

*Best practices
for the use of
reference materials
in the analysis of
organic residues and
contaminants
in foods and
environmental
matrices*

Reference Material Use in Trace Analysis

(draft in review – 1-13-2021)

by the NACRW Reference Materials Working Group



NACRW REFERENCE MATERIAL WORKING GROUP

Patricia Atkins	SPEX CertiPrep, USA
Rod Bennett	Winding Trails, LLC, USA
Janet Blount	U.S. Food and Drug Administration, USA
Jo Marie Cook	(formerly) Florida D. of Agriculture and C.S., USA
Jessica Krank	Colorado D. of Agriculture, USA
André de Kok	(formerly) Wageningen Food Safety Research, The Netherlands
Kelly Dorweiler	General Mills, USA
Håkan Emteborg	European Commission, Joint Research Centre, Belgium
Terry Grim	Cambridge Isotope Laboratories, Inc., USA
Kyle Heater	OMIC USA, USA
Brittany Holmes	Central Washington University, USA
Pearse McCarron	National Research Council, Canada
Joe Konschnik	Restek, USA
Alex Krynitsky	U.S. Food and Drug Administration-consultant, USA
Katerina Mastovska	Eurofins Scientific, USA
Francesca Mottola	Lab Instruments, Italy
Markus Obkircher	MilliporeSigma, Switzerland
Melissa Phillips	National Institute of Standards and Technology, USA
Mario Stefanelli	Lab Instruments, Italy
Kathy Stenerson	MilliporeSigma, USA
Jon Wong	U.S. Food and Drug Administration, USA

NACRW Reference Materials Working Group, October 2020

<https://nacrw.org/reference-materials>

© Copyright 2020-2030 North American Chemical Residue Workshop (NACRW)FLAG Works, Inc./NACRW-All Rights Reserved

Table of Contents

Table of Contents.....	1
1 INTRODUCTION.....	1
1.1 Reference materials (RMs) play an essential role.....	1
1.2 A RM is a material, sufficiently homogeneous and stable.....	1
1.3 A CRM provides metrological traceability	1
1.4 Calibrants and Quality Control Materials:	2
1.5 Different RMs.....	2
FIGURE 1. The Family of Reference Materials ⁴	3
1.6 Introduction References	5
2 ACCREDITATION AND ISO STANDARDS.....	7
2.1 Standards setting organizations.....	7
2.2 International Organization for Standardization (ISO).....	8
2.3 ISO/IEC 17025	8
2.4 ISO Guide 34 & ISO 17034.....	10
2.5 Scope of Accreditation.....	12
2.6 Impact of Changes on Laboratories	13
2.7 Accreditation References.....	15
3 APPLICATION AND USE OF RMS.....	14
3.1 RM Types.....	14
3.2 Method Development and Optimization.....	16
3.3 Method Validation	17
3.4 Method and/or Laboratory Comparison	18
3.5 Routine Analysis.....	18
3.6 Safety	21
3.7 RM Use References	21
4 RM DOCUMENTATION	22
4.1 The terminology.....	22
4.2 The definition of a CRM	22

Table of Contents

FIGURE 2. RM Documentation	22
4.3 An RM.....	23
4.4 An RMC	23
4.5 Additional information.....	23
4.6 Example of a typical RMC.....	23
FIGURE 3. Example of a RMC from a RMP	23
5 PROCESSING RAW MATERIALS FOR RMs.....	24
5.1 Sourcing Raw Materials	24
5.2 Materials Sampling	24
TABLE 1. Relative effect of particle size on mass (g) of material with uniform particle size and shape needed to achieve various uncertainty levels for representative test portions.....	26
5.3 Identification versus Verification of Materials.....	26
5.4 Materials Testing.....	26
5.5 Purities and Impurities	27
TABLE 2. Different expressions of purity and their significant figures.	28
5.6 Final Notes on Raw Materials	29
5.7 Raw Materials References	29
6 STABILITY AND INTERACTIONS of RMs	30
6.1 Neat Reference Standards	30
6.2 Individual Stock Standards and Matrix-Matched Standards	31
6.3 Stability of Multi-Component Mixtures	33
TABLE 3. Examples of pesticide chemical classes susceptible to degradation in solvent standards..	34
TABLE 4. Examples of precursors and known breakdown products for common pesticides.....	35
6.4 Matrix RMs.....	36
6.5 Stability Studies.....	37
6.6 Materials and Methods.....	38
6.7 Instrumental analysis tools	39
6.8 Stability and Interactions References	40
7 RM & CRM HANDLING and DISPOSITION	42
7.1 RM Handling Requirements	42

7.2	Transport.....	42
7.3	Receipt by the Laboratory.....	43
7.4	RM Handling.....	43
7.5	Storage	44
7.6	Expiry (or expiration) Date	44
7.7	Assessing RM validity	46
7.8	Handling and Disposition References	48
8	RM PREPARED IN-HOUSE	49
8.1	CRMs:	49
8.2	Laboratory prepared RMs	49
8.3	Using a CRM for Metrological Traceability of CALs.....	49
8.4	Considerations in Preparation of RMs	50
8.5	Preparation of CALs.....	52
8.6	Matrix RMs (QCMs).....	53
	FIGURE 4. AOAC food composition triangle.....	54
8.7	Preparation of QCMs	56
8.8	Characterization of RMs.....	57
8.9	In-House RM References.....	60
9	VERIFICATION USING RMs from a SECOND SOURCE	61
9.1	Is a Second Source Needed?	61
9.2	Acceptable second source quantitative verification criteria	61
9.3	The terminology “second source” is not always clear.	62
	TABLE 5. Second source RM descriptions	62
9.4	Second Source References.....	64
10	MEASUREMENT UNCERTAINTY	65
10.1	What is Uncertainty?	65
10.2	Bottom-Up Approach for Uncertainty Estimation	65
10.3	Top-Down Approach for Uncertainty Estimation	66
10.4	Assessment of Uncertainty associated with Certified Values.....	67

Table of Contents

FIGURE 5. Cause and effect diagram representing contributing factors to uncertainty in manufacture and qualification of a gravimetrically prepared standard solution.....	68
10.5 Uncertainty of the (In)Homogeneity.....	68
10.6 Uncertainty of the Stability.....	69
10.7 Uncertainties of Solutions, Mixtures and Matrix Materials.....	70
10.8 Reporting Uncertainty for a CRM.....	71
FIGURE 6. Steps in producing a CRM solution	71
10.9 Practical Use of Uncertainty Values.....	72
10.10 Measurement Uncertainty References.....	73
11 GLOSSARY.....	74
11.1 Glossary Sources	74
11.2 DEFINITIONS.....	74
11.3 Glossary References and Other Resources.....	87
12 COMPILED REFERENCES.....	89

1 INTRODUCTION

The NACRW Reference Material Working Group is pleased to present this best practices manual for use by scientists using reference materials in analytical measurement and quality control with a special emphasis on trace level analyses.

1.1 Reference materials (RMs) play an essential role in providing accurate, precise, verifiable and legally defensible testing results. An analytical chemistry RM defines a common standard of reference, similar to those used in metrology, by providing a sample with a content that is reliable and reproducible. The analysis of contaminants and residues in human and animal foods present 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-residue 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 for understanding and effectively using RM specific to complex trace level analysis.

1.2 A RM is 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.¹ The concepts of homogeneity and stability may be understood by comparison to the concepts of durability and equivalence of reference weight(s) used for balance calibration. The reference weight should be constant over time (i.e., durable or stable) and since many reference weights are produced for a specific mass, the difference in mass between them should be very small (i.e., equivalent or sufficiently homogeneous).

As an introduction to the terminology in this field, the complex nature of the term reference material will be described. Some types of RMs include:

1.2.1 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. RM producers can be accredited to International Organization for Standardization (ISO) standard Guide 17034.²

1.2.2 Proficiency Testing (PT) Material: Upon completion of proficiency testing, some PT materials are characterized as RMs. A PT 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 17043.³

Introduction

1.3 A CRM provides metrological traceability and should also fulfill the criteria of an RM in being sufficiently homogeneous and stable. A CRM is a RM, accompanied by documentation issued by an authoritative body of measurements which provide one or more specified property values with associated uncertainties and traceability obtained using valid procedures. RMs that are certified for a specific property will be accompanied by an RMC that describes the certified amount of the specified property and the uncertainty of that value. Metrological traceability must be stated on the certificate indicating that the property is traceable to the international system of units (SI) or to some other common standard or method. The traceability provides the basis for comparability of results.

1.4 Calibrants and Quality Control Materials: Besides PT materials and CRMs, calibrants (CALs) and quality control materials (QCMs) also belong to the RM family as described by Emons⁴ and illustrated with an emphasis on trace level analysis in FIGURE 1.

1.4.1 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.

1.4.2 CALs, such as an analytical standard or a calibration standard, are used to quantify instrument response during measurement. A CAL should have a metrologically traceable property value with an uncertainty suitable for the intended calibration.

1.4.3. QCMs, also known as non-certified RMs or in-house RMs, are characterized as sufficiently homogeneous and stable to be fit for the intended use. 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.

1.5 Different RMs in the form of pure chemicals, stable multi-analyte solutions and well characterized matrix materials are needed to support determination of trace level contaminants and residues in food, animal feed, and environmental materials. Target analytes include pesticides, veterinary drugs, natural toxins, toxic elements, heavy metals, environmental contaminants, processing contaminants, packaging migrants, unapproved additives, adulterants and others. A variety of analytical techniques including liquid and gas chromatography, typically coupled with mass spectrometry, are used to provide simultaneous identification and quantitation. These analytical determinations can be challenging due to the lack of information around stability and analyte interactions in multi-analyte mixtures containing a large number of compounds. Complexity and cost of analyses has increased due to routine use of isotopically labeled compounds as internal standards and C₁₄ radiolabeled compounds for metabolic studies. While many RMs are available commercially to support these methods, more are needed.

1.5.1 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 standards and

Reference Material Use in Trace Analysis

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. 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. Extensive characterization of neat chemicals is often required before RMPs can certify a CRM.

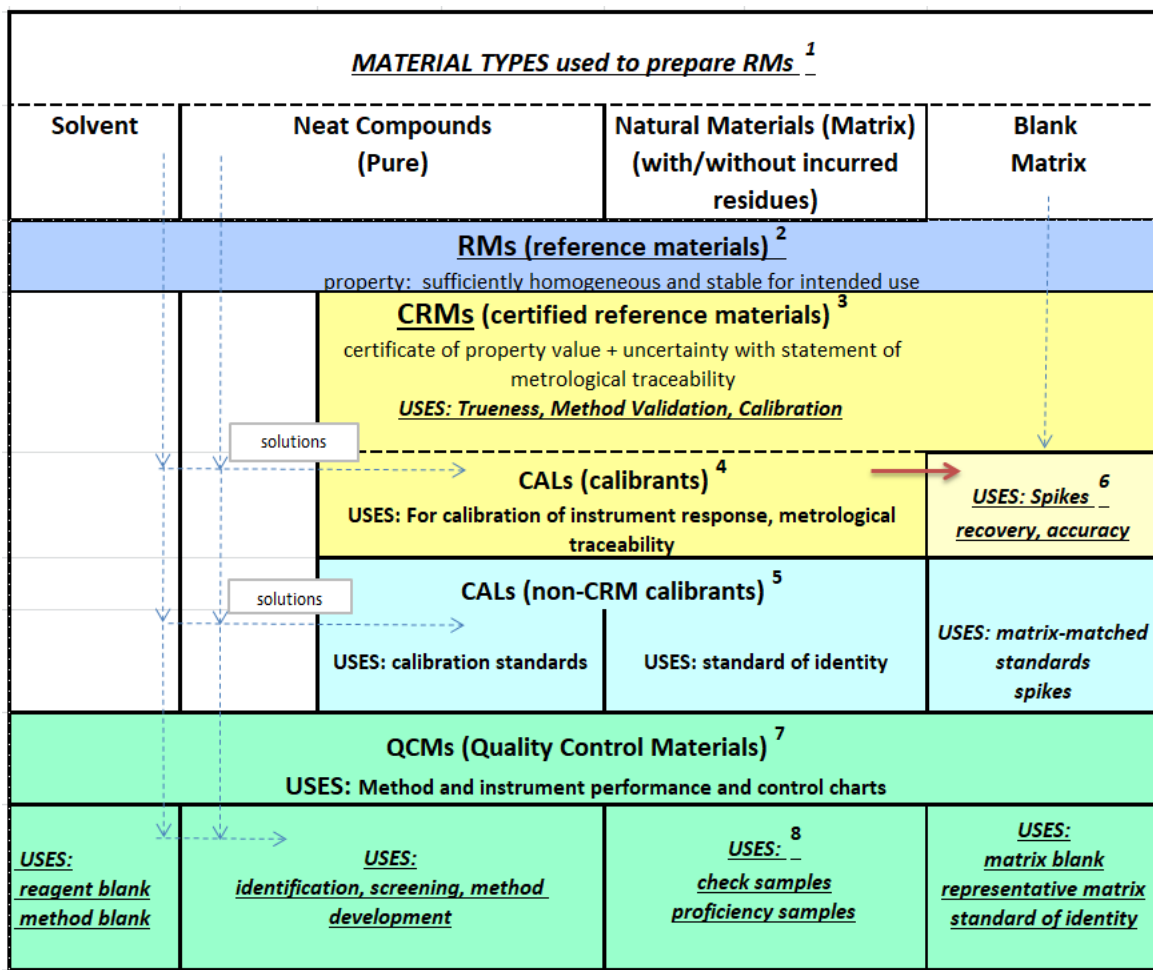


FIGURE 1. The Family of Reference Materials⁴

The diagram describes an overview of preparation and use of various RM types as well as RM inter-relationships. Beginning from the top:

1. RMs may be prepared using solvents, pure compounds, natural materials (matrices) with or without incurred residues and blank matrices, alone or in combination.
2. RMs are sufficiently homogeneous and stable with respect to the property of interest in the material for the intended use.

