratory to a full collaborative study of ≥8 labs). In general, multiple replicates (≥2) of multiple spike levels (≥2) are required. The number of matrices chosen depends on the scope of the method. Recovery ranges and repeatability criteria are similar to those mentioned in section 3.6.3 and vary based on level and desired confidence interval.

3.6.5 In organic trace analysis, in addition to spikes, suitable incurred laboratory samples can be used in method validation to evaluate precision of the entire method, including the initial laboratory sample homogenization, which is often a neglected step in the method validation when only spikes (or previously homogenized CRMs or PT samples) are used.

3.7 Method and/or Laboratory Comparison

3.7.1 Method comparison studies are conducted during method development when a new method is compared to a previously established method, such as an official or standard method. A matrix CRM, if available, should be used for this purpose. Note that multiple methods/techniques are often employed to characterize a matrix CRM.

3.7.2 PT programs involve interlaboratory comparison of participating laboratories using different methods for the analysis of the same homogeneous and sufficiently stable analytical sample. Interlaboratory validation studies (collaborative studies or multi-laboratory trials (MLTs)) are used to validate a method (mainly to establish method reproducibility) by applying the same method to the analysis of the same homogeneous and sufficiently stable analytical sample (or set of samples) in multiple independent laboratories. Analytical samples evaluated through PT and MLT studies are valuable materials, which sometimes may be further characterized to become CRMs.

3.8 Identification

3.8.1 In addition to quantitation, calibration standard solutions are used for analyte identification.

Identification of the measurand is a crucial step in the analysis of chemical residues and contaminants and should be performed before proceeding with analyte quantitation.

Identification is a crucial step in the analysis of organic chemical residues and contaminants and should be performed before proceeding with analyte quantitation. In chromatographic techniques with mass spectrometry detection (MS/ICP-MS), analyte identification is based on comparison of retention time and the MS spectrum for the test sample to those obtained for the calibration standard(s) analyzed in the same batch. Acceptance criteria differ based on the purpose of the analysis or the given regulatory requirement or guidance. Very useful examples include the SANTE guidance document for pesticide residue analysis and the U.S. FDA Guide 118 for the analysis of veterinary drug residues.[3]

3.9 Routine Analysis

3.9.1 Routine organic trace analysis can be either qualitative or quantitative. Both approaches employ calibration standard solutions (calibrants), but qualitative methods may use only a calibration level corresponding to the screening detection limit (or reporting limit) whereas quantitative methods typically employ a multi-point calibration.

3.9.2 Routine elemental trace analysis using ICP-MS (a qualitative method) may use the built-in instrument response curve to provide qualitative information about the sample composition. A calibration blank and a single point calibration plus the embedded instrument response curve is sometimes used to provide an estimate of quantitation. The accuracy of these quantifications should be evaluated.

3.9.3 Calibration of a quantitative measurement (determination) technique can be conducted in several ways for the analysis of organic and elemental trace analyses.

a) For *solvent-based calibration*, standard solutions are prepared in a solvent without any matrix present. For organic trace analysis, the solvent may be the solvent of the test solution at the end of the extraction procedure. For elemental trace analysis, the solvent may be the acid and water of the digestion procedure (discussed in more detail in point c, as the dilute acid solution is considered the matrix). This calibration is used if the measurement (determination) technique does not show any significant matrix effects (i.e., when the detector response of standards in solvent and in matrix extract differ less than 20%) or if any potential matrix effects are well compensated for by the use of stable isotopically labeled ISTDs or analyte protectants. Solvent-based calibration may be employed for screening purposes to obtain estimated levels of analytes in various matrices (especially when multiple matrices are analyzed in the same batch), followed by more accurate quantitation of positive results (mainly those close to a regulatory limit) by using, for example, the method of standard addition.

b) For *matrix-matched calibration*, standard solutions are prepared using the same or very similar matrix as the test (analytical) samples or using a representative matrix.

- **For organic trace analysis, matrix-matched calibration** is a commonly used approach to compensate for matrix effects. As compared to solvent-based calibration, preparation of the blank matrix extract is required. This process entails extra labor and practical considerations, including availability of a suitable matrix blank and potentially an increased number of calibration standard injections if multiple matrices are analyzed within the same batch. For this reason, matrix-matching using the same or very similar matrix is only practical if test (analytical) samples of the same matrix are analyzed in one batch, such as in certain monitoring programs (e.g., the USDA Pesticide Data Program) If multiple matrices are analyzed in one batch such as a pesticide regulatory program, then a representative matrix could be used for matrix-matching, but this approach should be validated, and positive hits close to the regulatory limits should be quantitated using a standard addition method. This consideration is especially important in LC-MS and HPLC-ICP-MS, where ion suppression or enhancement effects depend on analyte co-elution with some matrix components, which can vary significantly among matrices. Some regulatory agencies such as the US EPA require justification for the need to use matrix-matched standards by demonstrating signal suppression or enhancement greater than 30%. The evaluation of mass spectral signal and ion ratios for CALs prepared in solvent versus CALs prepared in matrix is a useful exercise during method development.
- **For elemental trace analysis, matrix matched standards** are generally recommended**.** For ICP-MS applications discussed in this manual, test portions introduced into the instrument are in liquid form, most commonly in a dilute acid solution. Standards which utilize similar components (typically water with similar acid concentration) should be used to ensure that the ionization effects in the ICP between the standards and samples are similar. Incomplete microwave digestions of analytical portions can lead to residual matrix components (e.g., carbon) that can interfere with analysis, which is further exacerbated by minimal sample dilution. Sometimes these issues cannot be avoided, therefore care should be taken to ensure all related quality control measures pass to ensure accurate results. Analytical solutions requiring different components, such as significantly differing acid components (e.g., sample group A needs HCl to stabilize Hg, but sample group B cannot include HCl as it can hinder analysis of Ag), should be analyzed with appropriate standards and are often analyzed in separate analytical batches. Elemental techniques commonly use internal standards to mitigate and/or indicate matrix effects. For these reasons, elemental techniques like ICP-MS are more amenable to analyzing multiple sample types within the same analytical batch.

c) For *procedural standard calibration*, standard solutions are prepared by spiking multiple aliquots of a blank matrix analytical sample prior to the analytical preparation (extraction) with the analyte(s) at multiple concentrations and then processing these test portions through the entire method along with the test samples. This approach can compensate for both matrix effects in the determination step and low recoveries, especially in cases where low recoveries are inherent to the analysis and

stable isotopically labeled ISTDs are not available or are too expensive. For instance, procedural standard calibration is often used in the analysis of veterinary drug residues. An important application of procedural calibration is a case where the analytes need to be derivatized and the derivatization product yield can be matrix dependent. If suitable (ideally stable isotopically labeled) ISTDs are available, then procedural standard calibration for the derivatized analytes may be prepared in a solvent blank instead of a matrix blank. If multiple matrices are analyzed in one batch, procedural calibration has the same limitations as matrix-matched calibration, because it does not correct for large variation in matrix effects with un-represented matrices.

d) In *standard addition calibration*, standards are added at multiple concentrations to test portion or test sample extract aliquots, and the analyte concentration in the unspiked test sample extract is extrapolated using linear regression based on the analyte responses and added concentrations. This procedure compensates for the matrix effects because the calibration standards are prepared in the exact same matrix as the test sample. If the standard addition is performed using test portion aliquots prior to the extraction, then it also compensates for potential recovery losses. Therefore, standard addition is a recommended procedure for accurate quantitation (confirmatory analysis) of test samples with analyte determinations that are close to the regulatory limits. This technique assumes some knowledge of the analyte concentration present in the test sample, such as a level estimated using a solvent-based calibration or a matrix-matched calibration with a representative matrix. For standard addition, a test sample (or sample extract) is divided in three or more portions (aliquots). One portion is analyzed directly and increasing amounts of the analyte are added to the other test portions immediately prior to extraction or the determinative step. For organic and hyphenated trace elemental techniques, if added to the extracts, the matrix concentration should be kept constant in all of the tested aliquots, including the unspiked extract. The amount of analyte added to the test portions should be 1-5 times the estimated amount of the analyte already present in the test sample. Standard addition is not often used in high throughput labs (except for clinical applications) since it reduces sample throughput and complicates the analysis. For those who have time and resources the method of standard additions is certainly a valuable approach.

f) *Isotope dilution* in hyphenated techniques: The isotope is an enriched preparation of an isotope which is typically present in low abundance. We refer to these as being “enriched isotopes”. See Chapter 13 for more information on isotope dilution.

3.9.4 In routine analyses, ISTDs should be used, when possible, in combination with any calibration approach to compensate for any volumetric variations.

- For organic mass spectrometry and hyphenated elemental speciation techniques, stable isotopically labeled ISTDs are especially useful because they can eliminate the need for matrix-based calibration approaches as discussed above.

- In elemental analysis, ISTDs are used to track changes in atomization and ionization as a consequence of changes in the sample matrix as well as instrument drift as the sample introduction instrument components are exposed to the sample matrix and become dirty.

3.9.5 Quality control (QC) is an essential part of routine analysis in laboratories testing chemical residues and contaminants. Materials used in routine QC include blanks (such as solvent blanks, procedural (method) blanks, or matrix blanks), spikes or laboratory control samples, and calibration check solutions. The laboratory control samples are typically in-house-prepared RMs, which have sufficient homogeneity and stability and have been characterized by the lab with respect to a mean value and acceptable ranges, which are monitored in the routine analysis using control charts.

3.10 Safety

It is essential that all scientific work is performed in a safe manner. All appropriate safety data sheets on the materials being used for the conduct of the study should be read and understood by all workers. The safety issues should be included in the methodology. All workers should be properly trained on the equipment, materials, and any other aspects of the study that they should know prior to working with any of the materials. A safety management program is one way to safeguard the operation of a laboratory and may be a requirement for accreditation. Proper Standard Operating Procedures (SOPs; also known as Safe Operating Procedures) should be required for all routine functions performed within the laboratory.

Chapter 3: References

1. SANTE/2021/11312, Analytical Quality Control and Method Validation Procedures for Pesticide Residue Analysis in Food and Feed, European Commission, Brussels, Belgium https://www.eurl-pesticides.eu/docs/public/tmplt_article.asp?CntID=727 (accessed 2/8/2023)
2. U.S. Food and Drug Administration, Guidelines for the Validation of Chemical Methods for the FDA FVM Program, Edition 3, https://www.fda.gov/media/81810/download (accessed 3/3/2023).
3. U.S. Food and Drug Administration, Guide 118, Mass Spectrometry for confirmation of the identity of Animal Drug Residues. FDA CVM, Rockville, MD, USA (2003), https://www.fda.gov/regulatory-information/search-fda-guidance-documents/cvm-gfi-118-mass-spectrometry-confirmation-identity-animal-drug-residues (accessed 3/3/2023).

4 RM DOCUMENTATION

4.1 User Beware

The terminology for documents which accompany RMs can sometimes be misleading or confusing. FIGURE 3 depicts multiple RM documents currently in use. While the requirements for RM and CRM documentation are clearly specified by ISO 17034:2016, the contents of other documentation such as certificates of analysis are less defined and can vary greatly. ISO Guide 31 states that an RM document shall contain sufficient information that users can decide if the RM meets their needs. RM documentation should contain essential information for the proper use of the RM. A CRM document shall contain all the information this is essential for the correct use of the CRM. RM documentation should include information describing homogeneity and stability with respect to one or more analytes or specified properties and establish fitness for purpose.

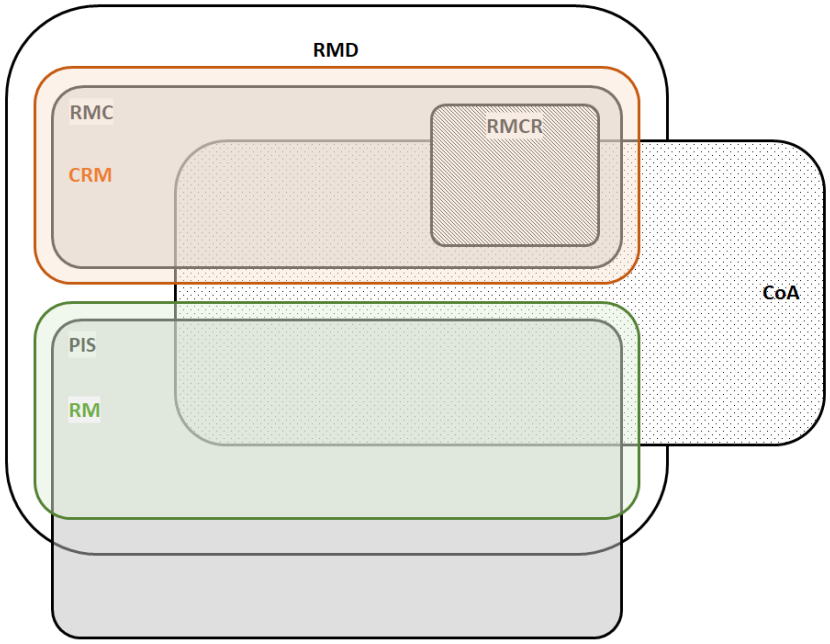


FIGURE 3. RM Documentation (RMD)

- A CRM shall be accompanied by a RMC. Many RMPs call this document a Certificate of Analysis (CoA). A CoA, however, may be provided with many different types of RMs and non-RM tested products, so presence of a CoA does not alone indicate that the material is a CRM.
- Additional CRM information such as analytical procedures, chromatograms, and other supporting documentation may be included in a Reference Material Certification Report (RMCR).
- A RM that is not certified can be accompanied by a Product Information Sheet (PIS) which can be known by other names such as an information sheet. Non-RM products can also be accompanied by a PIS.

4.2 Product Information Sheet (PIS)

A PIS is defined by ISO Guide 31 as a document containing all the information essential for using an RM. CRM documentation contains additional information. Some providers may refer to the PIS as a **Reference Material Information Sheet**. Some documents accompanying non-RM materials may also be referred to as PIS.

ISO 17034 states that all RMs be accompanied by a PIS containing the following information:

a) title of the document: e.g., RMC or PIS
b) RM unique identifier: e.g., lot #, product code, batch #
c) RM name: e.g., description that distinguishes RM for similar materials
d) RMP name and contact details: e.g., name, address, email
e) RM intended use: e.g., specify if independent or intended for a specific method
f) minimum sample size (whenever applicable): minimum analytical sample or test portion mass
g) period of validity: expiry date
h) storage information: e.g., temperature, exposure to light, etc.
i) instructions for handling and use: sufficient to ensure the integrity of the material
j) page number and the total number of pages
k) document version
l) information on commutability of the material (where appropriate); is RM intended for use with a specific method or may it be used with another?

4.3 Reference Material Certificate (RMC)

A RMC is defined by ISO Guide 31 as a document containing the essential information for the use of a CRM, confirming that the necessary procedures have been carried out to provide the validity and metrological traceability of the stated values. The contents of an RMC may include additional information as determined necessary by the RMP and may be provided in either hardcopy or electronic format.

ISO 17034 requires that a CRM be accompanied by a RMC and contain all the information required for a PIS plus the following additional information:

a) CRM description: additional detail on the physical appearance, chemical composition, matrix, interferences, etc.
b) property of interest, property value, and associated uncertainty: analytical details of the characterization of the CRM are sometimes provided
c) measurement procedure for operationally defined measurands: details of the method used to certify the CRM
d) metrological traceability of the certified values: measurement scale to which the certified value is traceable and measurement principles used to characterize the material
e) name and function of RMP's approving officer: person responsible for certification

Note: ISO Guide 31 provides more detailed information on required documentation.

4.4 Reference Material Certification Report (RMCR)

The RMCR contains detailed information in addition to that contained on the RMC, such as the preparation of the material, methods of measurement, factors affecting accuracy, statistical treatment of results, and the way in which metrological traceability was established.

4.5 Reference/Information Values

Reference or information values may be included with a CRM in a RMCR. The criteria used to assign these values as "reference" or "information" versus "certified" are listed on the RMCR, but in general represent a value with less confidence than a certified value. For example, they may be characterized using one analytical technique (rather than multiple techniques) or be expressed with no uncertainty due to insufficient information.

4.6 RM Label

The RM label of an individual unit shall uniquely identify the material and all the identification of the appropriate PIS or RMC.

4.7 Certificate of Analysis (CoA)

The term CoA can be used in multiple ways. A CRM RMC might be labeled as "Certified Reference Material Certificate of Analysis". There are currently many different titles for RM materials. Some RMs which are not yet compliant with ISO 17034 may still refer to the RMC as a CoA. However, the presence of a CoA does not necessarily mean the material is a CRM. Some RMPs may include a CoA with all RMs. Many non-CRM and non-RM materials may be accompanied by a CoA such as laboratory analysis report. ISO Guide 31 lists several other document names used for RMs.

4.8 National Metrology Institutes (NMI)

NMI provide critical measurement solutions and equitable standards in support of science, innovation, and industrial completeness. Reference materials provided by these institutes are considered the highest level of metrological traceability. MRIs are not required to be accredited to the ISO 17034 standard and their certificates may be labeled differently. For example, The U.S. National Institute of Science and Technology (NIST) provide "Standard Reference Materials (SRMs)" which is a trademark name for CRMs provided by NIST.

4.9 RMC Example

An example of a typical RMC is shown in FIGURE 4, illustrating where important information is commonly located including RMP, certified value, metrological traceability, identity and purity of compounds, stability, and associated uncertainties.

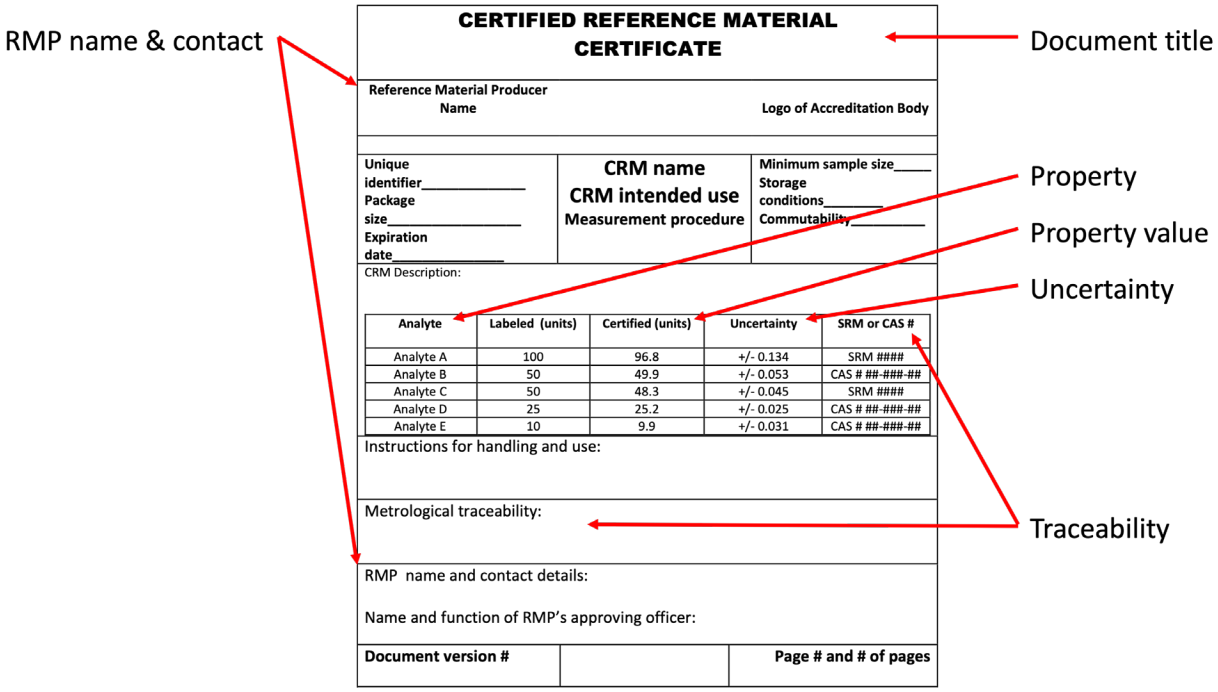


FIGURE 4. Example of a RMC from a RMP

4.10 Reporting Uncertainty for a CRM

The main differentiator between a RM and a CRM is the assessment of uncertainty and the metrological traceability statement. These components are not required for RMs, while they are a requirement for classification as a CRM. The RMC that accompanies a CRM, if constructed according to ISO Guide 31, should contain a certified concentration or mass fraction along with expanded uncertainty (U). In addition, information should be provided as to how U was determined, such as through combination of relevant uncertainties (u_c). An example of the steps in an uncertainty evaluation for a solution CRM is shown in Chapter 11, FIGURE 6.

The combined uncertainty is reported along with the contributions from groups of measured data such as mass of starting material, mass of batch solution, etc. The uncertainty value encompasses the range in which the true value can be predicted with a certain probability. The uncertainty should be reported for each parameter given on the RMC. If no uncertainty is given, the value reported is no longer certified and may be denoted as an information value or as additional characterization of the matrix. A proper evaluation of uncertainty provides information about the reliability of the results, and thus uncertainty values, related uncertainty information, and a statement of metrological traceability should be provided on an RMC. For more information on the assessment of measurement uncertainty refer to Chapter 11.

5 PROCESSING RAW MATERIALS FOR RMs

5.1 Sourcing Raw Materials

5.1.1 Raw materials are the natural matrices (food, environmental), commercial products (medicines, vitamins, nutritional supplements), or chemicals (neat or feedstock) used to produce a finished RM. The first and one of the most important steps in creating a RM is the sourcing, testing and qualification of raw materials. In cases of accreditation, a raw materials supplier is required to be vetted or certified by a procedure to provide the stated quality, purity, and identity of the sourced materials.

5.1.1.1 Traceability: According to ISO guidelines, traceability is the ability to identify and trace the history, distribution, location, and application of products, parts, and materials.[1]

5.1.2 Traceability is most often associated with the ability to document a product through production and trace back to a primary source. For raw materials, traceability includes the raw manufacturer's ability to prove through testing, the composition, purity, and overall quality of the material dictated by a quality plan such as ISO, GLP or other standardization or harmonization organizations tasked with laboratory quality plans.

5.1.3 Raw material receipt: An individual laboratory quality plan should include procedures for the isolation, receipt, and testing of raw materials prior to use. In the first step, the material is received and isolated from other materials. The labeling and paperwork for the shipment is checked and confirmed for identity, supplier, part numbers, etc. The packaging is examined for damage or contamination which can have occurred by broken seals, punctured containers, intentional or accidental tampering or contamination. All testing and conformance documents should be examined and logged appropriately to review available testing data.

5.1.4 Raw material sampling: The received raw materials are then sampled using a sampling protocol established by the receiving laboratory's quality procedure. Primary samples should be representative of the entire lot or batch from which they are taken. The terms lot and batch are often interchangeable with one another.

5.1.4.1 A production batch or lot, according to ISO, is a definite amount of material produced during a single manufacturing cycle and intended to have uniform character and quality.[2]

5.2 Materials Sampling

5.2.1 Sampling can be divided into two types: probability sampling (random) and nonprobability sampling.

5.2.1.1 In probability sampling, any unit or particle of the material being sampled has the same chance of being selected, no matter where that particle is located within the lot.

5.2.1.2 Nonprobability sampling is "grab sampling" or sampling when some increments are purposely selected, and the selection process does not allow all particles an equal chance of selection. Examples of nonprobability selection are selecting material only from the top of a container or selecting from just the first container of a multi-container lot. Sampling reports should include and specify the number of increments taken and which area of the container the increments are taken from, if applicable. The number of increments and total primary sample mass/volume should be based on the heterogeneity of the material being sampled, be independent of the size of the decision unit, and be written into a quality plan.

5.2.2 Processing: After selection of primary samples from raw material lots, each entire primary sample should be processed into an analytical sample in preparation for separation into test portions for testing. Processing may include grinding or dissolving material for appropriate testing. In some cases, the raw material may be in a form which is relatively homogenous such as a liquid or fine powder, but for materials consisting of larger particles, each step where there is mass reduction, appropriate processing and sampling methods should be used to achieve a sufficiently representative analytical sample.

While particle size reduction is an important tool to decrease heterogeneity, uncertainty must be empirically determined for all materials.

5.2.3 Reducing heterogeneity: One method for mitigating heterogeneity, sampling error, and uncertainty is grinding or comminution. Grinding laboratory samples reduces heterogeneity by decreasing particle size, and increasing the number of particles which allows for a reduced test portion mass/volume or increased accuracy and decreased uncertainty for the higher test portion/volume. In a study by Thiex et al., later adopted as ISO 6498:2012, smaller particle sizes were shown to require less test portion mass to achieve lower uncertainty in a test determination.[3] It is important to confirm that changes to the composition or the analyte do not occur from the particle reduction method, including loss of volatiles, introduction of contaminants or degradation of the analyte.

5.2.4 Caution: It is important to note that the relationship of test portion mass and particle size to uncertainty is only an estimate for materials with ideally uniform particle size and shape. Most food and environmental matrices are far from uniform. While particle size reduction is a very important tool to decrease heterogeneity, uncertainty must be empirically determined for all materials.

5.2.5 For example, if an ideal material has a particle size of 5 mm, that is about the size of a pencil eraser. If a laboratory required results within 5% uncertainty, 500 g of material would be needed for testing. But, if the particle size was reduced to less than 0.5 mm (the size of a fine point pen tip), the mass of test portion needed to achieve 5% uncertainty would drop to less than half a gram. See TABLE 1.

5.2.6 After laboratory samples are processed by the appropriate preparation method, a testing protocol must be instituted to validate the raw material against the quality protocol.

TABLE 1. Relative effect of particle size and mass on uncertainty

The table shows the relative mass (g) and particle size needed to achieve various uncertainty levels for representative test portions. It is based on a model material with uniform particle size and shape.

	Desired Uncertainty Level			
Particle Size	**15%**	**10%**	**5%**	**1%**
5 mm	56 g	125 g	500 g	12500 g
2 mm	4 g	8 g	32 g	400 g
1 mm	0.4 g	1 g	4 g	100 g
0.5 mm	0.1 g	0.1 g	0.5 g	12.5 g

5.3 Identification versus Verification of Materials

5.3.1 The goals of testing raw materials are to establish or verify the identity, quality, and purity of the materials. In the evaluation of a raw material for making a RM, a decision process should consider the purpose for the material, how it will be used, and specify whether or not the goal of the analysis is to verify or to establish the identification, purity or quality of a material.

5.3.2 Raw material identification matches the similarity in characteristics or spectral information, measures the fitness to the known identity, and estimates the error and uncertainty for the material.

5.3.3 Raw material verification uses some similar comparisons and tests but employs simpler pass-fail criteria to accept or reject the material. The pass-fail criteria are often based on comparison of the raw material manufacturer's data and verifying tests conducted in-house. Often data is accepted for a certified raw material from a known and trusted vendor with proper certifications and a receiving laboratory opts to verify that material rather than conduct full identification and purity testing. That material would come with documentation which the laboratory would check, then use as a reference against which to evaluate their result, making a pass or fail decision that the material meets criteria without necessarily undergoing all the tests required for mass balance calculations and uncertainty estimations.

5.3.4 The acceptance of a verification procedure over identification or qualification procedures does not mean that the material is not tested, just that the number and speed of tests are expedited, and weight is given to the data from the accompanying documentation. The end goal of both approaches is to accurately understand the identity, quality, and purity of the material. Physiochemical and instrumental tests can be performed to meet these goals.

5.4 Materials Testing

5.4.1 Physiochemical tests: The first tests performed on a raw material are often physiochemical tests for targets such as appearance (including applicable form, particle size, color). Many industries such as the pharmaceutical industry have guidelines regarding documenting the appearance of raw materials. Injectable raw materials should be free from visible particulates that can indicate contamination, lack of sterility, or foreign matter. Tests for appearance of liquids include visual inspection, clarity, turbidity, and

color. Solid raw materials can forgo tests such as turbidity and clarity in lieu of tests for particle size, crystal structure or chemical form (powder, crystal, liquid, etc.). Additional physiochemical tests include the entire spectrum of parameters from boiling point, melting point for purity evaluation, to water content. These physiochemical tests can aid in the verification of purity (or presence of impurities) and identification.

