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Guidance for the production of pure organic substance certified reference materials

Recommandations pour la production des matériaux de référence certifiés pour des substances organiques pures

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Contents

Page

Foreword

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

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

Introduction

Reference materials (RMs) play an important role in measurement processes and support sound, widely recognized measurement systems. ISO 17034 specifies general requirements to be met by reference material producers (RMPs), including for the production of certified reference materials (CRMs). CRMs play a key role in ensuring that measurements are comparable across time and space and are used by laboratories to establish metrological traceability of their measurement results to appropriate references.

This document outlines recommendations, which conform to general requirements of ISO 17034, for production of pure organic substance CRMs used to calibrate measuring instruments. These materials primarily comprise organic chemicals of specified, determinable structure. Guidance provided for characterization of pure organic chemical materials is also appropriate for those used to prepare pure organic substance solution CRMs. This document provides guidance on key aspects of the production of such CRMs, including the assessment of homogeneity and stability. Recommended approaches for characterization and assignment of certified purity values are described.

Guidance for the production of pure organic substance certified reference materials

1 Scope

This document notes the requirements of ISO 17034 and provides specific guidance on technical considerations for the production of pure organic substance certified reference materials (CRMs) that are used by laboratories to calibrate measurement equipment and procedures and to establish metrological traceability of the respective results. The guidance is relevant only to CRMs comprising organic compounds whose structures are specifically defined, where polymeric materials are not included.

In this document, reference to a CRM is limited to pure organic substance certified reference materials, including candidate materials, unless otherwise noted.

2 Normative references

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

ISO 9000, *Quality management systems — Fundamentals and vocabulary*

ISO/IEC 17000, *Conformity assessment — Vocabulary and general principles*

ISO 17034, *General requirements for the competence of reference material producers*

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

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

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 9000, ISO/IEC 17000, ISO Guide 30 and ISO/IEC Guide 99 and the following apply.

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

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

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

3.1
primary component
PC
principal chemical species of interest in the certified reference material

Note 1 to entry: A perfectly pure material is only an ideal concept because chemical species other than the PC will always exist in a material, even in very small amounts.

3.2
purity
quantity ratio of the primary component in the certified reference material

Note 1 to entry: Purity is usually expressed as the mass fraction, amount-of-substance fraction or amount content of the PC.

Note 2 to entry: Purity is ideally close to 1, but it can be considerably lower than 1.

4 Technical and production requirements

4.1 General

The production of a CRM requires diligent planning. The requirements can be found in ISO 17034, and recommendations can be found in ISO 33405.[1][1)] Central to this effort is clear specification of the intended uses of the CRM and characterization appropriate for these purposes. The following subclauses provide an overview of considerations relevant to the production of CRMs.

4.2 Production planning

The production of a CRM includes the following steps:

a) specification of the CRM and its measurand;

b) candidate material sourcing and assessment of suitability, including verification of PC identity and adequate purity;

c) product packaging and specification of conditions for storage and safe handling;

d) determination of approaches to purity assessment of the CRM;

e) development and validation of procedures to achieve target measurement uncertainty;

f) assessment of homogeneity;

g) assessment and monitoring of stability;

h) characterization of the CRM;

i) consideration of metrological traceability of the certified property value;

j) preparation of certificates.

4.3 Specification of the CRM and its measurand

4.3.1 General

The intended use and relevant properties of the CRM should be clearly specified at the outset of the production process. This can include, but is not limited to, measurement procedures or type of measuring systems for which it is intended to be used, properties to be characterized, target purity, appropriate metrological reference of the certified value and target measurement uncertainty. Special attention is needed for these topics, as described in the following subclauses.

4.3.2 Specification of purpose

It is important to consider the intended use of the CRM because it can affect various aspects of the CRM production process, including the verification of the suitability of the sourced material. Pure substances constitute the source of primary measurement standards and higher-order metrological traceability in most

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traceability chains in chemistry. The demand for such a CRM is usually recognized through engagement with the intended user community. The measurement needs that are commonly served include improved accuracy of relevant measurement procedures, establishment of metrological traceability and regulatory compliance for chemical testing laboratories.

Such CRMs are typically used for the calibration of measuring instruments and measurement systems. An impurity in a CRM can create interferences in a measurement; while the presence of such interferences would not generally invalidate the certified purity value of the CRM, it can render the CRM suboptimal for some measurement methods. When a CRM is intended to be used for preparing a multiple-component calibration solution, it should be characterized to account for all relevant chemical entities because an impurity in the CRM can be the PC of another material intended to be mixed, leading to biases in certified values derived from the preparation process of the multi-component calibration solution. Quantities of all compounds of interest in each of these materials, present as either a PC or impurities, should be evaluated during the purity assessment. The decision on which quantities are significant depends on the targets for production of the CRM and the producer should define them as part of the specification for the material. An example of quantities which need to be considered is the amount of substance that can interfere with the PCs (of the CRMs used to prepare a multiple-component calibration solution CRM) in a measurement method that is expected to be used.

CRMs can also be used in chemical identification and validation of procedures for elemental analysis.

4.3.3 Specification of the measurand

A clear and unambiguous specification of the measurand is key to the production planning. The certified purity value of a CRM is usually expressed as the mass fraction, amount-of-substance fraction or amount content of a structurally specified chemical or set of chemicals within the material. The measurand requires specification of the organic chemical structure(s), including the assigned stereochemistry, when applicable, and the relevant units for expressing composition.

4.3.4 Definition of the metrological reference

ISO 17034 requires the metrological traceability of certified values to be established for CRMs in accordance with ISO/IEC 17025.[2]

The appropriate metrological reference system is principally dependent upon the purpose of the CRM and the measurement community it is intended to serve. The SI, a coherent system widely used in commerce and science, is the most appropriate system of units for most chemical measurements. The certified purity value of a CRM is ideally obtained by, but not limited to, the practical realization of SI measurement units.

For certified values of nominal properties, traceability to appropriate chemical references should be carefully considered for each case. Some CRMs have certified values for chemical identity. Valid evidence linking this characterization to the chemical structure of the PC should be provided.

4.3.5 Fitness for purpose

Fitness for purpose of a measurement is the extent to which the measurement result meets the stated requirement for which the measurements are being made. Formal definitions can be found in various sources, such as Reference [11]. For the CRM to be fit for purpose, the uncertainty in the delivered certified value should be small enough to be useful. For example, it is not appropriate to use a CRM of certified purity with 10 % relative standard uncertainty for calibrating procedures that aim to produce results with 1 % relative standard uncertainty.

NOTE 1 A measured property value without associated uncertainty does not conform to the definition for the certified value of a CRM specified in ISO 17034.

NOTE 2 Some pure organic substance RMs and their intended use are covered by other standards, for example pharmacopoeia assay standards, and uncertainties in property values are not typically specified. Rather, they are treated as negligible in relation to the defined limits of the method-specific assays for which they are used.

4.3.6 Safety considerations

In regard to the workplace health and safety considerations, the reference material producer (RMP) should conduct a risk assessment, which can be replaced by the RMP's pre-established standard safety procedures, to establish that appropriate facilities and safeguards are in place to handle the candidate material.

4.3.7 Resources and approaches to purity analysis

Considerations for resource requirements are described in ISO 17034. CRM characterization should be fit for purpose and achievable with available laboratory resources, including labour, packaging materials and candidate materials. Allocation of these resources and anticipated cost recovery through CRM distribution are key considerations that govern the practicality of CRM production. Costs largely depend upon the rigour of analytical methods selected for characterization. For the CRMs, purity determination can be accomplished through either one or a combination of several basic approaches described in 4.6.

The target measurement uncertainty should be considered prior to attempting characterization.[12],[13] Use of two or more independent methods with different principles can evaluate possible systematic errors. Analyst expertise and preliminary experiments conducted for method development can generally inform realistic expectations of measurement uncertainty for specific measurement techniques and assist with experimental design for CRM characterization using either approach to purity analysis.

Statistical methods can also be employed to estimate optimal experiment design for a given set of constraints, including the target measurement uncertainty.[14] This experimental design should take into account sampling that is required to adequately assess homogeneity across the entire lot of candidate CRM.[1] As such, the number of units in the production lot should be known prior to development of methods for CRM characterization.

4.4 Candidate material sourcing and assessment of suitability, including verification of PC identity and adequate purity

4.4.1 Material sourcing

Candidate materials can be sourced commercially, through custom synthesis or from refinement of materials. Factors that should be considered in screening such materials include affordability, purity, homogeneity and stability.

Impurities can have a significant effect on the long-term stability of the material as well as on the accuracy of complex purity analyses. The RMP can conduct further purification of the sourced material when a sufficiently pure material cannot be sourced. The RMP should consider the advantages of purification against the recovery of PC during the process and any other potential changes to material composition of the sourced material during this process.