Introduction

3. CRMs are RMs in which a specific property is metrologically traceable to a common point of reference including the measurement uncertainty.
4. CALs should be characterized as CRMs including metrological traceability and measurement uncertainty.
CRM CALs should be used whenever possible.
5. Some CALs can be prepared from RMs without complete CRM-level characterization.
Non-CRM CALs should be thoroughly characterized in-house.
6. Matrices spiked with CRM CALs, which are sufficiently homogeneous and stable, may be used to check trueness.
7. QCMs are RMs that do not have CRM-level characterization but may be useful for method development or harmonization (e.g., control charting, interlaboratory comparisons).
8. PT samples are RMs during PT studies (i.e., fit for the intended purpose).
PT samples may be used as RMs after a PT study is concluded if supported by homogeneity and stability claims or characterized as CRMs with appropriate traceability and measurement uncertainty.

1.5.2 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 can 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 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 method bias. The laboratory should know any specific requirements that may be applicable to specific testing such as for enforcement methods and method validations. For example, The Code of Federal Regulations (CFR) Title 21, Chapter 58⁵ prescribes good laboratory practices (GLP) for conducting nonclinical laboratory studies related to products regulated by the U.S. Food and Drug Administration (FDA). The U.S. Environmental Protection Agency (EPA) also has GLP practices to safeguard the quality and integrity of data submitted to the EPA.^{6,7}

Definitions from ISO 17025, SANTE 12682, ISO/IEC Directives Part 2 and ILAC PT10 have been adopted for :

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

1.5.3 This “best practices” manual is a collection of information intended to provide chemists with practices that provide reliable, effective and efficient use of precious RMs whether purchased from a RMP or prepared in the laboratory. Information provided includes proper use and handling of RMs; recommendations to prevent analyte degradation or metabolite creation and identification of challenges in obtaining suitable RMs. A glossary of RM terminology is included to reduce ambiguity and clearly define important terms and concepts.

1.5.4 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.^{.8,9,10}

1.5.5 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.

1.6 Introduction References

¹ ISO Guide 30:2015, Reference materials — Selected terms and definitions. International Organization for Standardization, Geneva, Switzerland (2015).

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 ISO 17043:2010, Conformity assessment — General requirements for proficiency testing, <https://www.iso.org/standard/29366.html> (accessed 9-11-2020)

⁴ Emons, H. The ‘RM family’—Identification of all of its members. *Accred Qual Assur* 10 (2006) 690–691.

⁵ Code of Federal Regulations, Title 21, Part 58 – Good Laboratory Practice for Nonclinical Laboratory studies. <https://www.ecfr.gov>

Introduction

- ⁶ USEPA Good Laboratory Practices Standards (GLPS), <https://www.epa.gov/compliance/good-laboratory-practices-standards-compliance-monitoring-program> (reference date: September 08, 2020).
- ⁷ USEPA (1985) Final Enforcement Response Policy (ERP) for the Toxic Substances Control Act (TSCA) Good Laboratory Practice (GLP) Regulations, <https://www.epa.gov/enforcement/final-enforcement-response-policy-erp-toxic-substances-control-act-tsca-good-laboratory>
- ⁸ SANTE 12682:2019, Analytical Quality Control and Method Validation Procedures for Pesticide Residue Analysis in Food and Feed, EURL
- ⁹ ILAC PT10:07/2020 ILAC Policy on Metrological Traceability of Measurement Results, International Laboratory Accreditation Cooperation, <https://ilac.org/publications-and-resources/ilac-policy-series/> (accessed 10-2-2020)
- ¹⁰ ISO/IEC Directives Part 2 Principles and rules for the structure and drafting of ISO and IEC documents, Ed.8, 2018, https://www.iso.org/sites/directives/current/part2/index.xhtml#_idTextAnchor089 (accessed 9-12-2020)

2 ACCREDITATION AND ISO STANDARDS

2.1 Standards setting organizations

2.1.1 Laboratories around the world process and analyze hundreds of thousands of laboratory samples each day. Without an assurance of competency and accuracy, those results could be called into question as they potentially pose a risk to worldwide health, safety and economies.

2.1.2 *Accuracy* refers to the combination of *trueness* and *precision*. Measurement accuracy is the closeness of agreement between a measured quantity value and a true quantity value of a measurand. The glossary and references provide more information on the use of these and other related terms such as *measurement accuracy* and *bias*.

2.1.3 Many national and international standards organizations are focused on the development, implementation and amendment of technical standards to further improve existing processes or products. Most standards created by standards organizations are voluntary, in which the adopting entities agree to follow the standards without being mandated by regulations or laws. Mandatory standards are usually issued by governing bodies both at the national or international level and become part of the regulations and legislation for the industry. In some cases, voluntary standards may become mandatory standards.

2.1.4 Voluntary standards development organizations include ISO, Association of Official Analytical Collaboration (AOAC), American Society for Testing and Materials (ASTM), American National Standards Institute (ANSI), United States Pharmacopeial Convention (USP), European Standardization Committee (CEN), National Normalization Institutes (e.g. DIN, NEN, BIN) and others.

2.1.5 In the laboratory community, 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. For example the Good Laboratory Practice (GLP) standards¹, the Good Manufacturing Practice (GMP) standards², the National Institute of Standards and Technology (NIST)³ 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 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

Accreditation and ISO Standards

products or services and making a decision to whether or not the product or service meets passing 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.

2.2 International Organization for Standardization (ISO)

2.2.1 Since the 1940s, ISO has become one of the world's largest developers of voluntary international standards from 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.

2.2.2 Accreditation is the confirmation of the competence of a laboratory or an RMP by an unbiased independent third-party or external accreditation body to an ISO or other international standard. Each nation usually has at least one organization considered to be an accreditation body, and national organizations cooperate with a goal to provide international agreement between accreditation bodies. The primary purpose of International Laboratory Accreditation Cooperation (ILAC) is to establish international arrangement between member accreditation bodies based on peer evaluation and mutual acceptance of 100 accreditation body participants. A primary mission of ILAC is developing and harmonizing laboratory, inspection body and PT testing provider accreditation practices.

2.2.3 A laboratory or company can 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 normally 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. Proficiency should be periodically recertified.

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

2.3 ISO/IEC 17025

2.3.1 ISO/IEC 17025 "General requirements for the competence of testing and calibration laboratories"⁴ is the standard used by testing and calibration laboratories worldwide to demonstrate their technical competence. The standard was originally issued in 1999 and was followed by a second release 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.

2.3.2 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.

2.3.3 Some key points in ISO/IEC 17025:2017 are:

- Emphasis on impartiality and confidentiality
- Changes to document range and scope of laboratory activities
- Increased periodic review measures and control for environmental conditions (e.g., laboratory access, contamination, etc.)
- Expanded definition of equipment to include instruments, software, data, standards, RMs, reagents, consumables and other apparatuses
- 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 with regard to 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

Accreditation and ISO Standards

2.4 ISO Guide 34 & ISO 17034

2.4.1 ISO Guide 34, “General requirements for the competence of RM producers”⁵ was originally 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.

2.4.2 ISO Guide 34 was published to allow 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 verifications and evaluation or validation of measurement procedures. For the CRM producers, ISO Guide 34 required demonstrations of scientific and technical competence, which is shown by additional ISO/IEC 17025 accreditation. The guide also required certified values and supplementary information be provided about RMs including traceability statements, uncertainty, homogeneity, stability, preparation and methods of measurement.

2.4.3 Traceability describes the linkage of a product or service from the point of origin through the manufacturing or service process through to final analysis, delivery and receipt.

2.4.4 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.

2.4.5 RMPs should establish that the certified property values of a CRM can be traced back to a primary standard, one of the highest metrological value 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 RMs should, where possible, be traceable to SI units of measurement, or to CRMs. Koeber et.al.⁶ and ERM Application Note 3⁷ provide more explanations on the concept of metrological traceability.

2.4.6 The word “traceability” can also be used by RMPs to describe the linkage of a product or service from the point of origin through the manufacturing or service process through to final analysis, delivery and receipt. It is important not to confuse this with metrological traceability.

2.4.6 Measurement Uncertainty is a non-negative parameter characterizing the dispersion of the quantity values being attributed to a measurand, based on the information used. 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 confidence interval. It may also be termed “error of measurement”. Also refer to Chapter 10: Measurement Uncertainty.

2.4.7 Two categories of measurement uncertainties must be considered in the context of certified reference materials and validated analytical measurements.

- a. The uncertainty of the certified value is stated on the certificate and is provided by the RMP.
-

- b. The measurement uncertainty that is associated with normal method performance is estimated by the analytical laboratory (e.g. the expanded relative uncertainty of chloropyrifos in cucumber is 12 %).

2.4.8 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 confidence level (typically multiplied with a coverage factor of $k = 2$ for the 95% confidence level.) 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. Standard uncertainty is the term used for the uncertainty components 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 estimate uncertainty of certified values.⁸

2.4.9 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. For estimation of method related uncertainties, two different approaches; top-down and bottom-up as outlined in chapter 10. 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 errors or mistakes.

2.4.10 References on estimation of measurement uncertainty include two guides from Eurachem, Quantifying Uncertainty in Analytical Measurement⁹ and Terminology in Analytical Measurements¹⁰. In addition, a technical report from Nordtest provides practical advice on top down approaches which are more easily realized in normal analytical laboratories.¹¹

2.4.11 Homogeneity is the uniformity of a specified property value throughout a defined portion of a reference RM, and can refer to within-bottle homogeneity or between-bottle (or lot) homogeneity.

2.4.12 Within-bottle homogeneity means there is no precipitation or stratification of a bigger portion of the material than the minimal test portion size that cannot be rectified by following instructions for use. Some RMs can separate during storage but are still considered homogeneous if they can be re-blended by following the instructions for use (e.g., sonicate, heat, shake). Between-bottle or lot homogeneity is the homogeneity occurring between separate packaging units. It should be noted that powders are never completely homogeneous. RMs are assessed for their degree of homogeneity (inhomogeneity).

2.4.13 Stability is the characteristic of a RM, when stored under specified conditions, to maintain a specified property value within specified limits for a specified period.

Accreditation and ISO Standards

2.4.14 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, burn or explode under normal conditions and applications or might react with the matrix or with other components in the mixture. The label of a neat material or a material in a specified solvent can specify that it be “*kept frozen*” at a specified temperature or kept “*under a nitrogen atmosphere*”. 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 the certified parameter or may not such as in the case of inorganic elements. Moisture changes due to poor storage conditions can change property values so some materials are certified on a dry mass basis.



2.4.15 ISO 17034 includes references to several other documents including ISO/IEC 17025 (previously discussed), *ISO Guide 31: Reference Materials – General and statistical principles for certification* and *ISO/TR 16476: Establishing and expressing metrological traceability of quantity values assigned to reference materials*.

2.4.16 Many of the changes from the original 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.

2.4.17 Some of the 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 Scope of Accreditation and Certification

2.5.1 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.

2.5.2 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. These estimations of measurement uncertainty can vary in complexity with the type of uncertainty, namely Type A or Type B. Type A uncertainty is based on valid statistical methods, for example an analysis of measured quantity values using statistical methods associated with the analysis of variance (ANOVA). Type B uncertainty is based on scientific judgement made from previous experience and manufacturer's specifications by means other than the statistical analysis of series of observations. 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 the contributions to those uncertainties. Refer to the chapter on method uncertainty for more details.

2.6 Impact of Changes on Laboratories

2.6.1 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 to be traceable. If an RMP is not accredited to ISO 17034, signatories of the "Comité International des Poids et Mesures" (in French) Mutual Recognition Agreement (CIPM/MRA)¹² are equally acceptable providers of high-quality CRMs.

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.

Accreditation and ISO Standards

2.6.2 RMPs will now have to provide more detailed information regarding use, storage and stability in addition to instructions on use and storage 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.

2.7 Accreditation References

- ¹ Code of Federal Regulations, 21 CFR 58, Good Laboratory Practices for Nonclinical Laboratory Studies, <https://www.ecfr.gov> (accessed 9-10-2020)
- ² Code of Federal Regulations, 21 CFR 110 & 117, Current Good Manufacturing Practice in Manufacturing, Packing, or Holding Human Food, <https://www.ecfr.gov> (accessed 9-10-2020)
- ³ NIST, National Institute of Standards and Technology, <https://www.nist.gov/programs-projects/nist-food-safety-program> (accessed 9-10-2020)
- ⁴ ISO/IEC 17025:2017, *General requirements for the competence of testing and calibration laboratories*, <https://www.iso.org/standard/66912.html> (accessed 9-10-2020)
- ⁵ 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)
- ⁶ Koeber, R., Linsinger, P.J., Emons, T.H. An approach for more precise statements of metrological traceability on reference material certificates, *Accred Qual Assur* (2010) 15:255–262 DOI 10.1007/s00769-010-0644-2 (2009)
- ⁷ ERM App #3, *Using Reference Materials to Establish Metrological Traceability*, (2008-02)
- ⁸ ISO Guide 35:2017 *Reference materials*. International Organization for Standardization, Geneva, Switzerland (2017).
- ⁹ Eurachem/CITAC Guide CG4, *Quantifying Uncertainty in Analytical Measurement*, 3rd Edition (2012). https://www.eurachem.org/images/stories/Guides/pdf/QUAM2012_P1.pdf.
- ¹⁰ Eurachem, *Terminology in Analytical Measurement: Introduction to VIM 3* (2011)
- ¹¹ Nordtest TR 537 (2003-05) *Handbook for Calculation of Measurement Uncertainty in Environmental Laboratories*
- ¹² "Comité International des Poids et Mesures" (in French), International Committee for Weights and Measures (in English),

3 APPLICATION AND USE OF RMS

RMs used in the analysis of chemical residues and contaminants 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 preferably be characterized and certified for 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 the chapter *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.