5.4.2 Nuclear Magnetic Resonance (qNMR): Instrumental tests often help with identity confirmation in addition to determining impurities. The technique of choice, internationally recognized, to confirm the identity and to determine the purity of raw materials to be used in the preparation of CRM or RM (whether they are neat or solutions) is quantitative Nuclear Magnetic Resonance (qNMR). It is a metrologically valid calibration method capable of transferring the purity value of a measurement standard (CRM or SRM) to other materials. qNMR is the only technique capable of using the property value (e.g., purity) of a reference standard to determine the purity of any other organic compound. Since the purity value of the CRM or SRM used as a reference standard in the qNMR analysis is traceable to the International System of Units (SI), the purity values calculated for the other materials are also traceable to the SI.

5.4.3 Spectral analysis can identify and quantify elemental and molecular impurities and confirm identity with mass spectral fragments or by matching an elemental or spectral library. Typical instrumentation in raw materials testing includes familiar techniques such as spectrophotometry, mass spectrometry, and chromatography. Mass spectrometry techniques are very common and can be complementary analytical techniques such as qNMR in identification and determination of purity. The high resolution "untargeted" analysis can be used in the search for and identification of impurities. Other analytical techniques can be used as an alternative to qNMR for qualitative identification and determination of purity of a compound, but it is necessary to have a CRM or SRM for each of the compounds to which to attribute a property value traceable to the SI. Identity of materials is most often confirmed with multiple correlating data points across several techniques. For example, a liquid material may be identified by comparison to a NIST database using GC-MS and then confirmed by other tests such as boiling point, melting point, or FTIR. Usually, multiple points of identity are needed just as multiple techniques can be needed to confirm quality or purity.

5.5 Purities and Impurities

5.5.1 Purity: Not all raw materials are certified to the same standards of purity and quality. Some materials are issued a percent purity while materials like some metals are issued ‘nines’ as a measure of purity.

5.5.2 *“Nines”* are an informal notation for equivalency percentages very close to 100% which describes the number of consecutive nines in a percentage (Significant figures on 90% and 99%) A five nines copper material is said to be 99.999% pure. This notation scheme is a grading of the purity of raw materials. Purity is then defined as the absence of impurities or 100%. Some raw materials such as precious metals (Pt, Au, Ag) base purity on fineness, which is commonly seen imprinted on ingot material as 999 fine which corresponds to 99.9% or three nines. See TABLE 2.

TABLE 2. Expressions of purity and their significant figures.

Percent purity	Millesimal fineness	Number of nines
90.00%	900	1
99.00%	990	2
99.90%	999	3
99.97%	999.7	3.5
99.99%	999.9	4
100.00%	999.97	4.5
100.00%	999.99	5

5.5.3 Composite materials: High purity raw materials may be mixtures or composites of many compounds. For example, one may purchase glucose raw materials with a purity of 99.99% percent only to find that the target compound, D-glucose, has a much lower purity in that material. Impurities are any components (chemicals, molecules, elements, etc.) not desired in the target material. Some impurities are native to the manufacturing process for the material (e.g., trace solvents or trace elements) and other impurities are contaminants that are introduced into the material at various stages such as microbes or phthalates. The purity of a material is a sum of the calculated purities of all testing methods employed minus the sum of the impurities.

5.5.4 Mass balance equations: Often purity of a raw material is calculated using mass-balance equations, such as the example in Equation 1, which includes impurities from water and other solvents, inorganic impurities, and organic impurities. When making standards, a purity factor should be included with the standards calculation to correct for the actual purity accounted for by the mass balance equations.

$$\boldsymbol{Purity\ Factor} = \left\lfloor \frac{\{100-(wt\%\ solvents,water)-(wt\%\ inorganics)-(wt\%\ organics)\}\times(Purity,\geq 2\ methods)}{100} \right\rfloor \quad [1]$$

5.5.5 Isomers: In addition to the purity components discussed above, many compounds have isomeric forms that may be summed together as a total purity. Isomers are molecules that have the same molecular formula but a different arrangement of atoms. Depending on how differently the atoms are arranged, isomers can display similar or vastly different properties. Isomers are organized into two main groups depending on how they differ. Structural isomers are those that have their atoms connected to each other in different ways, while stereoisomers have the same arrangement of atoms but occupy 3-dimensional space differently. Depending on the desired analytical target, isomers can be considered impurities for analysis. In cases where single isomer purity is needed, more purification or isolation can be required to process a raw material into a usable constituent. The identification or separation of isomers is most commonly performed using chiral assays. It is good practice to specify the single isomer/enantiomer, and even when uncharacterized, to note the presence of isomers and enantiomers.

5.6 Final Notes on Raw Materials

After raw materials are received and qualified, the materials then should be properly stored to preserve the condition, purity, and quality of the material until use. This process may mean changing containers for long-term storage or adjusting storage conditions to preserve quality and purity. Materials should be protected from degradation and exposure to contamination that could alter their character or composition. The oldest raw materials should be used first to prevent changes over extended storage times. Materials that have been in storage for a prolonged period should be retested and reevaluated as fit for use and true to the original criteria used to accept the raw material upon receipt. Conditions which can cause degradation include, but are not limited to, light, heat, oxygen, humidity, and exposure to other chemicals in the storage compartment. Steps should be taken to prevent materials known to polymerize or oligomerize from doing so, use of stabilizers should be noted and if possible quantitated, and testing for polymerization or oligomerization should be included for these materials both upon receipt and prior to use after storage.

Chapter 5: References

1. ISO 9000, Definitions in plain English. International Organization for Standardization, Geneva, Switzerland (2015). https://asq.org/quality-resources/iso-9000 (accessed 9/13/2020)
2. ISO Guide 30:2015, Reference materials — Selected terms and definitions. International Organization for Standardization, Geneva, Switzerland (2015). https://www.iso.org/standard/46209.html (accessed 9/13/2020)
3. ISO Guide 6498:2012, Animal feeding stuffs — Guidelines for sample preparation, https://www.iso.org/standard/52285.html (accessed 9/13/2020)

6 STABILITY AND INTERACTIONS of RMs

6.1 Neat Reference Standards

6.1.1 Source material of satisfactory quality and purity may be selected for use as a reference standard from a batch or lot of the substance originating from the normal production process. Further purification techniques may be needed to render the material acceptable for use as a chemical reference standard; the requirements for which depend upon the intended use. A chemical reference standard proposed for an identification test does not require meticulous purification, since the presence of a small percentage of impurities in the substance often has no noticeable effect on the test. Alternatively, chemical reference standards that are to be used in quantitative assays should possess a high degree of purity. As a guiding principle, a purity of 99.5% or higher is desirable, calculated based on the material in its anhydrous form or free of volatile substances. When necessary, neat materials with purity from 98.0 – 99.5% may be used for preparation of CAL solutions after correction for purity. However, where the selectivity of the analytical procedure for which the chemical reference standard is required is high, such a degree of purity may not be necessary. For standards in which the purity is unknown, the analyte concentration can be analyzed along with a traceable reference material to ensure accurate quantitation.

ISO Guide 30:2015 describes *"STABILITY"* as the characteristic of a RM, when stored under specified conditions, to maintain a specified property value within specified limits for a specified period.

6.1.2 The suitability of a chemical reference substance is most influenced by the impact of impurities on the attribute measured in the assay when used in a non-specific assay procedure. Impurities with physicochemical characteristics like those of the main component will not diminish the usefulness of a chemical reference standard, whereas even traces of impurities with significantly different properties can render a substance unsuitable for use as a chemical reference standard. [1,2,3,4]

6.1.3 When a neat material to be used as a chemical reference standard is obtained from a vendor, the following information should accompany the material:

a) **RMC or PIS** with complete information on test methods employed, values found, number of replicates used, relevant spectra and/or chromatograms, purity factor (potency), and uncertainty on the purity factor (potency).
b) **Results of any accelerated stability studies**, including information about the more stable form (e.g., salt vs. free base).
c) **Optimal storage conditions** required to provide stability (e.g., temperature, humidity, light).
d) **Results of any hygroscopicity study** and/or statement of the hygroscopicity of the material.
e) **Identification of impurities** detected and/or specific information on the relative response factor as determined in compendial methods concerning the principal component, and/or the percentage mass of the impurity.

f) **Safety data sheet** outlining any health hazards associated with the material.

6.2 Individual Stock Standards and Matrix-Matched Standards

6.2.1 Neat Material Handling for Individual Standard Solutions: Air- or moisture-sensitive compounds should be handled in an inert atmosphere. Appropriate personal protective equipment should be used to handle toxic and highly labile compounds in a safety hood and/or in a glove box. Some materials require handling in an OSHA glovebox. A calibrated and checked balance should be used, appropriate for the amount to be weighed and calibrated with reference weights which are traceable to the kg of a SI system and certified according to schedule if used in an accredited environment. Adequate control of atmospheric conditions (vibration, air movement, temperature, static) is necessary, and if possible, weighing operations should be isolated from other operations. Weighing of larger amounts is generally achievable with higher accuracy than smaller amounts.

6.2.2 Matrix-Matched Standards: Matrix-matched standards are used to compensate for matrix-effects observed in both LC-MS and GC-MS. In LC-MS, the analytical response depends directly on the efficiency of converting the molecules in the eluent into gas phase ions. The charge introduced by the ionization system becomes distributed across all the species, meaning competition can exist between the compound of interest and all the other (frequently much more concentrated) compounds in the test sample. The result can be suppression of the signal from the compound of interest, which can be more than 50% reduction relative to the same compound in a standard solution. Unlike LC-MS, GC-MS often suffers from signal enhancement because reactive sites within the flow path can capture analytes. Under conditions where pure standards in solvent are injected, the loss of analyte molecules is uniform and reliable but can vary from injection to injection and be dependent on the concentration level. When matrix compounds are present, competition for the reactive sites by the matrix can be introduced and can allow more analyte molecules to pass through, however with a variable efficiency, depending on the type and concentration of matrix. While matrix-matched standard calibration is practical for multiresidue analysis, the major drawbacks with matrix-matched standards are the need for analyte-free matrix (which might not be possible) and additional work required for accurate quantitation for a wide range of matrices to be evaluated. The difficulty of selecting matrices that represent certain food groups such as high/low moisture, high lipid, high lipid/low moisture, acidic, and high pigmentation is also a challenge and generalization of these food groups might not be possible.

6.2.3 Matrix for elemental analysis: For elemental analysis, the liquid matrix entering the instrument is typically acidic, but sometimes basic, and is often the result of digested or extracted matrices.[5] Simpler matrices, such as water only require sample acidification and subsequent analysis.[6] Therefore, attempts should be made to match the estimated acid (or base) components of the calibration standards to those of the analytical test solutions. For example, if the analytical test sample was digested with HNO_3 and HCl, and eventually diluted to a final concentration of 5% (v/v) HNO_3 and 0.5% HCl (v/v), the standards should

be made with similar acid concentrations. Significant differences in acid concentrations can affect analyte ionization within the plasma leading to changes in instrument sensitivity. Often these can be corrected using an internal standard.

Note:

- 5% HNO_3 generally refers to 5 mL of 68% concentrated acid in 100 mL of water.
- It is common practice to assume that the sample digestion process consumes 50% of the acid volume and converts H_2O_2 to water.

6.2.4 Solvent for Organic Analysis: The choice of solvent and consideration of pH can best be illustrated by using sulfonylurea herbicides (SUs) as examples. SUs are a group of herbicides widely used for controlling weeds in several crops worldwide (e.g., rice, wheat, maize, barley, sugar beet and tomato). Use of SUs was widely accepted due to the high efficacy at low application rates (10–50 g/ha) and very low acute and chronic mammalian toxicities (the LD_{50} in rats is generally >5000 mg/kg). The analysis of SUs is quite challenging in that they hydrolyze under acidic conditions and in the presence of hydroxy compounds. One study showed the behavior of four SU herbicides (metsulfuron methyl, chlorsulfuron, chlorimuron ethyl, and bensulfuron methyl) in the presence of various hydroxy compounds.[7,8] When dissolved at 30 °C in simple primary, secondary, or tertiary alcohols (methanol, ethanol, isopropyl alcohol and tert-butyl alcohol) and in glycerol or in poly(ethylene glycol), most of these herbicides underwent rapid alcoholysis involving the breakdown of the urea part of the molecule. The corresponding sulfonyl carbamate is recovered in high yields, along with a small amount of sulfonylamide formed in the concomitant hydrolysis. Degradation rate constants and the selectivity of conversion were established. The addition of buffered water (pH 6.0) inhibited the alcoholysis reaction, leaving only hydrolysis, as already observed with concentrated saccharide solutions. In phenol solution, slight herbicide hydrolysis was primarily observed. The alcoholysis reactions only occurred under very particular conditions when SU herbicides were dissolved in pure alcohols, without buffered water. The above also applies to matrix-matched standard calibration.

6.2.5 Solvent for Elemental Analysis: For elemental analysis, the solvents used are typically water and acids but for some methods bases are used. For specific applications, organic solvents may be used, e.g., methanol or isopropyl to normalize carbon content of samples and/or to increase ionization of some analytes (arsenic and selenium). All solvents should be verified to contain low levels of elemental impurities. The highest purity is desired but can be expensive. Depending on the application, lower purity reagents could be substituted, but blank levels should be thoroughly assessed. Additionally, there is equipment available that can be used to distill acids of lower purities to create higher purity acids.

6.2.6 Potential Degradation of Organic RMs after Opening an Ampoule or Mixing Standards: A recent study showed the effect of opening ampoules from a GC kit, composed of 203 GC amenable compounds, and an LC kit, composed of 204 LC amenable compounds.[9] The ampoules were opened, and the contents transferred to the included deactivated vial and stored under recommended conditions (e.g.., 0 °C and 10 °C or colder). At different intervals, new vials were opened, and the stored vials were compared to the newly opened ones to determine the stability over varying periods ranging from 8 hours to 31 days. The GC kit had no failures (within 10% of label concentrations), whereas four failures were observed out of 204 compounds in the LC kit (not within 10% of label concentrations).

Laboratories should conduct their own stability studies and implement standard operating procedures (SOPs) describing use and handling of reference standards.

The study also investigated the stability of these compounds after combination into a mixture of 200+ pesticides. Many of the pesticides interacted with one another and did so at different rates. The above findings also applied to matrix-matched standard calibration (demonstrated with a spiked celery matrix).

6.2.7 Potential Degradation of Elemental RMs: The chemical stability of elemental RMs (referring to total elemental analytes, not species specific) is typically greater than organic RMs, as elemental components do not degrade to other elements. However, transpiration and human error are factors that have led to the general practice of elemental RMs having an expiration of less than one year. The expiration date is not to be confused with shelf life, which is used to describe storage of an unopened RM and is usually longer than the expiration date. Commercial providers of elemental RMs provide detailed guidance on these issues.[10,11]

It is common practice for laboratories to mix individual elemental CALs to form a multi-analyte CAL. Many individual elemental CALs are prepared in specific acid solutions, usually with HNO_3, HCl, and/or HF. When mixing CALs, it is important to realize that some acids are not compatible with certain elements. For example, titanium (prepared from $TiNO_3$) is not stable when diluted with HCl, while Ag standards are often prepared in >10% HCl solutions, therefore mixing these CALs could cause analyte precipitation leading to inaccurate results. There are several other examples; for a more in-depth look at these interactions, one can often find them on the websites of RM manufacturers.

6.2.8 Recommendations: Reference standards should include a RMC or PIS document indicating their expiration (expiry) or retest date under proper storage conditions, but only until the container is opened. Once a manufacturer's ampoule or vial is opened, it is the duty of the laboratory to assign an expiry or retest date based on the laboratory's experience and QC criteria. After opening, ampoule contents should immediately be transferred to a deactivated storage vial and properly stored until and between use. In the study described above, these kits were found to be stable, with a few exceptions, for up to 31 days after opening when properly packaged and stored. Certain analytes can degrade quickly and others over time when combined into a single mix because of chemical interactions. Therefore, working solutions of large multi-mixes may need to be prepared (combined into a single mix) as often as daily, depending on the established analyte stability in these solutions. The same is true for matrix-matched standards. Finally,

laboratories should conduct their own stability studies and implement standard operating procedures (SOPs) describing use and handling of RMs. Care should be taken to minimize evaporation of volatile analytes, and procedures should be implemented to determine when excessive evaporation (loss of solvent to the point where concentrations are outside specification range) has occurred.

Most elemental standards are sold and prepared in plastic bottles with resealable caps. Examples include, but are not limited to PFA (perfluoroalkoxy), HDPE (high-density polyethylene), LDPE (low-density polyethylene), PP (polypropylene). Glass should not be used to store or prepare elemental standards and should never be used with hydrofluoric acid. In general, most stock solutions have an expiration date of one year after opening, assuming proper storage conditions are followed. More dilute standards may have a shorter expiration date, for example a 1.0 ppb arsenic standard may only be stable for one month; these parameters are analyte and solution specific, and various combinations should be verified by the individual laboratories.

6.3 Stability of Multi-Component Mixtures

6.3.1 Multi-analyte mixes: Most pesticide testing laboratories utilize analytical calibration standard mixtures which can be comprised of tens to hundreds of components for routine testing. These standards not only streamline benchtop work for the chemist, but also offer on-going consistency. Laboratories have the option of purchasing standards in a variety of formats, such as commercially available kits and customized mixtures, or preparing within the laboratory. Laboratories should purchase analytical standards from ISO 17034 accredited manufacturers whenever possible and economically feasible. This section aims to provide guidance and acceptance criteria on development, storage, and use of multi-component standard mixtures.

It is common practice to use multi-elemental stock standards to cover a wide range of analytes. However, not all combinations are possible due to element specific interactions with various acids (as discussed previously). Depending on the elements included in multi-analyte mixture and their relative concentrations, the stability of the standard as a whole may be limited by the least stable element. For example, if Hg is included at 1 µg/mL along with other stable elements at higher concentrations (Pb for example), Hg will likely show instability before Pb.

6.3.2 Appendix 1 describes how the interactions of two analytes in a multi-analyte mix led to inaccurate quantitative measurements.

6.3.3 Acceptance Criteria: The following considerations are critical to provide stability of multi-component RM mixtures. Additional considerations are needed to provide stability of CRMs.

- **a)** Temperature control, and possibly reduced temperature (e.g., -20 °C), is used for storage.
- **b)** Individual stock standards should meet acceptance criteria.
- **c)** Solvents should be verified as fit for trace level analysis.
- **d)** Solvents should be compatible with no miscibility issues.
- **e)** Acids and bases for pH adjustments may have to be verified as fit for trace level analysis.

f) Vessels used for preparation and storage should maintain integrity of the RM.

g) Stability validation procedure in this guidance document (or equivalent) has been conducted and documented (see *6.5 Stability* Studies).

h) CRMs should maintain a specified property value within specified limits (of uncertainty) for a specified period, or as defined by ISO 17034. Typically, a ± 10% of original value criteria is sufficient, however the laboratory's QC procedures should specify such criteria based on the material type and intended uses.

6.3.4 Precursors and Breakdown Products: Some pesticides are known to degrade under certain conditions. TABLE 3 provides insight from user experience, but under alternative conditions these compounds can exhibit good stability. Additionally, TABLE 4 provides examples of pesticides known to degrade with corresponding products where known. Instability can be attributed to chemical lability with respect to solvent selection, pH conditions, storage conditions, time, and presence of other compounds within the mixture. Care should be taken to keep precursors and breakdown products in separate analytical standards to prevent artificial enhancement of breakdown products which can result in inaccurate measurements. While some breakdown products can be attributed to plant metabolism, degradation due to physical and chemical conditions can also occur within solvents. The RMP should research each analyte of interest to document known risks of instability during the production of the standard, and the user should be informed as well. Limited data are available to demonstrate accelerated degradation when combining tens or hundreds of analytes in a single mixture. Solvent selection, pH, and exposure to water or oxygen likely has a stronger impact on the stability of individual analytes within these mixtures. Additionally, exposure of analyte mixtures to matrix-matched extracts (in matrix-matched standards) might accelerate degradation, as previously described.

For total elemental analysis (e.g., total arsenic concentration versus determining concentration of individual arsenic species), there is no concern that elements will degrade to another element. Most stability-related concerns were addressed previously, with the primary issues being precipitation and evaporation. As it relates to speciation analysis, degradation is considered to be the change from one chemical species to another, which includes oxidation state (discussed later), complex, or molecular structure. These changes can be the result of many processes including oxidation/reduction, hydrolysis, heat, or light sensitivity, among others. Therefore, storage criteria are often stricter and include low temperature storage (sometimes <-60 °C), storage in the absence of light, or storage in the presence of a preservative, among others.

TABLE 3. Examples of pesticide chemical classes susceptible to degradation in solvent standards

Pesticide Chemical Class	Pesticide Examples	Conditions for Degradation
N-trihalomethylthio fungicides	Tolylfluanid, dichlofluonid, captan, folpet, captafol	Neutral/basic acetonitrile

Phenylurea herbicides	Diuron, linuron	
Sulfonylurea herbicides	Chlorsulfuron, Metsulfuron-methyl	Acidic conditions, methanol
Dimethyl phosphorothioates	Bromophos, Chlorpyrifos	Acidic aprotic solvents
Carbamates	Aldicarb, Benfuracarb	Acidic aprotic solvents
Acidic herbicides	Dicamba,	
Quaternary ammonium herbicides	Diquat, paraquat	
Zwitterionic herbicides	Glyphosate, glufosinate	
Organochlorinated insecticides	Chlordane	Highly basic conditions

6.3.5 Hydrolysis and Oxidation Potential: Certain pesticides are prone to hydrolysis or oxidation during the preparation of multi-component mixtures.[12,13] Typically, this degradation is of greater concern for multi-component standards prepared within a laboratory versus those purchased by a vendor with ISO 17034 accreditation, as accredited manufacturers are expected to have controls in place to monitor and verify for degradation. Laboratories preparing multi-component standards should be mindful of the individual standard stability from exposure to atmosphere. Once an ampoule is opened and its contents transferred to a vial, the exposure of the contents within the newly prepared standard, as well as the remaining unused standard that is stored for future use, should be considered (see *6.2 Individual Stock Standards and Matrix-Matched* Standards).

TABLE 4. Examples of precursors and known breakdown products for common pesticides

All reported in 0.1% acetic acid in acetonitrile.[12]

Precursor	Breakdown product(s)
Benfuracarb	Carbofuran
Demeton-*S*-methyl	Oxydemeton-methyl
Diuron	3,4-Dichloroaniline
Linuron	Monolinuron, 3,4-dichloroaniline
Neburon	3,4-Dichloroaniline

Fenitrothion	3-Methyl-4-nitrophenol
Aldicarb	Aldicarb sulfone, aldicarb sulfoxide
Thiofanox	Thiofanox sulfoxide, thiofanox sulfone

6.3.6 Multiple Oxidation States of Elemental Species: There are numerous elemental species that exist in multiple oxidation states. Some exhibit drastically differing toxicity while others have beneficial properties (e.g., chromium (III) is an essential element, while chromium (VI) is toxic). For others, the inorganic form is more toxic than the organic form and toxicities vary amongst inorganic forms (e.g., arsenic (III) is more toxic than arsenic (V), but both are more toxic than organic forms such as dimethylarsinic acid (DMA) or monomethlyarsonic acid (MMA).[14] Special care should be taken to preserve these species as intended, with special attention given to storage conditions. For example, commercial arsenic (III) standard solutions are in water, while arsenic (V) standards are stored in dilute nitric acid to reduce reduction.

6.3.7 Acquisition and Detection Systems: The benefits and limitations of different detection systems used for acquiring data from multi-component standards should be evaluated. While instrumental analysis of individual standards provides useful information about the purity of a single standard, laboratories performing multi-residue analysis often acquire information for tens of hundreds of analytes in a single injection. For this reason, one or more stability studies should be conducted with the full mixture intended for acquisition, especially in analyses utilizing non-specific detection techniques in which degradation is suspected or verified from stability studies. Mass spectrometers, especially those with high resolution capabilities, offer increased specificity over element-selective detectors and spectrophotometers. However, acquisition of data for hundreds of residues and contaminants within a single analysis can run the risk of suppression during atmospheric pressure ionization, particularly if chromatographic separation is not well achieved and large numbers of precursor breakdown products, and/or chemical interferences (e.g.., plasticizers), are ionized simultaneously.

For elemental analysis, ICP-MS is often selected versus ICP-OES due to its increased sensitivity. Additionally, ICP-MS instruments with different mass analyzers (single quadrupole MS, triple quadrupole MS, sector field high resolution, etc.) can also be used in combinations to derive complementary information regarding a sample. Primarily these analyzers are chosen for their abilities to address mass spectral interferences, which are discussed in detail in Chapter 8. As related to elemental speciation analysis, the same applies for the various mass analyzes, with the additional consideration for the chromatographic method used to separate the analytes of the given mass. Since detection with elemental analysis only confirms the eluting peak/species contains a given element (due to molecular structure being destroyed by the plasma), chromatographic resolution of the target analyte is critical. In the scenario where one separation could not resolve all potential interferences, an orthogonal separation mechanism should be used (e.g., use a combination of ion exchange and reversed-phase chromatography).

6.3.8 Detecting degradation: Depending upon the analysis, chromatographic methods should be carefully optimized to minimize coelution of analytes, particularly if degradation is suspected and breakdown products co-elute with known analytes of interest. While mass spectrometry is often a preferred detection technique, leveraging orthogonal techniques such as element selective and spectrophotometric detection can offer additional information about analyte behavior and can provide confirmatory data demonstrating stability or lack thereof within a multi-component standard.

As previously discussed, degradation as it relates to elemental analysis is relevant to elemental speciation. One way to assess degradation is to examine the peak areas of standards and compare the analyte peak area to those of any possible degradants. Once the ratio of the target analyte to the degradant becomes unacceptable, the standard should either be reprepared from a more stable source, or the peak areas can be used to adjust the purity accordingly to ensure accurate calculations. There are cases in which an impurity/degradant of one standard is the same as target analyte within another standard. If/when these solutions are used to prepare a standard mix, ensure that the sum of the appropriate compounds is accounted for when producing a calibration curve. For example, if an As(III) degrades to 95% As(III) and 5% As(V) and the arsenic As(III) standard is added to a mix including As(V), then the 5% As(V) from the As(III) standard should be added to the As(V) amount in the As(V) standard.

6.4 Matrix RMs

6.4.1 Why matrix match? The use of matrix RMs in trace residue and contaminant analysis provides valuable information during exploratory research, method development, validation, and verification. These materials aid in troubleshooting and offer insight into analyte extractability, method performance, and uncertainty.

6.4.2 Commercially available non-certified matrix RMs offer many advantages. ISO 17034 accredited manufacturers have the necessary equipment and resources to produce high quality products for this purpose. Whenever possible, matrix CRMs or RMs should be purchased from an accredited RMP, which are accompanied by a RMC or a PIS with the assigned values, uncertainties, storage conditions to maintain stability, and date range ensuring validity of assigned values in the material.[15] The analyte(s) of interest can be incurred (e.g., mycotoxins in cereal grain) or spiked (e.g., pesticides in animal fat). Matrix RMs can be available as the original matrix containing analytes or as an extract containing the matrix and analytes. In either case, accredited manufacturers are expected to characterize the stability of the material which is documented in a product information sheet.