When the candidate material is sourced as a coarse powder or pellets, the RMP can grind and sieve the bulk material to produce a fine powder that is more suitable for its intended use, for example one that should be sufficiently homogenous for a small minimum sample size. Moreover, a more homogeneous particle size distribution is less prone to spatial stratification during packaging and transportation. When solid-state properties are relevant for a particular certification study, i.e. when the RMP intends to characterize the crystalline composition of the material or when these properties substantially affect the handling of the powder (e.g. hygroscopicity, electrostatic effects, dissolution rate or flow behaviour), the RMP can carry out preliminary tests with the candidate material to evaluate its suitability. When the RMP decides to purify the candidate material using processes such as recrystallization or drying, an interval of time before packaging the candidate batch can be useful to allow the stabilization of the moisture content of the bulk material and avoid future stability issues with water mass fraction.

4.4.2 Verification of PC identity

The identity of the PC is critical for any chemical CRM. ISO 17034 requires the RMP to address the verification of the identity of the PC. In addition to verifying chemical bond connectivity between atoms, knowledge of the geometric arrangement of the PC can be critical for the intended use of the CRM. Techniques executed

to identify chemical components of the CRM should promote confident distinction of the PC from other inherent substances, especially those of similar structure.

The identity of the PC can be specified as a single precise organic chemical structure or as a closely related group of molecular entities. This structural specification should be governed by the intended use of the CRM to ensure that the measurand comprises only those chemical entities relevant for the intended use.

For example, when only the L arrangement of a chiral compound is biologically active, this quantity in a CRM should be specifically known, exclusive of the quantity of the compound having the D arrangement. Conversely, less specificity can define a measurand that includes related entities with slightly different structures, yet with similar or effectively the same properties, for example when the L and D arrangements serve the same purpose.

Specification of the measurand can consider the distinction of entities within the following classes of related chemical structures:

a) constitutional isomers – compounds with the same molecular formula, but different chemical bonding between atoms;

b) stereoisomers – compounds with the same molecular formula and bonding between atoms, but different three-dimensional spatial orientation of atoms within the molecule.

Tautomer and conformer structures of the PC should be considered if they are observed during candidate material characterization. When appropriate, they should be carefully attributed to the measurand.

NOTE Isotopologues, a molecular entity that differs only in isotopic composition (number of isotopic substitutions), for example CH_4, CH_3D, CH_2D_2, can be considered when required.

The RMP should utilize analytical techniques such as nuclear magnetic resonance (NMR), infrared (IR) spectroscopy and mass spectrometry (MS) to confirm the identity of the PC. NMR and MS can also support useful impurity identification. For crystalline compounds, a melting point determination can also be of value. As part of the planning process, it would be advantageous to source literature precedents for the structural identity of the analyte of interest.

For hydrate substances, identification should include determination of the ratio of water to the PC. For organic salts, identity of the counter ion should also be confirmed.

Examples of structure identification approaches can be found in Annex A.

4.4.3 Material suitability

A suitable candidate material is one that can be well characterized and has an acceptable level of impurities. A preliminary experimental effort should be made to verify that a candidate material meets acceptance criteria for purity. A suitability assessment of the candidate material should be conducted prior to material packaging using one or more analytical techniques available for purity determination and possibly other techniques to verify the absence of specific undesirable impurities. CRMs often contain significant impurities that have chemical structures similar to the PC. Suitable candidate materials typically contain a small proportion of these related impurities. The suitability assessment should be affordably conducted with the aim of verifying adequacy of a material and not necessarily conducted with the degree of rigour required for property value certification.

4.5 Product packaging and specification of conditions for storage and safe handling

4.5.1 General considerations

The nature of the product packaging, especially the primary packaging material, can widely affect the integrity of the material and the behaviour of the property values. Therefore, packaging should be selected carefully and studied under storage and transport conditions.

Factors that can influence the choice of packaging material include, but are not limited to:

— hygroscopicity and/or light sensitivity of the material;

— the physical state of the material (e.g. liquid, solid, viscous);

— the amount per unit to be packed;

— temperature conditions for storage and transport;

— inertness;

— leaching;

— conformity with transport requirements and regulations;

— conformity with safety requirements and regulations;

— handling aspects at the laboratory of the user.

4.5.2 Selection and treatment of packaging materials

Preliminary studies to assess candidate packaging materials or different storage conditions are recommended. The CRMs can be packaged in primary containment glass vessels, such as sealed ampoules or vials. In order to protect the material from environmental conditions that can impact the integrity of the CRM (e.g. light, heat and humidity), the following packaging options should be considered:

— amber or clear glass vials;

— screw cap or rubber septum lid with crimped aluminium cap;

— inert gas or air to fill the glass storage vial headspace.

Ampoules are generally sealed under an inert atmosphere (e.g. argon), whereas with bottles this is not the case.

The RMP should also consider any necessary pre-treatment of the packaging materials, such as cleaning.

To ensure that the candidate material is properly transferred to a selected packaging material upon production, the process should be controlled and documented, see ISO 17034.

After the establishment of a suitable process for packaging or filling, the selection of appropriate packaging material and storage conditions, ISO 17034 requires the RMP to conduct a stability assessment for the behaviour of all relevant properties of the CRM under expected storage and transport conditions. Details can be found in ISO 17034 and ISO 33405.[1]

4.5.3 Storage and transportation issues

Containers for CRM storage should sufficiently isolate the material from the environment. Isolation from light, moisture and temperature can be suitable examples for the selection for the storage conditions for many CRMs.

Equally important is the definition of the transport conditions. Heat-sensitive material could be shipped on dry ice or similar, or the shipment duration could be limited. The conditions of shipment, including the provision to provide all necessary documents (e.g. permits, statement of origin) for customs clearance, is the responsibility of the RMP.

4.5.4 Label of containers

ISO 17034 requires appropriate labels to be applied during product packaging. The guidance can be found in ISO 33401.[3]

4.6 Determination of approaches to purity assessment of the CRM

When purity assessment of candidate materials is conducted, ideally two or more independent methods should be used to determine purity. This allows an assessment of bias in one or both methods. It is generally recommended that one or more direct purity determination methods be used.

The RMP should plan a measurement strategy for measurement of purity of the candidate material that is fit for the intended use. Development of this strategy should consider measurement procedures that collectively ensure:

a) adequate specificity for PC measurement;

b) an adequate survey of impurities;

c) sufficient accuracy of measured purity value;

d) sound evidence for assigning a sufficiently small measurement uncertainty.

NOTE Numerous complementary instrumental techniques are available for purity assessment. Hence, it is possible for the strategy adopted to vary with the instrumentation and other resources available to the RMP.

The use of one or a combination of appropriate measurement procedures can meet the objectives for producing a CRM with metrologically traceable certified values.

Methods for organic chemical purity assessment are generally conducted through direct and indirect approaches to determining PC quantities.

Direct approach: determination of relative quantities, such as mass fraction, of the PC without necessarily quantifying all impurities. This approach commonly implements techniques such as quantitative nuclear magnetic resonance (qNMR), coulometry, titrimetry, freezing or melting point depression and stable isotope ratio mass spectrometry (ID-MS). qNMR, titrimetry and ID-MS methods require a pre-established CRM for comparison and assigning relative values For the pure organic substance CRMs, qNMR is a widely used technique for direct measurement of purity.

Indirect approach: determination of purity through a survey of impurity components, where the purity, w_{PC}, is calculated using Formula (1):

$$w_{\mathrm{PC}} = 1 - \sum w_{\mathrm{I}_i} \tag{1}$$

where

w_{PC} is the mass fraction of the PC;

w_{I_i} is the mass fraction of the i^{th} impurity component.

A thorough indirect approach can require more resources and varied expertise than a direct approach and this should be realistically accounted. Possible impurities to be investigated generally belong to the following classes of chemical components:

a) structurally related organic compounds;

b) volatile organic compounds;

c) water;

d) inorganic substances;

e) unrelated non-volatile organic impurities, for example polymeric compounds or biological substances.

Purity assessments of candidate materials can leverage information gathered with techniques employed for both approaches[15–17]. Ideally, direct and indirect determinations yield consistent results, though valuable insight about the material composition can be gained even if they do not. A rigorous assessment can directly

measure the PC quantity and collect important information about the entire material composition. This is especially useful if the material contains impurities that introduce systematic errors to the direct PC determination or that can give rise to potential interferences in the intended use of the CRM. Inconsistency between direct and indirect determination results indicate bias associated with either approach that should ideally be reconciled or accounted for in the measurement uncertainty.