3.1.2 Standard solutions are prepared from neat materials gravimetrically either in-house or by RMPs. Typically, the first step is 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 multiple compounds (composite standard solutions), such as for multiresidue analysis of pesticides or veterinary drugs or in multi-contaminant analyses, including the analysis of PCBs and dioxins, PAHs, mycotoxins etc. The composite standard solutions are prepared by gravimetrically or volumetrically mixing individual compound solutions or gravimetrically using multiple neat materials. The former process is typical for in-house preparation of composite (mixed) standard solutions, whereas the latter process is usually employed by RMPs. Reactivity and adsorption considerations of the solvent system and containment vessels should be evaluated for each test material. Some materials can require silanized glassware. 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 preferable used during method validation but can be quite expensive because their production often involves challenging processing certification processes. Therefore, laboratories usually do not use matrix CRMs for routine quality control but reserve them mainly for the estimation of bias in the method validation. Also, the availability is often limited for suitable matrix CRMs for the analysis of chemical residues and contaminants for various analyte/matrix combinations. As a result, laboratories employ alternative

Reference materials serve a variety of purposes in analysis of chemical residues and contaminants. Understanding the advantages and limitations of each will enable successful implementation in the laboratory.

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.

3.1.4 PT samples are primarily used for laboratory comparison during an actual testing round of limited duration in time. However, PT providers often sell unused PT materials, which were previously characterized in a PT interlaboratory comparison, including the information of assigned (mostly consensus mean value) and uncertainty. As opposed to a CRM, the PT samples are usually not characterized with metrological traceability or evaluation of long-term stability. Therefore, laboratories must not use PT samples for trueness evaluation. The consensus mean of a set of PT data has value, but participating laboratories can submit inaccurate values and, as a consequence, 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.

3.1.5 Spiked test portions are prepared by adding a known volume of a spiking solution containing the analyte(s) of interest to a test portion of a matrix test portion that ideally should be free of the target analyte(s) that are spiked. The spike volume should be small enough, so the solvent can be easily absorbed by the test portion matrix (e.g., Spiking a 10g test portion with 50-200 μL contributes less uncertainty than higher volumes). The test portion should be mixed thoroughly after spiking and then allowed to stand for at least 15 min before adding the extraction solvent to provide interaction of the added analytes with the matrix components. One drawback of this approach is that 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.

3.1.6 This best practices manual covers many important aspects related to neat materials, standard solutions and various matrix RMs, including their handling, stability considerations or selection of suitable solvents for standard solutions. The application of these RMs in the analysis of chemical residues and contaminants is crucial in all stages of a method life cycle: from the method development, through method validation to routine analysis, where they mainly serve for calibration of instruments, validation and monitoring of method performance, and for identification and quantitation of analytes.

Application and Use of RMs

3.2 Method Development and Optimization

3.2.1 Method development typically starts with 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 or LC-MS/MS methods, by optimizing compound-specific conditions for optimal sensitivity and selectivity. Ideally, single compound solutions should be used when developing a completely new method to prevent potential compound misidentification and to assess behavior of each individual compound, such as potential formation of degradation products (e.g., in the GC inlet) 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 Matrix effects (signal suppression or enhancement caused by co-extracted matrix components) on the instrument should be taken into account by injecting the analyte(s) of interest in solvent as well as in matrix. If mass spectrometry is used, a Total Ion Chromatogram (TIC) run 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 should be different for the various components or if this is not possible, the retention times should be established such that no overlap of signal is observed during actual test (analytical) sample 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.

3.2.3 The use of internal standards (ISTDs) is a well-established practice to control various steps in the analytical procedure. 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, including 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). If an ISTD is chosen that is not a stable isotope of the analyte.

3.2.4 Optimization: Once the initial determination method is established, the development and optimization of analytical method preparation steps, such as extraction, derivatization, 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, extraction time, temperature or mechanism. These incurred analytical samples do not need to be fully characterized because they serve for a relative comparison of the results obtained using different conditions.

3.2.5 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 or miscibility with the LC mobile phase. 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 taken into account by establishing a specific calibration curve for each instrument on each analysis run.

3.3 Method Validation

3.3.1 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. Validation of quantitative methods requires evaluation of the method accuracy (trueness and precision) and other important parameters, such as linearity, range, limit of quantitation (LOQ), specificity, robustness or matrix effects.

3.3.2 To validate method trueness, matrix CRMs should be used if available. For multiresidue or other multi-analyte methods, few matrix CRMs are available. Therefore, trueness for only a few analyte-matrix combinations can be evaluated with CRMs. Spikes using a calibrant using calibrants that are certified serve as the second best option for evaluation of accuracy (both spike recoveries and precision). The spike concentration levels and number of replicates depend on the purpose of the 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.

3.3.3 The SANTE 12682:2019 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

Application and Use of RMs

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 recoveries should be within the range of 70-120%, with an associated 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). Some regulatory bodies are considering adjusting the acceptable recovery range to 80-120% for some methods.

3.3.4 Incurred Samples: 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 already homogenized CRMs or PT samples) are used.

3.4 Method and/or Laboratory Comparison

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

3.4.2 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 very valuable materials, which sometimes may be further characterized to become CRMs.

3.5 Routine Analysis

3.5.1 Routine analysis of chemical residues and contaminants can be either qualitative or quantitative. Both approaches employ calibration standard solutions (calibrants) but the qualitative methods can use only a calibration level corresponding to the screening detection limit (or reporting limit) whereas quantitative methods typically employ a multi-point calibration.

3.5.2 Calibration of a quantitative measurement (determination) technique can be conducted in a number of ways for the analysis of chemical residues and contaminants.

a) In *solvent-based calibration*, standard solutions are prepared in a solvent (without any matrix present). 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 by the use of analyte protectants. Solvent-based calibration may be employed for screening purposes to obtain estimated levels of analytes in various matrices (especially when multiple different matrices are analyzed in the same batch), followed by more accurate quantitation of positive results (mainly those close to a regulatory limit), such as by using the method of standard addition.

b) In *matrix-matched calibration*, standard solutions are prepared in a blank matrix extract (of the same or very similar matrix as the test (analytical) samples or using a representative matrix). 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 different 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, 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 standard addition. This consideration is especially important in LC-MS, where matrix effects depend on the analyte co-elution with particular matrix components, which can vary significantly among different 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 signal and ion ratios for CALs prepared in solvent versus CALs prepared in matrix is a useful exercise during method development.

c) In *procedural standard calibration*, standard solutions are prepared by spiking multiple aliquots of a blank matrix analytical sample prior to the analytical preparation (prior extraction) with the analyte(s) at multiple concentration levels and then taking these test portions through the entire procedure together 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 too expensive. For instance, procedural standard calibration is often used in the analysis of veterinary drug residues. An important application of procedural calibration involves cases where the analytes need to be derivatized and the derivatization product yield can be matrix

Application and Use of RMs

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*, standards are added at multiple concentration levels to the 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 done using test portion aliquots prior to the extraction, then it also compensates for potential recovery losses. Therefore, standard addition is the 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. 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.

3.5.3 As noted previously, suitable ISTDs should be used in combination with any calibration approach to compensate for any volumetric variations whenever possible. Stable isotopically labeled ISTDs are especially useful because they can eliminate the need for matrix-based calibration approaches as discussed above.

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

3.5.4 Identification: In addition to quantitation, calibration standard solutions also are used for analyte identification. Identification is a crucial step in the analysis of chemical residues and contaminants and should be done before proceeding with analyte quantitation. In chromatographic techniques with mass spectrometry (MS) detection, analyte identification is based on comparison of retention time and the MS spectrum in the test sample with those obtained in 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 analysis¹ and the U.S. FDA Guide 118 for the analysis of veterinary drug residues².

3.5.5 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. 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.

3.6 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 lab and may be a requirement for accreditation of a lab. Proper Standard Operating Procedures (also known as Safe Operating Procedures) or SOPs should be required for all routine functions performed within the laboratory.

3.7 RM Use References

- 1 SANTE/2019/12682, Analytical Quality Control and Method Validation Procedures for Pesticide Residue Analysis in Food and Feed, European Commission, Brussels, Belgium (2020) https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkdoc_2019-12682.pdf.
- 2 U.S. Food and Drug Administration. Guide 118, Mass Spectrometry for Confirmation of the Identity of Animal Drug Residues. U.S. Food and Drug Administration, Center for Veterinary Medicine, Rockville, MD, USA (2003) <https://www.fda.gov/media/70154/download>.

4 RM DOCUMENTATION

4.1 The terminology for documents which accompany RMs can sometimes be misleading or confusing. FIGURE 2 depicts multiple RM documents currently in use. While the requirements for CRM documentation are clearly specified by ISO 17034-2018, the requirements for RM documentation are less defined and can vary greatly.

4.2 The definition of a CRM, found in both ISO 17034 and ISO Guide 30, describes the contents of a RM Certificate (RMC) which includes the value (stated, nominal or certified) of the specified property with an associated uncertainty and a statement of metrological traceability. Additional analytical information for a CRM may be provided in a RM Certification Report (RMCR). An RMC must accompany a CRM but is not required to accompany an RM.

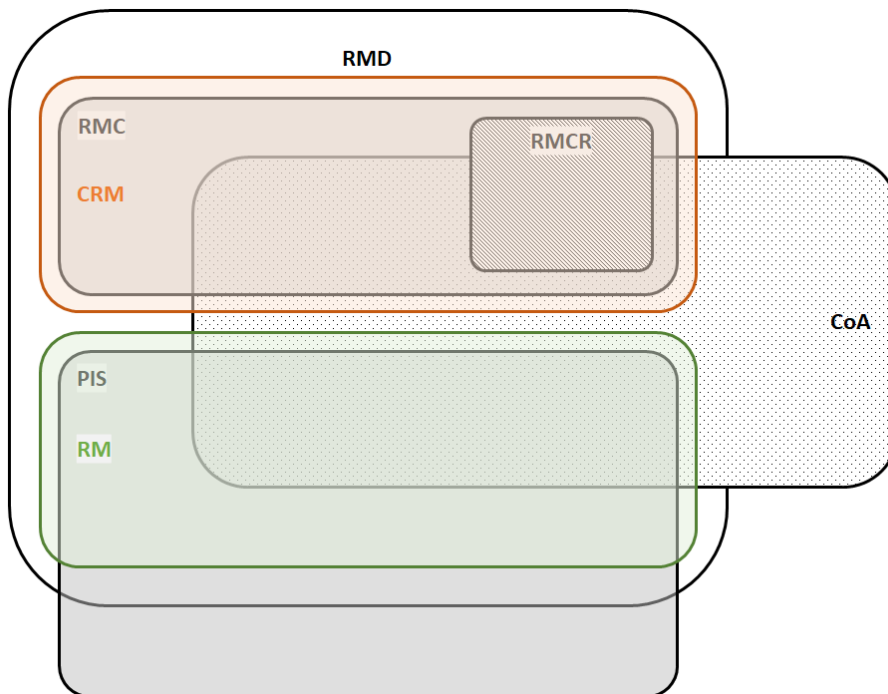


FIGURE 2. RM Documentation

- 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 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.

Reference Material Use in Trace Analysis

4.3 An RM should include documentation describing homogeneity and stability with respect to one or more specified properties and establish fitness for purpose. At the discretion of the RMP, a **Product Information Sheet (PIS)** may accompany an RM and can be called different names including **Certificate of Analysis (CoA)** and **RM Information Sheet (RMIS)**. The term CoA can also be used in other ways such as a report of laboratory non-RM results. Although some RMPs may include a CoA with an RM, the presence of a CoA does not necessarily mean the material is a CRM. The criteria specifically required for a GLP CRM may be provided on the RMC, RMCR or other document.

4.4 An RMC is defined by ISO 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 property values. The contents of an RMC may include additional information as determined necessary by the RMP and may be provided in either a hardcopy or electronic format.

4.5 Additional information may be included with a CRM in a **RMCR** 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. An expiry date (sometimes listed as a re-assay date) should be included for all GLP Certified Materials. If one is not given, one should be assigned per best scientific knowledge and specific Standard Operating Procedures (SOPs) should be in place explaining how this is to be done.

4.6 Example of a typical RMC is shown in Figure 3 illustrating where important information is typically located including RMP, certified value, metrological traceability, identity and purity of compounds, stability and associated uncertainties.