6.4.3 If not commercially available: Although commercially available materials are preferred, not all matrices or analytes of interest are available, and laboratories may find it necessary to create their own materials. In these cases, it is necessary to characterize both the matrix and the analytes. The matrix used in the characterization study needs to be sufficiently homogeneous for the purpose. For residue analysis, the matrix should be screened to be sure it is either free of the analyte of interest or to verify the level present is negligible or relatively small in comparison (~95-99%) to the level that is to be measured. For elemental analysis, the incurred levels of the materials need to be accurately quantified, as finding a material free of a given element might be very difficult. The stability of the matrix can supersede the analyte stability; for example, perishable goods require careful handling, processing, and storage to maintain the integrity of the original material. Enzymatic reactions can occur which can significantly alter the matrix composition or can accelerate degradation of analytes, affecting reference value determination. Attention should also be given to analytes susceptible to hydrolysis from the aqueous portion of the matrix, whether naturally present or added, as in the case of slurries (e.g., dried fruit). In addition to analyte degradation in matrix, semi-volatile analytes can prove difficult to maintain in matrices due to volatilization, even under temperature-controlled conditions.

6.4.4 Comminution: Proper comminution of the laboratory sample is required to achieve adequate homogenization of the matrix RM. Reduced particle size improves how precisely each test portion of the analytical sample represents the laboratory sample material. References providing information on theory of sampling, sample comminution, and laboratory sample preparation are available.[16,17,18,19,20,21]

6.4.5 Characterization: Analytical determination of the analyte reference value in a matrix RM ideally is obtained using more than one ISO 17025 accredited method of analysis, and preferably by multiple accredited laboratories. This reduces potential bias associated with a single method, equipment, instrumentation, analyst, etc.

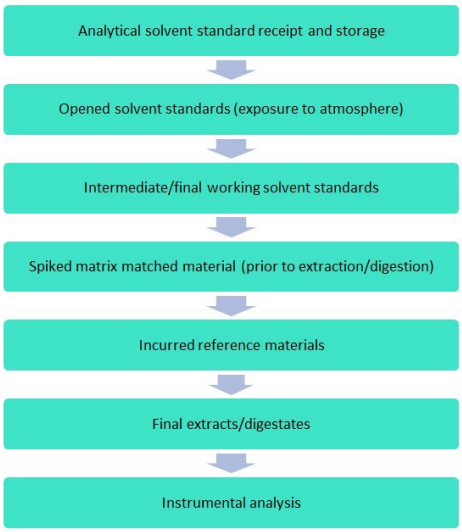
6.5 Stability Studies

6.5.1 Understanding the stability of the certified properties in RMs is a necessary aspect of method validation. The use of improperly stored analytical solvent standards, matrix matched standards, and incurred RMs is likely to compromise the validity of measured analytical results. On the other hand, understanding the shelf stability of these materials can prolong their use, helping to manage the cost of expensive standards by implementing a defensible recertification program well as minimizing unnecessary disposal. ISO Guide 35:2017 describes different experimental studies to evaluate RM stability.

6.5.2 Research Literature: References on stability generally derive from bioanalytical methods for pharmaceutical or forensics research.[22] Limited publications are available for trace level chemical residue

and contaminant stability;[13] though unpublished research has been presented on the topic in various forms. Stability discussions for various elemental standards are commonly presented through manufacturers of the standards.[23] Historically, numerous validated methods using in-house analytical standards omitted this step in their validation, but in recent years researchers who have published validated methods more commonly report the use of commercially available RMs from reputable RMPs.

6.5.3 Scope: Scope should be defined when developing a stability study; consider the simplified analytical process 'Opened solvent standards' (exposure to atmosphere) shown in the diagram.



6.5.4 All steps should be evaluated: In each of these steps, a stability study could be executed to demonstrate efficacy of the method overall. The outcome of each study may then be incorporated to determine method uncertainty, in addition to other factors such as fortification studies, instrument selectivity, etc. Ideally, stability studies should be performed in the order of the analytical process. Insights from solvent standard stability can help determine focus areas for subsequent work, particularly to understand the reason for loss of certain analytes. For example, loss of folpet in a final pesticide extract may be due to instability in the solvent, solvent extraction, or thermal lability during instrument acquisition.[13,24] All steps in the analytical process should be evaluated for stability and determine if skipping one or more of these steps poses a risk to the validity of the method.

6.6 Materials and Methods

6.6.1 Prior to conducting a stability study, the following guidance should be considered.

a) **Quality of materials**: Neat standards and single- or multi-component solvent standards purchased prior to use or developed in-house should follow the guidance previously outlined in this chapter. Refer to those sections for specific details.
b) **Number of analytes**: Include all analytes within the scope of the stability study.
c) **Chemical classes represented**: Be aware of the limitations when selecting representative analytes for a single chemistry class. Pesticides within a single class can have functional groups that behave differently under the same conditions (e.g., chlorpyrifos-methyl vs chlorpyrifos in aprotic solvents); a substituted atom can cause instability (chlorpyrifos vs chlorpyrifos-oxon).[8] Additionally, consider elemental interactions with storage solvents (i.e., acid compatibility) as previously discussed in order to minimize precipitation and other deleterious interactions for various elements.
d) **Accelerated vs real-time aging**: When possible, use real-time aging to evaluate stability of the analytes. If accelerated aging studies are needed, several sources of information are available for conducting these studies.[25] However, some assumptions can result in a highly conservative and

shortened shelf life. An accelerated aging study should be followed with a real-time aging study to evaluate the realistic behavior of analytes. If all analytes cannot be evaluated, such as those with long stability, assess those compounds of greater importance. For example, analytes with presumed short-term stability based on accelerated aging can have longer shelf lives under normal aging conditions. Rigorous recertification of standards is appropriate in cases where overly conservative/shortened shelf life is suspected.

e) ***Internal standards***: Multiple internal standards should be used, representing different chemical classes with different chromatographic retention times and ionization characteristics that differ from one another. Internal standard variety is helpful when evaluating response factors particularly if signal suppression or enhancement are observed for one of the internal standards used in the study. Internal standards used for elemental analysis can be added at the same concentration to each sample/standard solution prior to analysis or added during analysis (on-line) to all samples. Regardless of the addition method, be sure to choose internal standard elements that are not present in the samples at significant levels.

6.6.2 Minimizing Bias: Minimizing bias, where feasible, will improve the validity of the study. While analytical method validations require multiple analysts and multiple days to demonstrate that the method is robust, an effective stability study holds those variables constant.

- Enlist a single analyst to perform the work. How is work verified?
- As much as possible, perform the study on a single day.
- When using consumables, have sufficient quantities to keep lot codes the same.
- Start the study with an unopened bottle; if more than one is required, open them at the same time and alternate between them.
- Prepare sufficient volumes of internal standard solutions to cover the entire study.
- If evaluating the stability of analytes in a matrix-matched standard, prepare enough of the matrix matched extract to use across the study and store under conditions that maintain matrix stability.
- Randomize the order of preparation using a random number generator.
- Randomize the order of acquisition using a separate randomized list.
- Prepare replicates of each standard to be stored for the evaluation.
- Conduct isochronous stability studies.

6.6.3 Stability study example: For example, at t = 0, one might prepare or purchase three ampouled standards at t_0 and from each ampouled replicate three vialed replicates per instrument are prepared at the appropriate concentration with internal standard for a total of 9 replicates per instrument at t = 0. Statistically relevant data can still be produced, should one or two acquisitions fail. This process would be repeated for each time point. A stability study with five time points evaluated using an accelerated aging study would have a total of 45 vialed standards per instrument to analyze.

6.7 Instrumental Analysis Tools

6.7.1 Leverage orthogonal analytical techniques where feasible. Signal enhancement or suppression may suggest addition or degradation.

6.7.2 Significant method changes: If significant changes are made to one or more steps in the method, the laboratory should reassess to determine if an additional stability study should be conducted. Any one of the modifications below can directly impact analyte stability or result in pseudo-stability behaviors such as suppression or enhancement. Examples include:

- Changes in solvents, pH, and buffers
- Expansion of new matrices and effects from matrix matched standards (see *6.2.2 Matrix-Matched Standards*)
- Equipment, such as changes in chromatographic determination (GC to LC), detection (UV absorbance to MS), ionization (EI to NCI) or sensitivity or selectivity (triple quad to HRMS, or ICP-OES to ICP-MS)

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7 RM & CRM HANDLING and DISPOSITION

7.1 RM Handling Requirements

7.1.1 Improper use and handling of RMs may result in contamination or degradation of the RM material and can severely impact the reported values of analytical tests. This chapter aims to provide specific guidance on how to properly handle RMs to achieve data quality goals and meet analytical testing method requirements.

7.1.2 ISO/IEC 17025 Chapter 7.4 "Handling of test or calibration items" describes the requirements for the use and handling of test and calibration items.[1] Each laboratory is required to have a dedicated procedure for the receipt, handling, processing, protection, and storage of calibration items. Precautions should be taken by the laboratory against deterioration, contamination, loss, or damage of the item during handling, and "handling instructions provided with the item should be followed."

7.1.3 The management of RMs and CRMs is entrusted to the RMP, supplier and laboratory end-user. Each should properly control, through appropriate procedures, the entire management cycle of a RM, adopting all the precautions needed in order to prevent possible contamination or alteration of such materials either prior to or during use. The RMP should suggest measures to be adopted for avoiding the influence of environmental conditions on the quality of RM and CRM, and cross-contamination among different materials.[2] RMs should be packaged and stored in environmentally controlled areas (e.g., temperature, humidity, and light controlled) and in suitable containers to limit contamination, deterioration, volatilization, or interconversion, and to ensure the long-term stability of the material. Furthermore, precious, hazardous, or regulated materials should be stored in an access-controlled area and may require additional safety precautions for storage (e.g., fire, radioactive, or organic solvent safety cabinets). Transport, receipt, handling, processing, and storage should be carried out in accordance with the instructions provided by the RMP and reported in the materials' accompanying documents. When the instructions for the use and handling contained in a provider's documents are followed, the property values and associated uncertainties should remain consistent with RMP's specifications.

Improper use and handling of RMs can severely impact the reported values of analytical tests.

7.1.4 A stable RM or reference standard will retain its certified properties in the expected timescale in the presence of expected conditions of the application. An unstable material is one which will corrode, decompose, polymerize, interconvert, denature, burn, or explode under normal conditions and applications, or might react with the matrix or with other components in the mixture such that the original identification/ integrity of the property has been changed. The label of a neat material or a material in a specified solvent can specify that it be "*kept frozen*" at a specified temperature, kept "*under a nitrogen atmosphere*" or "*kept away from light sources*", as a few specific examples.

7.1.5 Degradation during storage: If the material is NOT kept under the specified conditions or is placed into another solvent system or different atmosphere, degradation or reactivity might occur. For example, degradation of a matrix material may result in decomposition of some constituents if stored inappropriately. This occurs more often with organic components. Inorganic components are not affected as often. Moisture changes due to poor storage conditions can also change property values, so some materials are certified on a dry-mass basis. When values are reported on a dry-mass basis, it is important to note the specific drying instructions and follow them to correct mass fraction values to a dry-mass value. Many metals RMs have specific and different drying instructions.

7.2 Transport

Specific temperatures may be required during the transport of thermally labile/unstable materials. Specific temperature requirements during transport can mean changes or additions to shipping materials, cooling agents or refrigerated transportation. Nevertheless, the carrier should be a qualified supplier or provider for such special shipments. It is advised to coordinate with the supplier to avoid shipments over weekends or holidays. Special consideration for customs clearance is also advised for international shipments requiring dry ice or other refrigeration methods.

7.3 Receipt by the Laboratory

When receiving the material, the following should be considered:

- Check compliance with the specifications declared by the manufacturer including the transport conditions established in the supply contract.
- Verify the presence of an adequate certificate and a safety data sheet. Missing documents should be immediately requested.
- Check for damage or overheating (in the case of shipments needing refrigeration methods). Even if the outside packaging appears uncompromised, contents can be damaged.
- Record information needed to guarantee the traceability of the material (e.g., product name, manufacturer data, product code, batch number, receipt date, expiry date, and location within the laboratory).
- Document persons handling the material during its lifetime in the lab.
- Document dates when aliquots / sub-samples are taken, and the mass removed.
- Document environmental conditions such as light, temperature, humidity, and pressure for sensitive materials.
- Record and file all documentation according to the QMS procedures.

7.4 RM Handling

7.4.1 Read the safety data sheet and the instructions for use provided by the manufacturer before unboxing the RM, to ensure safe handling of the material. The container should not be opened until a

thermal equilibrium with the environment has been reached in order to avoid possible moisture condensation, especially if the material has been stored at low temperatures.

RM container should not be opened until reaching a thermal equilibrium with the environment.

7.4.2 Withdrawal of material from the storage container represents the most critical step of handling. Remove material using tools that will not introduce contamination. Transfer material to containers that will not introduce contamination or degradation (e.g., glass vials for organics, clean plastic for metals, borosilicate for some light sensitive materials, etc).

- Mix RM as directed by the provider, prior to removing material.
- For a pure organic RM in a solid state, tap out the material needed or transfer using a spatula washed with a suitable solvent and dried carefully.
- For trace elemental analysis, transfer using an inert, metal-free spatula or pour material out onto a weighing paper.
- For liquids, transfer an aliquot to a clean container and withdraw a portion from the secondary container.
- Except in cases where the RMP considers weight or volume as a property value, users should never assume the liquid contents of a sealed ampoule are an exact volume and transfer the entire contents to volumetric glassware such as a volumetric flask without using a properly calibrated syringe or pipette to measure the amount required. RMPs may overfill ampoules to ensure the presence of enough material to properly extract the minimum volume needed for preparing a dilution. A volumetric measurement delivery device should be used to make such transfers.
- Any excess material should not be put back in the original container as this puts the whole RM at risk of contamination.
- For materials allowing multiple uses, the container should be securely closed to seal, weighed, and stored under the required environmental conditions. Compare weight before reuse to identify and calculate any loss due to transpiration of liquids.
- Opened ampoules should be discarded properly and contents transferred to a deactivated storage container and stored under environmental conditions required to preserve the RM.
- Discard unused material in a responsible and safe manner as deemed acceptable by the laboratory, state, and country protocols where the country legislation supersedes all others.

7.4.3 Pipetting: To ensure accurate volumes and low contamination, pipetting tools and techniques should be carefully chosen based on the intended use and the properties of the liquids to be pipetted. For example, air cushion pipettes are suitable for trace metals but not for volatile organic solvents which require positive displacement pipettes. The proper use of pipettes and demonstration of analyst competency should never be overlooked in a quality control program. Pipette suppliers provide good information on their products but there are many products and other considerations.

7.4.4 Repacking: When a RM is either a single component solution or a mixture at a high concentration, repackaging the remaining material may be necessary. Instructions provided by the manufacturer should be followed; otherwise, the material can become unreliable.

For example:

- **Transfer to capillary vials** could be efficient for minimizing the risk of contamination and evaporation while avoiding concentration changes.
- **Glass storage containers for organic materials** should also be deactivated to provide an inside surface of the vial that is as inert as possible to prevent reactions with the contents. Deactivated storage containers such as screw cap vials or bottles supplied by RMPs should be used for repackaging and storage.
- **Glass storage containers for inorganic arsenic materials** should be thoroughly cleaned with dilute acid (acid washing) to remove any As(V) that may leach from the glass.
- **Materials which are sensitive to light** should be stored in opaque or amber storage containers.

7.4.5 Minimum test portion sizes or larger, recommended by the RMP, should be utilized as smaller test portions can be unrepresentative of the RM due to its heterogeneity. Re-blending or other processing needed to provide a uniform material may also be necessary before selecting a test portion in order to guarantee the validity of values and uncertainties stated on the certificate. Conversely, so-called "single-shot" or "single-use" materials should be used for one measurement only and therefore should not be subdivided.

7.4.6 Subdividing RMs: Whenever a laboratory is comprised of several distant or distinct sites, the subdivision of the same RM into several aliquots to be assigned to the various locations is not recommended. If subdivided, the laboratory should prove that this RM transfer does not invalidate the material or cause differences among the aliquots. It is suggested that laboratories needing identical RMs request multiple aliquots from the same production lot of RM from their RMP for each site rather than subdivide one RM.

7.5 Storage

RMs should be stored in clean, controlled areas with regulated humidity and temperature (e.g., no higher than 20 °C and possibly without direct light). The RMP should properly store and evaluate the stability of a material, such as a RM or CRM, for the duration of the shelf life prior to shipment to a customer. Once the material is shipped to and received by a laboratory, the end user assumes responsibility to properly store, handle, and monitor the stability of the material. Chemically incompatible materials should not be stored together.

7.6 Expiry (or expiration) Date

7.6.1 Expiry dates: Most RMs have assigned expiry dates after which their efficacy or stability cannot be guaranteed. If a material is not properly packaged and stored in accordance with manufacturer's storage

guidelines, the expiry date listed may no longer be accurate. Some RMs may refer to the expiry date as a re-assay date.

For example:

- Organic RMs can, over time, begin to degrade into various metabolites, which a detection technique used for the parent compound analysis might be unable to detect and identify.
- Elemental species may degrade into other chemical species of the same element.
- Elemental RMs may lose volatile analytes.
- Changes in moisture can change inorganic and organic materials.

7.6.2 RM Stability is the characteristic of a RM, when stored under specified conditions, to maintain a specified property value within specified limits (or uncertainty) for a specified period.[3]

7.6.3 RM Period of Validity is the period of time during which a RMP warrants RM stability expressed as a date or time period within the lifetime of the RM.[3,4]

7.6.4 CRM Stability Studies are periodic experiments conducted to assess the period of validity or lifetime of an RM for a specified property value of the RM and uncertainty for specified time duration, under specific conditions of temperature and packaging. Studies may assess stability during short- and long-term storage, transportation, and applicable conditions of use.[5]

7.6.4 RM Expiry or Expiration Date may be used to define the period of validity of an RM. The fitness of purpose of a material cannot be guaranteed beyond the period of validity or date.[6]

7.6.5 RM Lifetime (or Storage Shelf Life) is the time interval during which a RM is guaranteed to retain assigned property values within their associated uncertainties if handled according to the certificate accompanying RM.[4,5]

7.6.6 Retest date is the date a RM should be re-examined to ensure that it is still suitable for use. If a raw material or a RM is stored for an extended period of time, it is recommended to verify that it remains fit-for-use and meets its original property values.[6]

7.6.7 Monitor the validity of results: ISO Guide 33:2015 section 7.2 requires that the expiry date on the RMC should be respected, and CRMs should not be used beyond this date.[7] ISO 17025:2017 section 6.4.13 requires that a laboratory retain records documenting the period of validity of RMs. Use of RMs outside the period of validity must be fit for the purpose.

7.6.8 Expiry dates are conditional. Expiry dates apply only if the RM is handled and stored under RMP specified conditions. Given the variety of materials sold as RMs and CRMs, including neat materials, single analyte solutions, mixtures of analytes in solvent, or mixtures of analytes in matrix, RMPs may specify different storage and handling conditions to guarantee the expiry dates.

Expiry dates may or may not apply after a product's packaging is opened.

7.6.9 Expiry dates may be extended. Some RMPs refer to the expiry date as the re-assay date. If a RM has passed its expiry or re-assay date, some RMPs may test, recertify, and issue a new certificate extending the expiry date. Contact your RMP for the most current certificate.

7.6.9 Variations in the stability among many different analytes and their potential reactivity during storage will determine differences in the period of validity. For example, polychlorinated biphenyls (PCBs) will remain stable for a very long time, whereas organophosphorus pesticides degrade more quickly. There is a great variation in how RMPs conduct stability studies and establish an expiry date, as well as the information RMPs provide to the user of their product. This is because some RMs are packaged and recommended for single-use, while others can be reused after opening. It is best to comply with guidance from a RMP for each product based on the characteristics of each material and its packaging. Such expiry guidance may be described either in a RMC, or other documentation supplied by the RMP. When expiry guidance has not been included with your product documentation, contact of your RMP for such guidance is recommended. When RMs are not procured from a RMP, and are instead prepared in-house by a laboratory, refer to sections 6.2.6 to 6.2.8 of this document for guidance.

7.6.10 Sealed ampoules: To maintain stability, some materials are blanketed using an inert gas such as nitrogen, prior to sealing in an ampoule to protect the contents from degradation. The period of validity is dependent on how these materials are handled once the ampoule is opened. RMPs may not guarantee the stability of some RMs once the ampoule is opened. For further guidance, refer to section 7.4 of this document for best practices on handling a RM. Follow storage and expiry guidance and recommendations contained in the documentation supplied by the RMP. When such guidance has not been supplied in the vendor documentation, contact your RMP for a recommendation.

7.6.11 To fully define expiry dates, RMPs should describe for the user how to interpret and apply their product expiry date.

Different expiry date qualifications include:

- Expiry dates may only apply while stored under specific conditions.
- RMPs may not guarantee the expiry date after the product packaging is opened. This may be specified in the “use” section of the RMC.
- Expiry date may apply after the date of product packaging is opened under specified conditions.
- Expiry date may be extended by RMP recertification.
- User may define the expiry date based on internal stability studies using valid RM or CRM for each analyte to be tested.
- While expiry dates are always recommended, some non-certified RMs, such as metals or solid materials, are considered very stable and may not be provided with an expiry date.

7.7 Assessing RM Validity

7.7.1 Users should conduct their own stability studies to determine the period of validity of materials in use in their laboratories. When stored beyond the most current expiry date provided by the RMP, follow recommendations in section 6.5 of this document.

7.7.2 Alternative storage: Storage guidelines given by manufacturers are typically *minimum* storage requirements. For example, a pesticide RM that is recommended to be stored in a refrigerator (0-7°C) will generally be okay if stored at colder temperatures like a freezer (<-20°C). However, it is important to consult with the RMP before using alternate storage conditions to assure that colder temperatures will not change the homogeneity of the RM. Following an alternative storage procedure is acceptable if validation demonstrates similar efficacy. It is important to understand that the CRM-stated property value uncertainties may vary depending on whether proper storage conditions (e.g., duration and temperature) are maintained. Manufacturer-provided expiry dates may sometimes be replaced or extended when storage conditions exceed the recommendation.[8] However, this should only be done when the laboratory's QMS describes procedures and criteria for doing so. Some RMPs may offer such expiry extensions while others may not, depending on their QMS policies.

7.7.3 Re-characterizing for in-house use: An inappropriately stored RM which has not yet expired or an RM for which the listed expiry date has passed can no longer be trusted without being replaced or re-characterized. In some cases, re-characterization of a RM may be conducted by comparison to a secondary RM or CRM source which has not yet expired (based on the laboratory's QC criteria). Replicate analyses of a suspect RM and a known RM, can indicate if a percent difference between materials is indicative of a failure to meet QC criteria. For organic trace level analysis, a ±10% of original value criterion is usually sufficient but inorganic analyses require a significantly tighter acceptance criterion. The laboratory's SOPs should specify such criteria based on the material type and intended uses. For a CRM to be re-characterized, duplicate testing should establish that a specified property value is maintained within specified limits of uncertainty for a specified period, or as defined by ISO 17034. Different analytical techniques and applications may have more, or less, strict guidelines, and users should achieve compliance with their own applicable QC allowances. Suspect material should be discarded in compliance with local, regional, or national safety and waste procedures or taken out of service for quantitative measurements.

7.7.4 Evaluate purity: Another approach to determining the quality of a suspect RM is by evaluating the purity of the starting or neat material. Refer to section 5.3 for recommendations on the identification and verification of raw materials.

7.7.5 Alternative uses: As an alternative to disposal, a material that has failed a recertification, may still be useful and repurposed.

Some possible alternative uses for RM materials include:

- being used in non-quantitative applications

- as screening method internal standards to track instrument performance
- to verify retention times for new methods or new instrumentation
- as tuning solutions for mass spectrometry applications
- Materials that have failed quality control guidelines or criteria could also potentially be used as negative controls against which future materials can be tested.

7.7.6 Disposal: In the event that a RM fails to be recertified for use and cannot be used for other non-quantitative or diagnostic purposes, it should be properly disposed of in accordance with local, state, provincial, parliamentary, and federal regulations. Certain materials may require very specific means of disposal that can only be performed by licensed organizations. Materials that are listed with keywords of "Warning" or "Danger" on their SDS forms should be handled with extra care and precaution during disposal. The US EPA specifies the identification and listing of hazardous waste in 40 CFR 261. Many laboratory RM materials may require specialized disposal methods.

Chapter 7: References

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8 CONTAMINATION IN ELEMENTAL ANALYSIS

8.1 RM Contamination Issues

Many of the contamination issues discussed in Chapter 7 regarding handling and storage are also applicable to metals analysis. However, because elemental impurities are ubiquitous, eliminating them completely is impossible.

For elemental analysis, controlling contamination usually focuses on laboratory sample preparation and quality control as it relates to detection limits, quantitation limits, reporting limits, uncertainty of the test measurement, etc. These aspects have been covered extensively in the scientific literature and are more geared towards method development and assessment.[1-3]

This chapter discusses best practices to minimize contamination of RMs in elemental analysis by identifying possible sources, describing mitigation procedures, and suggesting tests to detect such contamination.

8.2 Source of Contamination

8.2.1 Water: Part of understanding contamination control is simply realizing the number of sources that could potentially be contaminated with elemental impurities. One such source is the initial water, as it is the most used reagent. The current recommended practice utilizes reverse osmosis and ion exchange to remove contaminants. The resulting water should then meet ASTM Type I requirements which includes a maximum conductivity of 20 µS/cm at 25 °C, among other requirements.[4] Even though the water meets ASTM criteria, impurities may still exist including Si, B, and Zn. One aspect that may cause issues is the age of the filters within the system as they have been shown to release Si, B, Al, Ba, and U.[1] High purity water can also be purchased from various suppliers with claims of low levels of impurities, but this may not be cost effective due to the sheer volume of water needed for larger labs.

8.2.2 Acid: The next most common reagent in elemental analysis is likely acid. These can include, but are not limited to nitric acid, hydrochloric acid, and hydrofluoric acid. Various qualities can be purchased from vendors. In general, “trace metal grade” acid is relatively affordable while exhibiting minimal elemental impurities. These acids are used for cleaning glass and plasticware as well as sample digestion when ultralow detection limits are not necessary. They are not commonly used when preparing reference materials to be used as calibration standards (CALs). For preparation of CALs, ultrapure acids are used. However, these high-quality acids are expensive which may limit their use. Some labs employ a system of acid sub-boiling distillation which can be used to purify trace metal grade acids to a quality comparable to ultrapure acid.

8.2.3 Contaminants within the RM: During preparation of an RM, such as combining single element RMs into a multi-element calibration standard, it is possible that other elements (without certified values) could be present in the RM. This may be unavoidable as some elements are obtained from closely related sources. Additionally, trace levels of elements may inadvertently enter the RM during preparation. For example, trace levels (parts per billion) of thallium could be in a RM with higher (part per million) concentrations. While such elements are low in concentration, their high sensitivity for detection by ICP-MS could allow them to be detected. This is more important when mixed with another thallium standard (as part of a multi-element standard) as it could contribute to the instrument response and bias the results. It may be possible that their concentrations are negligible, but this should be considered when preparing multi-element calibration mixes. Therefore, it may be a good idea to analyze standards individually prior to mixing to ensure that an RM does not contribute to the elemental concentration of another standard. This is a more common issue for elemental speciation standards as species are synthesized and often difficult to make highly pure. For example, dimethylarsonic acid (DMA) is typically synthesized using arsenate, therefore trace levels of arsenate are commonly present in the DMA standard and can contribute to the arsenate levels when mixed with an arsenate standard as part of preparing a calibration mix. Depending on the level, it may be necessary to consider the arsenate contribution from the DMA to total arsenate level of the calibration mix.