The selection of methods for purity measurement should leverage information about material composition gathered during the suitability analysis. A preliminary investigation of impurities should indicate the most appropriate methods for purity measurement. Furthermore, the achievable uncertainty in the certified value is likely larger for materials with complex impurity profiles than for those with few impurities. Especially for an indirect determination, purity assessment of a material with many impurities requires confident measurement of several substances. Effort should be made to ensure that these impurities do not interfere with accurate measurement of the PC. When applying the mass balance approach to assess purity of a candidate material, the time and effort required to identify and quantify each impurity increases as the number of impurities increases. If such an analysis becomes too complicated or costly, the RMP should consider the purification of the candidate material to remove as many impurities as possible and reduce the effort required for purity assessment. Materials of higher purity are also generally more suitable for the purposes of the end user.

More detailed information for the purity assessment can be found in Annex B.

4.7 Development and validation of procedures for characterization, including achieving target measurement uncertainty

4.7.1 General

Specification of acceptable uncertainty in the certified purity value should be made with a clear understanding of the intended use of the CRM.

The certified purity value of a metrologically traceable CRM should have an associated uncertainty that contributes a reasonably small fraction of the total uncertainty budget in results achieved through this application. The certified purity value of a CRM that is used to establish metrological traceability for a measurement result should have an associated uncertainty that is fit for intended use compared to the total uncertainty of the measurement result. It is also crucial that the uncertainty of the certified purity value realistically reflects the homogeneity and stability of the production batch. General guidance for the evaluation of measurement uncertainty is provided in ISO/IEC Guide 98-3 (GUM)[4] and its supplements.[5],[6] Assessment of batch homogeneity and stability for CRM production is discussed in ISO 33405.[1] These sources of uncertainty should be approximated during material suitability tests and considered when planning experiments. Characterization of a candidate material should be conducted after it is packaged into individual units.

An optimal experiment design is one which uses a minimum amount of resources to provide a sufficient amount of information. For CRMs, this means efficiently conducting measurements to achieve results with suitably small uncertainty. Expert judgement should ultimately decide the combination of appropriate analytical procedures best suited to the nature and composition of the material. In addition to achieving the smallest practical measurement uncertainty of a certified value, the execution of a plan for adequate characterization is contingent upon resources available to the RMP. Batch size is an important factor in sampling schemes and experiment design. General guidance on approaches to experiment design and adequate material sampling to achieve target measurement uncertainty under these criteria is available.[1],[10],[14],[18]

Examples of purity analyses for characterization of CRMs are provided in Annex C.

4.7.2 Multiple methods for purity determination

When well-characterized CRMs are needed, the use of multiple methods is strongly recommended.

Multiple independent measurements of purity can provide a collective body of evidence that is complementary or self-validating. When multiple measurement results are consistent, confidence gained

through this corroboration can be reflected in the evaluation of uncertainty of a certified purity value. Information collected through several procedures should be combined in a way that is chemically and metrologically justifiable.[15],[16],[19] This is crucial when results from different methods are inconsistent or the composition of the material is complex. For these reasons, conducting multiple measurements of purity for CRM characterization using both a direct and an indirect mass balance approach is recommended, when feasible. Such an approach provides a variety of useful information about chemical composition.

NOTE Consistency of results from multiple methods does not guarantee that they are unbiased.

4.7.3 Property value boundaries

The RMP should consider the natural limit of chemical purity, 1 kg/kg (i.e. 100 %), during the characterization of CRMs. This is particularly relevant to candidate materials with very few low-level impurities. Estimates of purity that realize SI measurement units should ideally observe this limit. Generally, the entire coverage interval defined by the uncertainty of a certified value should lie between 0 kg/kg and 1 kg/kg.

When a material is known to have a small mass fraction of total impurities, the variability of data from a direct measurement (e.g. qNMR) can yield purity estimates that are not entirely within this interval. For such cases, the measurement function or coverage interval can be constrained to observe the natural limits. This can be achieved in accordance with approaches described in the GUM and others.[15],[20]–[23] Additional resources for the practical treatment of asymmetric coverage intervals near natural limits is available.[24]–[26]

Similar considerations should be made for measurements of w_{I_i} that are near limits of detection. If the uncertainty of a high purity value determined by a mass balance method is expected to be small, it should be substantiated by evidence from appropriately sensitive procedures used to determine w_{I_i}.

4.8 Assessment of homogeneity

4.8.1 General

For the assessment of homogeneity in CRMs, the following items should be considered in addition to the requirements stated in ISO 17034 and recommendations described in ISO 33405.[1]

Candidate materials prepared for the CRMs are expected to have a high degree of homogeneity. Certified values for such materials are often expected to have very small uncertainties, making even a small amount of heterogeneity potentially important.

Generally, only the homogeneity of the PC should be assessed. Depending on the circumstances, other aspects should be assessed. For example if the content of an impurity is also a certified value of the CRM, the homogeneity of the impurity should be assessed as well. Assessments should be conducted with consideration of the hygroscopicity and the particle size distribution of the material.

4.8.2 Preliminary assessment of homogeneity

Before packaging, it is recommended that a preliminary assessment of homogeneity be performed to ensure that the candidate material is suitably uniform. This is particularly beneficial if the sample is an agglomerated powder or granular. In the event that the candidate material demonstrates a practically significant degree of heterogeneity, the RMP can consider further homogenization of the sample. To avoid unnecessary risk of contamination and exposure to reactive environments, homogenization should only be performed when the variance due to heterogeneity would otherwise be too large to achieve an acceptable uncertainty in the certified value. If there is an unacceptable risk of contamination or environmental exposure expected during homogenization, a new candidate material should be sought.

4.8.3 Sampling strategy

When sampling crystalline solids or agglomerated powders, the position of the samples within the material container should be considered to assess impurity content stratification throughout the unit, in addition to the need for adequately representative sampling across units of the produced batch. For example, the

material should be collected from the upper, middle and lower portions of the container, from the inside and outside portions of the bulk, or from portions of the container with disparate particle size.

4.8.4 Minimum sample size

The minimum sample size can be set using other data or by experience.[1] The stated minimum sample size for the CRM should satisfy the requirements of the intended use of the CRM, which should not be smaller than the sample intake size for which homogeneity has been demonstrated.

4.8.5 Experimental method of homogeneity assessment

Generally, one of the methods used for characterization is selected as the experimental method for the assessment of homogeneity. A method with suitable repeatability and selectivity should be implemented. Experimental design enabling evaluation of in-homogeneity sources should be implemented. More detailed discussions and recommendations are described in ISO 33405.[1]

4.9 Assessment and monitoring of stability

4.9.1 General

ISO 33405[1] states the importance of demonstrated stability under long-term storage conditions, under transport conditions and, where applicable, the storage and usage conditions at the laboratory of the CRM user. For the assessment of the stability, including stability monitoring of CRMs, the following items should be considered in addition to the requirements stated in ISO 17034, and recommendations described in ISO 33405.[1]

4.9.2 Sources of instability

Adsorption or desorption of water is a common cause of instability.[27] This is dependent upon the hygroscopicity of the material and how well the potential effect is controlled by methods of packaging and storage conditions. For bulk material packaged in sample bottles, vials or jars, the RMP should monitor the uptake of water. Hygroscopicity should be monitored under appropriate conditions of use and can be measured through experimental determination of moisture sorption isotherms.

NOTE 1 Demonstrated stability of moisture content in the chosen packaging does not guarantee that the material is stable when opened and used in laboratory conditions.

Instability can also be caused by PC degradation (e.g. oxidation, hydrolysis). The stability of the PC and changes in impurity profile should be monitored using appropriate analytical techniques. This can be achieved using chromatographic or spectroscopic (e.g. NMR) techniques.

NOTE 2 While degradation of the PC to an impurity of related structure is more common, the degradation of an impurity leading to the formation of PC is also possible, which in turn results in an increase in purity.

If the material has been shown to contain residual volatile solvent impurity, the RMP should monitor for change in the assigned mass fraction. This can be done with either thermogravimetric analysis (TGA), gas chromatography (GC) or ^{1}H NMR spectroscopy (ideally run under quantitative NMR conditions). For measuring quantities of specific solvents, GC and qNMR techniques are often preferred. If the volatile solvent content is reasonably low, the uncertainty of the measured PC purity value can be evaluated such that the interval conservatively accounts for potential volatilization of this impurity.

NOTE 3 Statistical treatments of stability data are detailed in ISO 33405.[1]

4.9.3 Repeated use stability

When the CRM unit is permitted for repeated use, ISO 17034 requires the RMP to assess stability of the material during repeated use. This study should simulate routine use of the CRM unit, with repeated cycles of opening, weighing, closing and storing the vial in long-term storage conditions. Adsorption or desorption

of water is a common cause of instability.[27] The assessment of stability after repeated sampling can be run in parallel with classic stability studies or the RMP can adopt another design for this evaluation.