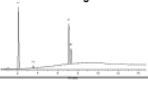
Company Name and Logo		CERTIFIED REFERENCE MATERIAL CERTIFICATE			Company Name and Logo	
Reference Material Producer (RMP)		Authoritative Body who issued Accreditation to RMP, with ILAC Mutual Recognition Number & logo				
Product/Catalog No.:	Product Lot No.:	Package Size/Amount:				
Product Name/Description:	Container Size:	Expiration Date:				
Storage Conditions:	Metrological Traceability of the certified values:					
Compound Name	Elution Order / RT (min)	Concentration (mg/L)	CAS No.	Lot No.	Purity %	Expanded Uncertainty (95% C.I.; K=2)
Compound A	1 / 2:25	100	76-44-8	W-239-19	99.6	+/- 0.7294
Compound B	2 / 3:58	50	309-00-2	X-678-19	98.9	+/- 0.7687
Compound C	3 / 7:15	20	72-20-8	Y-338-19	97.9	+/- 0.7162
Compound D	4 / 7:36	30	672-99-1	Z-994-20	99.2	+/- 5.3346
Certification Statement		Certified By: _____	Certified On: _____	Signature: _____		
Run Conditions Column: _____ Flow Rate: _____ Mobile Phase Composition: _____		Mobile Phase A: _____ Mobile Phase B: _____ Det. Type: _____		Chromatogram 		
Additional information provided in CRM Notes:						
Certified Reference Material Notes, Definitions, Instructions, Etc.						

FIGURE 3. Example of a RMC from a RMP

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.

5.1.2 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

5.1.3 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.

5.1.4 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.

5.1.5 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.

5.1.5 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.

a) 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.

b) 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 portions and 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 and sampling error 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 6498:2012³, smaller the particle sizes were shown to require less test portion mass to achieve lower uncertainty in a test determination.⁵

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

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.

Processing Raw Materials for RMs

TABLE 1. Relative effect of particle size on mass (g) of material with uniform particle size and shape needed to achieve various uncertainty levels for representative test portions.

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

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 standard, 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 an identification or qualification procedure 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)⁴. 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. The purity value of the CRM or SRM used as reference standards in the qNMR analysis, being 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 used in laboratories and can be complementary analytical techniques to qNMR in the identification and determination of the purity value. The high resolution "untargeted" analysis can be used in the search and identification of impurities. Other analytical techniques can be used as an alternative to qNMR for the qualitative identification and for the determination of the 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

Processing Raw Materials for RMs

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. Different 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, 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.

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

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.

5.7 Raw Materials 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 can 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,

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.

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.

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 compendia 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. The use of 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 a SI system, and certified according to schedule if used in 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 Solvent

The choice of solvent and consideration of pH can best be illustrated with 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

Stability and Interactions of RMs

toxicities (the LD₅₀ 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.^{5,6} 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 sulfonamide 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.

6.2.4 Potential Degradation 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.⁷ The ampoules were opened and the contents transferred to the included deactivated vial and stored under recommended conditions (i.e., 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 (< ± 10% of label concentrations), whereas four failures were observed out of 204 compounds in the LC kit (> ± 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 stability of these compounds when after combination into a single 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).

6.2.5 Recommendations

Reference standards should include a RMC or PIS document indicating their 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 reference standards. Care should be taken to minimize evaporation of volatile analytes, and procedures should be implemented to determine when excessive (loss of solvent to the point where concentrations are outside specification range) evaporation has occurred.

6.3 Stability of Multi-Component Mixtures

6.3.1 Most pesticide testing laboratories utilize analytical 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.

6.3.2 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 $\pm 10\%$ of original value criteria is sufficient, however the laboratory's QC procedures should specify such criteria based on the material type and intended uses.

6.3.3 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,

Stability and Interactions of RMs

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 risk of instability across the production of the standard, and the user should be informed as well. Limited data is 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.

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

Pesticide Chemical Class	Pesticide Examples	Conditions for Degradation
N-trihalomethylthio fungicides	Tolyfluanid, 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

6.3.4 Hydrolysis and Oxidation Potential

Certain pesticides are prone to hydrolysis or oxidation during the preparation of multi-component mixtures.^{9,8} 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 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 acetonitrile⁹

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

6.3.5 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 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's breakdown product, and/or chemical interferences (e.g., plasticizers) are ionized simultaneously.

6.3.6 Detecting breakdown: 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

Stability and Interactions of RMs

spectrophotometric detection can offer additional information about analyte behavior and can provide confirmatory data demonstrating stability or lack thereof within a multi-component standard.

6.4 Matrix RMs

6.4.1 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 product information sheet with the assigned values, uncertainties, storage conditions to maintain stability and date range ensuring validity of assigned values in the material.¹⁰ 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 and

screened to be sure it is free of the analyte of interest, or verify the level present is negligible or relatively small in comparison (~95-99%) to the level that is to be measured. The stability of the matrix can supersede the analyte stability; 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.^{11,12,13,14,15,16}

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 a 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 programs well as minimizing unnecessary disposal. ISO Guide 35:2017 describes different experimental studies to access RM stability.

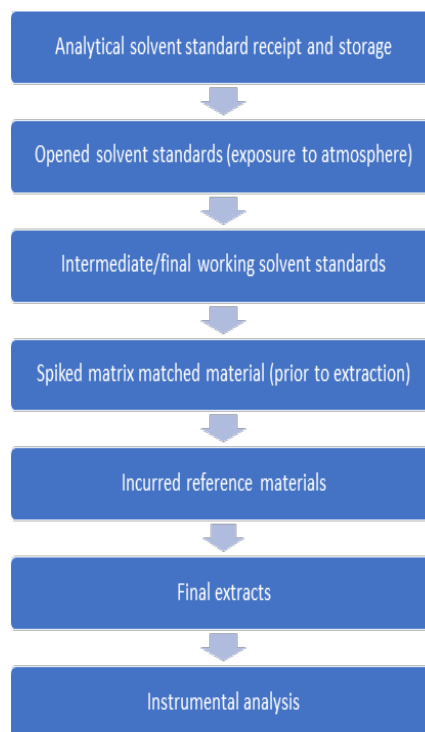
6.5.1 Research Literature

References on stability generally derive from bioanalytical methods for pharmaceutical or forensics research.¹⁷ Limited publications are available for trace level chemical residue and contaminant stability,⁹ though unpublished research has been presented on the topic in various fora. 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.

6.5.2 Scope

Scope should be defined when developing a stability study; consider the simplified analytical process shown in the diagram.

6.5.3 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.^{9,18} 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 The following guidance should be considered prior to conduct any type of stability study:

- 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.
- 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.^{9,9,19} 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.¹⁹ 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.

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 enough 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 it at 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 vialled 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 four time points evaluated using an accelerated aging study would have a total of 36 vialled 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)
-

Stability and Interactions of RMs

6.8 Stability and Interactions References

- 1 WHO Expert Committee on Specifications for Pharmaceutical Preparations: Forty-first Report. WHO Technical Report Series 943, World Health Organization, Geneva, Switzerland (2006).
- 2 Dilek, I., Gates, K., Johnson, R., Pogue, S., Rettinger, M., Sreenivasan, U. Certified Solution Standards and Reagents for Therapeutic Drug Monitoring Applications. Presented at Midwest Association for Toxicology and Therapeutic Drug Monitoring (2009).
- 3 Codex Alimentarius Commission. Guidelines on Good Laboratory Practice in Residue Analysis CXG 40-1993. Joint FAO/WHO Food Standards Programme, Rome, Italy (2010).
- 4 SANTE/2019/12682, Analytical Quality Control and Method Validation Procedures for Pesticide Residue Analysis in Food and Feed, European Commission, Brussels, Belgium (2020) https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkdoc_2019-12682.pdf.
- 5 Sabadie, J. Behavior of Four Sulfonylurea Herbicides in the Presence of Hydroxy Compounds. *J. Agric. Food Chem.* 48 (2000) 4752–4756.
- 6 Boschini, G., D'Agostina, A., Antonioni, C., Locati, D., Arnoldi, A. Hydrolytic degradation of azimsulfuron, a sulfonylurea herbicide. *Chemosphere* 68 (2007) 1312–1317.
- 7 Konschnik, J., Fisher, J., Wiest, L., Rousova Hepner, J., Risha, K. Unlocking the Mystery of Pesticides CRM Stability for Food Analysis. Presented at the European Pesticide Residue Workshop, Virtual (2020).
- 8 Besamusca, E.W., Vreeker, C.P., Toonen, A.A., deKok, A. Validation stability of pesticide standards. Presented at the European Pesticide Residue Workshop, Alkmaar, the Netherlands (1996).
- 9 Dorweiler, K.J., Gurav, J.N., Walbridge, J.S., Ghatge, V.S., Savant, R.H. Determination of Stability from Multicomponent Pesticide Mixes. *J. Agric. Food Chem* 64 (2016) 6108–6124.
- 10 Gates, K., Chang, N., Dilek, I., Jian, H., Pogue, S., Sreenivasan, U. The Uncertainty of Reference Standards—A Guide to Understanding Factors Impacting Uncertainty, Uncertainty Calculations, and Vendor Certifications. *J. Anal. Tox* 33 (2009) 532-539.
- 11 Thiex, N.J., Ramsey, C.A. Taking and Testing GOODSamples: A Systematic Approach for Representative Sampling from Field to Test Portion. *J Regulatory Sci* 02 (2016) 1–8. Also available at <https://www.aafco.org/Publications/GOODSamples>.

- 12 Ambrus, A. Global Harmonization of Maximum Residue Limits for Pesticides. *J. Agric. Food Chem.* 64 (2016) 6071-6081.
- 13 Fussell, R.J., Hetmanski, M.T., Macarthur, R., Findlay, D., Smith, F., Ambrus, A., Brodesser, P.J. Measurement Uncertainty Associated with Sample Processing of Oranges and Tomatoes for Pesticide Residue Analysis. *J. Agric. Food Chem.* 55 (2007) 1062-1070.
- 14 Fussell, R.J., Hetmanski, M.T., Colyer, A., Caldow, M., Smith, F., Findlay, D., Assessment of the stability of pesticides during the cryogenic processing of fruits and vegetables. *Food Additives & Contaminants* 2007, 24 (11), 1247-1256.
- 15 Stephany, R.W., van Ginkel, L.A. Quality criteria for residue analysis and reference materials. Relationship between legal procedures and materials. *Fres. J. Anal. Chem.* 338 (1990) 370-377.
- 16 Whitaker, T.B. Sampling Foods for Mycotoxins. *Food Additives & Cont.* 23 (2006) 50-61.
- 17 Van de Merbel, N., Savoie, N., Yadav, M., Ohtsu, Y., White, J., Riccio, M.F., Dong, K., de Vries, R., Diancin, J. Stability: recommendation for best practices and harmonization from the Global Bioanalysis Consortium Harmonization Team. *The AAPS Journal* 16 (2014) 392-399.
- 18 Mastovska, K., Lehotay, S. Evaluation of common organic solvents for gas chromatographic analysis and stability of multiclass pesticide residues. *J. Chromatogr. A* 1040 (2004) 259–272.
- 19 Schueneman, H., Campos, J. Arrhenius Equation Demystified. Presented at FMS Reliability Webinar (2014). https://slidelegend.com/queue/arrhenius-equation-demystified-westpak_5ae78ab37f8b9a1a798b4585.html (accessed 9-10-2020)

7 RM & CRM HANDLING and DISPOSITION

7.1 RM Handling Requirements

7.1.1 Improper use and handling of RMs 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 analytical testing method requirements are met.

7.1.2 ISO/IEC 17025 chapter 7.4 “Handling of test or calibration items” describes the requirements for using and handling of test and calibration items.¹ Each laboratory is required to have a dedicated procedure for the receipt, handling, 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 adequately packaged and stored in safe areas to prevent damage or deterioration of any material between characterization and distribution to users. Transport, receipt, handling 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.

7.2 Transport

Specific temperature requirements may be required for shipping materials unstable at ambient temperatures. Temperature requirements 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 the information needed to guarantee the traceability of the material at any time (e.g., product name, manufacturer data, product code, batch number, receipt date, expiry date, and location within the laboratory).
- Record and file all documentation according to the QMS procedures.

7.4 RM Handling

7.4.1 Before unboxing the RM, a user should read the safety data sheet and the instructions provided by the manufacturer to safeguard 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 is stored at low temperatures.

7.4.2 Withdrawal of material from the storage container represents the most critical step of handling.

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

For a pure RM in a solid state, the spatula should be washed with a suitable solvent and dried carefully. For liquids, an aliquot should be transferred to a clean container and a test portion withdrawn 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 can 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. The residual amount should not be put back in the original container unless proper closure for storage can be provided. Opened ampoules should be discarded and contents transferred to a deactivated storage container. For materials allowing multiple uses, the container should be securely closed to seal.

7.4.3 Repacking: When a pesticide 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 instance, the use of capillary vials could be efficient for minimizing the risk of contamination and evaporation while

RM & CRM Handling and Disposition

avoiding concentration changes. Glass storage containers 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. Materials with light sensitivity should be stored in opaque or amber storage containers.

7.4.4 Minimum test portion sizes recommended by the RMP should be respected as smaller test portions can be unrepresentative. Re-homogenization of the 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.

7.4.5 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 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 and monitor the stability of the material.

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. For example, a RM 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. 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.

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.⁵

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.^{5,6}

7.6.4 CRM Stability Studies are experiments conducted to assess the period of validity or lifetime of an RM for a specified property value and uncertainty under specific conditions of

time, temperature and packaging. Studies may assess stability during short and long term storage, transportation and applicable conditions of use.⁷

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.⁸

7.6.5 RM Lifetime (or Storage Shelf Life) is the time interval during which RM properties retain their assigned values within their associated uncertainties.^{6,7}

7.6.6 Retest date is the date a RM should be re-examined to ensure that it is still suitable for use.⁸

7.6.7 Monitor the validity of results: ISO Guide 33 7.2 requires that the expiry date on the RMC should be respected and CRMs should not be used beyond this date.³ ISO 17025 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 which may be either a neat material, single component solution, mixture of components in solvent, or mixture of components 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 Variations in the stability among many different compounds 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 is not included with your product documentation, contacting 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 6.2.4 and 6.2.5. 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 sections 7.4.2. and 7.4.3 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 is not supplied in their documentation, contacting your RMP is recommended.