8.3 Preparation Tools

Review Chapter 7 for general information on proper use and handling of RMs. More in-depth information related to elemental analysis is presented here. During primary sampling and comminuting of RMs, metal tools should be avoided. Often grinding mills employ stainless steel or other metal blades. If titanium is not an analyte of interest, many manufacturers offer titanium blades that could be used. Other manufacturers offer coated blades or impactors. Note that while a material might be labeled as “metal-free”, it does not mean that it does not contain other elemental impurities. When possible, material should be cleaned with dilute acid, referred to as acid washing, for the purpose of washing or leaching out elemental impurities. Acid content is typically in the range of 1-10% depending on the application. After rinsing, these solutions could be analyzed to assess the level of contamination. Pipettes are a common tool utilized for diluting liquid RMs and should be plastic. Some pipettes utilize metal pieces to eject the plastic tips. Ensure that metal pipette components do not contact the sample material. Additionally, new tips should be used when changing solutions and should never be inserted directly into a stock solution. Increased handling of RMs has been shown to lead to detectable levels of contamination for various reasons.[1-2] Dispensers, like pipets, should have no metal to liquid contact as this could lead to contamination. Knowledge of the internal dispenser components may be useful. Any re-usable container that encounters the sample material should be thoroughly cleaned between usages.

8.4 Storage Containers

Once RMs have been prepared, they should be stored in appropriate containers. Often plastic is the bottle of choice with the most common being Teflon, polymethyl pentene, polyethylene, perfluoroalkoxy, polystyrene, or polypropylene. Many of these containers are produced by manufacturers specifically for

elemental analysis and often contain insignificant levels of impurities. It is recommended that a given product line and lot be tested upon initial receipt. It may be necessary to acid wash as discussed previously. In general, plastic is favored rather than glass, but glass can be used with more emphasis on acid washing. For ultra-trace analysis, glass is seldom appropriate. Additionally, it is wise to maximize the volume-to-surface ratio of a container as this could help minimize the contributions from leached contaminants. Note that the higher the acid content of an RM within a container, the more impurities will leach from the container. Leaching also increases with increasing temperature. Acid content should be low enough to minimize leaching, while high enough to keep analytes stable as discussed in Chapter 6. The history of a container should also be considered. For example, if a bottle was used to store a concentrated lead (Pb) solution, it should not be used later for a solution with low level Pb without extensive cleaning and/or testing to ensure carryover is not an issue. The same goes for extraction or digestion vessels as highly contaminated samples could lead to carryover without proper cleaning. For example, iodine and arsenic stick to fluoroplastic vessels and will give erratic results so it is better to use perfluoroalkoxy vessels. Manufacturers often provide detailed cleaning instructions to minimize carryover in microwave digestion vessels.

8.5 Environmental Contaminants

While most contaminations mentioned above are somewhat obvious or more often considered based on their direct contact with the sample, other sources of contamination may not be as evident. These include several sources from within the laboratory. Due to acid fumes being corrosive and the common use of acids in elemental laboratories, there is a chance of increased rust prevalence on cabinets, drawers, and other surfaces that may be near the preparation areas, leading to the potential for their particulates ending up in samples. Therefore, these types of rust areas should be removed or covered. Additionally, particulates from ceiling tiles and general dust have been shown to enter solutions that are not covered.

Suggestions for minimizing environmental contamination include:

- While it is common practice to leave samples open to the air during analysis on the autosampler, it may be necessary to cover the autosampler and keep it dust free.
- Additional air circulation and filtering with HEPA filters may be needed.
- As previously mentioned, be cognizant of materials that were prepared previously in the area. It may be recommended to keep comminution areas separate from sample dilution areas, or to keep concentrated standard solutions away from those of lower concentrations used for external calibration.
- Routinely clean the laboratory areas to minimize dust and other contamination. This can be done by wiping surfaces down with water.
- When possible, keep receipt of laboratory samples, comminution, preparation of analytical portions, RM and CAL preparation, and instrumental analysis areas separate.

8.6 Clean Rooms

Depending on the application, different clean room environments should be utilized for elemental analysis.[5] A Class 100 clean room (<100 particles with diameter of 0.5 mm per cubic foot of air) is chosen for the highest level of elemental contaminate control (e.g., for use by the semi-conductor industry or other ultra-trace applications). For most typical food or environmental laboratories, Class 1000 or even Class 10,000 can be used, often with smaller preparation areas (clean boxes) within the Class 100 specification.

8.7 Analyst-based Contamination

One aspect that often is overlooked is contamination via the analysts. Some materials commonly used by analysts including wipes and gloves have been shown to contain and leach elemental impurities. Gloves are mainly used to protect the analyst from hazards, but they also protect the RMs from the analysts as direct contact with skin has been shown to transfer impurities such as sodium and lead.[2] Additional potential sources of elemental contaminants include cosmetics, jewelry, and hair dye. Fibers and other particulate from clothing or lab coats can be sources, however most lab coats are generally sufficient for routine applications. For applications requiring ultra-trace levels of contaminants, additional personal protective equipment may be needed.

8.8 Contamination Testing

8.8.1 Detection of contamination often occurs after analysis of a sample. Ideally, sources of contamination should be evaluated prior to starting an analysis, but this may not always be practical. Once contamination is found, it can be difficult to determine the source for remediation. Often meticulous experimental investigation is necessary to pinpoint the failure. One of the best ways to do so is to include method blanks along with samples and reference materials that proceed through the entire sample preparation process.

8.8.2 Specific troubleshooting strategies include:

- Comparing a blank solution test result concentration, to the concentrations found in method blanks can help determine if the sample preparation process introduced contamination. For example, a calibration blank should have the lowest concentration in the analytical batch.
- Isolate various components of the laboratory sample and RM preparation processes and analyze them. For example:
- Evaluate containers used for analytical sample dilution by filling them with dilute acid solution matching the concentration in CALs (e.g., 5% HNO_3, 0.5% HCl), cap and allow to soak overnight. If the test containers are compatible with the instrument autosampler, analyze them directly by placing them in the appropriate autosampler rack positions. Although more labor intensive, test containers could manually be analyzed directly by inserting the sample uptake tubing into the test containers. Avoiding unnecessary pipetting and/or solution transfers helps to focus in on

the true contamination source. If pipetting and/or solution transfer are needed, ensure the materials are clean prior to use.

- Dilute various reagents used for analytical preparation and analyze and compare their relative analyte concentrations to determine significant sources of contamination.
- Analyze a solution before and after filtering (to remove undigested particulates) to test contamination from filters/syringes.
- Analyze method blanks for contamination or interferences over a long period of time to provide data on concentration range and frequency.

8.8.3 Example of contamination testing: Many experiments that provide insights into the source of contamination result from isolating various testing parameters and analyzing each separately.

One example is tracking apparent lead (Pb) contamination in digested samples: Three test portions of analytical sample A were digested using nitric acid with microwave assisted extraction. After digestion, the samples were diluted and analyzed by ICP-MS. One replicate had a significantly higher Pb concentration than the other two.

1. Check the method blanks analyzed alongside the test samples. Were they all free of detectable lead?
   - Yes: Contamination likely related to Sample A only
   - No: Possible contamination from any of the components in the preparation process.
2. Did any other analytical samples analyzed in the same batch have similar differences in Pb concentrations between replicates?
   - Yes: Maybe the contamination is related to this type of sample, or the contamination may be more widespread than expected.
   - No: Contamination likely related to Sample A only.
3. If suspected to be related to Sample A only, it could be related to:
   - sample heterogeneity, which can be remediated by using a larger test portion mass for digestion to better represent the sample as a whole or by comminuting the laboratory sample to a smaller and more uniform particle size (e.g., blended, ground, etc.).
   - spot contamination of the suspect analytical sample (e.g., dirty microwave vessel, dilution tube, other environment contamination, etc.), which can be remediated by repreparation and reanalysis of replicate analytical samples with emphasis on cleaning vessels and tubes, with the anticipation that the “random” contamination will not occur again.
   - instrumental error, which can be investigated by reanalysis of the test portion to confirm solution is contaminated. It is important to recognize that if there is significant variability in the instrumental testing process, that the contamination problem may remain unidentified.
4. If contamination is detected in several samples in an analytical batch, all components of the sample preparation should be isolated and checked for contamination. Analyze the most “common” component, verify it is clean, then move on.

- Analyze reagent water. Be sure to use a clean container. It may need to be acid washed. If the water and tubes are clean, move on to the next component,
- Analyze acids and reagents used for digestion or dilution. Dilute with a previously verified-clean water source. If clean, move to the next component.
- Analyze sampling tubes. Soak in acid as previously described and analyze directly. If clean, move to the next component.

Repeat the process with other reagents and laboratory equipment that contact the sample. If any components are found to be dirty, clean and retest or replace and retest. Continue this process for other components until contamination is located.

8.9 Remediating Contamination

As previously mentioned, there are several sources of potential contamination.[6,7] Understanding the sources and determining their contributions are the initials steps. Ultimately the contamination should be remediated. Implementing the changes discussed above will help remediate most common sources of contamination, however, not all issues were discussed and there may be no way to resolve all sources of contamination.

The following are general strategies to remediate contamination:

- Ensure that the work areas are routinely and effectively cleaned.
- Methodically check any materials that come in contact with RMs, CALs or samples and remove/clean them as appropriate.
- If a source of contamination is suspected, try swapping that component (for example a CAL that had been opened and used many times) or switch to a different lot.
- It is possible that a single contamination source is not the sole contributor to the contamination, therefore assess as many components as possible.
- It will likely be impossible to eliminate all measurable contamination, therefore it may be necessary to assess analytical needs (target analyte concentration, desired reporting limits, etc.) and determine your laboratory's acceptable limits of contamination.

8.10 Physical and Chemical Effects and their Impact on Elemental Analysis

Instrument-based interferences could produce artificially high concentrations that may appear to be contamination related. Both physical and chemical effects can adversely impact the accuracy and precision of the analytical measurement and need to be addressed. There are several ways to reduce these effects including:

- Sample introduction component selection
- Matrix matching
- Internal standard addition

Every analytical technique has some unique challenges associated with it in terms of the types of interferences and ways in which these can be handled. Depending upon the analytical technique, these can be corrected via:

- Matrix separation and analyte pre-concentration
- Mathematical corrections
- Cell, plasma, or additional gases (ICP-MS)
- Hardware (e.g., additional quadrupoles in ICP-MS)

The best approach to evaluate the validity of these corrective measures is to use an independent analytical technique to validate the results. However, this approach is often time-consuming, expensive and may require additional personnel or expertise to be brought in-house.

An alternative and relatively inexpensive way to establish accuracy of a particular method is via the selection of an appropriate reference material which is similar in analyte(s), matrix(es), and concentration(s) to your samples. Reference materials can be viewed as supporting the laboratory in two key ways:

1. Validating the calibration of the measurement system and the techniques used (e.g., correction equations, reaction gases) to address interferences.
2. Validating the performance of the measurement system.

In selecting a reference material, it's quite important to ensure that the matrix of your samples and the reference material do not significantly differ as this may skew the results. A good question to ask is, How might the levels of some components in the matrix affect or interfere with the measurement of my analyte of interest? For example, if I'm interested in evaluating trace elements in seawater, How would the sodium chloride in the samples impact the analysis using ICP-MS and what measures, such as matrix matching, selection of an appropriate seawater CRM or internal standardization, could help overcome this issue? In terms of food analysis, we can consider a high fat sample versus a low-fat CRM. What would be the mathematical relationship between the amount of the interfering substance and that of the component/element of interest?

Though sometimes difficult to gauge, there is guidance which exists in literature. For example, NIST Special Publication 260-181, *The ABCs of Using Standard Reference Materials in the Analysis of Foods and Dietary Supplements: A Practical Guide*[8] provides valuable information on how to select reference materials for the analysis of foods and dietary supplements.

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9 MITIGATING ELEMENTAL INTERFERENCES

9.1 Introduction

In addition to contamination control, elemental analysis laboratories also need to be aware of the possibility of their analysis being affected by various types of interferences. A range of atomic spectroscopy techniques can be used for elemental analysis, of which Atomic Absorption Spectrometry (AAS) and Inductively Coupled Plasma (ICP) spectroscopy are the most widely used. AAS with a flame source (FAAS) can be used for fast, cost-effective analysis at concentration levels in the µg/L (ppb) range and above. AAS with a graphite furnace source (GFAAS) or with Hydride Generation (HG-AAS) can be used for lower analyte concentrations down to ng/L (ppt) levels. AAS has a limited dynamic range of about 2 or 3 orders of magnitude.

In a similar manner, ICP techniques can use an Optical Emission Spectrometer (ICP-OES) for relatively high (0.1 ppb and above) concentrations. Alternatively, the ICP can be connected to a Mass Spectrometer (ICP-MS) for much lower-level analysis, down to pg/L (ppq) concentrations if sufficiently high purity reagents and a clean laboratory environment are available. ICP-MS has the broadest elemental coverage of any of the techniques (up to 78 elements), and the widest detector linear dynamic range (up to 11 orders of magnitude, from ppq to 1000s ppm). Both ICP techniques provide fast, multielement analysis and are widely used in laboratories that perform routine, high throughput analysis according to regulated methods, where 60 elements or more may be measured in each sample.

Each technique has its own pros and cons including considerations regarding the type and severity of the interferences that might occur in any given application. In general terms, AAS is used to determine a single element in each measurement, while ICP techniques measure multiple elements in each analysis. This means that an ICP user may need to consider a wider range of potential interferences, due to the larger number of analytes being determined. Similarly, GFAAS and ICP-MS can perform measurements at much lower concentrations than FAAS and ICP-OES, respectively, so interferences may be more apparent for the more sensitive techniques, due to the lower analyte levels determined.

In this chapter we will provide a high-level overview on the three predominantly utilized atomic spectroscopic techniques, namely AAS, ICP-OES and ICP-MS techniques, the types of interferences which affect them, and discuss some of the ways in which they can be addressed.

9.2 Atomic Absorption Spectroscopy (F/AAS)

In atomic absorption spectroscopy the atom cloud is produced via aspirating a sample solution into a flame, where the metals in solution undergo desolvation, liquefaction, vaporization, atomization, excitation, and ionization. Flame temperature is an important metric governing the effectiveness of atomization, where cooler flames are more likely to be affected by interferences resulting from inadequate atomization. This flame is aligned with a light beam, where the amount of light absorbed is

directly related to the number of ground state metal atoms formed. The concentration of each element in a sample is measured by analyzing the absorption spectrum of the atoms in question. Atomic Absorption Spectroscopy is a very specific technique, possessing very few interferences which are all well-defined. The interferences that do exist, however, are caused by other elements in the sample or in the flame that can absorb the same wavelengths of light as the element being analyzed. This can lead to inaccurate readings and affect the sensitivity and selectivity of the measurement.

There are two main types of interferences in AAS, namely non-spectral and spectral interferences.

9.2.1 Non-spectral interferences: Non-spectral interferences affect the formation of analyte atoms and can be further divided into matrix effects, chemical interferences, and ionization interferences.

- **Matrix interferences.** If the sample composition is vastly different from the calibration standards to a point where nebulization efficiency differs, there may be a resulting difference in absorbance efficiency. Thus, the physical parameters of the matrix will have resulted in analytical bias. To address this issue, matrix matching is essential. If the interference is too pronounced, the use of Method of Standard Additions may be recommended.
- **Chemical interferences** are caused by the presence of other elements in the sample matrix that can form thermally stable compounds with the analyte, preventing atomization and consequently resulting in reduced signal intensity. Here, a matrix-modifier can be added which preferentially forms a thermally stable compound with the interferent. Alternatively, a hotter flame can be used to promote the dissociation of atoms in the compound.
- **Ionization interferences.** This interference is common in hot flames where excessive energy is supplied to the analyte atom such that it forms positive ions instead of the desired ground state atoms which are needed for light absorbance, reducing sensitivity. By changing to a cooler flame, this issue can be resolved for most elements except for alkali metals and alkaline earth metals. An alternative approach is to add an ionization suppressant, such as an excess of an easily ionized element, to the sample which results in a large number of electrons in the flame which in turn suppresses the ionization of the analyte.

9.2.2 Spectral interferences: Spectral interferences occur when the absorption spectrum of the interfering element overlaps with the absorption spectrum of the element being analyzed, making it difficult to distinguish between the two, resulting in a decrease in sensitivity or inaccurate results.

9.2.3 Background absorption: This type of interference arises from incomplete atomization of all matrix materials in a sample. Although uncommon, undissociated molecular forms of matrix materials may scatter light over a wide wavelength region. The resolution for this issue involves the use of a background correction technique which measures the background absorption and subtracts it from the total measured absorption to determine the true value. Approaches to this include Continuum Source background correction and Zeeman background correction. Continuum source background correction automatically measures and compensates for background components which might be present in an atomic absorption measurement. This correction technique incorporates a continuum light source within the optical system. Continuum source background correction is widely used for most flame AA applications. However, this technique can adversely affect graphite furnace atomic absorption, making

Zeeman correction a superior choice. Zeeman background correction involves the use of a strong magnetic field. When the atomic absorption profile is observed with polarized light, the atomic absorption profile is split into two symmetrical components on either side of the normal position. The spectra of the background absorption are usually unaffected by the magnetic field. DC Zeeman systems use a permanent magnet and a rotating or vibrating polarizer to separate the combined and background only signals, while AC Zeeman systems use an electromagnet that is turned on and off to measure the combined and background only signals.

9.3 Inductively Coupled Plasma Spectroscopy

Both ICP-OES and ICP-MS are mainly used for the analysis of liquid samples, although solids and gases can be measured using a suitable accessory, for example, laser ablation for direct sampling of solid materials. Quantitative analysis is usually performed by preparing a synthetic multielement calibration standard (or standards) from certified and traceable materials. The measured signal for each analyte in the sample is compared to the signal for that element in the known-concentration standard to allow a concentration for each element in the sample to be calculated.

Both ICP-OES and ICP-MS techniques use a similar plasma source, and the sample goes through the process of desolvation (liquids only), atomization, excitation, and ionization. The key difference between these technologies is that ICP-MS only measures ions, while ICP-OES measures both atomic and ionic emission lines. These two techniques and their respective interferences will be briefly discussed below.

9.4 Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)

ICP-OES measures the emission spectra produced from excited atoms and ions of the elements in the sample. In the argon plasma, atoms and ions from the sample go from their ground state to an excited state. Here they are not stable and return to their ground state, emitting energy in the form of light. It is this light which is being measured. Since samples are typically complex and contain multiple elements, the atomic or ionic emission line of one element can overlap that of another, leading to bias in the determined concentrations. Below are the main types of interferences encountered in ICP-OES and mechanisms to address them.

9.4.1 Background correction: Argon emission lines and a continuum which extends over the wavelength range are characteristic of ICP-OES technology. Sample matrix elements can further complicate the background emission spectra, which consequently demands that the background is compensated for either by measuring a single point on the continuum background adjacent to determine the net intensity or by using advanced algorithms. The background intensity decreases as the sample enters the plasma and this causes the plasma to cool down.

9.4.2 Non-spectral interferences: There are a number of non-spectral interferences which are common to both ICP-OES and ICP-MS. These will be covered in the ICP-MS section to avoid duplication.

9.4.3 Spectral interferences: Spectral interferences occur when the analyte emission lines overlap with other spectral lines or broad-band emission. This type of interference occurs when multiple elements in the sample emit light at similar wavelengths, making it challenging to differentiate the signal from the analyte of interest from the background. Spectral interferences can be overcome by:

- **Choosing an alternative wavelength** which does not have an interference on or near it.
- **Matrix matching** is typically used to compensate for non-spectral interferences but can also be useful in addressing spectral interferences, such as when the interferent concentration and/or intensity is constant in all solutions of interest.
- **Inter-element correction (IEC).** While one of the oldest approaches to removing interferences in ICP-OES, IEC is still used in many situations today to compensate for direct spectral overlaps. By using a wavelength of the interferent which is free of interferences and evaluating the ratio between a single element solution of this clean wavelength and the analyte wavelength, the interferent can be subtracted from the total signal intensity. This approach is not particularly suited to the resolution of partial overlaps. As an overall technique, since IEC is additive, the analytical error increases in accordance with the law of propagation and can adversely impact reproducibility and limits of detection. The inter-element correction factor relies upon the excitation conditions, as is the case when one line is in atomic transition whereas the other is in the ionic transition and so it is important that the plasma temperature and nebulizer flow remains constant, and contamination mitigated.
- **Correction using multivariate regression or multi-component spectral fitting** is used to address the issue of partial overlaps and involves measuring the spectra of the sample solution and fitting a mathematical model to the data. A major benefit to this approach is its independence from changing excitation conditions and therefore, it is suitable for complex spectra, delivering improved reproducibility and limits of detection. This model takes into account the effect of interfering species on the signal of the element of interest. The model can then be used to correct the measured values for the presence of interferences, providing more accurate results. With this approach, it is important to ensure that contamination is limited (as this can skew the results), the interferent can be identified, and the concentration of the model solution is not too low.

9.5 Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

ICP-MS uses an inductively coupled plasma source like the ICP used in ICP-OES, but with the key difference that the desired outcome is to promote the formation of positive ions in the argon plasma from the atoms present in the sample. The ions formed in the plasma are extracted into a vacuum chamber, separated by mass (or mass to charge ratio, in the case of a quadrupole mass spectrometer) and passed to a detector for measurement.

Common interference issues encountered in many ICP-MS laboratories can be divided into chemical effects, matrix effects including ionization suppression and space charge, and spectral overlaps. ICP-MS matrix effects can be broadly divided into two types, physical and instrumental effects. These types of

interferences are discussed in the following sections, together with suggested approaches to deal with them.

9.5.1 Chemical effects: Some interferences in ICP-MS are caused by factors related to the sample composition, rather than the instrument itself. This can cause poor signal stability, long washout times, poor calibration linearity, and low recoveries. The remedy for this sort of chemical effect is to ensure that elemental solutions are prepared in a way which ensures all the analytes are present in stable chemical forms. For typical ICP-MS applications, the samples are usually prepared and/or stabilized in an acidified aqueous solution. Historically, ICP-MS users were encouraged to use only nitric acid (HNO3) and avoid other acids such as HCl or H_2SO_4, which could give rise to Cl-based and S-based interferences, respectively. However, modern instruments with collision/reaction cells (CRCs) can easily remove these spectral interferences, so adding HCl to stabilize elements such as Hg is now a standard part of most laboratories' methodologies. Using a mix of HNO_3 and HCl to stabilize samples and for the rinse solutions also helps avoid issues of slow wash-in (stabilization time) and washout (carryover) for several elements.

9.5.2 Physical matrix effects: Physical effects include factors that alter the flow rate of the sample solution through the sample uptake tubing, the nebulization processes, and transport of the aerosol to the plasma. For example, a sample with higher viscosity or surface tension will typically flow more slowly through the uptake tubing and will be converted to droplets in a different way at the nebulizer tip. A viscous solution will form fewer, larger droplets that will evaporate more slowly and are more likely to be filtered out in the spray chamber, impacting the aerosol droplet size distribution reaching the plasma. A change as simple as increasing the level of acid used to stabilize samples from 0.1% to 1%, for example, can affect the solution viscosity and surface tension enough to cause a significant change in the measured signal. Because physical matrix effects typically change the total mass of sample that is delivered to the plasma, they affect all analytes more-or-less equally, so the signal changes can usually be corrected effectively by using internal standards. Nevertheless, it should be noted that it is considered good practice to try to prepare the synthetic calibration solutions in an acid mix that closely matches the sample composition.

Another type of physical matrix effect that may be observed, especially when high matrix samples are analyzed, is signal loss and long-term drift due to deposition of the sample matrix on the ICP-MS interface cones. Matrix deposition can be minimized by ensuring the sample is appropriately diluted to a level that the plasma can tolerate. Historically, the maximum amount of total dissolved solids (TDS) that ICP-MS systems could tolerate was 0.2%, or 2000 ppm. Higher matrix samples had to be diluted to below this level, either manually or using an auto-dilutor. However, the development of aerosol dilution systems now allows much higher matrix levels to be introduced, with the "dilution" occurring in the aerosol state using argon gas. Aerosol dilution reduces the risk of sample handling errors and contamination from manual sample dilution, while avoiding the cost and complexity of an auto-dilutor.

An alternative way of addressing matrix-related spectral interferences that may be applicable for certain sample types is to use sample clean-up or matrix elimination using a chelating column, which can also provide the added benefit of preconcentrating the analytes.[1] However, this approach is often not suitable for all the required analytes, so multiple separations may be required to cover all the elements of interest.

In routine analysis, column pre-treatment is not usually practical due to the time and cost required and is typically reserved for specific applications.

9.5.3 Instrumental matrix effects: These interferences occur due to instrumental factors, such as the way the sample aerosol droplets are processed in the plasma, and the way the ions are formed, extracted, and focused through the instrument.

- **Ionization.** ICP-MS requires that the atoms of the elements being analyzed are converted to ions, so the process of ionization is critical to the measurement. The degree of ionization of an element (the percentage of an element's atoms that lose an electron to become singly-charged ions) depends on the element's first ionization potential (1st IP). The 1st IP, measured in electron volts (eV), indicates how much plasma energy is needed to remove the atom's outermost electron. Ionization is affected by several instrument parameters, most importantly the energy available in the central channel of the plasma, the density and size of the aerosol droplets (the aerosol "loading"), and the amount of time the atom spends passing through the plasma, known as the "residence time". Together, these parameters control the "effective plasma temperature" which is the amount of energy the analyte atoms are exposed to as they pass through the plasma. A "hotter" plasma promotes conversion of atoms to ions, so will give higher overall sensitivity. Plasma "temperature" (or energy) is monitored using the ratio of CeO+ to Ce+, which shows the ability of the plasma to break the strongly bound Ce-O molecule. A lower CeO/Ce ratio indicates a hotter, more robust plasma and can have a great effect on the sensitivity of elements that are more difficult to ionize. It should be noted, however, that while a hotter plasma may improve sensitivity for elements which are difficult to ionize, such conditions may also promote the formation of doubly-charged ions, especially for ions which have low 2nd ionization potentials if the plasma conditions remain the same.
- **Ionization suppression.** This interference effect is caused when a high concentration of easily ionized elements (such as Na, K, Li, etc.) is present in the plasma, which leads to the plasma being flooded with a lot of free electrons. These free electrons will preferentially recombine with the positively charged ions of elements which have a high 1st IP, meaning they have a strong affinity for electrons. A high concentration of an easily ionized matrix element therefore suppresses the ionization, and consequently the signal, for poorly ionized analytes. If the matrix is well-characterized and consistent for all samples, ionization suppression can be mitigated through matrix-matching the calibration standards so that the suppression effect is the same for standards and samples. Using internal standards that are matched for 1st IP as well as mass can also help to reduce errors caused by ionization suppression. Optimizing the plasma for robust conditions also helps to control ionization suppression. This approach is particularly useful when the sample matrix varies significantly or when it is not practical to matrix-match the sample and calibration standards.
- **Ionization enhancement.** In sample types that contain a high level of carbon, the signal for some elements, notably As and Se, may be increased. This so-called "carbon-enhancement effect" occurs when the high level of carbon in the sample increases the ionization of the poorly ionized

elements, leading to high recoveries relative to the low carbon calibration standards. A simple solution to address this effect is to match the carbon content of the samples by adding an excess of carbon to the standards as well. Around 2% carbon, e.g., as an organic solvent such as butanol added to the internal standard solution, provides uniform ionization for As and Se in the standards and samples.

- **Space charge.** Space charge is the term used to describe the ion dispersion effects which occur after the ion beam passes into the vacuum region behind the skimmer cone. The plasma is electrically neutral but, after the ion beam is sampled into the vacuum system, the highly mobile electrons quickly diffuse out of the extracted ion beam, leaving the positively charged ions. The positive ions try to repel each other, which results in the beam becoming dispersed and defocused. Since lighter ions are more easily deflected, they tend to migrate to the edges of the ion beam, while heavier ions are less easily deflected and so remain near the center resulting in lower relative transmission of light masses, or mass bias. Space charge effects are influenced by the geometry of the vacuum interface and ion lens, and the voltages applied to steer the ions through the intermediate vacuum region.