4.9.4 Stability monitoring

Generally, organic compounds should be stored out of direct sunlight in a relatively dry environment (<50 % humidity). The recommended storage temperature depends on the stability of the compound in question. Many organic compounds can be appropriately stored at room temperature (≤25 °C), in a refrigerator (e.g. 2 °C to 6 °C) or in a freezer at approximately −20 °C.

Stability should be monitored during the lifetime of the CRM. When an indirect method, such as mass balance, is used, the RMP should ensure that the classes of impurities prone to variation or degradation are determined. Particular attention to water, solvents and impurities of related structure is warranted.

Non-volatile inorganic salt content is unlikely to change over time; however, its reactivity with the PC should be considered in the stability assessment of the CRM.

NOTE Comprehensive guidance on stability monitoring can be found in ISO 33405.[1] Monitoring of stability throughout the period of certificate validity provides insight into the long-term impact of the storage and, where applicable, usage conditions.

4.10 Characterization of the CRM

4.10.1 General

ISO 17034 requires the RMP to clearly specify the measurand and perform the respective quantitative characterization of the PC in the candidate material. For this document, the specified measurand should be independent of any specific measurement procedure. ISO 17034 requires the RMP to select a characterization strategy appropriate for the intended use of the CRM. Ideally, two or more independent characterization methods should be conducted with the aim of assessing bias in one or both methods.

When the intended use of the CRM is to combine with other CRMs, for example, in the preparation of multiple-component calibration solutions, impurities having the same structures as PCs of the other CRMs should be quantified.

ISO 17034 requires the RMP to use documented procedures for the assignment of property values. For certified purity values, ISO 17034 requires the RMP to identify all uncertainty contributions to be included in the assigned uncertainty. ISO 17034 requires the RMP to also define the probability or level of confidence for calculating the expanded uncertainty.

NOTE ISO 33405[1] and the GUM can provide guidance on uncertainty evaluation.

4.10.2 Direct determination

Annex B describes the direct determination of purity of the candidate material. It is important to consider the possibility of interferences from the impurities. When interference is expected, the amount of such impurities should be independently evaluated by another method to assess bias caused by the impurities. For example, the RMP can evaluate impurities using chromatographic techniques and detectors suitable for impurity identification and quantification.

4.10.3 Indirect determination

Indirect methods have been widely applied for the assessment of organic chemical purity. There are two common approaches for the indirect purity determination:

— mass balance method, whereby the relative amount of each impurity is measured and their total subtracted from 1 kg/kg;

— thermal methods, whereby measurement of a single physical property is used to infer the total relative amount of all impurities.

The mass balance approach can be practical when direct methods are not viable or adequate and when knowledge of impurity composition is important for the intended use of the CRM. The thermal methods described in Annex B, such as freezing point depression, measure the total impurity content that depresses the freezing point of the candidate CRM, provided the material meets the conditions for this type of analysis.

NOTE Knowledge of the exact chemical structure of an organic impurity is not always necessary. The impurity and PC structures can be similar enough that the relationship between detector response and chemical quantity can be treated as effectively the same for both compounds. The extent to which an impurity is identified needs to be established. It is not always necessary to know the exact structure, thus it can be sufficient to establish that the impurity has a similar structure to the PC and that the relationship between quantity and magnitude of detector response is effectively the same for both compounds.

4.10.4 Characterization for use in multi-component solutions

When the intended use of the CRM includes the preparation of multi-component calibration solutions, the impurity of one CRM can have the same structure as the PC of another. For such cases, significant quantities of all relevant impurities should be determined for each CRM used in the mixture.

4.11 Metrological traceability of the certified property value

Establishing metrological traceability is a key part of the production of any CRM. The detailed requirements for establishing metrological traceability of the certified property value can be found in ISO 17034 and further information can be found in ISO 33405[1] and ISO/TR 16476.[7] A pure CRM often represents the practical realization of a PC mass fraction unit.

4.12 Preparation of certificates

The preparation, authorization and issuance of RM certificates, as well as making them available to the users, are mandatory steps for the production of a CRM in accordance with ISO 17034.

Two of the most important pieces of information about the RM certificate are the assigned purity value and the associated uncertainty. They are calculated using results from characterization, homogeneity and stability studies to ensure the reliability of the assigned value and associated uncertainty for all units within a batch during the period of validity of the certificate.

NOTE Further information concerning the contents of RM certificates, labels and accompanying documentation can be found in ISO 33401.[3]

In addition to the information listed in ISO 17034, the following information can be useful:

— CAS Registry Number®[2] (where available);

— measurement methods for method-independent measurands;[3]

— health and safety information;[3]

— subcontractors;[3]

— indicative values;[3]

— legal notice;[3]

— reference to a certification report;[3]

— chemical formula;

— chemical structure;

— chromatograms;

2) Chemical Abstracts Service (CAS) Registry Number® is a trademark of the American Chemical Society (ACS). This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

— NMR spectra and chemical shifts;

— other characterization data relevant to the intended use of the CRM;

— solubility data;

— unit size (mass);

— isotope atom fractions;

— contents of delivery;

— reference to a safety data sheet.

Annex A
(informative)

Examples of structure identification approaches

A.1 Considerations for structure identification

The RMP should ensure that sufficient experimental evidence is collected for identification of the PC and impurities. To ensure that compounds are identified with an adequate level of confidence, especially those having complex structures or those that should be distinguished from other similar structures (such as isomers), multiple techniques for structure examination should be applied.

Structure identification can be performed by comparison with reference spectra or interpretation from first principles. Reference spectra can be sourced from either a standard database or published articles, preferably peer reviewed.

Knowledge of the synthesis would also aid structure identification of the PC and impurities.

A.2 Examples for structure identification methods

A.2.1 General

The following subclauses summarize common approaches to chemical identification using analytical techniques that are routinely used for structure elucidation and/or confirmation. Chemical identification of a PC is ideally inferred through the implementation of more than one of these techniques.

A.2.2 Mass spectrometry

MS techniques can be used for the characterization of organic and inorganic chemical composition.

Generally, the most useful peak in the mass spectrum is the *m/z* peak corresponding to the relative molecular mass of the analyte in question, although it should be recognized that this peak is not always evident due to facile fragmentation. In cases where the molecular ion is not evident or in low abundance, the fragmentation peaks provide useful information about molecular connectivity. Derivatisation can be a way of enhancing the molecular ion. Structural information describing the spatial construction of the molecule is not provided in most cases, and isomeric structures in most cases are not identified with complete certainty.

MS is often coupled with a chromatographic technique. This facilitates the separation of the PC and impurities, which in turn provides a more selective mass spectrum of each component.

A.2.3 Nuclear magnetic resonance

NMR spectroscopy is a powerful technique that is routinely used for chemical structure elucidation. NMR can aid identification of chemicals through observation of quantum mechanical properties of nuclei. NMR can assist structure elucidation through assessment of the relative amounts, atom connectivity and spatial arrangement of observed constituent nuclei. This can be achieved through evaluation of the corresponding resonance frequencies and magnitudes, coupling constants and spectral patterns. Furthermore, data analysis can be performed by the interpretation of each spectrum from first principles.[28]

A.2.4 Molecular spectroscopy

Complementary information about functional groups present in the PC can be obtained through both IR spectroscopy, using dispersive or Fourier-transform techniques, and Raman spectroscopy.

UV/Vis spectroscopy has a limited role in chemical identity examination and is rarely, if ever, used on its own to elucidate structure. For these purposes, it often serves as a means of detection for chromatographic techniques that provide greater chemical selectivity.

Circular dichroism spectroscopy can be applied to investigate the chirality of components of the CRM and distinguish optical isomers

A.2.5 Chromatography

Comparison of chromatographic retention times of the PC in a candidate material to that of an authentic sample of the same analyte provides some evidence supporting chemical identification of the PC, demonstrated co-elution being the most conclusive. Confidence in the PC identification is further improved when the mass spectrum of a mixed sample of the candidate and authentic materials is indistinguishable from the individual spectra. This approach can also be used to identify and quantify impurities of related structure.

Ionic impurity species, both inorganic and organic, can be assayed using ion chromatography with an appropriate detector, for example a conductivity detector. Such procedures can provide comparable and complementary evidence for the measurement of inorganic metal ions, as well as many other non-volatile and inorganic substances. The use of ion chromatography is especially useful for the characterization of organic salt CRMs. The advantages of this application include direct measurement of counter ions to the PC not observable via NMR, confident assessment of salt substance identity and composition (stoichiometry) for rigorous mass balance approaches to purity determination and measurement of ionic impurities giving rise to interferences in titrimetric purity measurement procedures. This technique can also be used to assess counter ion to the PC in a salt form candidate material.