RM & CRM Handling and Disposition

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.
- 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 compound to be tested.
- Non-certified RMs may not provide 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 expiry date provided by the RMP, follow recommendations in section 6.2.5. 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). Following an alternative storage procedure is acceptable if validation demonstrates similar efficacy. It is important to understand that CRMs stated property value uncertainties may vary depending on whether proper storage conditions (e.g. time and temperature) are maintained. Manufacturer provided expiry dates may sometimes be replaced or extended when storage conditions exceed the recommendation.⁴ 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: 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). Duplicate 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. Typically a $\pm 10\%$ of original value criterion is sufficient, however 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 analysis 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 safety and waste procedures or taken out of service for quantitative measurements.

7.7.4 Evaluate purity: Another approach to the quality of a suspect RM is by determining the purity of the starting or neat material. Refer to Chapter 5, Section 5.3. for recommendations on 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 on 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 for future materials to be tested against.

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 disposition. The United States Environmental Protection Agency (US EPA) lists in 40 CFR 261 Subpart D many materials that require specialized disposal methods.



7.8 Handling and Disposition References

- 1 ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories. International Organization for Standardization, Geneva, Switzerland (2017).
- 2 ISO 17034:2016(E) General requirements for the competence of reference material producers. International Organization for Standardization, Geneva, Switzerland (2016).
- 3 ISO Guide 33:2015 Reference materials – Good practice in using reference materials. International Organization for Standardization, Geneva, Switzerland (2015).
- 4 SANTE/2019/12682, Analytical Quality Control and Method Validation Procedures for Pesticide Residue Analysis in Food and Feed, European Commission, Brussels, Belgium (2020)
- 5 ISO Guide 30:2015, Reference materials — Selected terms and definitions. International Organization for Standardization, Geneva, Switzerland (2015).
- 6 ISO Guide 31:2015 Reference materials – Contents of certificates, labels, and accompanying documentation. International Organization for Standardization, Geneva, Switzerland (2015).
- 7 ISO Guide 35:2017 Reference materials. International Organization for Standardization, Geneva, Switzerland (2017).
- 8 OECD GLP #19 Draft OECD GLP advisory document #19 on the management, characterization and use of test items (4 May 2017)

8 RMs PREPARED IN-HOUSE

8.1 CRMs: As described earlier, CRMs are produced in compliance with the guides and standards, ISO 17034¹ 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.

8.2 Laboratory prepared 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). They can be easily adapted for a method modification. A new analyte CAL can be prepared for confirmation of a qualitative identification. Two types of RMs produced in the laboratory are described in the introduction (FIGURE 1).

8.2.1 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 useful for calibration. CALs are usually solvent solutions.

8.2.2 QCMs are used for measurement system for 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.

8.2.3 Both CALs and QCMs should be suitably homogeneous and stable with respect to one or more properties to meet the intended purpose. ISO Guide 80³ 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.

8.3 Using a CRM for Metrological Traceability of CALs

8.3.1 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.

8.3.2 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

RMs Prepared In-house

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.

8.4 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 natural a material which, after processing, is referred to as a matrix.

8.4.1 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 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.
- 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 the chapter on *Second Source Materials*, a compound might only be produced by one manufacturer, so a 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. University 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 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.

8.4.2 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 80³ and in the chapter on *Stability and Interactions of Reference Materials*.

8.4.3 Packaging and Storage

The 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 RT 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

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

RMs Prepared In-house

standards may not be completely soluble at lower temperatures than the ones at which they were prepared.

8.5 Preparation of CALs

8.5.1. 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 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 and making volumetric dilutions.



a) Weighing. Quantitative measurements with an analytical balance calibrated with traceable reference weights are essential. 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 usually agree within 10%. 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 crystallize 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 pesticides 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 affect a smaller number of analytes; less stable analytes can be prepared more often; and concentrates can be 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. 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 a single, weekly or monthly use depending on the laboratory needs and verified stability.
- f) **Working solutions.** Aliquots of intermediate solutions may be vialled for immediate use or added to matrix for in-matrix standards.

8.5.2 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.

8.5.3 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 blank matrix and added to a testing sequence, 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.

8.6 Matrix RMs (QCMs)

8.6.1 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. (see Figure 1)

8.6.2 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 analytical sample

RM's Prepared In-house

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.

8.6.3 AOAC Food Triangle

AOAC 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 4). The developers conceptualized that 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 9 sectors of the food triangle are available to use in conjunction with CALs and QCMs to verify method performance.⁶

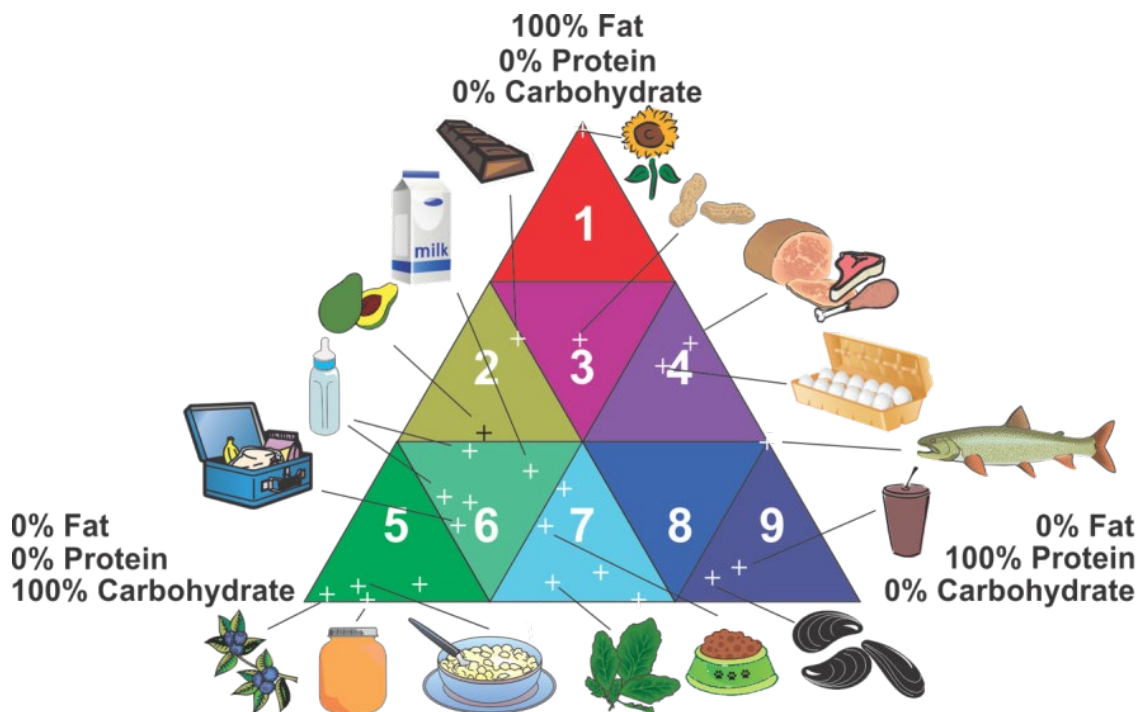


FIGURE 4. AOAC food composition triangle

Reprinted courtesy of the National Institute of Standards and Technology, U.S. Department of Commerce. Not copyrightable in the United States.

8.6.4 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 methods¹⁵, the SANTE guidance document divides food and feed commodities into groups and provides typical commodity categories within each group and also 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

8.6.5 Natural Material Variations

Natural materials (e.g., fruits, vegetables, herbs, spices, dietary supplements, *Cannabis*, 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.

RM's Prepared In-house

8.6.6 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.

8.7 Preparation of QCMs

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

8.7.1 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 can provide a pesticide free matrix but it does not account for possible variations in any natural product.

8.7.2 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 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.

8.7.3 Comminution

Natural materials should be comminuted (e.g., ground, blended, milled, sieved) to a fine particle size to produce sufficient homogeneity. The applicable particle size is often dependent on the test portion size. Test method precision is improved with smaller particle sizes and larger test portion mass.⁸ If processed foods are used, multiple jars or cans should be mixed and comminuted into a uniform batch.

8.7.4 Representative portion sampling

Once comminuted, QCMs are usually aliquoted into multiple storage containers for future use. 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 Samples⁹ and GOOD Test Portions.⁸

8.8 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.

8.8.1 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.

8.8.2 Accurate Concentration

If QCMs are used only to evaluate ongoing method trueness & precision (accuracy) determination is not necessary. For testing to demonstrate compliance with regulatory limits, however, accurate quantitation is necessary. The accuracy of concentration for each analyte may not be determined unless analyzed and verified in comparison to a CRM.¹⁰ 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, then 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.

8.8.3 Homogeneity

A newly prepared QCM is characterized by analyzing multiple replicate portions (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.^{2,3,11} 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).¹²

RM's Prepared In-house

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

- Select at least 10 RM units randomly over the whole batch.
- Measure each RM unit in duplicate.
- 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.
- Conduct all measurements under repeatability conditions (e.g., on the same day, same instrument and same analyst, where possible).
- Correct measurements for analytical drift, as needed.
- Evaluate the between-unit variation using one-way ANOVA. With a well-prepared material, homogeneity 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.).
- When beginning to use a new portion of an RM, compare to the previous RM portion.

8.8.4 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 can 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.

8.8.5 Stability

Storage stability is an essential part of RM characterization. ISO Guides 35 and 80 as well as Lamberty provide detailed instructions for evaluation of stability.^{2,3,13} Often homogeneity and stability may be evaluated together from the same experimental data set.

Even when purchased from a CRM provider, concentrated pesticide 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.

8.8.6 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.^{3,14}

8.8.7 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. Review Chapter 4: RM Documentation for more information.

RMs Prepared In-house

8.9 In-House RM References

- 1 ISO 17034:2016(E) General requirements for the competence of reference material producers. International Organization for Standardization, Geneva, Switzerland (2016).
- 2 ISO Guide 35:2017 Reference materials. International Organization for Standardization, Geneva, Switzerland (2017).
- 3 ISO Guide 80:2014 Guidance for the in-house preparation of quality control materials (QCMs). International Organization for Standardization, Geneva, Switzerland (2017).
- 4 Emons, H. The 'RM family'—Identification of all of its members. *Accred Qual Assur* 10 (2006) 690–691.
- 5 Official Methods of Analysis, 21st Edition, Appendix F. AOAC INTERNATIONAL, Gaithersburg, Maryland, USA (2019).
- 6 Sharpless, K.E., Greenberg, R.R., Schantz, M.M., Welch, M.J., Wise, S.A., Ihnat, M. Filling the AOAC triangle with food-matrix standard reference materials. *Anal. Bioanal. Chem.* 378 (2004) 1161–1167.
- 7 SANTE/2019/12682, Analytical Quality Control and Method Validation Procedures for Pesticide Residue Analysis in Food and Feed, European Commission, Brussels, Belgium (2020)
https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkdoc_2019-12682.pdf.
- 8 AAFCO, Guidance on Obtaining Defensible Test Portions: GOOD Test Portions, AAFCO, Champaign, Illinois, USA (2018). <https://www.aafco.org/Publications/GoodTestPortions>.
- 9 AAFCO, Guidance on Obtaining Defensible Samples: GOOD Samples, AAFCO, Champaign, Illinois, USA (2015). <https://www.aafco.org/Publications/GOODSamples>.
- 10 Linsinger, T. Application Note 1, Comparison of a Measurement Result with the Certified Value. European Reference Materials, European Commission - Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel, Belgium (2010).
- 11 Pauwels, J., Lamberty, A. Homogeneity testing of reference materials. *Accredit Qual Assur* 3 (1998) 51–55.
- 12 Dabrio, M., Grimalt Brea, S., Shegunova, P., Harbeck, S., Sejerøe-Olsen, B., Fernández-Alba, A.R., Seghers, J. The certification of the mass fraction of pesticides in cucumber: ERM®-BC403. European Commission Joint Research Centre Directorate F – Health, Consumers and Reference Materials, Geel, Belgium (2018).
- 13 Lamberty, A., Schimmel, H., Pauwels, J. The study of the stability of reference materials by isochronous measurements. *Fresenius J Anal Chem* 360 (1998) 359–361.
- 14 ISO Guide 31:2015 Reference materials – Contents of certificates, labels, and accompanying documentation. International Organization for Standardization, Geneva, Switzerland (2015).
- 15 Guidance Document on Pesticide Residue Analytical Methods Series on Testing and Assessment **No. 72** / Series on Pesticides No. 39 [ENV/JM/MONO\(2007\)17](ENV/JM/MONO(2007)17) (accessed 9-10-2020)

9 VERIFICATION USING RMs from a SECOND SOURCE

9.1 Is a Second Source Needed?

9.1.1 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.

9.1.2 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.

9.2 Acceptable second source quantitative verification criteria

9.2.1 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.

9.2.2 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.

Second Source RMs

- Identify analyte interactions in a complex mixture.
- Verify storage stability in complex mixtures.

9.3 The terminology “second source” is not always clear.

9.3.1 A second lot is sometimes used to refer to a second source RM or CRM. ISO Guide 30(E) 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(E) definition of “lot”

9.3.2 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*), 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.

9.3.3 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 $\pm 15\%$ 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.

9.3.4 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 plant, 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.

9.3.5 General Observations and Recommendations

- If calibration solutions (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.