9.5.4 Spectral Overlaps: ICP-MS has a number of well-characterized spectral interferences which are generated from the plasma gases, matrix ions, and the solvents or acids being used, impacting limits of detection and accuracy. Spectral interferences can be separated into isobaric, polyatomic, and doubly charged ion interferences. Incomplete resolution of an intense peak adjacent to the analyte mass can also cause peak-tail overlaps. Some of the ways to address these types of interferences are discussed briefly below.

9.5.5 Polyatomic ions: Polyatomic ion overlaps are by far the most prevalent interferences in most typical ICP-MS applications and a lot of attention has gone into developing hardware, software, and methods to address them. These interferences occur when two or more atoms combine to form an ion at the same mass as the analyte ion. Numerous approaches have been used to address polyatomic ion overlaps, some of which have been superseded by later hardware and software developments:

- **Alternative isotope selection.** Many isobaric and polyatomic interferences can be addressed by simply choosing an alternative isotope that does not have an interference on it. If the alternative isotope has a much lower abundance and therefore lower sensitivity than the preferred isotope, detection limits can be compromised. Moreover, in the case of monoisotopic elements and those at ultra-low concentrations, the choice of an alternative isotope is not always possible.
- **Correction equations**. Correction equations were historically the main approach used to address spectral interferences in ICP-MS.[2] Correction equations can be effective for isobaric and doubly charged overlaps as well as polyatomic interferences. However, correction equations rely on measuring the source of the interference and assuming a polyatomic ion formation ratio or measuring a “free” isotope of another element. As a consequence, the results can be unreliable, especially in samples with complex or variable matrices. Nevertheless, correction equations are

still defined in some regulatory methods, notably those that were originally written before collision/reaction cells became universally available.

- **Cold/cool plasma**. Argon-based interferences and intense N2+ and O2+ backgrounds can be addressed using cold/cool plasma conditions. The reduced plasma energy prevents the ionization of the background species, enabling trace analysis of interfered elements such as Ca (mass 40, overlapped by Ar-40) and Fe (mass 56, overlapped by ArO). Cool plasma conditions provide the added benefit of reducing space charge effects, giving extremely high sensitivity for low mass analytes. However, the lower power plasma is less robust, so ionization of poorly ionized analytes is reduced, and matrix effects are higher, so this approach is not applicable to all scenarios.
- **Robust plasma conditions**. As with addressing matrix effects, a higher plasma temperature offers a more generally applicable approach to reducing the formation of many polyatomic ions. Optimizing the plasma for more robust, low CeO/Ce conditions gives a similar or greater reduction in many other, less strongly bound matrix oxides, such as SiO, CaO, ZrO, MoO, and WO. Lower CeO/Ce conditions also reduce the level of other polyatomic ions, including ClO, SO, S_2O, etc.
- **Collision mode in a collision/reaction cell**. A collision/reaction cell is an enclosed chamber or cell, which can be pressurized with a gas to process the ion beam prior to the ions entering the analyzer quadrupole. Collision mode uses a non-reactive gas, typically helium, and works on the principle that analyte ions always have a smaller ionic cross-section than polyatomic ions at the same mass. All ions passing through the pressurized cell undergo collisions and lose kinetic energy, but the polyatomic ions undergo more collisions, lowering their kinetic energy relative to the analyte ions. Using a positive bias voltage at the cell exit, the lower energy (polyatomic) ions can be prevented from passing to the quadrupole, while the higher energy (analyte) ions can overcome the bias voltage and pass through to be measured, a process known as kinetic energy discrimination (KED).[3]

  The large number of collisions required for effective KED mean that there is some scattering and loss of transmission for low mass analyte ions. But for most ICP-MS analytes (above about mass 40), the polyatomic ion contribution can be reduced by several orders of magnitude relative to the analyte signal. Collision mode is a physical process, so it works for all analyte/polyatomic ion combinations, even where multiple polyatomic ions contribute at the same analyte mass. As a result, it is typically used as a "universal approach" for multi-element analysis in complex or variable sample matrices with unknown compositions, where the contribution from the polyatomic interference(s) is typically no more than about 4 orders of magnitude higher than the analyte signal.
- **Reaction Mode**. A reactive cell gas provides a highly selective way to resolve many polyatomic and other spectral interferences, as the ion-molecule reactions are very fast and efficient compared to KED.[4] Reaction mode is ideally suited to cases where the analyte is in low concentrations and the sample matrix is well-characterized, so the interfering ions can be predicted, and an appropriate reaction gas selected. Reactive cell gases may also be used in cases where the contribution from the interference exceeds four orders of magnitude, for example N2+, O2+, Ar+, ArC+, ArO+, Ar2+, and so on. In reaction mode, a reactive gas suited to addressing the specific interference is introduced into the cell at an appropriate flow rate for the reaction to take

place. Here, the reaction gas can either react with the analyte ion, shifting its mass to a higher mass where there is no interference, or the gas can react with the interference such than it is neutralized or removed to a different mass.

It should be noted that reactive cell gases have limited applicability on single quadrupole ICP-MS, where no mass selection occurs before the collision/reaction cell. When multiple analytes enter the cell, the cell gas can react with other, non-target ions in the cell to form new, reaction product ion interferences. A reaction cell that contains a quadrupole ion guide can use a bandpass mass transmission window to control the formation of reaction product ions, provided the intermediate ions are sufficiently distant in mass to be rejected by the cell bandpass settings. However, product ion overlaps that form from other ions that are close in mass to the analyte cannot be rejected reliably, which means reaction mode is not generally applicable for single quadrupole ICP-MS applications that involve the analysis of high matrix or variable composition samples.

- **Reaction mode with tandem MS (MS/MS)**. The addition of a further quadrupole mass filter before the collision/reaction cell allows the selection of the specific mass of ions that are allowed to enter the cell. Using MS/MS mode, therefore, ensures that only the analyte ions and on-mass interferences can take part in the reactions. ICP-MS/MS is widely and successfully used across many applications, particularly where ultratrace level analytes are measured, and intense spectral overlaps must be resolved. The main benefit of the double mass selection approach is that it ensures selectivity of the reactions, while eliminating the possibility of non-target ions being involved in the reactions, or of existing ions being present at the mass of an analyte product ion. For example, when analyzing As (m/z = 75), only m/z 75 is allowed into the cell (As-75, 40Ar35Cl, 150Sm2+/150Nd2+), but only As will react with O_2 being introduced into the cell and produce an ion at m/z 91.

9.5.6 Isobaric overlaps: In ICP-MS, an isobaric overlap refers to an elemental ion that appears at the same mass as the analyte isotope of interest but is derived from a different element, for example Ar-40 overlaps Ca-40. Mass spectrometry analysts benefit from the fortunate fact that all naturally occurring elements except indium possess at least one isotope that occupies a unique mass, free from overlap by an isotope of any other element. For each analyte, the most abundant free isotope is typically selected as the preferred isotope for ICP-MS analysis, giving analysts a simple way to avoid most isobaric overlaps. However, the most abundant isotope of an analyte may suffer an isobaric overlap, as in the case of Ca-40 mentioned above. If the isobaric overlap can be removed, measuring the more abundant isotope would improve the detection limit. Also, analysts sometimes need to measure analyte isotopes that are affected by isobaric overlaps, for example for stable isotope ratio analysis (e.g., Sr-87/Rb-87) or when long-lived radionuclides such as Sr-90, Sm-151, and I-129 are measured. These isotopes are all overlapped by naturally occurring isotopes of other elements (Zr-90, Eu-151, and Xe-129, respectively). Isobaric overlaps are too close in mass to be resolved using a sector field “high resolution” ICP-MS, and they cannot be resolved using collision mode. While reaction mode with a reaction gas on a single quadrupole system may help to reduce the interference, best results are achieved on an ICP-MS/MS instrument using a reaction gas that reacts with one of the isobars and not with the other. In this way, many overlaps and

interferences can be resolved, enabling novel applications in geochemistry, environmental monitoring, radiopharmaceuticals, and nuclear science.

9.5.7 Doubly-charged ions: If an element has a low enough 2nd IP, it will lose 2 electrons to form a doubly charged ion (M2+), rather than the usual singly charged ion (M+). Most elements have a 2nd IP that is too high for them to form a significant proportion of M2+ ions, but some, have a 2nd IP that is below the 1st IP of Ar, so these elements can form a few % of M2+ ions.

A quadrupole mass spectrometer separates ions based on their mass to charge ratio (m/z), rather than their actual mass, so doubly charged ions appear in the mass spectrum at half their true mass. Doubly charged ion overlaps can sometimes be avoided by selecting a different analyte isotope. For example, Mg-26 is not affected by doubly charged ions of Ti that overlap Mg-24 and Mg-25 (49Ti2+ and 50Ti2+). Alternatively, a half-mass correction equation can be defined with an odd-numbered isotope used as the reference mass for the overlap, where the M2+ ion is measured at a half mass position. This approach requires the quadrupole to be operated under increased resolution conditions to allow access to the half-mass peaks. However, the most effective means of resolving doubly charged ion interferences is by using ICP-MS/MS with reaction chemistry to remove the M2+ overlap or mass-shift the analyte ions away from the overlap (as explained in the section "Reaction mode with tandem MS (MS/MS)".

9.5.8 Peak tail interferences: In a few applications, a trace analyte needs to be measured at a mass adjacent to a high intensity peak, where the "tails" on either side of the intense peak may extend far enough to overlap the adjacent mass. Peak tailing is measured by the abundance sensitivity (AS) of the spectrometer. The quadrupole mass filters, most commonly used in ICP-MS instruments, are extremely good at rejecting off-mass ions and a typical AS specification for a commercial quadrupole ICP-MS instrument is 10^{-7}. This means that, for every 10 million ions present at mass M, only 1 ion appears (erroneously) at the adjacent masses M-1 and M+1.

This AS performance is sufficient to separate most adjacent mass overlaps, but in some cases the difference in intensity is much more than 7 orders of magnitude, so the peak tail overlaps can be significant. In these cases, the double mass selection of tandem mass spectrometer instruments offers a solution, as the overall AS is the product of the two mass filters, so $10^{-7} \times 10^{-7}$. In practice, this means that MS/MS can effectively eliminate peak tail overlaps.

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10 RMs PREPARED IN-HOUSE

10.1 CRMs

As described earlier, CRMs are produced in compliance with ISO 17034[1] and ISO Guide 35.[2] CRMs are provided with an RMC stating certified values with their respective uncertainties together with a statement of metrological traceability. Such materials provide the best estimate of the true value of the amount of an analyte in a matrix. CRMs can be used for trueness checks when validating a method and on-going calibrations.

10.2 Laboratory-prepared RMs

Laboratory-prepared RMs, known as in-house RMs, can be custom designed to match the needs of the laboratory testing method (e.g., solvent, analyte combinations, concentration ranges and matrices of interest). An in-house RM may be needed because no CRM or RM currently exists, an RM that more closely represents routine samples is needed, or an RM is needed to maintain traceability at lower cost. A new analyte CAL can be prepared for confirmation of a qualitative identification. Two types of RMs produced in the laboratory are described in Chapter 1.

10.2.1 CALs are used for measurement system calibration such as analytical standards prepared in a suitable solvent or matrix extract and used in instrument calibration and analyte measurement. In-matrix CALs also include matrix spiked with analytical standards and carried through the extraction test method. Calibrants should have established metrologically traceable analyte values and uncertainty suitable for calibration.

10.2.2 QCMs are used for measurement system quality control with materials used as reagent, method, and matrix blanks; matrix with naturally incurred analytes (preferred); matrix spiked with analytes of interest; and inter-laboratory test samples. QCMs can typically be used to generate control charts for a specific method once its stability has been established. A CRM can be used at a regular interval, once a month or once every so many analyses, to continue to verify that the in-house RM is still in control.

10.2.3 Suitably homogeneous and stable: Both CALs and QCMs should be suitably homogeneous and stable with respect to one or more properties to meet the intended purpose. ISO Guide 80[3] for preparation of in-house RMs provides useful guidance. In conjunction with CRMs, CALs can provide a measure of accuracy and QCMs provide an ongoing assessment of method performance.

10.3 Using a CRM for Metrological Traceability of CALs

10.3.1 Metrological traceability is the property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty.

10.3.2 Metrological traceability requires an established calibration hierarchy. For example, if the measurement result is given in mg/kg, this mass fraction is traceable to the SI system (i.e., kg). The metrological traceability is the basis for comparison between measurements in time and in space. Preparation of the CAL using a CRM (such as a multi-analyte solvent mixture) enables a level of traceability and a spiked matrix using a CAL that is a CRM offers the possibility of a trueness check and recovery test. Multi-level standard solutions prepared by dilution of a CRM solution provide calibration accuracy. A spike prepared with a CRM solution provides a measure of recovery. Metrological traceability enables a result for chlorpyrifos in a German fruit from 2012 that can be compared with a result in a US fruit in 2020.

10.4 Considerations in Preparation of RMs

Preparation of a CAL using a CRM (such as a multi-analyte solvent mixture) enables a level of traceability and determination of trueness second to using a CRM.

In describing the preparation of RMs for trace analysis, first consider CALs prepared in solvent which are assumed to be acceptably homogeneous. The role of CALs as RMs is sometimes overlooked by analysts. CALs also often play an important role in the preparation of QCMs. Second, consider QCMs (except for solvent blanks) which are prepared to mimic analytical sample matrix under testing conditions. Most QCMs are prepared from a natural material which, after processing, is referred to as a matrix.

10.4.1 Neat Chemicals. Neat chemicals used to prepare CALs can be obtained from a variety of sources including the manufacturer, which leads to some unique challenges.

Examples of challenges with neat chemicals are described below.

- The laboratory should use chemicals with known and confirmed quality, however it is the user's responsibility to verify that the information on a CoA is accurate.
- The laboratory might not receive a CoA or some other information that verifies the identity and purity of the chemical, or the uncertainty of the purity value. The user is responsible for assessing purity and uncertainty when using uncertified materials. Verifying identification of solid and neat materials may require infrared and qNMR spectroscopy and will not be discussed here.
- The laboratory might not receive information on the stability and solubility of the compound. For example: Some arsenic species RMs have been provided with misleading purity information. It was necessary to determine the total arsenic content to eliminate issues of insolubility, followed by a purity check for each analyte using LC-ICP-MS.
- Some compounds can be particularly unstable and require shipment and storage frozen or kept away from ultraviolet light.
- The laboratory may find unexpected metabolites or breakdown products are present.
- Isomer concentrations might not be specified.
- As discussed in Chapter 10, a compound might only be produced by one manufacturer, so a suitable second source might not be available for comparison.

- When working with very novel compounds or newly identified metabolites, a laboratory may prepare or receive a nearly pure material from a research laboratory. Because preparation of a highly pure material is extremely complex, the laboratory should assume the purity is unknown until proven otherwise. Quantitative NMR may be used for purity assessment if measures of both trueness and precision (accuracy) are needed. Universities and some private laboratories may provide purity assessments.
- For materials that are not well characterized, some laboratories examine the full mass spectra of diluted neat materials to detect significant contaminants or breakdown products. This approach does not provide a full picture of the purity of the material as inorganic, and many volatile or large compounds might not be detected by the chromatography method or the mass range of the instrument.
- Stable isotopically labeled calibration standards are frequently used as internal standards (ISTDs) for quantitative measurement. These ISTDs can be expensive to purchase and are sometimes custom made or prepared in the laboratory. When using ISTDs in this way, the user should verify that the labeled material (at the concentration used in the test sample extracts and CALS) does not contribute interfering quantities (typically >1% of LOQ) of the native, unlabeled compound. Similarly, the user should confirm that no labeled material is present (detected) in the test sample extract. The number and position of the isotopes on the molecule can be important. Characterizing isotopically labeled standards can be verified by high resolution mass spectroscopy. When added to calibration standards, the labeled material should be at a concentration near the middle of the calibration range.

10.4.2 Analyte Integrity. Analyte integrity is an important consideration in the preparation of both CALs and QCMs. Degradation of labile materials from heat, UV light or oxygen in the surrounding air should be prevented. Many pesticide residue analysts have begun preparing natural materials by cryogenic comminution using -80 °C freezing, liquid nitrogen, or dry ice, but some pesticides can be sensitive to freezing. Special precautions should be used in storage of some compounds and materials. Dry materials might be useable for a longer period. Procedures for stability determination are discussed in ISO Guide 80[3] and in Chapter 6.

10.4.3 Packaging and Storage. Proper packaging and storage conditions should be determined in order to maintain analyte concentration and integrity over the lifetime of both CALs and QCMs. Storage considerations include the type of container (e.g., glass, plastic), temperature (e.g., room temperature, reduced temperature), exclusion of light, storage under inert gas and constant humidity, among others. Standards stored at reduced temperatures should be carefully brought back to room temperature to reduce introduction of water through condensation on the container, especially in humid environments. Care should be taken to re-establish homogeneity after restoring from reduced temperature, as some standards may not be completely soluble at lower temperatures than the ones at which they were prepared.

Care should be taken to re-establish homogeneity after restoring RM from reduced temperature.

10.5 Preparation of CALs

10.5.1. A neat chemical used to prepare a CAL should have a known purity value, established metrological traceability, and uncertainty suitable for the intended purpose.[4] For chemicals containing multiple isotopes or species, the purity and concentration of each analyte may need to be verified. A CAL can be prepared in-house by dilution of CRMs on calibrated balances using pure solvents. The uncertainty of the CAL includes variations in weighing or making volumetric dilutions.

a) **Weighing.** Quantitative measurements with an analytical balance calibrated with traceable reference weights are essential. Balances should be calibrated annually by an accredited calibration company and calibrations should be checked daily with certified weights. Weights, temperature, humidity, and pressure should be recorded. Weighing should be made on an analytical balance of sufficient accuracy and at a controlled temperature and humidity, as some neat materials can absorb water in a humid environment. A good practice when preparing new solutions is to compare quantitation using two solutions prepared independently, such as comparing new CALs to ones currently in use. Quantitation results should agree within a range fit-for-purpose (usually within 10% for trace organic analyses and much better agreement for elemental analyses). When results don't agree, it should be determined whether the current standard has drifted out of specification, or whether the new one was not prepared to specification, or both.

b) **Solubility**. All compounds should be soluble in solution, both at room temperature and while stored at lower temperatures. Compounds which crystalize out of solution at freezer temperatures might not easily dissolve when brought to room temperature. Additionally, some compounds might not be soluble when combined into a large mixture containing other compounds. Storage stability studies and quantitative verifications are recommended to determine accuracy when calibration standards are put into service at a later time.

c) **Concentrated mixtures**. Many laboratories prepare or purchase solutions containing 5 to 25 compounds at concentrations about 25- to 100-fold higher than needed for a stock solution for preparation of CALs and spiking solutions. These mixtures may be prepared in various solvents depending on their solubility and stability, and a small amount of a stabilizer might be added. Benefits to preparing mixtures containing a smaller number of compounds include the ability to prepare the CAL in a single day; errors affecting a smaller number of analytes; less stable analytes being prepared more often; and concentrates being made in solvents that are most compatible with the analyte solubility.

d) **Stock solutions**. Aliquots from several different multi-analyte concentrated solutions may be used together to prepare a composite stock solution in the solvent needed for analysis. Larger volumes of stored solutions are less susceptible to solvent evaporation or absorption of contaminants. Stored

solutions should be weighed before and after aliquots are taken and a calculation made to adjust concentrations of analytes for evaporation/transpiration if necessary. Solutions at this concentration might also be used to prepare spikes.

e) **Intermediate solutions**. Dilutions of the stock solution to several different concentrations may be prepared for daily use. These dilutions may be for a single, weekly, or monthly use depending on the laboratory needs and verified stability.

f) **Working solutions**. Aliquots of intermediate solutions may be vialed for immediate use or added to matrix for calibration standards, internal standards, or matrix matched spikes.

10.5.2 CALs in Matrix. To compensate for instrumental interferences, as well as signal enhancement or suppression, CALs are often prepared in a matrix extract which mimics the matrix being tested. Some methods require CALs to be spiked into blank matrix and carried through the test method (procedural CALs) to compensate for losses during extraction or derivatization.

10.5.3 CALs Used to Prepare Spikes. Quality control spikes are not usually RMs but are prepared by adding known amounts of CAL solution to a test portion of matrix and adding to a testing sequence to be extracted and measured in the same manner as the test samples. The purpose of quality control spikes is to evaluate the on-going ability of the test method to recover the analytes of interest. The CALs and spikes used in a method should be prepared from different CRMs. If spikes are prepared from the same solutions as the CALs, bias in the CALs cannot be detected. Ideally, a separate spiking solution should be prepared from a second source CRM of high quality, with known purity and uncertainty. If only one CAL is available, the spike should be prepared from a separately prepared solution, possibly prepared more recently to detect any analyte degradation.

10.6 Matrix RMs (QCMs)

10.6.1 Incurred Analytes Perform Differently. Many trace level analytes perform differently in solvent than as incurred residues. For that reason, QCMs are often prepared using representative matrices. Materials containing naturally incurred analytes are preferred and can be used as QCMs directly, diluted with blank matrix or spiked with additional analytes of interest. If incurred residue material is unavailable, blank matrix can be spiked with analytes of interest. If prepared to a suitable homogeneity and stability, QCMs are very useful in providing on-going assessment of measurement precision, and when combined with CALs that are CRMs, they can be used to evaluate trueness.

10.6.2 Choosing a Representative Matrix. Residue chemists are asked to analyze for pesticides, veterinary drugs, and other contaminants in a wide variety of fresh and processed human and animal foods and food supplements. The analytical sample matrices and analytes to be analyzed should be identified and a material of similar matrix and analyte levels selected as a QCM. Multiple RMs may be needed to represent varied matrices in analytical samples, as thousands of analyte/matrix combinations are possible. For that reason, a representative matrix is often chosen that behaves in a manner similar to the analytical sample matrices.

10.6.3 AOAC Food Triangle. AOAC INTERNATIONAL developed a model for classification of foods into groups with similar composition.[5] The AOAC food triangle is based on the relative levels of fat, protein, and carbohydrate and is divided into nine sectors, where each corner of the triangle represents 100% of one component (FIGURE 5). The developers conceptualized that those foods within the same sector will offer similar analytical challenges. While developed for the analysis of nutrients, the food triangle can be used to choose appropriate matrices for use in preparing calibration standards, quality control spikes, and matrix blanks. AOAC recommends that methods validated for 2 matrices in any section of the pyramid can demonstrate method performance for other foods with similar characteristics. CRMs for each of the 10 sectors of the food triangle are available to use in conjunction with CALs and QCMs to verify method performance.[6]

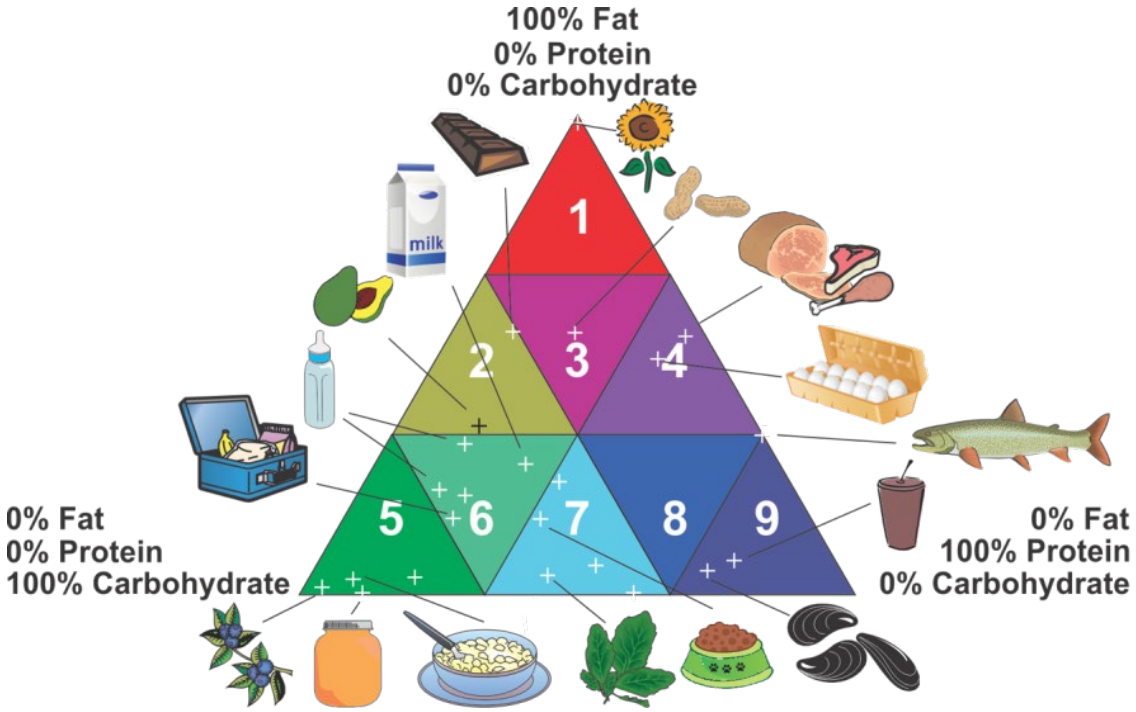

FIGURE 5. AOAC Food Composition Triangle

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10.6.4 OECD/SANTE Commodity Groups. Similarly, the European Commission's Directorate on Health and Food Safety (DG SANTE) developed a guidance on the validation of analytical procedures for pesticides, which is regularly updated.[7] On the basis of the OECD guidelines for pesticide residue analytical methods[8], the SANTE guidance document divides food and feed commodities into groups and provides typical commodity categories within each group, and typical representative commodities within each category. For vegetables and fruits, cereals, and food of animal origin, ten commodity groups are distinguished based on composition and/or origin:

- high water content
- high acid content and high water content
- high sugar and low water content
- high oil content and very low water content
- high starch and/or protein content with low water and fat content
- difficult and unique commodities
- meat (muscle) and seafood
- milk and milk products
- eggs
- fat from food of animal origin

10.6.5 Natural Material Variations. Natural materials (e.g., fruits, vegetables, herbs, spices, dietary supplements, C*annabis*, soil) can be especially challenging because they can vary widely in both composition and concentration of active ingredients. A given food commodity can vary greatly with variety, freshness, growing season, geographic growing location, ripening method, and multiple other factors that are not well known. An evaluation of these variables may be necessary to choose the most representative matrix.

The composition of a food commodity can vary greatly with variety, freshness, growing season, geographic growing location, ripening method, and multiple other factors that are not well known.

Natural-matrix RMs should behave in the same manner as test samples with the designated test method. Re-characterization may be necessary when adopting a new method. A given QCM can be suitable for one method and not for another. Some important food CRMs are available which have been extensively studied and may be used to confirm composition, residues, and contaminants. If available, these CRMs can provide verification that a method performs similarly to other methods but might not perfectly represent the response of all test sample matrices tested.

10.6.6 Authenticity. When obtaining a fresh or processed natural material to prepare as an in-house RM, the source and composition should be verified. It is important to obtain natural products from a reliable source that can verify authenticity. Natural materials might be obtained from multiple sources at different times of the year and compared to characterize variety and seasonal variability. Interlaboratory comparisons may be useful to verify that the RMs used are accurately characterized.

Some foods, such as orange juice and honey, have standards of identity which include specific tests to confirm composition. These standards of identity have been developed in recognition of the variability in food composition as well as the need to assure that products sold are accurately represented.

10.7 Preparation of QCMs

After selecting the material to be used as a RM, the following steps in preparation of the QCMs apply.