A.2.6 Other approaches

Elemental microanalysis and melting-point determination can be used as supporting evidence for the structural assignment, particularly in high-purity materials.

NOTE For hydrates, elemental analysis can be used to support the determination of water content. It is recommended that water content is determined via Karl Fischer titration (KFT) and verified using elemental analysis as supporting evidence.

The inductively coupled plasma–mass spectrometry (ICP-MS) and ICP with optical emission spectrometry (ICP-OES) can be used to analyse the inorganic elements, including those present as an integral part of organic compound (e.g. phosphorus, sulfur and organometallic compounds) in the candidate material. If the mass fraction of an element is significant, it can be further determined by ion chromatography or other methods.

X-Ray fluorescence (XRF) for metals and size exclusion chromatography for oligomeric or polymeric materials are other useful techniques to confirm the presence of such elements or materials in candidate materials.

Annex B
(informative)

Examples of measurement procedures for assessment of purity

B.1 Preamble

Methods for organic chemical purity assessment are generally conducted through direct and indirect approaches to determining PC quantities. Though typical approaches are described here, a more comprehensive description of the purity assessments can be found in Reference [29].

B.2 Direct determination of purity

B.2.1 General

The procedures for purity determination listed in this clause allow for the determination of the PC without the need to quantify all impurities. These procedures can yield purity results that are metrologically traceable to a reference through a single comparison.

B.2.2 Quantitative nuclear magnetic resonance

RMPs with access to NMR capabilities can implement qNMR for direct purity determination.[8] This technique provides a measure of the amount-of-substance fraction of chemical moieties in solution – typically through observation of respective ^{1}H nuclei. As such, qNMR is broadly applicable for organic chemical purity analysis.

QNMR is especially useful if resource constraints allow for only one measurement procedure to be executed for characterization of the PC. However, obtaining confirmatory evidence from other methods is recommended when feasible. Use of qNMR can also be preferable for the characterization of organic salt materials, which by nature contain a smaller mass fraction of organic PC than the corresponding free-base. Unlike an indirect (i.e. mass balance) approach, the qNMR direct determination supports quantification of the organic ion PC. This readily supports the determination of a metrologically traceable result for measurement of the PC mass fraction without the need to confidently quantify the counter ion or make potentially inaccurate assumptions about the stoichiometric proportion of the ionic components, and thus the molar mass of the salt.

Scientific literature describes applications of several different types of quantitative NMR spectroscopy techniques. Variations of the qNMR method can be found in References [24] to [26] and [30] to [33].

B.2.3 Titrimetry

There are several classes of chemical titration that make use of the chemical reactivity associated with certain functional groups.[34]-[36] Chemically active impurities or other contaminants of the procedure can give rise to bias in results determined by titrimetric procedures. Since selectivity of titrimetric procedures is based on chemical reactivity rather than structure, identity should be verified by other analytical techniques and diligence should be exercised to minimize chemical interferences.

For the characterization of the PC, coulometric titrations are occasionally used to directly measure quantities of an electrochemically viable PC. Coulometric titrimetry does not rely upon the use of characterized chemical standards and, when rigorously performed, can yield very precise results. These electrolytic procedures are used to determine the amount of PC in a sample that undergoes complete charge transfer via applied constant current.[37] Metrological traceability of these results is established to reference systems, including the SI, through appropriate calibration of the electrolysis procedure.

Coulometric titration techniques can only be implemented for materials with an electrochemically reactive PC and should only be considered for characterization of candidate materials known to contain a very small impurity proportion.

B.3 Indirect determination of purity

B.3.1 General

Purity determinations of organic substances, especially when not possible via titrimetry, have traditionally relied upon the quantification of impurities. Assuming a practically comprehensive assay of all impurities, purity w_{PC} can be calculated according to Formula (B.1):

$$w_{PC} = 1 - \sum w_{I_i} \quad \text{(B.1)}$$

where

w_{PC} is the mass fraction of the PC;

w_{I_i} is the mass fraction of the i^{th} impurity component.

This is the fundamental model for a mass balance method of organic chemical purity determination. The procedures executed for purity assessment should confidently quantify all impurities and be selective enough to distinguish them from the PC.

B.3.2 Mass balance method

B.3.2.1 General

Possible impurities to be investigated generally belong to the following classes of chemical components:

a) structurally related organic compounds;

b) volatile organic compounds;

c) water;

d) inorganic substances;

e) unrelated non-volatile organic impurities, for example polymeric compounds, biological substances.

Different techniques are required to perform analyses for each of these classes of chemical components. The complementary results are combined to determine the total impurity content $\left(\sum w_{I_i}\right)$ and ultimately derive an estimate of purity with appropriate uncertainty according to Formula (B.1). In order to determine PC purity results that are traceable to SI measurement units through this method, evaluation of impurity composition should be rigorous and care should be taken to ensure that all components of the analysis are under adequate control.

B.3.2.2 Structural related organic impurities

An extensive array of analytical technologies can be implemented for the measurement of structurally similar organic chemical impurities. This can be the most critical and challenging effort of CRM characterization due to the large number of possible impurity structures. Selection of appropriate methods generally depends upon the physical properties of the PC and composition of the material. Prior knowledge regarding material production and provenance should inform practical approaches the RMP should take to quantify known impurities and survey a range of likely and plausible impurities.

Chromatographic separation techniques coupled with adequately sensitive detectors are mainstays of organic chemical analysis. HPLC, GC and ultra-high performance liquid chromatography (UHPLC) can be

utilized to achieve adequate resolution of organic chemical species present in the candidate material.[15],[38] This directly supports observation, identification and quantification of structurally similar impurities. It would be prudent to verify the evident impurity profile using an alternative chromatographic method and/or NMR.

The principles of qNMR procedures used for direct PC quantification can be applied to the quantification of impurities. NMR data can provide crucial structural information for the identification of similar impurities to complement a survey by LC and GC assays. If a candidate material has a large number of very low-level or indistinguishable similar impurities, NMR cannot be a reliable independent technique for total impurity quantification. Very small impurity proportions (multiple orders of magnitude less than the PC) are preferred for CRM candidates and the use of NMR for accurate impurity measurement can be limited for such materials.

B.3.2.3 Volatile organic impurities

Measurement of volatile organic impurities can be accomplished through several procedures, especially those implementing GC. Such assays can be performed via extraction of volatile organics from CRM sample headspace or by direct injection of test solutions containing the candidate material onto an appropriate GC column. Solid-phase micro-extraction (SPME) techniques and/or direct injection are often implemented with GC using an MS detector and a flame ionization detector (FID). This approach supports identification and quantification of significant volatile organic impurities.[39]-[41]

TGA can also be implemented for the determination of volatile impurities. Based on mass loss as a function of temperature, such assays allow direct measurement of the mass fraction of volatilized substances. However, the use of this technique for the measurement of organic solvent content has the following limitations:

1) TGA is not viable for CRMs having a PC that volatilizes, sublimes or degrades at temperatures required for solvent assay.

2) Evidence for the identification of impurities includes only the temperatures at which mass loss occurs, unless the TGA instrumentation is coupled to a detector that provides structural information, such as IR or MS.

3) Occluded substances should possibly not be volatilized through heating at suitable temperatures.

Organic solvents can also be identified and quantified via NMR. Very small organic solvent quantities or species with unobservable resonances due to spectral overlap are less suitable for measurement via qNMR.

B.3.2.4 Water

A large number of organic compounds contain occluded water; in many cases, this can be the largest impurity. Therefore, for many CRMs, determination of water content and its variation throughout the produced batch can be a critical part of the characterization. Water accumulation can impact the stability and homogeneity of CRMs, especially of those that are very hygroscopic.

Coulometric KFT procedures can be employed for the direct assay of water content. The oven transfer technique is more appropriate for materials that react with or are not soluble in the KFT reagent. However, the RMP should be mindful of possible bias caused by crystallization or occluded water that was not released at elevated temperatures. One option to minimize this problem is to set the temperature of the oven above the melting point of the candidate material, although care also needs to be taken to avoid thermal degradation.

Mass loss on drying and TGA can also be used to deduce water content. These methods are relatively simple and inexpensive but are not selective for water determination. Furthermore, occluded water can possibly not volatilize at temperatures below the point of thermal degradation of some substances. These procedures can be used for the determination of the total water and volatile content of viable materials if the selectivity of the impurity determination is fit for purpose for the characterization.

B.3.2.5 Inorganics or non-volatile residue

A broad range of non-volatile and inorganic substances can be present within a candidate material. Similar to the assessment of structurally similar impurities, a practical approach to characterization should consider prior knowledge of the material's origin and attempt to survey a range of plausible impurities. Measurement of various organic species can be accomplished through several viable techniques.