Second Source RMs

9.4 Second Source References

- 1 EPA-600/4-88/039 Methods for the Determination of Organic Compounds in Drinking Water. Environmental Monitoring Systems Laboratory, Office of Research and Development, US Environmental Protection Agency, Cincinnati, Ohio, USA (1991).
- 2 Constitution, Bylaws, and Standards. National Environmental Laboratory Accreditation Conference. US Environmental Protection Agency, Washington, DC, USA (1997).
- 3 Constitution, Bylaws, and Standards. National Environmental Laboratory Accreditation Conference. US Environmental Protection Agency, Washington, DC, USA (1999).
- 4 AFCEE Long-Term Monitoring Optimization Guide Version 2.0. Air Force Center for Environmental Excellence, Brooks Air Force Base, Brooks City, Texas, USA (1997).
- 5 Smith, R.K. Benzidine? Really? Presented at the 14th Annual Water Testing & Quality Assurance Symposium (WTQA), Arlington, Virginia, USA (1998).
- 6 Environmental Quality: Guidance for Evaluating Performance-based Chemical Data EM-200-1-10. U.S. Army Corps of Engineers (USACE), Washington, DC, USA (2005).
- 7 EPA Method 200.8: Determination of Trace Elements in Waters and Wastes by Inductively Coupled Plasma – Mass Spectrometry (Revision 5.4). US Environmental Protection Agency, Cincinnati, Ohio, USA (1994).
- 8 ISO Guide 30:2015, Reference materials — Selected terms and definitions. International Organization for Standardization, Geneva, Switzerland (2015).
- 9 ISO Guide 31:2015 Reference materials – Contents of certificates, labels, and accompanying documentation. International Organization for Standardization, Geneva, Switzerland (2015).

10 MEASUREMENT UNCERTAINTY

10.1 What is Uncertainty?

One of the most important properties of a CRM is the statement of a certified value including an associated combined uncertainty of the certified property value.

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” according to the Eurachem/CITAC Guide,¹

Uncertainty in a measurement quantity is a result both 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 To estimate the overall uncertainty, the contribution from each source, termed an uncertainty component, can be determined in a bottom-up approach.

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. In section 10.9 both of these uncertainties are used and explained. **Other factors which contribute to measurement uncertainty are sampling uncertainty and human error. Unfortunately these factors are frequently not evaluated when estimating measurement uncertainty but 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.**

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.

10.2 Bottom-Up Approach for Uncertainty Estimation

Uncertainty components are described as a **standard uncertainty (u_j)**, which is a measurement uncertainty expressed as a standard deviation. The uncertainty then of a final measurement is a result of combining all these uncertainty components expressed as relative standard uncertainties. This combination of uncertainty components is referred to as **combined standard uncertainty (u_c)**.

10.2.1 In analytical chemistry, the range in which a final measured true value lies should be known with a high level of confidence. To obtain this, the combined standard uncertainty (u_c) is multiplied by a **coverage factor (k)** to obtain **expanded uncertainty (U)**. The coverage factor (k) is chosen based on the

Measurement Uncertainty

level of confidence desired. For example, for a commonly used 95% confidence limit, k is set to a value of 2.

10.2.2 Uncertainty is not the same as bias, although the two are typically confused, and thus uncertainty should not be used to correct analytical results. Bias is the difference between a measured average value and the true value. Uncertainty, describes dispersion in a measurement as a result of a contribution of factors.¹

10.2.3 Estimation of combined standard uncertainty (u_c) can be described as

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

u_c combined standard uncertainty
 u_j standard uncertainty
 j number of uncertainty components

where j is the number of uncertainty components and u_j is the standard uncertainty for each of these contributions. To estimate u_c , each source of uncertainty component should be identified and u_j determined.

10.2.4 The combined standard uncertainty of a CRM comes typically from contributions of characterization, homogeneity and stability as described in Equation 3 and the variables below.

$$u_{CRM} = \sqrt{u_{char}^2 + u_{hom}^2 + u_{stab}^2} \quad [3]$$

u_{CRM} Combined standard uncertainty for a certified value of a CRM
 u_{char} Uncertainty deriving from the characterization measurements
 u_{hom} Uncertainty from inhomogeneity 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, which is in compliance with the ISO norms. The combined standard uncertainty will be multiplied by a coverage factor k (Equation 4).

$$U_{CRM} = u_{CRM} \times k \quad [4]$$

k coverage factor (t student factor can also be used)

In most cases, a normal distribution with a sufficient number of measurements can be applied and a confidence interval of 95% ($k = 2$) is used. Alternatively, the student t factor can be used as the coverage factor.

10.3 Top-Down Approach for Uncertainty Estimation

While the bottom-up approach for uncertainty estimation is the gold standard, such an empirical evaluation is often difficult in a practical setting for solutions, mixtures and matrix materials.

The top-down approach can be used, provided the sources of uncertainty are adequately identified and accounted for in the estimation of combined standards uncertainty. The reader can find an example of this approach described in section 3 of the Nordtest Report TR 537e⁷.

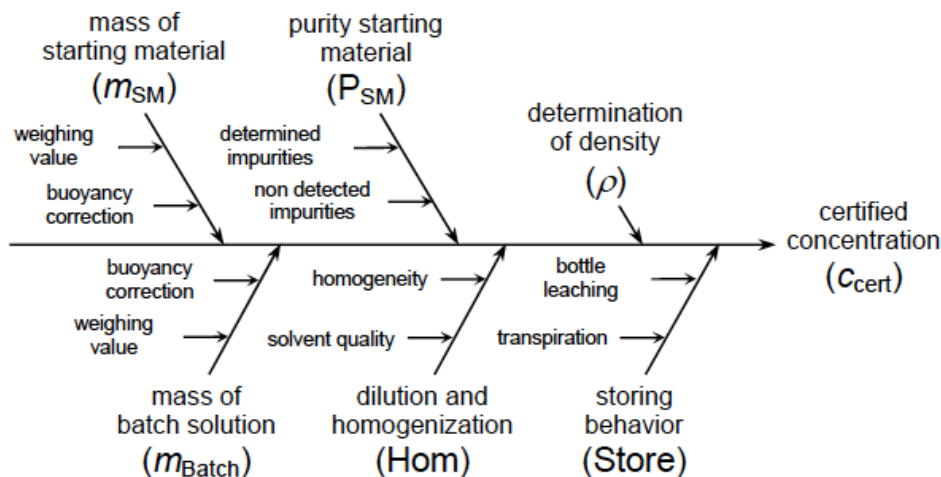
10.4 Assessment of Uncertainty associated with Certified Values

10.4.1 RMP requirements: ISO 17034 accreditation confirms competence as a RMP for a specific scope.³ Developed and published by ISO, ISO 17034 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.⁴

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

10.4.2 Statistical data: ISO Guide 35 outlines the principles for the estimation of uncertainty for a certified value.⁵ 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. This data includes 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. This measurement data set is used in the process of calculating combined standard uncertainty (u_c) for CRM mass fractions or concentrations. The expanded uncertainty (U) for the CRM is then

estimated from the combined standard uncertainty (u_c) by applying the coverage factor (k) described previously.



Measurement Uncertainty

FIGURE 5. Cause and effect diagram representing contributing factors to uncertainty in manufacture and qualification of a gravimetrically prepared standard solution

10.4.3 Contributing factors: An example of this approach for a gravimetrically prepared CRM is illustrated below in

FIGURE 5. The certified concentration is the value for which U should be determined. The individual components that contribute to the combined standard uncertainty (u_c) of the CRM concentration have been identified and grouped based on measured data. For example, buoyancy correction and weighing value are measurements that can be used in determination of the contribution to u_c from mass of the starting material and mass of the batch solution (in the case of a CRM which is a solution).

10.5 Uncertainty of the (In)Homogeneity

10.5.1 Contributions to in-homogeneity: In general, the in-homogeneity of an analytical sample cannot be looked at as an isolated attribute although it is possible to quantify heterogeneity down to a specific level dictated by the precision of the measurement method.⁶ The deviation or variance of measurement data always includes contributions from the measurement and inhomogeneity. For that reason, methods with the highest precision should be applied for the determination of the homogeneity. These methods can differ from that used for characterization of the material. Very small inhomogeneities can only be determined with high precision measurement techniques such as coulometry, isotope dilution MS, titrimetric approach or quantitative NMR. In addition to the measurement technique, appropriate test portion size and number of repetitions per test sample are crucial to achieve an accurate determination of the homogeneity. Through analysis of variances (ANOVA), uncertainty contributions can be determined very precisely.

10.5.2 within bottle homogeneity (u_{wb}): ISO Guide 35 describes two inhomogeneities that may be present in a produced lot: inhomogeneity between bottles or units and inhomogeneity within bottles or units. The **within bottle homogeneity (u_{wb})** is very closely related to the minimal test portion size in 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.

10.5.3 Between bottle homogeneity (u_{hom}): In addition to the determination of the inhomogeneity within a unit, the homogeneity between bottles should be determined (u_{hom}) as seen in equation 3. According to ISO Guide 35, for a normal batch size, 10 to 30 test portions are chosen randomly from the packaged lot. Lot size dependent sample numbers are between the cubic root of the number of units produced and three times this number and preferable 10 units as a minimum. Any analytical data set

used for the assessment of an uncertainty contribution from inhomogeneity should be free from artifacts and the measurement data should not show significant trending. The measurements should be performed in random order with respect to the test portion's position in the filling sequence. Repeatability conditions are preferred for as high precision as possible. If trends in the analytical sequence are detected by plotting the data in the order of analysis, it is possible to correct for analytical drift before the final heterogeneity is quantified.

10.5.4 Factorial analysis: The determination of the uncertainty contribution from inhomogeneity is based on a factorial analysis of variances as described in the equations below. Evaluation of homogeneity testing is easy to do using one-way ANOVA in Excel.

$$n_0 = \frac{1}{a-1} \left[\sum_{i=1}^a n_i - \frac{\sum_{i=1}^a n_i^2}{\sum_{i=1}^a n_i} \right] \quad [5]$$

In the simplest example the uncertainty from inhomogeneity is equal to u_{bb} and

$$u_{bb}^2(\text{max}) = s_{bb}^2 + \frac{s_r^2}{n} \quad [6]$$

s_{bb}	Deviation from inhomogeneity of test portions between bottles
s_r	Standard deviation
u_{bb}	Uncertainty from inhomogeneity between bottles
n, n_0	Number of measurements

10.6 Uncertainty of the Stability

10.6.1 Uncertainty includes storage and transport: Throughout the entire shelf life of a product, the certified property value should remain within the range of its overall uncertainty. In order to assess the impact of storage and transport to the overall uncertainty, stability studies should be performed.

10.6.2 Factors: 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). The most critical factor however is storage and shipping temperature.

10.6.3 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 testing⁷, in which units exposed to difference 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

Measurement Uncertainty

the possibility of detecting potential trends originating from ever so slight degradation of the certified parameters.⁵

10.6.3 Long & short-term stability: ISO 17034:2016 describes two ways to address stability assessments, which differ in the thermal stress for the analytical sample.³ All experiments should be carried out according to the same guidance as the characterization and assessment of the inhomogeneity.

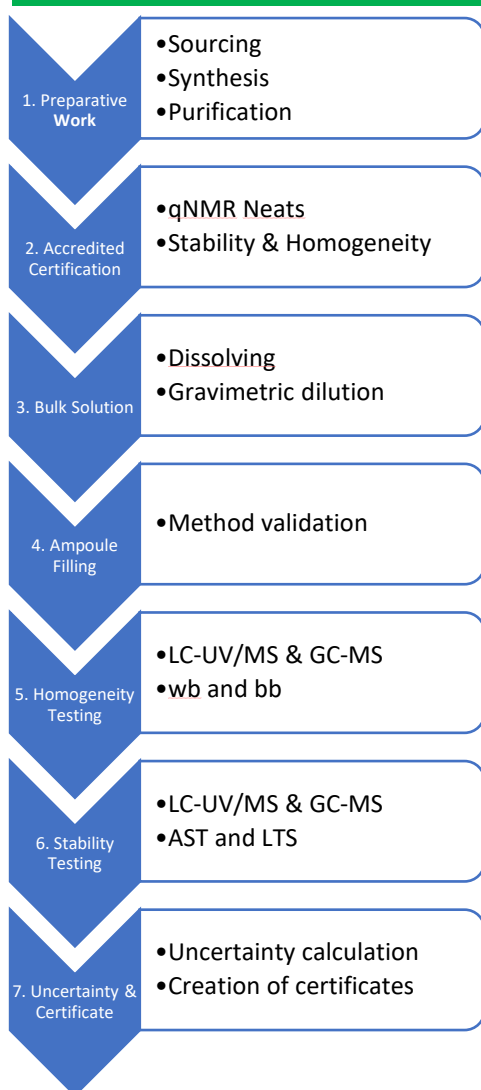
A long-term stability study that covers the shelf life of the CRM.

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.

10.6.4 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 was 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.

10.7 Uncertainties of Solutions, Mixtures and Matrix Materials

10.7.1 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.⁸



10.7.2 Lot and mixture uncertainty: Each of these steps 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.^{6,9}

10.8 Reporting Uncertainty for a CRM

The main differentiator between a RM and a CRM is the assessment of uncertainty and the metrological traceability statement.¹⁰ 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).¹¹ 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 FIGURE 6.¹²

FIGURE 6. Steps in producing a CRM solution

The combined uncertainty is reported along with the contributions from groups of measured data such as mass of starting material (m_{sm}), mass of batch solution (m_{batch}), etc. In this example, 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.