10.7.1 Bulk QCMs: For method development, validation, routine calibration, and quality control, large quantities of a matrix material can be prepared and characterized in-house. Usually, RMs are prepared as bulk homogenates and then divided into smaller portions that are suitable for single or multiple uses; the smaller portions are then characterized and stored for future use. For example, organic foods and baby foods might be used to prepare matrix-matched calibration standards, blanks, and quality control spikes. This may provide a pesticide-free matrix, but it does not account for possible variations in any natural product.

10.7.2 Batch Size: The batch size should be determined by considering the stability of the analytes, the frequency of use and how much material is needed for each analysis. This consideration should include RMs used for initial RM characterization work, in preparing spikes or blanks, and for development, validation and calibration standards. Determine the amount in each use portion and how many will be used. For a laboratory very dependent on matrix-matched RMs for on-going method performance QCMs, a good strategy might be to purchase a large quantity of a chosen matrix, comminute at low temperature and keep frozen at -20 °C or below until needed so that, at the very least, the matrix being used will be consistent from test to test.

10.7.3 Comminution: Natural materials should be comminuted (e.g., ground, blended, milled, sieved) to a fine particle size to produce sufficient homogeneity. If processed foods are used, multiple jars or cans should be mixed and comminuted into a uniform batch. The applicable particle size is often dependent on the test portion size. Test method precision is improved with smaller particle sizes and the largest test portion mass fit for the analytical method being used.[9]

10.7.4 Comminution for elemental analysis: In elemental analysis, contamination of the sample with metals from the grinding apparatus is a major concern. If comminuting with a metal grinding apparatus, any metals imparted into the ground material become part of any subsequent QCM (e.g., Cr, Fe, Ni, or possibly Zn). Therefore, when measuring for these elements, it is imperative to characterize the QCM for purity and uncertainty. If extraneous elemental impurities are undesired, then comminution using non-metal implements is necessary.

10.7.5 Representative portion sampling: Once comminuted, QCMs are usually aliquoted into multiple storage containers for future use. Containers for storage should be for single or short-term use, not bulk storage containers. Storage containers should be of a material known not to absorb analytes or leach elemental or organic contaminates.

Too often laboratories assume that simple mixing, blending, and sub-division will produce portions of a material that are sufficiently identical for their purposes. The order in which the QCM aliquots were prepared, packaged (fill order, box order) and analyzed should be logged. Before preparing RMs, laboratories should become familiar with the selection of representative portions as described in GOOD Samples[10] and GOOD Test Portions.[9]

10.8 Characterization of RMs

When an in-house RM has been prepared the next step is characterization of the material to demonstrate that the produced material is fit for its intended purpose. Characterization results should be summarized in the final documentation associated with the in-house RM.

10.8.1 Identity: While initial identity is established from the source material used in the preparation (e.g., natural material, neat chemical, or CRM), analyte identity should be verified. Due to the complexity of multi-analyte CALs and QCMs, incurred or spiked residues might degrade. In some cases, verification of hundreds of analytes in a single CAL or QCM can be challenging (e.g., pesticides) and techniques capable of multi-analyte analysis (e.g., gas or liquid chromatography with mass spectrometry) might be needed. Additional screening using full scan MS analysis can identify transformation products.

10.8.2 Accurate Concentration: For testing to demonstrate compliance with regulatory limits, accurate quantitation is necessary. The accuracy of concentration for each analyte may not be determined unless analyzed and verified in comparison to a CRM.[11] When preparing solvent mixes of hundreds of compounds, however, comparing all of them to CRMs can be difficult. If using CRMs to prepare in-house CALs, the concentration should be determined by gravimetry (i.e., the dilutions should have been done on balances, and the final concentration determined by weight). This preparation may then be verified by comparing with a duplicate preparation or previous in-house CALs that were prepared in the same way. An in-house mixture may also be compared by analysis of concentrated mixtures prepared by an accredited provider. If a CRM is unavailable, comparison to a second laboratory analysis using a different method and instrument provides additional certainty of accuracy.

10.8.3 Homogeneity: A newly prepared QCM is characterized by analyzing multiple replicate portions using a stratified random sampling scheme across the entire lot (at least 10) for the analytes of interest using a well-defined method. The mean and standard deviation of the characterization analyses provides the precision for each analyte. ISO Guides 35 and 80 as well as Pauwels provide detailed instructions for evaluation of homogeneity.[12] Examples of homogeneity evaluations may be found in the certification reports of the European Commission Joint Research Center CRMs such as ERM-BC403 Cucumber (pesticides).[13]

Brief recommendations are given below to assist in evaluating homogeneity of trace level analytes in complex matrices.

- Select at least 10 - 30 RM units from a stratified random sampling scheme over the whole batch.
- Measure each RM unit in duplicate.
- Measurements should be performed in random order with respect to the test portion's position in the filling sequence.
- For large, multi-analyte RMs, measure analytes representative of the different chemistries in the test method. Measurement of every analyte might not be possible but understanding the homogeneity of all analytes is important.

- Use the most precise test method and instrument available.
- Analytical measurements should not show significant trending.
- Where possible, evaluate with a second test method.
- Correct measurements for analytical drift, as needed.
- Evaluate the between-unit variation using one-way ANOVA. With a well-prepared material, heterogeneity is negligible (i.e., within test sample variability is no greater than between bottle variability).
- Between-unit variation should meet laboratory requirements (i.e., method performance criteria).
- If testing indicates unacceptable levels of heterogeneity, potential causes should be investigated (e.g., fill order, losses during handling, analysis order, etc.).
- It may be acceptable to characterize one or two analytes with a higher uncertainty.
- When beginning to use a new portion of an RM, compare it to the previous RM portion or a CRM.

10.8.4 Minimal test portion size: The within bottle homogeneity is very closely related to the minimal test portion size of the intended use. If the recommended minimal test portion size is equal or larger than the amount used in the certification process of the material, no further investigation is necessary. If this is not the case, additional test series should prove that the deviation of the measurement results of the certification cover the results with smaller test portion sizes. Normally, the variance increases with decreasing test portion amount.

10.8.5 Incurred Residue Extractability: Some procedures to determine incurred analyte extractability include:

- Compare to incurred residue CRMs with similar analytes and matrices.
- Compare to incurred residue proficiency samples with similar analytes and matrices.
- During method development, the same material should be extracted multiple times or with different solvents to determine if any analyte remains. Some testing methods employ repeat extractions to demonstrate complete extractability.
- Extract using a different, more exhaustive testing chemistry.
- Evaluate radiolabeled incurred residues. For example, when evaluating new agrichemicals for registration, radiolabeled pesticides are applied to growing food crops. Evaluations of residual radioactivity can be used to determine analyte extractability.

10.8.6 For RMs that contain various species of a given element, there are two aspects to consider:

- Analyze the total elemental concentration of the extract of an RM and compare that to the total elemental concentration of RM. This will evaluate the extraction efficiency for a given element.

- Compare the sum of the individual species of an element to the total elemental concentration of the RM to assess the mass balance (i.e., Does the speciation analysis account for all the elemental species in the RM?).

10.8.7 Stability: Storage stability is an essential part of RM characterization. ISO Guides 35 and 80 as well as Lamberty[14] provide detailed instructions for evaluation of stability. In order to assess the impact of storage and transportation to the overall uncertainty, stability studies should be performed. Often homogeneity and stability may be evaluated together from the same experimental data set.

Several factors can influence the mass fraction value or concentration of a product, such as choice of packaging material, light, oxygen, or humidity. On the basis of literature data, existing measurement results, and preliminary tests, several features of the product design may be assessed (e.g., use of an inert gas or brown glass bottles). However, the most critical factors are storage and shipping temperature.

10.8.9 Stability determinations over time: For the determination of the stability, ideally the same method may be applied as for the characterization provided the same quantity is measured. Values that are generated during the stability testing do need to have similar requirements as for the characterization, but in some cases may be determined against a stable relative reference point. The mass fraction or concentration will be measured over a previously defined interval and frequency and compared against the starting value at time (t) = 0. Isochronous stability testing[14], in which units exposed to different storage conditions and times are tested, offers the additional advantage of analyzing all time points at the same time. This means the analysis is performed under repeatability conditions thereby increasing the possibility of detecting potential trends originating from ever so slight degradation of the certified parameters.[5]

10.8.10 Long & short-term stability: ISO Guide 80:2014[3] describes two ways to address stability assessments, which differ in the thermal stress for the analytical sample. All experiments should be carried out according to the same guidance as the characterization and assessment of the heterogeneity.

- A ***long-term stability study*** that covers the shelf life of the CRM.
- A ***short-term stability study*** that simulates the temperature influence during the transport from the warehouse to the customer (transport stability) and may be used for extrapolation of the shelf life.

10.8.11 Stability contribution to uncertainty: The uncertainty contributions from the stability studies can be incorporated in two ways. If possible, an additional contribution can be added, which is estimated according to ISO Guide 35 after the data are assessed against a trend analysis and significance of a potential instability. Alternatively, if all measurement values of the stability studies lay within the measurement uncertainty of the used method (k = 2), the additional contribution can be omitted. If possible, a storage temperature of a CRM is assigned in a way that for a given shelf life no significant changes in content or concentration occur. The RMP may choose to implement post-certification

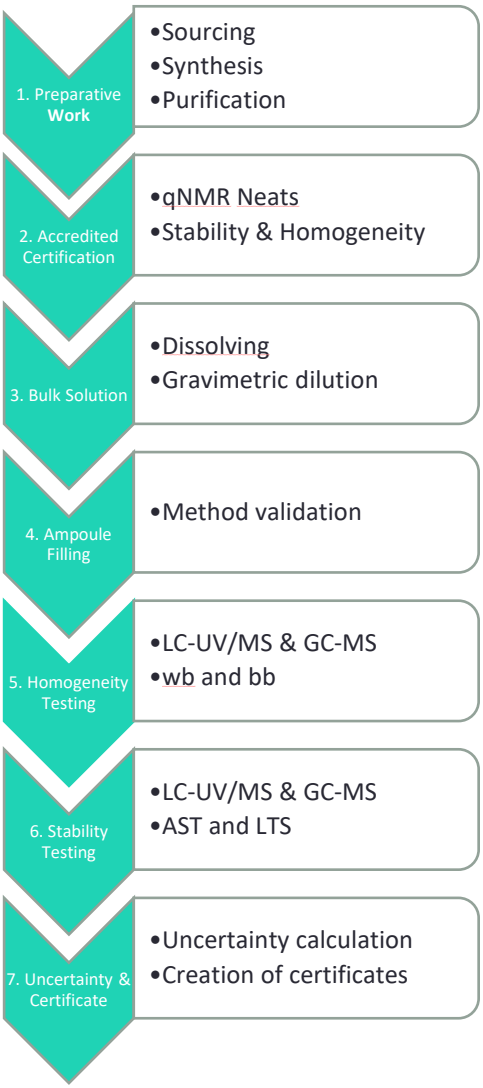
monitoring programs of its stock of CRMs. This is to make sure that the sold CRMs are still valid until its date of expiry.

10.8.12 Stability of purchased RMs and CRMs: Even when purchased from a CRM provider, concentrated pesticides and other organic analyte mixes are prone to analyte degradation. However, adhering to recommendations of proper storage conditions and handling should normally be sufficient. Once new RMs have demonstrated stability for several months, equivalent stability of a replacement material can be assumed as long as the new material was prepared in a similar way from similar materials.

Often, extra vials of new RMs are stored at low temperature for storage stability studies. Periodically, stored vials are analyzed and compared to working solutions stored at refrigerated or room temperature to verify on-going stability. Working solutions, often used for a month or more, are prone to evaporation, contamination, and other forms of degradation.

10.9 Uncertainties of Solutions, Mixtures, and Matrix Materials

10.9.1 CRM uncertainty: Establishing a certified value and an appropriate uncertainty becomes more complex for materials in solution, mixtures or matrices compared to neat or pure CRM. In order to realize a certified concentration without bias, the raw material for the preparation of these formats needs to be characterized the same way as the pure RMs, including homogeneity and stability during the time between characterization of the components and the preparation of the solution or mixture. Additionally, the overall process involves not only the steps mentioned before but the preparation of the bulk solution, mixture, or material; the filling into an appropriate packaging format (e.g., ampoules); followed by homogeneity and stability testing. The steps in such a process are illustrated in FIGURE 6 below.



10.9.2 Lot and mixture uncertainty: Each step results in individual uncertainty contributions for the combined standard uncertainty of each product. Depending on the components in the preparation of the solution, mixture, or matrix material, further dilution and filling should be validated and applied to similar product lines. The homogeneity and stability testing should be performed for every new product lot and mixture; to not only assess the stability of the component with the solvent or the matrix, but also potential interactions between the individual components.

FIGURE 6: Steps in determining a RM certified value and uncertainty.

10.9.3 Labelling: Many laboratories develop a code system for labeling each RM portion and a logbook system (manual or digital) for tracking complete chain of custody. Uniquely label each in-house CAL or QCM portion with:

- Name
- Unique identifier
- Preparation date
- Portion number
- Expiry date

Information might also include:

- Storage location
- Analyst name
- Laboratory
- Known hazards

The unique RM identification should be recorded at the time of preparation, use, or disposal. Where applicable, the unique identification should be traceable to information describing source material used in the preparation.[15]

10.9.4 Documentation: Records documenting the preparation and characterization of a RM should include the source of the material (e.g., natural material or CRM), preparation date, preparer's name, comminution procedure, portion selection procedure, packaging, storage conditions and estimated and/or assigned expiry date. Documentation should also include assigned values as well as the methods used, and results of analyses conducted to characterize the RM. For more information, review Chapter 4: RM Documentation.

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11 VERIFICATION USING RMs from a SECOND SOURCE

11.1 Is a Second Source Needed?

11.1.1 History of Second Source: Since the 1980's, environmental laboratories have been required to verify the identity and/or concentration of analytes in their calibration standards using an independently prepared RM from a second source.[1,2,3,4] This requirement is now part of many state regulations, accreditation guidelines and internal quality systems in order to prevent errors such as the misidentification of an analyte as reported in 1998.[5] A second source RM may be recommended when a new testing method is validated or for verification of the initial calibration.[3,6] Calibration standards should be prepared from one source and QCMs from a second source.[7] Without multiple sources of neat compounds from different origins, there may be a systematic error in results which is difficult to detect.

11.1.2 In today's multi-residue methods where 50 to 500 analytes can be present in a single calibration standard, verification of the individual analyte starting materials by the RMP for identity and purity is critical to demonstrate the accuracy of the calibration standard solution. Do these complex mixtures need to be verified with a second source? Use of a second source complex mixture may not be necessary for screening methods, as the presence and identity of the analytes in a complex mixture may be verified with a mass spectrometer or other specific detection technique. For multi-residue methods that detect significant numbers of actionable analytes, confirming the concentration of screening calibration standards using a second source is a worthwhile exercise to avoid unnecessary confirmatory quantitative testing. For regulatory work, non-compliant findings that may result in regulatory action require confirmatory testing and may also require verification with a second source RM.

11.2 Acceptable Second Source Quantitative Verification Criteria

11.2.1 Second source verification criteria vary depending on the purpose and type of testing, as well as the analyte and instrumentation. The establishment of quantitative acceptance criteria for a second source verification is often left up to the laboratories' QC procedures.

11.2.2 A second source RM may be used to:

- Confirm the identity of the analyte being measured.
- Verify the quantity of the analyte being measured Including potential dilution errors.
- Verify identity and retention time of isomers.
- Verify peak ratios and other spectral data.
- Check for degradation of primary source calibration standards.
- Validate the performance of a new testing methodology.
- Verify the identity and quantity of analytes in newly prepared calibration standards.
- Confirm non-compliant regulatory findings.
- Identify analyte interactions in a complex mixture.

- Verify storage stability in complex mixtures.

11.3 The Terminology "Second Source" is Not Always Clear

11.3.1 A second lot is sometimes used to refer to a second source RM or CRM. ISO Guide 30 defines lot as a definite amount of material produced during a single manufacturing cycle and intended to have uniform character and quality.[8] Other possible second source RM descriptions are listed in TABLE 5 in order of uniqueness.

TABLE 5. Second source RM descriptions

Class	A second source RM may be prepared from:
A	a neat chemical (or solution prepared from it) produced from a different lot of raw materials by a different chemical company.
B	a neat chemical (or solution prepared from it) produced from a different lot of raw materials by the same chemical company.
C	a neat chemical (or solution prepared from it) produced from the same lot of raw materials by the same chemical company at a different time.
D	a solution prepared from the same neat chemical by a different RMP or laboratory.
E	a solution prepared from the same neat chemical by the same RMP or laboratory, at a different time and/or analyst.
F	A solution prepared from a second lot according to the ISO Guide 30 definition of "lot"

11.3.2 Describing 2nd Source: ISO Guide 31 requires that second source RMs should be clearly identified by the RMP and/or the laboratory.[9] While some quality assurance manuals and programs have required the use of second source RMs prepared from different starting materials, ISO does not. One might prefer class A as described in TABLE 5 (*a neat chemical produced from a different lot of raw materials by a different chemical company*) but meeting this description can be challenging. For example, some starting materials are only available from a single chemical company, or third-party supplier, or are no longer being manufactured, so the only available second source starting material is a second portion of the same chemical lot or batch supplied by the same manufacturer. The manufacturer may or may not test the new portion of the same lot for purity and identity. Also, many chemical manufacturers do not produce chemicals for the specific use as starting materials for RMs, but instead for industrial applications and might not be highly purified. In many cases, starting material manufacturers do not possess ISO accreditations specific to RM manufacture, although they may have some accreditations for manufacturing, health and safety, or other unrelated credentials. Many laboratories resort to purchasing

the same chemical from a different RM provider or obtaining a second portion of the neat chemical and preparing working standards in-house. In every case, the RM documentation should identify the starting material source.

11.3.3 Quality of 2nd Source: Differences exist, as with all chemicals, in the quality of RMs. Raw materials vary by compound, purity, price, and availability. In procuring a second source raw material, the purity can be lower, the cost significantly higher, the quality questionable when only available from a non-accredited supplier, and availability within a reasonable timeframe might not be possible. If a laboratory purchases RMs from different providers, results may not agree within ±15% because the raw materials were of different purities and the preparations were not the same or because the RMP may not have adjusted for purity differences following starting material characterization. In some cases, following starting material characterization, purity of some materials may vary by more than 10-20% from what appears on the label of the material. Purchasing from a second source for a large multi-analyte calibration solution can also be difficult, however some RMPs offer them as custom products. Custom products might not fit the needs of all customers, but instead are prepared to meet specific needs of fewer users, or even a single user.

A second source material of the same purity & documented characterization may not be available.

11.3.4 The primary reference material source of a specific metabolite may be from the isolation of the metabolite (often using radiolabeled material and fractionation techniques) from plants, animal tissues, or soil. The material is isolated, purified, characterized, and assigned a purity. A second batch of the isolate should be prepared to demonstrate the ruggedness of the isolation technique as well as to act as a secondary source of the reference material.

11.3.5 General Observations and Recommendations:

- If CALs are prepared from CRMs, a second source material may not be necessary.
- In some cases, the only available second source material might not be of sufficient purity and/or identity to be used as a comparison to a CRM, or for the preparation of a CRM.
- RM documents should provide accurate information concerning the source, identity and purity of the neat and raw materials used, ensuring traceability.
- Neat and/or starting materials used to prepare RMs should be characterized for purity and identity.
- The concentrations of analytes in mixtures should be corrected for purity of the starting material.
-
- RM certificates should contain the information outlined in the Chapter 4: RM Documentation.

Chapter 11: References

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12 Measurement Uncertainty

12.1 What is Measurement Uncertainty?

Measurement Uncertainty is defined as "a parameter associated with the result of a measurement, that characterizes the dispersion of the values that could reasonably be attributed to the measurand".[1]

Uncertainty in a measurement quantity is a result of our incomplete knowledge of the "true" value of the measured quantity and of the factors influencing it. The sources of uncertainty are not necessarily independent and some or all can contribute to the variations in repeated observations.[2] It is important to realize that a method used in a laboratory has an uncertainty associated with the measurement result obtained which should be estimated during method validation. This method-specific uncertainty should not be confused with the uncertainty reported on the CRM certificate for a certified parameter. In section 12.9 both uncertainties are used and explained.

Uncertainty is not the same as bias and should not be used to correct analytical results in a systematic manner. Bias is the difference between a measured average value and the true value.

Sampling errors are frequently not evaluated, but should not be excluded, when estimating measurement uncertainty and may significantly contribute to the global uncertainty which includes both total sampling uncertainty and total analytical uncertainty. Refer to Chapter 5 for further discussion on sampling contributions to measurement uncertainty.

Any accredited ISO 17034 facility producing RMs should calculate and report uncertainty measurements for all certified parameters.

Human errors (blunders) are explicitly excluded from the calculation of measurement uncertainty and should be corrected when identified.

It is also important to note that the method-specific measurement uncertainty must be part of the decision rule for accepting or rejecting a value based on pre-set criteria as mandated in ISO/IEC 17025:2017 and ISO 10576.

In the context of CRMs, an estimate of the uncertainty is a required aspect of the certification of the assigned quantity value and must be included on the RMC. This chapter provides an overview of what reference material producers must do to assess the uncertainty of the certified values given on the certificate.

Estimations of measurement uncertainty can be obtained either by quantifying and combining all significant sources, commonly referred to as a bottom-up (predictive or modeling) approach, or by

statistical evaluation of relevant measurement results, commonly referred to as a top-down (retrospective or empirical) approach.

12.2 Bottom-Up Approach for Uncertainty Estimation

12.2.1 The bottom-up approach involves identifying, quantifying, and combining all significant sources contributing to the measurement uncertainty. A cause-and-effect (fishbone) diagram can be a helpful tool in identifying such influence factors. An example of a cause-and-effect diagram is presented in FIGURE 6. The construction of an uncertainty budget in spreadsheet form can help clarify the relative contribution of each influence factor.

12.2.2 Uncertainty component(s): Each identified uncertainty component is represented as a **standard uncertainty (u_j),** which is expressed as a standard deviation. Standard uncertainties may be derived from statistical data (preferentially, termed Type A uncertainties), or from assumed probability distributions (termed Type B uncertainties). Type A uncertainties are typically calculated according to Equation 12.1 and Type B uncertainties according to Equations 12.2 to 12.4.

Type A: $u_j = s / \sqrt{n}$ **[12.1]**

Type B (normal distribution): $u_j = a / \sqrt{9}$ **[12.2]**

Type B (triangular distribution): $u_j = a / \sqrt{6}$ **[12.3]**

Type B (rectangular distribution): $u_j = a / \sqrt{3}$ **[12.4]**

(where a is the ½ width of the tolerance interval)

12.2.3 Combined uncertainty: The **combined standard uncertainty (u_c)** is then obtained by combining all significant uncertainty components expressed as relative standard uncertainties according to the root-sum-square (RSS) equation shown in Equation 12.5.

$$u_c = \sqrt{\sum_{j=1}^{J} u_j^2} \quad \textbf{[12.5]}$$

where u_j is the standard uncertainty for the jth component. To estimate u_c, each source of uncertainty component should be identified and u_j quantified.

12.2.4 Expanded Uncertainty: The combined standard uncertainty (u_c) is multiplied by a **coverage factor (k)** to obtain the **expanded uncertainty (U)** *as shown in Equation 12.6*. The coverage factor (k) is chosen based on the desired coverage probability. For example, for a commonly used 95% coverage probability, k is set to a value of 2.

U = $u_c \bullet k$ **[12.6]**

Note that the selection of a k of 2 for 95% coverage probability relies on the principles of the Central Limit Theorem which suggest that when three or more randomly variable probability distributions are

Expanded uncertainty is the most common measurement uncertainty used in analytical chemistry.

propagated, the resultant probability distribution tends towards normality. Where very small data sets are used or less than three input variables are included, it is considered more appropriate to use the corresponding *Student's t*-value as an estimator of the expansion factor (*k*).

12.2.5 The combined standard uncertainty of a CRM is typically derived from contributions of characterization, heterogeneity, and stability (including storage and transportation) as described Equation 12.7.

$$u_{CRM} = \sqrt{u_{char}^2 + u_{het}^2 + u_{stab}^2} \qquad [12.7]$$

u_{CRM} Combined standard uncertainty for a certified value of a CRM.
u_{char} Uncertainty deriving from the characterization measurements.
u_{het} Uncertainty from heterogeneity of the parameter to be certified in the material.
u_{stab} Uncertainty deriving from instability of the parameter to be certified in the material.

In some cases (e.g., solutions, mixtures, or matrix materials) additional contributions to the uncertainty may be included.

12.2.6 The expanded uncertainty of a certified value in a CRM: The combined standard uncertainty is multiplied by a coverage factor *k* to obtain the expanded uncertainty, U_{CRM} (Equation 12.8).

$$U_{CRM} = u_{CRM} \times k \qquad [12.8]$$

12.3 Top-Down Approach for Uncertainty Estimation

While the bottom-up approach for uncertainty estimation is considered universally applicable, its determination is often difficult in a practical setting including evaluation of sample preparation steps (e.g., extraction, clean-up, evaporation, reconstitution), analyte interactions in mixtures, and matrix effects.

> Note: NordTest Report TR 537e[3] describes a top-down approach which can be used to estimate the measurement uncertainty from existing performance data. The process described in the NordTest TR 537e is consistent with approaches described in ISO 1132:2012 and 21748:2017.

12.3.1 Important Considerations:

- The measuring system (method) must be well-developed, fit-for-purpose, and under statistical (quality) control.
- Uncertainty associated with sampling (and any other processing not included in the analytical method) must be estimated separately and combined with the analytical measurement uncertainty prior to calculating the Expanded Uncertainty (U).

12.3.2 Estimation based on within laboratory reproducibility data considers all (known or unknown) influence factors to be included in two terms and is calculated using the Equation 12.9.

$$u_c = \sqrt{R_W{}^2 + u_{bias}{}^2} \qquad [12.9]$$

Within Laboratory Reproducibility (R_W), usually referred to as intermediate precision, is generally taken from a statistically significant number of repeated measurements of a representative QCM, pooled replicate sample analysis, or pooled replicate fortified sample analysis over a period of time.

Laboratory (and method) Bias (u_{bias}) is generally derived from a statistically significant number of repeated measurements of CRMs, interlaboratory comparisons, or recovery studies. Note that even when bias can be quantified and corrected, the uncertainty associated with bias must be included in the combined uncertainty estimation. The Laboratory Bias (u_{bias}) is calculated from two influence factors using Equation 12.10. Uncertainty for trueness is another term that is sometimes used for the description given above.

$$u_{bias} = \sqrt{RMS_{bias}{}^2 + u_{Cref}{}^2} \qquad [12.10]$$

The standard uncertainty of the bias (RMS_{bias}) is the root mean square sum of the individual bias estimates. The standard uncertainty of the Reference (u_{Cref}) is generally taken directly from the relevant RMC(s), interlaboratory comparison reports, or recovery study report. Where multiple CRMs (or consensus values) were evaluated to compute the bias, the standard uncertainties associated with each are combined for inclusion in the u_{cref} term.

12.3.3 Estimation based on Between Laboratory Reproducibility Data considers all (known or unknown) influence factors to be included in a single term, namely the precision under reproducibility conditions. The combined standard uncertainty (u_c) is equivalent to the mean reproducibility standard deviation (s_R) from (preferably six or more) interlaboratory collaborations (*e.g.*, proficiency tests or multi-laboratory validation studies).

12.4 Assessment of Uncertainty associated with Certified Values

12.4.1 RMP requirements: Recognizing that there is uncertainty associated with the assigned quantity values for all CRMs, ISO 17034 places particular requirements on RMPs for the establishment, documentation, and communication of metrological traceability (including measurement uncertainty) of certified quantity values. ISO guide 35 outlines detailed examples and principles for the evaluation of uncertainty with particular focus on the characterization, homogeneity, and stability of CRMs as shown in Equation 12.7.