The use of ICP-MS or ICP-OES, as well as XRF spectroscopy, is common for the characterization of CRMs with significant inorganic impurities. These procedures provide reasonably sensitive multi-element surveys of metal element species that could be present (e.g. Na, Ca, Fe, Si). However, the relative measurement uncertainty of quantity estimates for materials with less than 0,1 mg/g of inorganic impurities could be relatively large.

Non-volatile impurity quantities can be determined through ashing experiments. These classical techniques determine the mass of residue after the total combustion of a material. This measurement indicates a lower limit of inorganic impurity content; however, it provides little information regarding chemical identity. The measurement of non-volatile impurities by ashing experiments is robust and relatively inexpensive. To ensure complete combustion of the organic component(s), a sufficiently high temperature (e.g. 650 °C) should be attained in the presence of air. In the case of organic salt CRMs, the non-volatile residue can contain the inorganic counter ions associated with the PC and therefore cannot be attributed to impurities only.

B.3.3 Thermal methods

Colligative properties of some crystalline materials can allow direct determination of the relative amount (mol/mol) of impurity substance within the candidate material. This quantity can be converted to w_I if the impurities have been identified. Procedures such as differential scanning calorimetry (DSC) and those for freezing and melting point depression methods monitor dynamics of phase changes with respect to temperature.[42]–[44] The relative amount of total impurity content can be determined by these methods through observed deviations in phase change characteristics with respect to a completely (100 %) pure substance.

These assumptions are valid for purity measurement of crystalline materials that have PCs with experimentally viable phase transition temperatures. The materials should also give rise to clear and sharp thermogram peaks that do not shift with different heating rates, have sufficiently high PC purity [for DSC, ≥ 98,5 % (mol/mol)] and contain only impurities that are insoluble in the solid phase and soluble in the liquid phase.[9]

Annex C
(informative)

Examples of production of pure substance organic certified reference materials by reference material producers

C.1 Preamble

There is no single approach for implementing the requirements of this document, but the following examples illustrate the ways in which several RMPs have addressed them. The examples were compiled by asking the RMPs to complete a template structured according to the relevant clauses of this document. They are not intended to be used as model answers. However, they clearly demonstrate that it is possible to address the requirements in a variety of ways, depending on both the nature of the candidate material and the resources available to the RMP.

C.2 The certification of the purity of benzo[*a*]pyrene

C.2.1 Information regarding the example

Target analyte (material): benzo[*a*]pyrene.

Methods used:

— GC with flame ionization and mass spectrometric detection;

— comprehensive two-dimensional GC with flame ionization detection;

— HPLC with ultraviolet (UV) detection;

— differential scanning calorimetry;

— inductively coupled plasma optical emission and mass spectrometry;

— combustion with subsequent UV fluorescence or thermal conductivity detection;

— qNMR spectroscopy;

— interlaboratory collaborative purity assignment work.

C.2.2 Sourcing

Benzo[*a*]pyrene was synthesized on demand for this project.

C.2.3 Identity

The identity of the substance is established through knowledge of the steps of the custom synthesis, by agreement of the NMR spectra with literature data, agreement of the elemental composition with the theoretical composition as well as the agreement of the chromatographic retention time of the PC with the retention times of standard solutions.

C.2.4 Strategies

The basic goal of the project was to obtain materials of a purity above 95 %. In this case, impurity quantities expressed as amount-of-substance fraction, mass fraction and response fraction (chromatographic area per cent) were assumed to be roughly equivalent, as even response factors differing by an order of magnitude

were not expected to have a large impact on the final assessment of purity. If this is ensured, results from different methods can be combined even if they contain unidentified impurities.

Multiple laboratories were used to provide a consensus value of purity evaluation of this candidate material. The methods used for the mass-balance approach aimed at the quantification of impurities of related structure, inorganic impurities and residual solvents. Unfortunately, none of the laboratories were able to identify the impurities, therefore results were reported in area-percent for the chromatographic methods and mass fractions for the inorganic impurities. In addition, quantitative NMR was carried out and the certified value is a combination of the results obtained by qNMR and the mass balance approach.

C.2.5 Assessment of homogeneity

C.2.5.1 Between-vial homogeneity

The number of vials selected corresponds to approximately the cube root of the total number of vials produced. For the benzo[*a*]pyrene CRM, 15 vials were selected using a random stratified sampling scheme covering the whole batch, which was divided into groups with an equal number of vials per group (one vial was selected randomly from each group).

Triplicate (independent) analyses were performed on each vial of the benzo[*a*]pyrene CRM by DSC.

Regression analysis was performed; no trends in the filling sequence or the analytical sequence were observed at the 95 % confidence level. The datasets were assessed for consistency using Grubbs outlier tests at a confidence level of 99 % on the individual results and on the vial means; no outliers were detected.

The distribution of the mean values per vial was judged to be normal by visual inspection using histograms and normal probability plots. Quantification of between-vial inhomogeneity was undertaken by analysis of variance (ANOVA).

The results of the evaluation of the between-vial variation are summarized in Table C.1.

Table C.1 — Results of the homogeneity study

Between-vial relative standard deviation $s_{bb,rel}$ %	Relative uncertainty that could be hidden by method repeatability $u^*_{bb,rel}$ %	Relative uncertainty related to potential between-vial inhomogeneity $u_{bb,rel}$ %
—[a]	0,002	0,002

[a] Cannot be calculated as $MS_{among} < MS_{within}$, where:

MS_{among} is mean of squares between-vial from ANOVA;

MS_{within} is mean of squares within-vial from ANOVA.

The between-vial standard deviation can be used as an estimate of between-vial uncertainty, u_{bb}.[1],[45] As the influence of measurement variability (u^*_{bb}) sets the limits of the study to detect inhomogeneity, the larger value of the two values s_{bb} and u^*_{bb} was adopted as the uncertainty contribution to account for potential inhomogeneity.

C.2.5.2 Within-vial homogeneity and minimum sample size

As the laboratories applying the mass balance approach carried out determinations on six different subsamples of two different vials, this can be considered good evidence that the sample sizes used by the laboratories were appropriate. Typically, sample sizes of 0,25 mg to 5 mg have been used. The homogeneity data by DSC was obtained using 1,16 mg to 1,80 mg samples. Summarizing these findings, a minimum sample size of 1 mg is established for use of the CRM.

C.2.6 Assessment and monitoring of stability

C.2.6.1 General

The stability studies were carried out using an isochronous design.

C.2.6.2 Short-term stability study

For the short-term stability study, samples were stored at 18 °C and 60 °C for 0, 1, 2 and 4 weeks (at each temperature). The reference temperature was set to −20 °C. Two vials of the benzo[*a*]pyrene CRM per storage time were selected using a random stratified sampling scheme. From each vial, two samples were measured by DSC. The measurements were performed under repeatability conditions.

The results were screened for outliers using the single and double Grubbs test at a confidence level of 99 %. One outlying individual result was found for the benzo[*a*]pyrene CRM. As no technical reason for the outliers could be found, all data were retained for statistical analysis.

In addition, the data were evaluated against storage time, and regression lines of amount-of-substance fraction versus time were calculated, to test for potential increases or decreases in the purity due to shipping conditions. The slopes of the regression lines were tested for statistical significance. None of the trends was statistically significant at a 95 % confidence level for any of the temperatures.

The samples can be safely dispatched under conditions where the temperatures do not exceed 60 °C for up to 4 weeks, i.e. at ambient temperature.

C.2.6.3 Long-term stability study

For the long-term stability study, samples were stored at 4 °C and evaluated after 0, 4, 8 and 12 months. The reference temperature was set to −20 °C. Three vials per storage time were selected using a random stratified sampling scheme. From each vial, two samples were measured by DSC. The measurements were performed under repeatability conditions.

The results were screened for outliers using the single and double Grubbs test at a confidence level of 99 %. One outlying result was found for the benzo[*a*]pyrene CRM. As no technical reason for the outlier could be found, all data were retained for statistical analysis.

The data were plotted against storage time and linear regression lines of amount-of-substance fraction versus time were calculated. The slopes of the regression lines were tested for statistical significance (loss or increase due to storage). No significant trend was detected at a 95 % confidence level. The material can therefore be stored at 4 °C.