Measurement Uncertainty

10.9 Practical Use of Uncertainty Values

10.9.1 Use CRM to evaluate method performance: The uncertainty value reported for a CRM can be used as part of an evaluation of method performance. 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 Linsinger.¹³

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

$$\Delta_m = |C_m - C_{CRM}| \quad [7]$$

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

$$u_\Delta = \sqrt{u_m^2 + u_{CRM}^2} \quad [8]$$

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

$$U_\Delta = 2 \times u_\Delta \quad [9]$$

4. **Compare Δ_m to U_Δ .**
5. **If $\Delta_m < U_\Delta$, the difference between the measured and certified values is insignificant.**

10.9.2 Example using CRM uncertainty: In another scenario, if the overall measurement uncertainty for a specific analytical method is to be estimated, the contribution from any RMs used for quantitation should be included. As described previously, if a CRM is used, the uncertainty value can be obtained from the RMC. To use this value in estimation of overall method uncertainty, the value may need to be converted into a combined standard uncertainty (u_c), for example, if the value is expressed as an expanded uncertainty (U) or as a confidence interval. For a 95% confidence level, assuming $k = 2$, the standard uncertainty is taken to be one half of the expanded uncertainty. For example, the RMC of a CRM solution states the combined expanded uncertainty, reported at a 95% confidence level, is (100 ± 0.6) mg/L. To estimate the combined standard uncertainty (u_c) for the CRM,

$$u_c = \frac{U}{2} = \frac{0.6 \text{ mg/L}}{2} = 0.3 \text{ mg/L} \quad [10]$$

In percent, the uncertainty would be

$$u_c = \frac{0.3 \text{ mg/L}}{100 \text{ mg/L}} \times 100\% = 0.3\% \quad [11]$$

When estimating the combined standard uncertainty for the entire method in which the CRM is used, the contribution from the CRM in this case is 0.3%. This value can then be used along with other standard uncertainties to estimate u_c for the measurement made using the analytical method.

10.10 Measurement Uncertainty References

- 1 Eurachem/CITAC Guide CG4, Quantifying Uncertainty in Analytical Measurement, 3rd Edition (2012). https://www.eurachem.org/images/stories/Guides/pdf/QUAM2012_P1.pdf.
 - 2 G104 - A2LA Guide for Estimation of Measurement Uncertainty in Testing. A2LA, Frederick, Maryland, USA (2019).
 - 3 ISO 17034:2016(E) General requirements for the competence of reference material producers. International Organization for Standardization, Geneva, Switzerland (2016).
 - 4 Kiefer, K., Walbridge, J. Consumer Beware: Is your Certified Reference Material Really Certified? Presented at BERM, National Harbor, Maryland, USA (2015).
 - 5 ISO Guide 35:2017 Reference materials. International Organization for Standardization, Geneva, Switzerland (2017).
 - 6 Linsinger, T.P.J. Evaluation of CRM Homogeneity in Cases of Insufficient Method Repeatability: Comparison of Bayesian Analysis with Substitutes for ANOVA Based Estimates. *Anal. Chim. Acta*: X 5 (2020) 100049.
 - 7 Lamberty, A., Schimmel, H., Pauwels, J. The Study of the Stability of Reference Materials by Isochronous Measurements. *Fresenius J Anal Chem* 360 (1998) 359–361.
 - 8 Magnusson, B.; Mäykki, T.; Hovind, H.; Krysell, M.; Nordtest Report TR 537. *Handbook for Calculation of Measurement Uncertainty in Environmental Laboratories*. Finland, 2003. <http://www.nordtest.info/index.php/technical-reports/item/handbook-for-calculation-of-measurement-uncertainty-in-environmental-laboratories-nt-tr-537-edition-3.html>
 - 9 Gates, K., Chang, N., Dilek, I., Jian, H., Pogue, S., Sreenivasan, U. The Uncertainty of Reference Standards—A Guide to Understanding Factors Impacting Uncertainty, Uncertainty Calculations, and Vendor Certifications. *J. Anal. Tox* 33 (2009) 532-539.
 - 10 How to Choose the Correct Reference Material Quality Grade for Your Needs? MilliporeSigma (accessed 2020) <https://www.sigmaaldrich.com/technical-documents/articles/analytical/how-to-choose-the-correct-reference-material-quality-grade.html>.
 - 11 ISO Guide 31:2015 Reference materials – Contents of certificates, labels, and accompanying documentation. International Organization for Standardization, Geneva, Switzerland (2015).
 - 12 Kocherlakota, N., Obenauf, R., Thomas, R. A Statistical Approach to Reporting Uncertainty on Certified Values of Chemical Reference Materials for Trace Metal Analysis. SPEX CertiPrep, Metuchen, New Jersey, USA (2017). <https://www.spexcertiprep.com/knowledge-base/files/WhitePaper-StatisticalApproachToReportingUncertaintyOnCertifiedValuesOfCRMs.pdf>.
 - 13 Linsinger, T. Application Note 1, Comparison of a Measurement Result with the Certified Value. European Reference Materials, European Commission - Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel, Belgium (2010).
-

11 GLOSSARY

11.1 Glossary Sources

The glossary contained in the following pages is a collection of terms deemed relevant to this document and its user group has been assembled from various resources, including the *RM Guidelines* published by the AOAC Technical Division on Reference Materials¹, the Eurachem Guides on *The Selection and Use of Reference Materials* and *Terminology in Analytical Measurement*^{2,3}, Guidelines for the Validation of Chemical Methods for the FDA Foods Program⁴, International Vocabulary of Metrology (VIM)⁵, ISO 17034:2016(E)⁶, and ISO Guide 30⁷. 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.

11.2 DEFINITIONS

Term	Definition	Source
Accuracy	<ul style="list-style-type: none"> • 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 test method, accuracy refers to a combination of trueness and precision. • Note that it is common practice to refer to both “<i>accuracy and precision</i>” 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.” • <u>In this document, accuracy = trueness & precision</u> 	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

Reference Material Use in Trace Analysis

Term	Definition	Source
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.	GTP ¹⁴
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. Blanks can be further classified as method blanks, matrix blanks, reagent blanks, instrument blanks, and field blanks.	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 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.	

Glossary

Term	Definition	Source
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 produced and distributed by the National Institute of Standards and Technology (NIST).	ISO 17034 ⁶ ISO GUIDE 30 ⁷
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
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	Committe 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)	
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 confidence level.	VIM ⁵
Coverage Probability	Probability that the set of true quantity values of a measurand is contained within a specified coverage interval.	VIM ⁵
Error	Measured quantity value minus a reference quantity value.	VIM ⁵
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

Reference Material Use in Trace Analysis

Term	Definition	Source
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
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	Uniformity of a specified property value throughout a defined portion of a RM.	ISO GUIDE 30 ⁷
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
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

Glossary

Term	Definition	Source
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)	<p>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 by the internal standard. The internal standard should have similar physico-chemical properties to those of the analyte.</p> <p>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.</p>	<p>FDA⁴</p> <p>SANTE 12682:2019¹⁰</p>
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 ¹⁰
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

Reference Material Use in Trace Analysis

Term	Definition	Source
Laboratory Sample	The material receive by the laboratory	GTP ¹⁴
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 <i>Minimum Detectable Concentration</i> .	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	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

Glossary

Term	Definition	Source
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
Method 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

Reference Material Use in Trace Analysis

Term	Definition	Source
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)	means an absolute requirement (<i>within this document</i>) Must not means an absolute no.	SANTE 12682-2019 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.	
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

Glossary

Term	Definition	Source
Portion	An amount, section or part of the whole (i.e. of the material being sampled)	Macmillan Dictionary.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 <i>Random Error</i> . Precision may be further classified as Repeatability, Intermediate Precision, and Reproducibility.	FDA ⁴
Primary Sample	The material selected from a decision unit	GTP ¹⁴
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
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.	
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 Emons
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

Reference Material Use in Trace Analysis

Term	Definition	Source
Random Error	Component of measurement error that in replicate measurements varies in an unpredictable manner. See also <i>Precision</i> .	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.	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 marginal recovery based only on the added analyte (the native analyte is subtracted from both the numerator and denominator).	FDA ⁴ AOAC ¹
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 homogeneity and stability testing, confirmation of identity, and binary testing results (yes/no or presence/absence) related to the overall fitness for purpose of the material.	
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

Glossary

Term	Definition	Source
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.	
Reference Standard (Measurement Standard or Standard)	<p>A substance of known identity and purity, generally with a certificate of quality from an authoritative body and used to prepare calibration standards.</p> <p>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</p>	FDA ⁴ 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

Reference Material Use in Trace Analysis

Term	Definition	Source
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 ¹²
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 <i>Secondary Source</i> .	
Secondary Standard	Measurement standard whose property value is assigned by comparison with a primary measurement standard of the same property or quantity. See also <i>Secondary Source</i> .	ISO GUIDE 30 ⁷

Glossary

Term	Definition	Source
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 (<i>In this document it will be used only when referring to an accreditation standard or an official government regulation.</i>)	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
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
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

Reference Material Use in Trace Analysis

Term	Definition	Source
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
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	GTP ¹⁴
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
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
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

11.3 Glossary References and Other Resources

1. RM Guidelines. AOAC INTERNATIONAL Technical Division on Reference Materials (TDRM), Gaithersburg, Maryland, USA (2015). <https://www.aoac.org/membership/tdrm/>
2. Eurachem (2002) <https://www.eurachem.org/index.php/publications/guides/usingrm>.
3. Barwick, V.J., Prichard, E., Eds. Eurachem Guide: Terminology in Analytical Measurement – Introduction to VIM 3. Eurachem (2011) <https://www.eurachem.org/index.php/publications/guides/terminology-in-analytical-measurement>.
4. Guidelines for the Validation of Chemical Methods in Food, Feed, Cosmetics and Veterinary Products, 3rd ed., US Food and Drug Administration Foods Program, Rockville, Maryland, USA (2019) <https://www.fda.gov/media/81810/download>.
5. 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>.

Glossary

6. ISO 17034:2016(E) General requirements for the competence of reference material producers. International Organization for Standardization, Geneva, Switzerland (2016).
 7. ISO Guide 30:2015, Reference materials — Selected terms and definitions. International Organization for Standardization, Geneva, Switzerland (2015).
 8. Emons, H. The 'RM family'—Identification of all of its members. *Accred Qual Assur* 10 (2006) 690–691.
 9. Magnusson, B., Näykk, T., Hovind, H., Krysell, M. *NORDTEST Handbook for calculation of measurement uncertainty in environmental laboratories (NT TR 537 - Edition 3.1)*. Taastrup, Denmark (2012). <http://www.nordtest.info/index.php/technical-reports/item/handbook-for-calculation-of-measurement-uncertainty-in-environmental-laboratories-nt-tr-537-edition-3.html>.
 10. SANTE 12682:2019, Analytical Quality Control and Method Validation Procedures for Pesticide Residue Analysis in Food and Feed, EURL
 11. SI Units. National Institute of Standards and Technology (NIST), Gaithersburg, Maryland, USA (2019). <https://www.nist.gov/pml/weights-and-measures/metric-si/si-units>.
 12. Official Methods of Analysis, 21st Edition, Definition of Terms and Explanatory Notes. AOAC INTERNATIONAL, Gaithersburg, Maryland, USA (2012). <http://eoma.aoac.org>.
 13. Thiex, N.J., Ramsey, C.A. Taking and Testing GOODSamples: A Systematic Approach for Representative Sampling from Field to Test Portion. *J Regulatory Sci* 02 (2016) 1–8. Also available at <https://www.aafco.org/Publications>.
 14. Sharpless, K.E., Lippa, K.A., Duewer, D.L., Ruhkin, A.L. NIST Special Publication 260-181: The ABCs of Using Standard Reference Materials in the Analysis of Foods and Dietary Supplements: A Practical Guide. National Institute of Standards and Technology (NIST), Gaithersburg, Maryland, USA (2014) <https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-181.pdf>.
 15. ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories. International Organization for Standardization, Geneva, Switzerland (2017).
 16. Official Methods of Analysis, Appendix E, 2005, Laboratory Quality Assurance, AOAC INTERNATIONAL, Gaithersburg, Maryland, USA http://www.eoma.aoac.org/app_e.pdf
 17. OECD GLP #19 Draft (4 May 2017) OECD GLP advisory document #19 on the management, characterization and use of test items
 18. ISO Guide 35:2017 Reference materials. International Organization for Standardization, Geneva, Switzerland (2017).
 19. U.S. Dept. of Energy, OSIT, (19 May-2017) Guideline on Isotope Dilution Mass Spectrometry, DOI: [10.2172/1358328](https://doi.org/10.2172/1358328) .
 20. Oxford Dictionary (on-line), <https://www.oxfordlearnersdictionaries.com/definition/english/aliquot>
-

12 COMPILED REFERENCES

AAFCO, Guidance on Obtaining Defensible Test Portions: GOOD Test Portions, AAFCO, Champaign, Illinois, USA (2018). <https://www.aafco.org/Publications/GoodTestPortions>.

AAFCO, Guidance on Obtaining Defensible Samples: GOOD Samples, AAFCO, Champaign, Illinois, USA (2018). <https://www.aafco.org/Publications/GOODSamples>.

AFCEE Long-Term Monitoring Optimization Guide Version 2.0. Air Force Center for Environmental Excellence, Brooks Air Force Base, Brooks City, Texas, USA (1997).

Ambrus, A. Global Harmonization of Maximum Residue Limits for Pesticides. *J. Agric. Food Chem.* 64 (2016) 6071-6081.

Barwick, V.J., Prichard, E., Eds. Eurachem Guide: Terminology in Analytical Measurement – Introduction to VIM 3. Eurachem (2011) <https://www.eurachem.org/index.php/publications/guides/terminology-in-analytical-measurement>.