12.4.2 Contributing factors: An example of a cause-and-effect diagram for the preparation of a multi-unit CRM is illustrated in FIGURE 6. The certified concentration is the quantity value, x, for which *U* should be determined. Some individual components (u_j) that may contribute to the combined standard uncertainty (u_c) of the measurand have been identified and grouped based on which parts of the measurement process they influence. For example, balance uncertainty is an influence factor which contributes to u_c

associated with the mass of the starting material, within unit heterogeneity, and between unit heterogeneity.

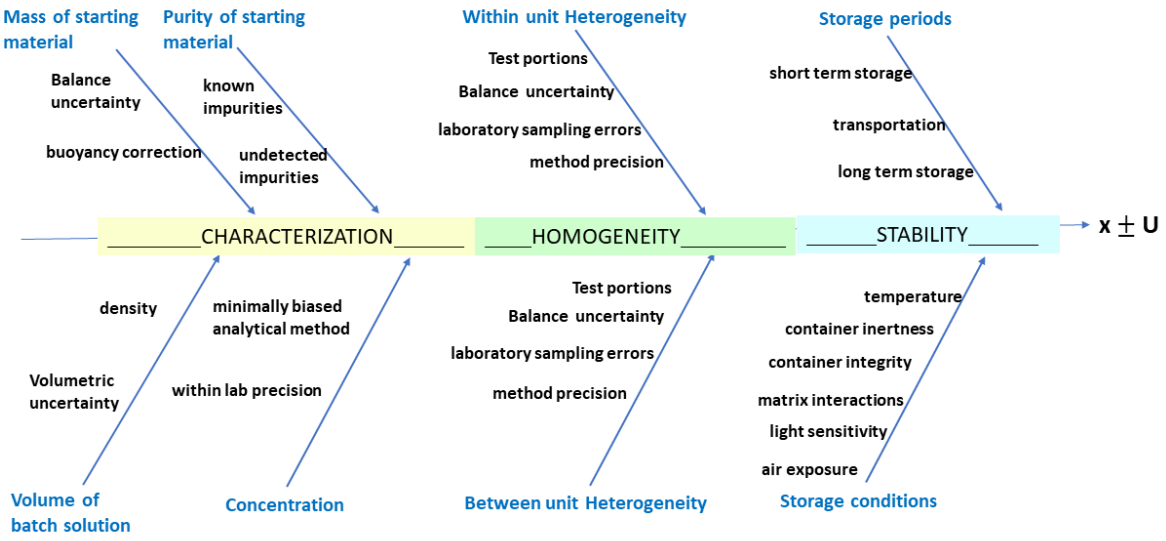

FIGURE 6. Cause and effect (fish bone) diagram representing examples of the influence factors contributing to the uncertainty (U) of each analyte concentration (x) in the manufacture and qualification of multiple units of a Certified Reference Material.

12.5 Uncertainty of the Characterization

12.5.1 Contributions to Variability in Characterization: Because the characterization (assignment of quantity value) can be accomplished in a wide variety of ways, the corresponding estimation of uncertainty must be chosen to appropriately reflect the pertinent significant sources of variability. Frequently the sources of variability will include the measurement procedure(s), CRM(s) concentrations, bias estimates, within laboratory precision, component concentrations and/or the proportioning process. ISO guide 35 describes a number of common approaches, while recognizing that others may be applicable. If the assigned property value comes from an inter-laboratory comparison based on a sufficient number of normally distributed mean values, an RMP can, for example, use the standard error of the mean of all data sets to estimate the uncertainty contribution from the characterization.

When formulation principles are applied (i.e., calculation from the known concentrations/purity of the components), establishing a certified value and an appropriate uncertainty can become quite complex for multi-analytes in solution, material mixtures or materials in matrix compared to neat or pure RMs. In order to produce a certified concentration without bias, the raw material for the preparation of these formats needs to be characterized the same way as the pure RMs, including homogeneity and stability during the

time between characterization of the components and the preparation of the solution or mixture. Additionally, the overall process involves not only the steps mentioned before, but also the preparation of the bulk solution, mixture, or material, consideration of potential interactions, and the filling into an appropriate packaging format (e.g., ampoules).

12.6 Uncertainty of Homogeneity

12.6.1 Assessing homogeneity: Definitionally speaking, a substance (i.e., an RM) is either homogenous (all the same) or heterogenous (not the same) on the scale at which it is sampled for a specific parameter.

> **Homogeneity** is the degree to which a property or a constituent is uniformly distributed throughout a quantity of material. A material may be homogeneous with respect to one analyte or property but heterogeneous with respect to another for the same test portion.
>
> **Heterogeneity** (the opposite of homogeneity) is the determining factor of sampling error.

As a practical matter, chemists characterize a substance's heterogeneity (less than perfect homogeneity) as a function of the residual variance of repeated measurements of a statistically significant number of replicate sample portions discounting the variance associated with the measurement process. Homogeneity studies are typically carried out using a defined minimum test portion; basic, randomized block, or balanced nested designs; and, where available, high-precision analytical methods since the method precision defines the lowest limit to which the homogeneity can be evaluated.[6,7] Very small heterogeneities can only be determined with high precision measurement techniques such as coulometry, isotope dilution MS, titrimetric approaches, or quantitative NMR. Ideally, a measurement technique with a low repeatability precision is preferable such that $s_r/\sqrt{n} \leq u_{target}/3$.

ISO Guide 35 defines the uncertainty associated with RM homogeneity (u_{hom}) as the combination of two components: uncertainty arising from within unit/bottle heterogeneity (u_{wb}) and uncertainty arising from between unit/bottle heterogeneity (u_{bb}), combined using equation 12.11:

$$u_{hom} = \sqrt{u_{wb}^2 + u_{bb}^2} \qquad [12.11]$$

Note: In general, the components of u_{hom} (u_{wb} and u_{bb}) are expected to be less than a third of u_{char} if the material is to be considered sufficiently homogenous to serve as a reference material.

12.6.2 Within bottle homogeneity (u_{wb}): u_{wb} may be based on the within bottle standard deviation of a homogeneity study (s_{wb}) or on the standard deviation of the characterization results (s_{char}) (if the specified minimum analytical portion is greater than or equal to the portion used for characterization) but must not be reported as less than u_{bb}. Note that u_{wb} may be omitted when the RM minimum portion has been specified under certain conditions specified in ISO Guide 35.

12.6.3 Between bottle homogeneity (u_{bb}): u_{bb} is based on the between bottle standard deviation of a homogeneity study (s_{bb}). Note that u_{bb} may be omitted when s_{bb} is much smaller than u_{char} or u_{stab}.

12.6.4 Estimates of s_{bb} and s_{wb}: The determination of the standard deviations of between bottle homogeneity ($M_{between}$) and within bottle homogeneity (M_{within}) is based on a factorial analysis of variances (ANOVA) as described in the equations below. ANOVA can be readily performed using commercially available statistical software or spreadsheet programs (e.g., Microsoft Excel with Data Analysis add in module).

When the homogeneity study follows a basic design (ISO Guide 35 section 7.6.2), one-way (single factor) ANOVA is applied:

$$s_{wb} = \sqrt{M_{within}} \quad [12.12]$$

$$s_{bb} = \sqrt{(M_{between} - M_{within})/n_0} \quad [12.13]$$

$$n_0 = \frac{1}{a-1}\left[\sum_{i=1}^{a} n_i - \frac{\sum_{i=1}^{a} n_i^2}{\sum_{i=1}^{a} n_i}\right] \quad [12.14]$$

Note: if the study design is balanced and no data points are excluded as outliers, n_0 simplifies to n (i.e., the number of test portions tested per unit of RM) in Equation 12.14.

When the study follows a randomized block design (ISO Guide 35 section 7.6.3) two-way (two factor) ANOVA without replication is applied. When the study follows a balanced nested design (ISO Guide 35 section 7.6.4) two-way (two factor) ANOVA with replication is applied. In both cases:

$$s_{wb} = \sqrt{M_{within}} \quad [12.15]$$

$$s_{bb} = \sqrt{(M_{between} - M_{within})/n_0} \quad [12.16]$$

$$n_0 = \frac{\sum_{i=1}^{p}\sum_{j=1}^{bj} n_{ij} - \sum_{i=1}^{p}\left[\frac{\sum_{j=1}^{bj} n_{ij}^2}{\sum_{j=1}^{bj} n_{ij}}\right]}{\sum_{i=1}^{p} b_i - p} \quad [12.17]$$

Note: if the study design is balanced and no data points are excluded as outliers, n_0 simplifies to n (i.e., the number of test portions tested per unit of RM) in Equation 12.16.

Several other homogeneity study designs are possible for which a multi-variate analysis of variance (MANOVA), restricted maximum likelihood estimate (REML) or Bayesian estimate method should be applied (detailed explanations of such estimates are beyond the scope of this guide).

Note: Outliers should be identified using statistical tests (i.e., Grubb's test), however, discarded outliers may result in the study becoming unbalanced and consideration should be given to replacing the discarded data points if it can be done reasonably. Preferably, exclusion of outliers should be based on technical grounds.

Note: Care should be taken to ensure that trends in measurement data are not confused for variance. If trends are observed in the measurement data, steps should be taken to eliminate the affected data or to correct for the effect (especially from the analytical sequence) before the ANOVA is processed.

12.7 Uncertainty of the Stability

12.7.1 Uncertainty associated with stability (u_{stab}) addresses the potential for changes in the certified quantity value over the post-production shelf life of the RM.

12.7.2 Evaluation of the need to include uncertainty associated with stability (u_{stab}): When the results of a stability study indicate a non-negligible change in the certified quantity value, within the expiration period to be published on the certificate, the uncertainty in the prediction of the change must be included in the combined uncertainty reported on the RMC. As a general rule, the change in the certified quantity may be considered negligible if it is significantly smaller than the combined uncertainty calculated from the characterization and homogeneity.

$$|x_{CRM} - x_{ti}| < u_{CRM}/3 \qquad [12.18]$$

$$|x_{CRM} - x_{ti}| \leq k \times \sqrt{u_{CRM}^2 + u(x)_{ti}^2} \qquad [12.19]$$

Note: The $u(x)_{ti}$ term represents the uncertainty of the measured quantity at the decision point in the stability study and should include components corresponding to repeatability precision, between-unit precision and/or reproducibility precision were applicable.

Note: The coverage factor (k) is set to correspond to the desired coverage probability (i.e., for 95%, K=2).

When the change at the final state is concluded to be insignificant, the uncertainty corresponding to that component may also be considered insignificant and not included in the estimation of uncertainty associated with stability.

Note: It is expected that rigorous estimates of the stability contribution to the uncertainty of a certified quantity will be relatively uncommon as RMPs often set the period of validity so as to preclude the need for a correction.

12.7.3 Components of uncertainty associated with stability: The stability under storage conditions at the RMPs facility, often termed "long term stability" **(u_{lts})** and the stability under transport conditions to the end user, often termed "short term stability" **(u_{tran})** each contribute uncertainty to the certified quantity by the time it is obtained for use by the end user. The contributions of these two sets of conditions to the uncertainty associated with stability can be represented by equation 12.21:

$$u_{stab} = \sqrt{u_{lts}^2 + u_{tran}^2} \qquad [12.20]$$

12.7.4 Estimation of uncertainty associated with storage conditions (u_{lts}) and transportation (u_{tran}): While u_{lts} and u_{tran} represent distinct influences on the certified quantity, the uncertainty associated with each can be estimated in similar ways.

When a stability study indicates that the rate of change can be modeled using a linear equation (i.e., $y_i = b_i x_i + b_0 + \varepsilon_i$), the associated uncertainty can be estimated directly from the standard error of the slope of the linear equation.

$$u_{lts} \text{ or } u_{tran} = s_{bi} \qquad [12.21]$$

In such cases, the significance of the change can be evaluated by comparing the t-statistic against the critical value for the two-tailed student's t statistic at the desired confidence (typically 95%) with the appropriate degrees of freedom (generally n-2 for linear equations).

$$t_{bi} = |b_i|/s_{bi} \qquad [12.22]$$

Note: If $t_{bi} \leq t_{\alpha,v}$, then Δ_x can be considered statistically negligible.

When a stability study indicates that the rate of change can be modeled using a polynomial equation (i.e., $y_i = {}_k x_n^k + b_0 + \varepsilon_i$), the associated uncertainty can be estimated from the combination of the standard errors of the coefficients (including covariance) of the polynomial equation.

$$u_{lts} \text{ or } u_{tran} = \sqrt{b_k} \qquad [12.23]$$

Other more complex models may be required to adequately describe the observed change in the quantity of interest during a stability study and the uncertainty in each term of such equations would be combined in similar fashion. When a stability study indicates that the rate of change is subject to one or more inflection points, the equation designed to model the behavior may require that the uncertainty be individually estimated and communicated for each of the time intervals indicated by the inflection points.

12.8 Reporting Uncertainty for a certified value of a CRM

ISO 17034 and Guide 35 provide guidance on the required contents of RMCs. Uncertainty estimates are most frequently given in terms of Expanded Uncertainty and should be stated in the same units and with the same significance as the certified property value. The uncertainty estimation accompanying an RMC should be complete enough for the end user to understand how it was derived and to calculate back to the combined standard uncertainty with coverage probability (which usually means both U and k should be clearly stated). For additional information, consult Chapter 4.

12.9 Practical Use of Uncertainty Values

12.9.1 Use of CRM to evaluate method performance: CRMs are frequently used in assessments or evaluations of the accuracy (precision and bias) of analytical methodology (in both method development and quality control context).

For assessment of method precision, the measured intermediate precision (the within-laboratory standard deviation under intermediate precision conditions, s_w) is compared against a required or reference precision (σ_{wo}) by computing the chi-squared ratio (χ_m^2) of the method and comparing to the appropriate critical value (χ_c^2).

$$\chi_m^2 = s_w^2 / \sigma_{wo}^2 \quad [12.24]$$

$$\chi_c^2 = \chi^2_{(n-1);\alpha} / (n-1) \quad [12.25]$$

Note: values for $\chi^2_{(n-1);\alpha}$ are readily available from reference tables or may be computed.

Note: α is most frequently set to 0.05 for 95% coverage probability.

Acceptable method precision is indicated when $\chi_m^2 \leq \chi_c^2$.

12.9.2 Assessment of method bias: For the assessment of method bias, the measured value (preferably a mean value of numerous measurements) is compared to the certified value incorporating an allowance for the measurement uncertainty. When the property of a CRM is measured and this value is compared to the certified value, the uncertainties in both values should be taken into account when deciding if the measured value is acceptable. The sequence below is summarized from an application note issued by the European Union's Joint Research Centre and is consistent with ISO Guide 33.[13]

1. Calculate the absolute difference (Δ_m) between the average measured value (C_m) and certified value (C_{CRM}) reported on the RMC.

$$\Delta_m = |C_m - C_{CRM}| \quad [12.26]$$

2. Estimate the uncertainty of the bias estimate (u_Δ) from the combined uncertainties of the measured result (u_m) and the CRM's certified value (u_{CRM}).

$$u_\Delta = \sqrt{u_m^2 + u_{CRM}^2} \quad [12.27]$$

3. Estimate the expanded uncertainty (U_Δ) corresponding to a coverage probability of about 95% by using k = 2.

$$U_\Delta = 2 \times u_\Delta \quad [12.28]$$

4. Compare Δ_m to U_Δ.

5. If $\Delta_m < U_\Delta$, the difference between the measured and certified values is insignificant.

12.9.3 Use of CRMs in Calibration: CRMs (or solutions prepared from CRMs) are frequently used as calibrators for measurement systems.

When the uncertainty of the certified property value provided on the RMC is presented as an expanded uncertainty (U) or as coverage probability, it must be converted into a combined standard uncertainty (u_c).

$$u_{CRM} = U_{CRM} / k \quad [12.29]$$

$$u_{CRM} = a / k; \quad \text{where } a \text{ is the half-width of the coverage probability interval} \quad [12.30]$$

$$u(CRM)_{rel} = (u_{CRM} / C_{CRM}) \bullet 100\% \quad [12.31]$$

This value can then be used along with other standard uncertainties to estimate u_c for the measurement made using the analytical method.

When the working calibration solutions are prepared from CRMs (e.g., by dilution), the potential for variability in the preparation process must be accounted for.

$$u_{cal} = \mu_{bias} = \sqrt{u_{CRM}^2 + u_{prep}^2} \quad [12.32]$$

Note: In the common case, when least-squares linear regression provides a suitable calibration model, the standard uncertainty associated with the calibration model can usually be estimated by the combination of the standard error of the slope and intercept:

$$u_{cal} = \sqrt{se_{slope}{}^2 + se_{intercept}{}^2} \quad [12.33]$$

It is important to understand that while the use of Equation 12.33 does not directly use the certified values of the CRMs or calibrators, it is important to use CRMs in order to maintain traceability.

12.9.3 Use of CRMs to assign values to other RMs: Sometimes referred to as value transfer, the assignment of values to newly prepared or previously uncharacterized RMs should also include an estimate of the associated uncertainty. The uncertainty should be evaluated using the principles described for the characterization (12.5), homogeneity (12.6), and stability (12.7) of CRMs.

Note: It is common practice, when RMs are prepared by dilution of CRM(s) in liquid diluents to rely on the principle of mass dispersion to avoid the need for homogeneity evaluations.

Note: It is common practice for working RMs to be assigned short periods of validity (based on previous experience) in order avoid the need for routine stability testing.

When the value assignment is based on formulation principles (often by gravimetry or volumetry), it is best practice to verify the acceptability by measuring the new RM solution in comparison to a previous valid RM solution. The new RM preparation can be considered verified if the observed difference is within the expected expanded uncertainty:

$$|x_{RM2} - x_{RM1}| \leq k \times \sqrt{u_{RM2}{}^2 + u_{RM1}{}^2} \qquad [12.34]$$

Verification of a newly prepared RM calibrant solution may also be accomplished by the acceptable recovery of an independent CRM analyzed by equipment calibrated using the new RM calibrant solution.

12.9.4 Use of CRMs to establish metrological traceability: CRMs are also useful to establish Metrological Traceability of measurement results to the S.I. system. This is often accomplished by using CRMs (or calibrators prepared from CRMs) in the calibration of the measurement device or measurement system and in the best case, the availability of a certified matrix reference material that can be subjected to the whole sample preparation process.

12.9.5 Estimation of measurement uncertainty for a self-developed method or during method verification of an existing standard method: The method-specific measurement uncertainty is the parameter of main focus for any analytical laboratory as the uncertainties of certified values are simply taken form the RMCs. The method-specific measurement uncertainty should be estimated for the measurement results either using bottom-up or top-down approaches as described in sections 12.2 and 12.3. Once the measurement uncertainty is estimated, it should be stated on all analytical reports for the validated matrix/matrices to provide information to the end user about the reliability of the measurement results.

12.9.6 Free On-Line Course: Recently, the Joint Research Centre of the European Commission launched a free online course[8] that consists of eight modules whereof the most important modules are listed here. Reference 8 provides a link to this free on-line resource which is hosted by EU-learn which is the European Commission's platform for online training courses.

Module 1. Estimation of Measurement Uncertainty

Module 2 Use of CRMs to prove laboratory and method performance

Module 4 Use of CRMs in method validation

Module 7 Traceability of measurement results

Chapter 12: References

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13 Glossary

13.1 Glossary Sources

The glossary contained in the following pages is a collection of terms deemed relevant to this document and its user group and has been assembled from various resources, including the *RM Guidelines* published by the AOAC Technical Division on Reference Materials[1], the Eurachem Guides on *The Selection and Use of Reference Materials*[2] and *Terminology in Analytical Measurement*[3], Guidelines for the Validation of Chemical Methods for the FDA Foods Program[4], International Vocabulary of Metrology (VIM)[5], ISO 17034:2016[6], and ISO Guide 30[7]. No specific references to ISO Guide 31 are included because the terms included in that Guide are referenced to other sources that have already been included. While sources for definitions are given where applicable, complementary and appropriate definitions from other sources can be available. Other regulatory agencies have their own glossary definitions such as the USEPA QA Glossary for the Environmental Monitoring and Assessment Program. Specific terminology should be referenced when used in association with specific analytical methods as relevant to the intended audience.

13.2 DEFINITIONS

Term	Definition	Source
Accuracy	• Eurachem VIM and FDA: Closeness of agreement between a measured quantity value (test result) and a true quantity value (accepted reference value) of a measurand. When applied to test results, accuracy includes a combination of random and systematic error. When applied to a test method, accuracy refers to a combination of trueness and precision. • Note that it is common practice to refer to both "*accuracy and precision*" when describing the performance of a method to emphasize that two parameters (i.e., mean and standard deviation) are necessary to report accuracy. • In AOAC, accuracy is a synonym of bias and precision is reported as a separate parameter. AOAC states that "methods may be precise without being accurate or accurate without being precise." • **In this document, accuracy = trueness & precision**	FDA[4] VIM[5] AOAC[1]
Action Level	Level of concern or target level for an analyte that must be reliably identified or quantified in a test sample.	FDA[4]
Aliquot	A portion taken from a larger whole, especially a test portion taken for chemical analysis	Oxford dictionary[20]

Analyte	The chemical substance measured and/or identified in a test sample by the method of analysis.	FDA[4]
Analytical Batch	An analytical batch consists of samples, standards, quality controls, and blanks which are analyzed together with the same method sequence and same lots of reagents and with the manipulations common to each sample within the same period (usually within one day) or in continuous sequential periods.	FDA[4]
Analytical Sample	The material from which the test portion is selected. Also called the test sample.	Thiex[13]
Bias	The difference between the expectation of the test result and the true value or accepted reference value. Bias is the total systematic error for a measurement for a laboratory or for an analytical method, and there can be one or more systematic error components contributing to the bias.	FDA[4]
Blank	A substance that is intended to not contain the analytes of interest and is subjected to the usual measurement process.	FDA[4]
Calibration	Determination of the relationship between the observed analyte signal generated by the measuring/detection system and the quantity of analyte present in the sample measured. Typically, this is accomplished with calibration standards containing known amounts of analyte.	FDA[4]
Calibration Blank	A calibration blank is a calibration standard that does not contain the analyte(s) of interest at a detectable level. It may be a solvent or matrix blank.	Eurachem [24]
Calibration Standard (Calibrant, CAL)	A known amount or concentration of analyte used to calibrate the measuring/detection system. May be matrix matched for specific sample matrices. Amount or concentration is known through purity evaluation of the pure substance or neat material.	FDA[4] Emons[8]
Can	Word that indicates a possibility or capability.	ISO 17025[15] ISO 17034[6]
Carryover (Memory)	Residual analyte from a previous sample or standard which is retained in the analytical system and measured in subsequent samples. Also called Memory.	FDA[4]
Certificate of Analysis (CoA)	An official document that shows the results of scientific tests on a product. Commonly issued as part of quality control of an individual batch of a product and may be used to confirm that a regulated product meets its product specification.	This document
Certified Reference Material (CRM)	RM characterized by a metrologically valid procedure for one or more specified properties, accompanied by a RM certificate issued by an authoritative body that provides the value of the specified property, its associated uncertainty, and a statement of metrological traceability. Note: Standard Reference Material (SRM) is the trademark name of CRMs	ISO 17034[6] ISO GUIDE 30[7]

	produced and distributed by the National Institute of Standards and Technology (NIST). A certified reference material is a reference material, accompanied by documentation issued by an authoritative body and providing one or more specified property values with associated uncertainties and traceabilities, using valid procedures.	VIM[5]
Certified Value	Value, assigned to a property of a RM that is accompanied by an uncertainty statement and a statement of metrological traceability, identified as such in the RM certificate.	ISO 17034[6] ISO GUIDE 30[7]
Characterization	Determination of the property values or attributes of a reference material, as part of the production process.	ISO GUIDE 30[7]
Check Analysis	Result from a second independent analysis which is compared with the result from the initial analysis. Typically, check analyses are performed by a different analyst using the same method.	FDA[4]
CIPM	Bureau International des Poids et Mesures" (in French), International Committee for Weights and Measures (in English), https://www.bipm.org/en/committees/cipm/ (accessed 10-2-2020)	
Clean Sample	A sample of a natural or synthetic matrix containing no detectable amount of the analyte of interest and no interfering material.	EPA [22]
Combined Standard Measurement Uncertainty	Mathematical combination of several standard measurement uncertainties. The procedure for combining standard uncertainties is often called the "law of propagation of uncertainties" and in common parlance the "root-sum-of-squares" (RSS) method.	VIM[5] GUM[25]
Commutability	Property of a RM, demonstrated by the equivalence of the mathematical relationships among the results of different measurement procedures for a RM and for representative samples of the type intended to be measured.	ISO Guide 30[7]
Confirmatory Analysis/Method	Independent analysis/method used to confirm the result from an initial or screening analysis. A different method is often used in confirmation of screening results.	FDA[4]
Coverage Factor, (k)	Number larger than one by which a combined standard measurement uncertainty is multiplied to obtain an expanded measurement uncertainty at a specified coverage probability.	VIM[5]
Coverage Probability	Probability that the set of true quantity values of a measurand is contained within a specified coverage interval.	VIM[5]
Dynamic Blank	A sample-collection material or device (e.g., filter or reagent solution) that is not exposed to the material to be selectively captured but is transported and processed in the same manner as the sample.	EPA [22]

Equipment Blank	A clean matrix or solvent processed through the equipment steps of the analytical preparation process; used to determine instrument contamination.	
Error	Measured quantity value minus a reference quantity value.	VIM[5]
Expanded Measurement Uncertainty	Product of a combined standard measurement uncertainty and a coverage factor larger than the number one. The coverage factor depends upon the type of probability distribution of the output quantity in a measurement model and on the selected coverage probability. Provides an interval within which the value of the measurand is believed to lie with a higher level of confidence and is obtained by multiplying the combined standard measurement uncertainty by a coverage factor. The choice of the coverage factor is based on the level of coverage probability desired.	GUM[25]
Expiry Date (Expiration Date)	The designated time during which a test item is expected to remain within established shelf life specifications if stored under defined conditions, and after which it should not be used.	OECD GLP #19[17]
False Negative Rate	In qualitative analysis, a measure of how often a test result indicates that an analyte is not present, when in fact it is present or is present in an amount greater than a threshold or designated cut-off concentration.	FDA[4]
False Positive Rate	In qualitative analysis, a measure of how often a test result indicates that an analyte is present when in fact it is not present or is present in an amount less than a threshold or designated cut-off concentration.	FDA[4]
Field Blank	A clean sample (e.g., distilled water), carried to the sampling site, exposed to sampling conditions (e.g., bottle caps removed, preservatives added) and returned to the laboratory and treated as an environmental sample. Field blanks are used to check for analytical artifacts and/or background introduces by sampling and analytical procedures.	EPA [22 &23]
Fitness for Purpose	Degree to which data produced by a measurement process enables a user to make technically and administratively correct decisions for a stated purpose.	FDA[4]
Guidance Level	Level of concern or action level issued under good guidance practices that must be reliably identified or quantified in a sample.	FDA[4]
Homogeneity and **Heterogeneity**	Uniformity of a specified property value throughout a defined portion of a RM. The degree to which a property or a constituent is uniformly distributed throughout a quantity of material. A material may be homogeneous with respect to one analyte or property but heterogeneous with respect to another. The degree of **heterogeneity** (the opposite of homogeneity or inhomogeneity) is the determining factor of sampling error.	ISO GUIDE 30[7] The Gold Book[21]