C.2.6.4 Estimation of uncertainties

The uncertainty of the linear regression line with a slope of zero was calculated. The uncertainty contributions u_{sts} and u_{lts} were calculated as the product of the chosen transport time or shelf life and the uncertainty of the regression lines according to Formulae (C.1) and (C.2):

$$u_{sts,rel} = \frac{s_{rel}}{\sqrt{\sum (t_i - \bar{t})^2}} \cdot t_{tt} \quad \text{(C.1)}$$

$$u_{lts,rel} = \frac{s_{rel}}{\sqrt{\sum (t_i - \bar{t})^2}} \cdot t_{sl} \quad \text{(C.2)}$$

where

$u_{sts,rel}$ is relative standard uncertainty calculated as the product of the chosen transport time;

$u_{lts,rel}$ is relative standard uncertainty calculated as the product of the chosen shelf life;

s_{rel} is relative standard deviation of all results of the stability study;

t_i is time elapsed at time point i;

$\bar{t}$ is the mean of all t_i;

t_{tt} is chosen transport time (1 week at 60 °C);

t_{sl} is chosen shelf life (24 months at 4 °C).

After the certification study, the material was included in the producer's regular stability monitoring programme, to control its further stability.

C.2.6.5 Additional confirmation of stability

For the previous polycyclic aromatic hydrocarbons (PAH) CRMs, no instability was detected over a period of 17 years to 27 years. This confirms the finding of the long-term stability study.

Since the characterization exercise took longer than planned, it was decided to check the material stability beyond the established 24-month shelf life. This was done by comparative analysis of CRMs stored at the normal storage temperature (4 °C) and at the reference temperature (−20 °C).

Measurements were performed by qNMR. The average purities at the two storage temperatures with their expanded uncertainties are summarized in Table C.2.

Table C.2 — Purities of samples stored at +4 °C and −20 °C as determined by qNMR

	Purity P kg/kg	Expanded uncertainty[a] U kg/kg
−20 °C	0,987	0,010
4 °C	0,988	0,010
[a] Coverage factors used $k = 2$.		

Purities for samples stored at +4 °C agreed with those of samples stored at −20 °C (Table C.2), demonstrating that no detectable change had occurred for 12 years.

C.2.7 Consideration of metrological traceability

C.2.7.1 Identity

An established synthetic route was used to deliver benzo[*a*]pyrene. This was confirmed by agreement of the NMR spectra with literature data, agreement of the elemental composition with the theoretical composition, as well as co-elution of the primary component with the PC of standard solutions. Accordingly, the identity of the PC was established beyond any doubt.

C.2.7.2 Quantity value

The purity values have been obtained by a combination of subtraction of the sum of impurities from unity and direct quantification by qNMR. The sum of impurities as such is not traceable to the SI, as it was impossible to identify and calibrate each measurement for the individual impurities. Still, the purity of the CRM can be considered SI-traceable, as the fraction of impurities was small and associated with a conservative uncertainty estimate.

The values by qNMR are traceable to the SI by the traceability of the standards used for quantification.

The purity values are therefore SI-traceable, as they are combinations of two SI-traceable values.

C.2.8 Characterization using direct methods

C.2.8.1 qNMR

qNMR measurements were performed by three laboratories using different internal standards and solvents. The values of the three laboratories agree within their respective uncertainties (Table C.3).

Table C.3 — Purity of the benzo[*a*]pyrene as determined by qNMR

	Purity *P* kg/kg	Expanded uncertainty[a] *U* kg/kg
L1	0,981 8	0,010 0
L5	0,987 3	0,009 9
L7	0,986 8	0,008 4
[a] Coverage factors used $k = 2$.		

The unweighted mean of the three laboratory means was used as the combined value for qNMR. Based on estimates submitted by the laboratories, it was apparent that uncertainty in integration and weighing of the samples were the main components of measurement uncertainty. These factors are independent of one another and therefore reduced by the square root of the number of results averaged. The combined uncertainty was therefore estimated according to Formula (C.3):

$$u_{\mathrm{qNMR}} = \sqrt{\frac{u_{\mathrm{L1}}^2 + u_{\mathrm{L5}}^2 + u_{\mathrm{L7}}^2}{3^2}} \tag{C.3}$$

where

u_{qNMR} is the combined standard uncertainty for qNMR result;

u_{L1} is standard uncertainty of the result from the laboratory 1;

u_{L5} is standard uncertainty of the result from the laboratory 5;

u_{L7} is standard uncertainty of the result from the laboratory 7.

Note that the denominator 3 is the sensitivity coefficient of 1/3, which comes from the division by 3 in averaging three results for each laboratory. The combined purity as determined by qNMR is shown in Table C.4.

Table C.4 — Purity of the benzo[*a*]pyrene CRM by qNMR

Purity *P* kg/kg	Expanded uncertainty[a] *U* kg/kg
0,985 3	0,005 4
[a] Coverage factors used $k = 2$.	

C.2.8.2 Differential scanning calorimetry

DSC measurements were used as additional confirmation, but not taken into account for the calculation of the certified value. DSC does not detect impurities that form solid solutions with the main component. As it was to be expected that most of the impurities would be structurally very similar by-products of the synthesis of the PAHs, it could be assumed that some impurities would not be detected by DSC. Comparing the DSC results with those of the other methods, it was apparent that DSC overestimated the purity of the PAHs.

C.2.9 Characterization using indirect methods

C.2.9.1 General

The mass balance approach was used. Accurate determination of the mass fraction of impurities for the chromatographic methods would require identification of all impurities, calibration (or at least knowledge of their relative molecular mass and sensitivity) and subsequent quantification. Participants were requested to identify and quantify impurities. However, as it was not possible to identify the impurities, results were reported in area per cent for the chromatographic methods and mass fractions for the inorganic impurities.

In the absence of reliable sensitivity factors for the impurities, area-fractions and amount-of-substance fractions were converted into mass fractions using a conversion factor of one. This is justified because of the sufficiently high purity of the main compound: uncertainty of the response factors for the individual impurities results in a large relative uncertainty for each of them. However, the relative amounts of these impurities are small and they are not major components of uncertainty of purity of the main compound.

In order to obtain an overall estimate of the purity using the mass balance approach, the impurity fractions detected by GC-based methods, by HPLC-UV methods and the inorganic impurities were summed and subtracted from unity.

C.2.9.2 Organic impurities

An assumption was made that GC-based methods would detect a different set of impurities than HPLC-UV. Therefore, the impurities detected by GC-based methods were added to the impurities detected by HPLC-UV to obtain an estimate of the total impurity content. This assumption was based on the different separation principles of the methods. This can only be an approximation because it is likely that some impurities are detected by both methods. The final stated purity of the PAH was probably underestimated, due to the calculation approach chosen, but could be considered as best estimate that can be achieved with the available data.

To estimate the mass fraction of impurities detected by a particular method (e.g. GC-based methods or HPLC-based methods), the following approach was taken.

The different datasets, including the chromatograms provided by the different laboratories, were considered, choosing the dataset that detected the largest number of impurities (not necessarily being identical with the largest mass fraction of impurities). The impurities detected in this dataset were then taken as the best estimate of the total impurities detectable by this method. In order to obtain an estimate for the uncertainty associated with the purity estimate, a symmetric uncertainty interval was chosen such that it would cover any value reported in other datasets using the same method. This uncertainty interval was then considered to follow a rectangular distribution and consequently converted into a standard uncertainty. This standard uncertainty was then used as an estimate of the uncertainty of the impurity determination with this particular method. The uncertainty of the total impurities was calculated as the square root of the sum of squares of the uncertainties of the different methods contributing to the total impurities.

As a best estimate of impurities detectable by GC-based methods, GC-FID data was used. FID was considered as the most likely detector to elicit consistent responses for the different PAH compounds. Additionally, the largest number of impurities was detected using GC-FID.

C.2.9.3 Inorganic impurities

Results of the elemental analysis of nitrogen and sulfur are summed with the inorganic impurity content determined by ICP-MS and ICP-OES to obtain an estimate of total inorganic impurity content.

The mass fractions of all detected elements were summed. The relative standard uncertainty for this sum was evaluated as 10 %.

The sum of the limits of detection (LOD) of the non-detected elements was taken as the upper limit of the mass fraction of the non-detected elements. For the calculation, the mass fraction of all non-detected elements was set to zero. This is justified as the sum of the LODs of non-detected elements was only 1/5 to 1/20 of the sum of the detected impurities. The standard uncertainty of this estimate was assumed to follow

a rectangular distribution between zero and the sum of all LODs and was therefore obtained by dividing the sum of all LODs by $\sqrt{3}$.

The final value for inorganic impurities was the sum of all detected impurities. The standard uncertainty of inorganic impurities was the combined uncertainty of the detected and non-detected impurities as described previously.

C.2.9.4 Residual solvents

Residual solvents were assessed only in a qualitative manner by DSC analysis starting at room temperature. Evaporation of solvent leads to perturbation of the heat flow curve due to its evaporation enthalpy. The observed heat flow curve was smooth up to the melting point. Therefore, solvent residues were not observed.