Besamusca, E.W., Vreeker, C.P., Toonen, A.A., deKok, A. Validation Stability of Pesticide Standards. Presented at the European Pesticide Residue Workshop, Alkmaar, the Netherlands (1996).

Boschin, G., D'Agostina, A., Antonioni, C., Locati, D., Arnoldi, A. Hydrolytic Degradation of Azimsulfuron, a Sulfonylurea Herbicide. *Chemosphere* 68 (2007) 1312–1317.

Code of Federal Regulations, Title 21, Part 58 – Good Laboratory Practice for Nonclinical Laboratory studies. <https://www.ecfr.gov> (accessed 9-21-2020)

Codex Alimentarius Commission. Guidelines on Good Laboratory Practice in Residue Analysis CXG 40-1993. Joint FAO/WHO Food Standards Programme, Rome, Italy (2010).

Constitution, Bylaws, and Standards. National Environmental Laboratory Accreditation Conference. US Environmental Protection Agency, Washington, DC, USA (1997).

Constitution, Bylaws, and Standards. National Environmental Laboratory Accreditation Conference. US Environmental Protection Agency, Washington, DC, USA (1999).

Dabrio, M., Grimalt Brea, S., Shegunova, P., Harbeck, S., Sejerøe-Olsen, B., Fernández-Alba, A.R., Seghers, J. The Certification of the Mass Fraction of Pesticides in Cucumber: ERM[®]-BC403. European Commission Joint Research Centre Directorate F – Health, Consumers and Reference Materials, Geel, Belgium (2018).

Compiled References

Dilek, I., Gates, K., Johnson, R., Pogue, S., Rettinger, M., Sreenivasan, U. Certified Solution Standards and Reagents for Therapeutic Drug Monitoring Applications. Presented at Midwest Association for Toxicology and Therapeutic Drug Monitoring (2009).

Dorweiler, K.J., Gurav, J.N., Walbridge, J.S., Ghatge, V.S., Savant, R.H. Determination of Stability from Multicomponent Pesticide Mixes. *J. Agric. Food Chem* 64 (2016) 6108–6124.

Emons, H. The 'RM family'—Identification of A(?)ll of its Members. *Accred Qual Assur* 10 (2006) 690–691.

Environmental Quality: Guidance for Evaluating Performance-Based Chemical Data EM-200-1-10. U.S. Army Corps of Engineers (USACE), Washington, DC, USA (2005).

EPA Method 200.8: Determination of Trace Elements in Waters and Wastes by Inductively Coupled Plasma – Mass Spectrometry (Revision 5.4). US Environmental Protection Agency, Cincinnati, Ohio, USA (1994).

EPA-600/4-88/039 Methods for the Determination of Organic Compounds in Drinking Water. Environmental Monitoring Systems Laboratory, Office of Research and Development, US Environmental Protection Agency, Cincinnati, Ohio, USA (1991).

ERM App #3, Using Reference Materials to Establish Metrological Traceability, (2008-02)
<http://www.erm-crm.org/>

Eurachem Guide: The Selection and Use of Reference Materials. Eurachem (2002)
<https://www.eurachem.org/index.php/publications/guides/usingrm>.

Eurachem/CITAC Guide CG4, Quantifying Uncertainty in Analytical Measurement, 3rd Edition (2012).
https://www.eurachem.org/images/stories/Guides/pdf/QUAM2012_P1.pdf.

Eurachem, Terminology in Analytical Measurement: Introduction to VIM 3 (2011)
https://www.eurachem.org/images/stories/Guides/pdf/TAM_2011_Final_web.pdf

Fussell, R.J., Hetmanski, M.T., Macarthur, R., Findlay, D., Smith, F., Ambrus, A., Brodesser, P.J. Measurement Uncertainty Associated with Sample Processing of Oranges and Tomatoes for Pesticide Residue Analysis. *J. Agric. Food Chem.* 55 (2007) 1062-1070.

Fussell, R.J., Hetmanski, M.T., Colyer, A., Caldow, M., Smith, F., Findlay, D.,. Assessment of the stability of pesticides during the cryogenic processing of fruits and vegetables. *Food Additives & Contaminants* 2007, 24 (11), 1247-1256.

G104 - A2LA Guide for Estimation of Measurement Uncertainty in Testing. A2LA, Frederick, Maryland, USA (2019).

Gates, K., Chang, N., Dilek, I., Jian, H., Pogue, S., Sreenivasan, U. The Uncertainty of Reference Standards—A Guide to Understanding Factors Impacting Uncertainty, Uncertainty Calculations, and Vendor Certifications. *J. Anal. Tox* 33 (2009) 532-539.

Guidelines for the Validation of Chemical Methods for the FDA FVM Program, 2nd Ed. US Food & Drug Administration Office of Foods and Veterinary Medicine, Rockville, Maryland, USA (2015).

How to Choose the Correct Reference Material Quality Grade for Your Needs? MilliporeSigma (accessed 2020) <https://www.sigmaaldrich.com/technical-documents/articles/analytical/how-to-choose-the-correct-reference-material-quality-grade.html>. ISO Guide 31:2015 Reference materials – Contents of certificates, labels, and accompanying documentation. International Organization for Standardization, Geneva, Switzerland (2015).

ILAC PT10:07/2020 ILAC Policy on Metrological Traceability of Measurement Results, International Laboratory Accreditation Cooperation, file:///C:/Users/jomar/AppData/Local/Temp/ILAC_P10_07_2020.pdf (accessed 9-12-2020)

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>.

ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories. International Organization for Standardization, Geneva, Switzerland (2017).

ISO/IEC Directives Part 2 Principles and rules for the structure and drafting of ISO and IEC documents, Ed.8, 2018, https://www.iso.org/sites/directives/current/part2/index.xhtml#_idTextAnchor089 (accessed 9-12-2020)

ISO 17034:2016(E) General requirements for the competence of reference material producers. International Organization for Standardization, Geneva, Switzerland (2016).

ISO 9000, Definitions in Plain English. International Organization for Standardization, Geneva, Switzerland (2015).

ISO Guide 30:2015, Reference materials — Selected terms and definitions. International Organization for Standardization, Geneva, Switzerland (2015).

ISO Guide 31:2015 Reference materials – Contents of certificates, labels, and accompanying documentation. International Organization for Standardization, Geneva, Switzerland (2015).

ISO Guide 33:2015 Reference materials – Good practice in using reference materials. International Organization for Standardization, Geneva, Switzerland (2015).

Compiled References

ISO Guide 35:2017 Reference materials. International Organization for Standardization, Geneva, Switzerland (2017).

ISO Guide 80:2014 Guidance for the in-house preparation of quality control materials (QCMs). International Organization for Standardization, Geneva, Switzerland (2017).

Kiefer, K., Walbridge, J. Consumer Beware: Is your Certified Reference Material Really Certified? Presented at BERM, National Harbor, Maryland, USA (2015).

Koeber, R., Linsinger, P.J., Emons, T.H. An approach for more precise statements of metrological traceability on reference material certificates, *Accred Qual Assur* (2010) 15:255–262
DOI 10.1007/s00769-010-0644-2 (2009)

Kocherlakota, N., Obenauf, R., Thomas, R. A Statistical Approach to Reporting Uncertainty on Certified Values of Chemical Reference Materials for Trace Metal Analysis. SPEX CertiPrep, Metuchen, New Jersey, USA (2017). <https://www.spexcertiprep.com/knowledge-base/files/WhitePaper-StatisticalApproachToReportingUncertaintyOnCertifiedValuesOfCRMs.pdf>.

Konschnik, J., Fisher, J., Wiest, L., Rousova Hepner, J., Risha, K. Unlocking the Mystery of Pesticides CRM Stability for Food Analysis. Presented at the European Pesticide Residue Workshop, Virtual (2020).

Lamberty, A., Schimmel, H., Pauwels, J. The Study of the Stability of Reference Materials by Isochronous Measurements. *Fresenius J Anal Chem* 360 (1998) 359–361.

Linsinger, T. Application Note 1, Comparison of a Measurement Result with the Certified Value. European Reference Materials, European Commission - Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel, Belgium (2010).

Linsinger, T.P.J. Evaluation of CRM Homogeneity in Cases of Insufficient Method Repeatability: Comparison of Bayesian Analysis with Substitutes for ANOVA Based Estimates. *Anal. Chim. Acta: X* 5 (2020) 100049.

Mastovska, K., Lehotay, S. Evaluation of Common Organic Solvents for Gas Chromatographic Analysis and Stability of Multiclass Pesticide Residues. *J. Chromatogr. A* 1040 (2004) 259–272.

Magnusson, B., Näykk, T., Hovind, H., Krysell, M. NORDTEST Handbook for Calculation of Measurement Uncertainty in Environmental Laboratories (NT TR 537 - Edition 3.1). Taastrup, Denmark (2012). <http://www.nordtest.info/index.php/technical-reports/item/handbook-for-calculation-of-measurement-uncertainty-in-environmental-laboratories-nt-tr-537-edition-3.html>.

Nordtest TR 537 (2003-05) Handbook for Calculation of Measurement Uncertainty in Environmental Laboratories

Reference Material Use in Trace Analysis

OECD (2017) Draft OECD GLP advisory document #19 on the management, characterization and use of test items, 4 May 2017

Official Methods of Analysis, 21st Edition, Appendix F. AOAC INTERNATIONAL, Gaithersburg, Maryland, USA (2019).

Official Methods of Analysis, 21st Edition, Definition of Terms and Explanatory Notes. AOAC INTERNATIONAL, Gaithersburg, Maryland, USA (2012). <http://eoma.aoac.org>.

Pauwels, J., Lamberty, A. Homogeneity Testing of Reference Materials. *Accredit Qual Assur* 3 (1998) 51–55.

Raynie DE. Know Your Sample: Size Matters. <http://www.chromatographyonline.com/know-your-sample-size-matters-0> (Accessed June 24, 2020).

RM Guidelines. AOAC INTERNATIONAL Technical Division on Reference Materials (TDRM), Gaithersburg, Maryland, USA (2015). http://tdrm.aoac.org/rm_docs/RM%20Documents/RMG.pdf

Sabadie, J. Behavior of Four Sulfonylurea Herbicides in the Presence of Hydroxy Compounds. *J. Agric. Food Chem.* 48 (2000) 4752–4756.

SANTE/12682/2019, Analytical Quality Control and Method Validation Procedures for Pesticide Residue Analysis in Food and Feed, European Commission, Brussels, Belgium (2020)
https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkdoc_2019-12682.pdf.

Schueneman, H., Campos, J. Arrhenius Equation Demystified. Presented at FMS Reliability Webinar (2014). https://slidelegend.com/queue/arrhenius-equation-demystified-westpak_5ae78ab37f8b9a1a798b4585.html (accessed 9-10-2020)

Sharpless, K.E., Greenberg, R.R., Schantz, M.M., Welch, M.J., Wise, S.A., Ihnat, M. Filling the AOAC triangle with food-matrix standard reference materials. *Anal. Bioanal. Chem.* 378 (2004) 1161–1167.

Sharpless, K.E., Lippa, K.A., Duewer, D.L., Ruhkin, A.L. NIST Special Publication 260-181: The ABCs of Using Standard Reference Materials in the Analysis of Foods and Dietary Supplements: A Practical Guide. National Institute of Standards and Technology (NIST), Gaithersburg, Maryland, USA (2014)
<https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-181.pdf>.

SI Units. National Institute of Standards and Technology (NIST), Gaithersburg, Maryland, USA (2019). <https://www.nist.gov/pml/weights-and-measures/metric-si/si-units>.

Smith, R.K. Benzidine? Really? Presented at the 14th Annual Water Testing & Quality Assurance Symposium (WTQA), Arlington, Virginia, USA (1998).

Compiled References

Stephany, R.W., van Ginkel, L.A. Quality Criteria for Residue Analysis and Reference Materials. Relationship between legal procedures and materials. *Fres. J. Anal. Chem.* 338 (1990) 370-377.

Thiex, N.J., Ramsey, C.A. Taking and Testing GOODSamples: A Systematic Approach for Representative Sampling from Field to Test Portion. *J Regulatory Sci* 02 (2016) 1–8. Also available at <https://www.aafco.org/Publications/GOODSamples>.

U.S.EPA Good Laboratory Practices Standards (GLPS), <https://www.epa.gov/compliance/good-laboratory-practices-standards-compliance-monitoring-program> (accessed 9-8-2020).

U.S.EPA (1985) Final Enforcement Response Policy (ERP) for the Toxic Substances Control Act (TSCA) Good Laboratory Practice (GLP) Regulations, <https://www.epa.gov/enforcement/final-enforcement-response-policy-erp-toxic-substances-control-act-tsca-good-laboratory> (accessed 9-8-2020).

U.S. Food and Drug Administration. Guide 118, Mass Spectrometry for Confirmation of the Identity of Animal Drug Residues. U.S. Food and Drug Administration, Center for Veterinary Medicine, Rockville, MD, USA (2003) <https://www.fda.gov/media/70154/download>.

Van de Merbel, N., Savoie, N., Yadav, M., Ohtsu, Y., White, J., Riccio, M.F., Dong, K., de Vries, R., Diancin, J. Stability: Recommendation for Best Practices and Harmonization from the Global Bioanalysis Consortium Harmonization Team. *The AAPS Journal* 16 (2014) 392-399.

Whitaker, T.B. Sampling Foods for Mycotoxins. *Food Additives & Cont.* 23 (2006) 50-61.

WHO Expert Committee on Specifications for Pharmaceutical Preparations: Forty-first Report. WHO Technical Report Series 943, World Health Organization, Geneva, Switzerland (2006).