Identity (Chemical)	Unambiguous structure attributed to a measured analytical feature, supported by evidence, within a defined scope (e.g., isomers). Best determined by qNMR for a pure material and required for traceability to SI. For mixtures or in matrix, often confirmed by a highly specific technique such as mass spectrometry or by demonstration of results from two or more independent analyses in agreement. Used to determine selectivity and sensitivity of a method for the measurand.	FDA[4]
Incurred Samples	Samples that contain the analyte(s) of interest, which were not derived from laboratory fortification but from sources such as exogenous exposure (e.g., pesticide use, consumption by an animal, environmental exposure) or endogenous origin.	FDA[4]
Indicative Value	Value of a quantity or property of a RM which is provided for information only. An indicative value cannot be used as a reference in a metrological traceability chain.	ISO GUIDE 30[7]
Instrument Blank (see Dynamic Blank)	A clean sample processed through the instrumental steps of the measurement process; used to determine instrument contamination.	EPA [22]
Instrument Matrix Blank	A substance that closely matches the samples being analyzed with regard to matrix components. Ideally, the matrix blank does not contain the analyte(s) of interest but is subjected to all instrumental testing steps used to analyze the test samples. It is often used to determine instrumental matrix effects. (See *Matrix Blank*)	
Interference	A positive or negative response or effect on response produced by a substance other than the analyte. Includes spectral, physical, and chemical interferences which result in a less certain or accurate measurement of the analyte.	FDA[4]
Interlaboratory Comparison	General term for a collaborative study for either method performance, laboratory performance (proficiency testing), or material certification. A common tool for evaluation of reproducibility and/or ruggedness testing for a laboratory or method. Samples used in an interlaboratory comparison are RMs for the duration of the study and excess materials may be qualified for use beyond the study if extended stability is confirmed.	NORDTEST[9]
Intermediate Precision	Measurement precision under a set of conditions that includes the same measurement procedure, same location, and replicate measurements on the same or similar objects over an extended period of time but may include other conditions involving changes. Part of repeatability testing for a laboratory or method.	VIM[5]
Internal Standard (ISTD)	A chemical added to the sample, in known quantity, at a specified stage in the analysis to facilitate quantitation of the analyte. Internal standards are used as procedure or injection ISTD; to correct for matrix effects or incomplete spike recoveries and as quality and process control checks. Analyte concentration is deduced from its response relative to that produced	FDA[4]

	by the internal standard. The internal standard should have similar physico-chemical properties to those of the analyte. An internal standard (IS) is a chemical compound added to the sample test portion or sample extract in a known quantity at a specified stage of the analysis, in order to check the correct execution of (part of) the analytical method. The IS should be chemically stable and/or typically show the same behavior as of the target analyte.	SANTE 11312:2021[10]
International System of Units (SI)	The system of metric units which has been adopted by agreement in all major countries for use in science, medicine, industry, and commerce. SI is a coherent system based on the seven basic quantities of length (meter, m), mass (kilogram, kg), time interval (second, s), electric current (ampere, A), thermodynamic temperature (degree Kelvin, K), luminous intensity (candela, cd) and amount of substance (mole, mol).	VIM[5] NIST[11]
Isochronous Stability Study	Experimental study of reference material stability in which units exposed to different storage conditions and times are measured in a short period of time.	ISO Guide 35[18]
Isotope Dilution	Isotope Dilution Mass Spectrometry IDMS) is used to determine the concentration of a compound of interest in a matrix. It is a destructive analysis technique that is applicable to a wide range of analytes and sample types. With this method, a known amount of a compound containing enriched levels of certain isotopes of atoms in the compound of interest is added to a known amount of sample. The compound of interest is chemically purified from the matrix, the isotope ratio of the spiked sample is measured by mass spectrometry, and the concentration of the compound of interest is calculated from this result. https://www.osti.gov/biblio/1358328	US D. of Energy[19]
Laboratory Sample	The material received by the laboratory.	Thiex[13]
Level of Concern	Level of concern is the concentration of an analyte in a sample that must be exceeded before the sample can be considered violative. This concentration may be a regulatory tolerance, safe level, action level, guidance level or a laboratory performance level.	FDA[4]
Lifetime	Time interval during which RM properties retain their assigned values within their associated uncertainties.	ISO Guide 30[7]
Limit of Detection (LOD)	The minimum amount or concentration of analyte that can be reliably distinguished from zero. The term is usually restricted to the response of the detection system and is often referred to as the Detection Limit. When applied to the complete analytical method it is often referred to as the Method Detection Limit (MDL). (Some organizations such as EPA set specific criteria such as 99% probability of detection using specified analytical procedures.) See also *Minimum Detectable Concentration*.	FDA[4]

Limit of Quantitation (LOQ)	The minimum amount or concentration of analyte in the test sample that can be quantified with acceptable accuracy. Limit of quantitation (or quantification) is variously defined but must be a value greater than the MDL and should apply to the complete analytical method.	FDA[4]
Limit Test (Binary Test, Pass/Fail Test)	A type of semi-quantitative screening method in which analyte(s) has a defined level of concern. Also called a Binary Test or a Pass/Fail Test.	FDA[4]
Linearity	The ability of a method, within a certain range, to provide an instrumental response or test results proportional to the quantity of analyte to be determined in the test sample.	FDA[4]
Matrix	All the constituents of the test sample with the exception of the analytes.	FDA[4]
Matrix Blank **(see *Clean Sample, Sample Blank*)**	A substance that closely matches the samples being analyzed with regard to matrix components. Ideally, the matrix blank does not contain the analyte(s) of interest but is subjected to all sample processing operations including all reagents used to analyze the test samples. The matrix blank is used to demonstrate the absence of significant interference due to matrix, reagents, and equipment used in the analysis.	FDA[4]
Matrix Effect	An influence of one or more components from the sample matrix on the measurement of the analyte concentration or mass. Matrix effects may be observed as increased or decreased detector responses, compared with those produced by simple solvent solutions of the analyte.	FDA[4]
Matrix Reference Material	RM that is characteristic of a real sample.	ISO GUIDE 30[7]
Matrix Source	The origin of a test matrix used in method validation. A sample matrix may have variability due to its source. Different food matrix sources may be defined as different commercial brands, matrices from different suppliers, or in some cases different matrices altogether. For example, if a variety of food matrices with differing physical and chemical properties are selected, the number of sources for each food sample matrix may be one or more.	FDA[4]
Matrix Spike (Laboratory Fortified Matrix)	An aliquot of a sample prepared by adding a known amount of analyte(s) to a specified amount of matrix. A matrix spike is subjected to the entire analytical procedure to establish if the method is appropriate for the analysis of a specific analyte(s) in a particular matrix. Also called a Laboratory Fortified Matrix.	FDA[4]
May	Indicates a permission.	ISO 17025[15] ISO 17034[6]
Measurand	Quantifiable property of an analyte to be measured.	
Measurement	Process of experimentally obtaining one or more quantity values that can reasonably be attributed to a quantity.	VIM[5]

Measurement Accuracy	Closeness of agreement between a measured quantity value and a true quantity value of a measurand. Accuracy includes the effect of both precision and trueness	VIM[5]
Measurement Procedure	Detailed description of a measurement according to one or more measurement principles and to a given measurement method, based on a measurement model and including any calculation to obtain a measurement result.	VIM[5]
Measurement Traceability (Traceability)	Property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty.	VIM[5]
Measurement Uncertainty (MU) (Uncertainty)	Non-negative parameter characterizing the dispersion of the quantity values being attributed to a measurand, based on the information used.	VIM[5]
Media Blank	Unexposed samples not taken to the field or shipped. Media blank results are used for background correction of sample readings and for recovery studies.	EPA [23]
Method Blank **(see matrix blank)**	A substance that does not contain the analyte(s) of interest but is subjected to all sample processing operations including all reagents used to analyze the test samples. An aliquot of reagent water is often used as a method blank in the absence of a suitable analyte-free matrix blank.	FDA[4]
Method blank spike, (Blank spike), (Laboratory fortified method blank)	A method blank spike is a test portion prepared by adding a known amount of analyte(s) to a specified amount of blank substance. A method blank spike is subjected to the entire analytical procedure to establish if the method is appropriate for the analysis of a specific analyte(s).	This document
Method Detection Limit (MDL)	The minimum amount or concentration of analyte in the test sample that can be reliably distinguished from zero. MDL is dependent on sensitivity, instrumental noise, blank variability, sample matrix variability, and dilution factor.	FDA[4]
Method Development	The process of design, optimization, and preliminary assessment of the performance characteristics of a method.	FDA[4]
Method Validation	The process of demonstrating or confirming that a method is suitable for its intended purpose. Validation includes demonstrating performance characteristics such as trueness & precision (accuracy), specificity, limit of detection, limit of quantitation, linearity, range, ruggedness, and robustness.	FDA[4]
Method Verification	The process of demonstrating that a laboratory is capable of replicating a validated method with an acceptable level of performance.	FDA[4]
Metrology	Science of measurement and its application.	VIM[5]

Metrological Traceability Chain	Sequence of measurement standards and calibrations that is used to relate a measurement result to a reference.	VIM[5]
Minimum Detectable Concentration (MDC)	In qualitative analysis, an estimate of the minimum concentration of analyte that must be present in a sample to provide at a specified high probability (typically 95% or greater) that the measured response will exceed the detection threshold, leading one to correctly conclude that an analyte is present in the sample.	FDA[4]
Minimum RM Sample Size	Lower limit of the amount of a RM, usually expressed as a mass quantity, that can be used in a measurement process such that the values or attributes expressed in the corresponding RM documentation are valid.	ISO GUIDE 30[7]
Must (Shall)	Indicates an absolute requirement *(within this document)* **Must not** means an absolute no.	SANTE 11312-2021[10]
Neat Material (Pure Substance)	A material consisting of only one type of atom or molecule; free from impurities, and not in solution. Neat can describe solids, liquids, or gases.	This document
Nominal Value	Value of a quantity or property of a RM, which is the best representation of a true value but may not represent all sources of uncertainty or bias.	
Operationally Defined Measurand	A measurand that is defined by reference to a documented and widely accepted measurement procedure to which only results obtained by the same procedure can be compared.	ISO GUIDE 30[7]
Period of Validity (expiry date)	Period of time during which a RMP warrants an RM stability expressed a date or time period within the lifetime of the RM.	ISO GUIDE 30[7]
Portion	An amount, section, or part of the whole (i.e., of the material being sampled)	macmillandictionary.com
Precision	The closeness of agreement between independent test results obtained under specified conditions. The precision is described by statistical methods such as a standard deviation or confidence limit of test results. See also *Random Error*. Precision may be further classified as Repeatability, Intermediate Precision, and Reproducibility.	FDA[4]
Primary Sample	The material selected from a decision unit	GTP[13]
Primary Standard	Measurement standard that is designated or widely acknowledged as having the highest metrological qualities and whose property value is accepted without reference to other standards of the same property or quantity, within a specified context.	ISO GUIDE 30[7]
Procedural Blank	A procedural blank is a sample that does not contain the matrix, that is brought through the entire measurement procedure and analyzed in the same manner as a test sample. When preparing procedural blanks, water is often used in place of the matrix.	Eurachem 24

Product Information Sheet (PIS)	Document containing all the information that is essential for using an RM other than a CRM. (May also be called a *RM Information Sheet*.)	ISO GUIDE 30[7]
Production Batch or Lot	Specific traceable quantity of material produced during a single manufacturing cycle and intended to have uniform character, quality, and traceable QC data.	ISO GUIDE 30[7]
Purity	Compositional evaluation of a substance to determine the fraction of the substance that consists of the atom or molecule of interest. The acceptable purity of a substance may vary depending on intended scope for use of that substance.	This document
Qualitative Analysis/Method	Analysis/method in which substances are identified or classified on the basis of their chemical, biological, or physical properties. The test result is either the presence or absence of the analyte(s) in question.	FDA[4]
Quality Control Material (QCM) (In-House RM, Proficiency Testing Material)	A material that is stable, homogeneous, and similar in composition to the samples of interest, characterized by comparison to a CRM. Remainder samples from an interlaboratory comparison such as a proficiency test can be considered as QCMs for the duration of the comparison. Results from the comparison can be used to assign values to the QCM and remaining samples may be utilized as RMs. Depending on the accreditation level of the RMP and the documentation provided, QCMs may be upgraded to CRMs.	ISO Guide 80[26] Emons[8]
Quality Control Sample	A blank matrix spiked with known amounts of analytes from a source independent from the calibration standards. It is generally used to establish intralaboratory or analyst specific precision and bias or to assess the performance of all or a portion of the measurement system.	EPA [22]
Quantitative Analysis/Method	Analysis/method in which the amount or concentration of an analyte may be determined (or estimated) and expressed as a numerical value in appropriate units with acceptable trueness and precision (accuracy).	FDA[4]
Quantity Value	Number and reference together expressing magnitude of a quantity.	VIM[5]
Random Error	Component of measurement error that in replicate measurements varies in an unpredictable manner. See also *Precision*.	FDA[4]
Range	The interval of concentration over which the method provides suitable trueness and precision (accuracy) .	FDA[4]
Reagent Blank	Reagents used in the procedure taken through the entire method. Reagent Blanks are used to determine the absence of significant interference due to reagents or equipment used in the analysis. May also be called a Laboratory Blank.	FDA[4]
Recovery	The fraction or percentage (incurred or added) remaining at the point of the final determination from the analytical portion of the sample measured. Total recovery is based on recovery of the native plus added analyte, and	FDA[4] AOAC[1]

	marginal recovery based only on the added analyte (the native analyte is subtracted from both the numerator and denominator).	
Reference	Term assigned to materials (matrix, target analytes) or methods used for testing that have been designated by an authoritative body and are used as a source of information in order to perform analysis, such as an official method of analysis or material used for quantitation.	
Reference Material (RM)	A material, sufficiently homogenous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process or in examination of nominal properties. Uses may include calibration, validation, verification, or interlaboratory comparison.	FDA[4]
Reference Material Certificate (RMC)	Document containing the essential information for the use of a CRM, confirming that the necessary procedures have been carried out to safeguard the validity and metrological traceability of the stated property values.	ISO GUIDE 30[7]
Reference Material Certification Report (RMCR)	Document giving detailed information, in addition to that contained in a RM certificate, e.g., the preparation of the material, methods of measurement, factors affecting accuracy, statistical treatment of results, and the way in which metrological traceability was established.	ISO GUIDE 30[7]
Reference Material Characterization	Typically refers to assignment of quantity values through analytical testing but may also include other non-quantitative information such as confirmation of identity, and binary testing results (yes/no or presence/absence) related to the overall fitness for purpose of the material. ISO 17034 makes a distinction between characterization, homogeneity, and stability studies. See also *Characterization*.	ISO GUIDE 30[7] ISO 17034[6]
Reference Material Document (RMD)	Document containing all the information that is essential for using any RM, covering both the product information sheet and RM certificate.	ISO 17034[6]
RM Information Sheet	Document containing all the information that is essential for using an RM other than a CRM (May also be called a *Product Information Sheet*).	
Reference Material Producer (RMP)	Body (organization or company, public or private) that is fully responsible for project planning and management; assignment of, and decision on, property values and relevant uncertainties; authorization of property values; and issuance of a RM certificate or other statements for the RMs it produces.	ISO 17034[6] ISO GUIDE 30[7]
Reference Material Source	Body (organization or company, public or private) that is fully responsible for providing RMs and their accompanying documentation. May or may not be a RMP.	This document
Reference Standard (Measurement	A substance of known identity and purity, generally with a certificate of quality from an authoritative body and used to prepare calibration standards.	FDA[4]

Standard or Standard)	A measurement standard designated for the calibration of other measurement standards for quantities of a given kind in a given organization or at a given location.	VIM[5]
Repeatability	Precision obtained under observation conditions where independent test results are obtained with the same method on identical test items in the same test facility by the same operator using the same equipment, materials, solvents, and consumables within short time intervals.	FDA[4]
Repeatability Conditions	Conditions where independent test results are obtained with the same method on identical test items in the same laboratory by the same operator using the same equipment within short time intervals.	NORDTEST[9]
Repeatability Limit	Performance measure for a test method or a defined procedure when the test results are obtained under repeatability conditions.	NORDTEST[9]
Representative Analyte	An analyte used to assess probable analytical performance with respect to other analytes having similar physical and/or chemical characteristics. Acceptable data for a representative analyte are assumed to show that performance is satisfactory for the represented analytes. Representative analytes should include those for which the worst performance is expected. Representative analytes are used mostly for non-targeted analysis and unknown screening procedures.	FDA[4]
Representative Matrix	Matrix used to assess probable analytical performance with respect to other matrices, or for matrix-matched calibration, in the analysis of broadly similar commodities. For food matrices, similarity is usually based on the amount of water, fats, protein, and carbohydrates. Sample pH and salt content can also have a significant effect on some analytes.	FDA[4]
Reproducibility	Precision obtained under observation conditions where independent test results are obtained with the same method on identical test items in different test facilities with different operators using different equipment. May also include different lots of chemicals, target analytes, reagents, etc.	FDA[4]
Reproducibility Conditions	Conditions where test results are obtained with the same method on identical test items in different laboratories with different operators using different equipment.	NORDTEST[9]
Reproducibility Limit	Performance measure for a test method or procedure when the test results are obtained under reproducibility conditions.	NORDTEST[9]
Reproducibility Standard Deviation	Can be estimated from validation studies with many participating laboratories or from other interlaboratory comparisons (e.g., proficiency testing).	NORDTEST[9]
Resolution	Smallest change in a quantity being measured that causes a perceptible change in the corresponding quantity value provided by a measuring instrument or a measuring system.	VIM[5]

Retest Date	Date a test item should be re-examined to ensure that it is still suitable for use.	OECD GLP #19[17]
Ruggedness/ Robustness	A measure of the capacity of an analytical procedure to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.	FDA[4]
Sample	A portion (mass or volume) of a material selected from a larger mass or volume (batch) to intended to represent the whole.	Thiex[13]
Sample Blank (matrix blank)	Sample matrix with no analyte present	Eurachem [24]
Sampling Equipment Blank	A clean sample that is collected in a sample container with the sample-collection device and returned to the laboratory as a sample. Sampling equipment blanks are used to check the cleanliness of sampling devices.	EPA [22]
Screening Analysis/Method	An analysis/method intended to detect the presence of analyte in a sample at or above some specified concentration (action or target level). Screening methods typically attempt to use simplified methodology for decreased analysis time and increased sample throughput.	FDA[4]
Secondary Reference Material	A RM that maintains traceability through another RM used for calibration or other qualification. See also *Secondary Source.*	This document
Secondary Standard	Measurement standard whose property value is assigned by comparison with a primary measurement standard of the same property or quantity. See also *Secondary Source.*	ISO GUIDE 30[7]
Secondary Source	Alternate source for a material, either from a producer or manufacturer. Level of sourcing depends on scope and purpose of analytical test (e.g., regulatory vs. survey). Should be a different accredited provider (or lot number if provider not available), and often used to identify degradation or bias in materials.	FDA[4]
Selectivity	Property of a measuring system, used with a specified measurement procedure, providing measured quantity values for one or more measurands such that the values of each measurand are independent of other measurands or other quantities in the phenomenon, body, or substance being investigated. Typically determined using the measuring system that was used to determine the known identity (chemical) of the measurand.	VIM[5]
Sensitivity	The change in instrument response which corresponds to a change in the measured quantity (e.g., analyte concentration). Sensitivity is commonly defined as the gradient of the response curve or slope of the calibration curve at a level near the LOQ.	FDA[4]
Shall (Must)	Indicates a requirement *(In this document it will be used only when referring to an accreditation standard or an official government regulation.)*	ISO 17025[15] ISO 17034[6]

Shelf Life (storage lifetime)	The period of time within which a RM material is expected to remain acceptable for use (usually determined during stability studies) and the certified value should exist within the range of its overall uncertainty.	ISO 35:2017[18]
Should	Indicates a recommendation	ISO 17025[15] ISO 17034[6]
Simulated Blank	If a sample blank cannot be obtained, then, in certain cases it may be possible to create a simulation. Matrices such as ocean water lend themselves to the production of a simulated blank by the dissolution of appropriate mineral salts in water.	Eurachem 24
Solvent Blank	A solvent blank is made up from the solvent(s) contained in the solution presented to the instrument.	Eurachem 24
Specificity	In quantitative analysis, specificity is the ability of a method to measure analyte in the presence of components which may be expected to be present. The term Selectivity is generally preferred over Specificity.	FDA[4]
Spike Recovery	The fraction of analyte remaining at the point of final determination after it is added to a specified amount of matrix and subjected to the entire analytical procedure. Spike Recovery is typically expressed as a percentage. Spike recovery should be calculated for the method as written. For example, if the method prescribes using isotopically labeled internal standards or matrix-matched calibration standards, then the reported analyte recoveries should be calculated according to those procedures.	FDA[4]
Standard Measurement Uncertainty	Measurement uncertainty expressed as a standard deviation.	GUM[25]
Standard Reference Material (SRM)	A CRM issued by the National Institutes of Standards and Technology (NIST) in the United States (www.nist.gov/SRM).	FDA[4]
Storage Stability	Characteristic of a RM, when stored under specified conditions, to maintain a specified property value within specified limits for a specified period.	ISO GUIDE 30[7]
Systematic Error (Bias)	Component of measurement error that in replicate measurements remains constant or varies in a predictable manner. Also called Bias.	FDA[4]
Test Portion	The mass or volume of material selected from an analytical sample for a single test.	Thiex[13]
Threshold Value (Cut-off Concentration)	In qualitative analysis, the concentration of the analyte that is either statistically lower than the level of concern (for limit tests) or at which positive identification ceases (for confirmation of identity methods).	FDA[4]
Trace Analysis	A test measurement of a chemical analyte at a concentration less than 100 µg/g in a material. IUPAC defines concentrations as Major (>1%), minor (1-0.01%), trace (0.01-0.0001%), and ultra-trace(<0.0001%).	IUPAC[21]

Transportation Stability	Stability of a RM property for the period and conditions encountered in transportation to the user of the RM.	ISO GUIDE 30[7]
Trip Blank	A clean sample of matrix that is carried to the sampling site and transported to the laboratory for analysis without having been exposed to sampling procedures.	EPA [22]
Trueness	The degree of agreement of the mean value from a series of measurements with the true value or accepted reference value. This is related to systematic error (bias).	FDA[4]
Uncertainty (Measurement Uncertainty)	Non-negative parameter characterizing the dispersion of the values being attributed to the measured value.	FDA[4]
Working Standard	Measurement standard that is used routinely to calibrate or verify measuring instruments or measuring systems.	VIM[5]
Zero Level Calibrant	See *Calibration Blank*	

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14 Appendix 1: Challenge of adding new compounds into a multi-residue method.

Problem with introducing two new compounds into a validated multiresidue method

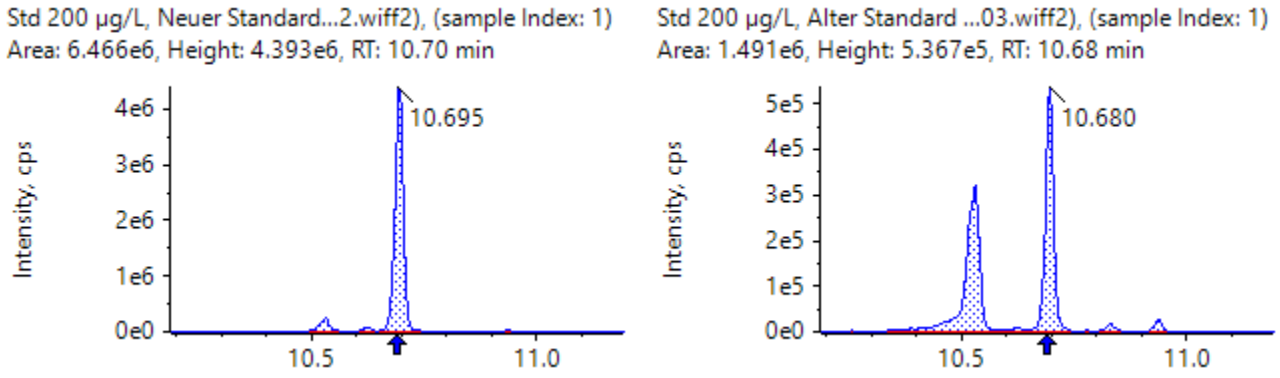
Anton Kaufmann, Official Food Control Authority of Zurich Switzerland, 08-27-2021

Problem:

We participated in a proficiency test (coccidiostats in freeze dried eggs).
We obtained four times too high results for two compounds (Salinomycin and Narasin).
Repeatability, linearity of calibration curve, recovery, and signal suppression were fine.
We therefore tested the mixed reference standard solution against a freshly prepared one.
Some analytes showed a significant peak area loss (see table).

X500 200116					
	Peak area			Difference to new Std %	
	Std newly prepared	Std set 1(4 months old)	Std set 2 (4months old)		
Arprinocid	1059000	1072000	1092000	1	3
Clopidol	435900	309900	307700	-29	-29
Decoquinat	7075000	2284000	3287000	-68	-54
Diaveridin	1778000	1691000	1817000	-5	2
Ethopabat	304900	300300	321900	-2	6
Halofuginon	357100	378200	390600	6	9
Lasalocid	1949000	1990000	2012000	2	3
Maduramycin	2277000	1946000	1858000	-15	-18
Monensin	6265000	5057000	4934000	-19	-21
Narasin	6466000	1509000	1396000	-77	-78
Robenidin	2345000	2330000	1920000	-1	-18
Salinomycin	3918000	826200	797300	-79	-80
Semduramicin	1173000	914600	899400	-22	-23
Diclazuril	1086000	960900	967200	-12	-11
Dinitolmid	343200	342000	352000	0	3
Dinitrocarbanilid	2285000	2373000	2290000	4	0
Nitromid	271000	260400	267800	-4	-1
Toltrazuril	2214000	2152000	2211000	-3	0
Toltrazuril sulfon	2132000	1983000	2155000	-7	1
Toltrazuril sulfoxid	1559000	1729000	1797000	11	15

The mass trace of salinomycin shows an additional peak (right, “Alter Standard”) as compared to a freshly prepared standard (left “Neuer Standard”)



We found that salinomycin degrades in mixed standard solutions (stored in the freezer at -20 ^{0}C). After two weeks about 50 % were degraded.

Reason:

The originally utilized analytical method included all the analytes listed above, except decoquinate and diaveridin. That original method has been propery validated and the stability of the mixed standard solution was investigated. It was the extension of the method with the two analytes (decoquinate and diaveridin) which lead to problems. These analytes are very poorly soluble. We normally produce stock solutions containing 1000 mg/L of analytes. Depending on the analyte we use mixtures of acetonitrile, methanol, water, and DMSO. One analyte (clopidol) could only be dissolved in 20 % DMSO; 2 % ammonium hydroxide (35 %) and 78 % acetonitrile.
The two additional analytes (decoquninate and diaveridin) were not sufficiently soluble in any of these solvents, that is why we dissolved:
Diaveridin in water containing 1% formic acid
Decoquinate in acteonitrile containing 10 % formic acid
This not only resulted in a low pH value in the two stock solutions, but also affected to a lesser degree the pH of the mixed standard solution (consisting of a total of 20 analytes). A pH value of 4 resulted. This drop was obviously too much and led to an instability of some analytes (marked in red in the table above).

Solution to the problem:

Decoquinate stock solution was produced by dissolving the analyte in 25% chloroform in methanol.
Diaveridin stock solution was produced by using 100% DMSO.
Pippeting these two stock solutions into the mixtures of the other 18 analytes does not anymore cause a drop of the pH value. The stability of the standard was therefore assured.

We have a number of multiresidue methods, where some stock solutions contain acids or alkalis. As a conclusion, we add first a relatively large volume of dilution solution into the volumetric flask. Then we add first the pH neutral stock solutions. This is followed by the non-neutral pH stock solutions. We pay attention that during the production step (this is done at room temperature) analytes are not exposed to a low or high pH environment. Finally we fill up to the mark with dilution solution (only a small volume is required, due to the initial adding of dilution solution into the volumetric flask).

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