C.2.9.5 Summary of mass balance approach

A summary of the mass balance approach is presented in Table C.5.

Table C.5 — Summary of impurities as determined by different methods and combined purity following the mass balance approach

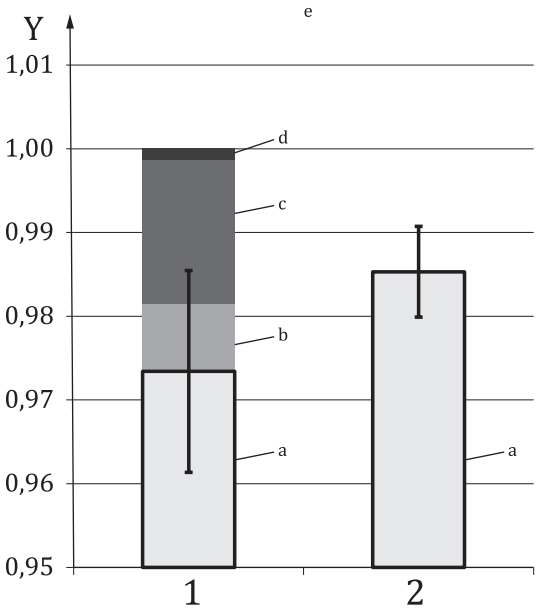
	Impurity/purity kg/kg	**Standard uncertainty** *u* kg/kg
Impurities detected by GC-based methods	0,008 1	0,000 6
Impurities detected by HPLC-based methods	0,017 2	0,005 6
Inorganic impurities	0,001 3	0,000 1
Total impurities	0,026 6	0,006 0
Purity	0,973 4	0,006 0

C.2.10 Treatment of non-detects in characterization

See C.2.9.3.

C.2.11 Combination of qNMR and mass balance approach

The results of the purity determined by the mass balance approach and by qNMR are shown in Figure C.1.



Key

Y	mass fraction (g/g)
1	mass balance
2	qNMR
a	Mass fraction of the PC.
b	Impurities detected by HPLC.
c	Impurities detected by GC.
d	Inorganic impurities.
e	Benzo[*a*]pyrene.

NOTE The error bar on the primary component is the expanded uncertainty (k = 2).

Figure C.1 — Comparison of the results of the purity determination by mass balance and qNMR

The results from the mass balance approach and from qNMR agree within the respective uncertainties. It is not known whether the difference between the central values of the estimates is partially due to a bias associated with double counting of some impurities in the mass-balance approach, an non-detected interference in qNMR or other random effects reflected in the uncertainties.

Since the purity obtained by mass balance and by qNMR agree, the purity of the PAH was calculated according to Formula (C.4):

$$w_{\text{purity}} = \frac{w_{\text{qNMR}} + w_{\text{MB}}}{2} \tag{C.4}$$

where

w_{purity} is combined purity of the candidate material;

w_{qNMR} is purity as determined by qNMR;

w_{MB} is purity as determined by the mass balance approach.

The uncertainty was calculated according to Formula (C.5). Note that the denominator 2 in Formula (C.5) is the sensitivity coefficient of 1/2, which comes from the division by 2 in Formula (C.4).

$$u_{\text{char}} = \sqrt{\frac{u_{\text{qNMR}}^2}{2^2} + \frac{u_{\text{MB}}^2}{2^2}} \tag{C.5}$$

where

u_{char} is standard uncertainty of characterization;

u^2_{qNMR} is standard uncertainty derived from the purity determination by qNMR;

u^2_{MB} is standard uncertainty derived from the purity determination by the mass balance approach.

The results are reported in Table C.6.

Table C.6 — Summary of the purity and uncertainty for the benzo[*a*]pyrene CRM

Purity	Combined standard uncertainty	Relative combined standard uncertainty
P	u_{char}	$u_{char,rel}$
kg/kg	kg/kg	%
0,979 4	0,003 3	0,34

C.2.12 Assignment of property value and uncertainty

The assigned uncertainty consists of uncertainties relating to characterization, u_{char}, potential between-vial inhomogeneity, u_{bb}, and potential degradation during transport, u_{sts}, and long-term storage, u_{lts}. These different contributions were combined to estimate the relative expanded uncertainty of the certified value ($U_{CRM,\,rel}$) with a coverage factor k = 2 as given in Formula (C.6):

$$U_{CRM,\,rel} = k\sqrt{u^2_{bb,\,rel} + u^2_{sts,\,rel} + u^2_{lts,\,rel} + u^2_{char,\,rel}} \tag{C.6}$$

$U_{CRM,\,rel}$ is the relative expanded uncertainty of the certified value;

k is coverage factor;

$u^2_{bb,\,rel}$ is relative standard uncertainty relating to potential between-vial inhomogeneity;

$u^2_{sts,\,rel}$ is relative standard uncertainty relating to potential degradation during transport;

$u^2_{lts,\,rel}$ is relative standard uncertainty relating to potential degradation during long-term storage;

$u^2_{char,\,rel}$ is relative standard uncertainty relating to characterization.

The certified value and uncertainty are summarized in Table C.7.

Table C.7 — Certified value and uncertainty for the benzo[*a*]pyrene CRM

Purity	Relative standard uncertainties				Expanded uncertainty of the certified value[a]
	Characterization	Potential between-vial inhomogeneity	Potential degradation during long-term storage	Potential degradation during transport	
P	$u_{char,rel}$	$u_{bb,rel}$	$u_{lts,rel}$	$u_{lts,rel}$	U_{CRM}
kg/kg	%	%	%	%	kg/kg
0,979	0,34	0,002	0,024	0,018	0,007

[a] Coverage factor used k = 2.

C.3 Purity assessment of benzoic acid

C.3.1 Information regarding the example

Target analyte (material): benzoic acid.

Methods used: q^1H-NMR with internal standard (q^1H-NMR_{IS}), qNMR with external standard (q^1H-NMR_{ES}), coulometric titration of H^+, mass balance, stable isotope ratio analysis.

C.3.2 Sourcing

Ultra-pure benzoic acid candidate material was sourced from a commercial supplier. A 1,8 kg synthesized batch of high-purity benzoic acid (>99 %) was micronized to release water occluded within the crystal structure and increase purity of the material. Suitability tests of chemical purity via q^1H-NMR and coulometry were performed before and after micronization. Once the material was verified to be adequately pure, units were packaged as two bottles, each containing 0,5 g of material. The screwcap bottles were flushed with inert gas prior to filling and sealing. The bottles were sealed (hermetically) within polymer pouches.

C.3.3 Identity

Identity of benzoic acid, having a relatively simple structure, was determined using multiple methods for structural analysis: ^{1}H, ^{13}C, and ^{1}H-^{13}C NMR (^{1}H resonance frequency at 400 MHz and 600 MHz), liquid chromatography/high resolution MS (LC-HRMS; orbitrap); assessment of isotopic composition through stable isotope analysis for H, C and O. Additionally, comparison of ^{1}H-NMR spectra with those of other well-characterized benzoic acid materials and published spectra confirmed identification of the PC.

C.3.4 Assessment of homogeneity

A uniform sampling of the production lot was assessed for certification measurements: 36 units for coulometric titration, 10 units for qNMR with internal standard, 12 units for KFT water assay, 5 units for qNMR with external standard. Within- and between-bottle homogeneity was assessed for 5 mg quantities via qNMR with an internal standard. Heterogeneity was not determined to be significant with respect to the uncertainty of the certified value and there was no trend in purity with respect to the filling order.

C.3.5 Assessment and monitoring of stability

Prior to bottling, tests for hygroscopicity and stability were performed at elevated temperatures and humidity for 6 months.

C.3.6 Consideration of metrological traceability

The procedures implemented to realize SI measurement units for chemical mass fraction included primary direct coulometric titration, primary ratio qNMR, primary indirect mass balance. Metrological traceability to the SI is established through the assessment of chemical structure (NMR, HRMS) and purity analyses. The purity results are to the SI unit for mass (kg) and the Faraday constant (s A mol^{-1}) (coulometry, NMR).

C.3.7 Characterization using direct methods

The following direct methods were performed:

— Coulometric titration of H^+.

— qNMR with internal standard ($qNMR_{IS}$): measurements using each of four different internal standards: dimethyl sulfone, dimethylmalonic acid, maleic acid, 3,5-dinitrobenzoic acid. This level of rigour, applied for characterization of a primary standard for qNMR, is usually not necessary for characterization of most CRM candidate materials.

— qNMR with external standard ($qNMR_{ES}$): comparison with benzoic acid external standard of known purity; results from this method were confirmatory and did not contribute to calculation of the certified purity value.

— Stable isotope ratio analysis of C, H, O for the determination of molecular weight and relative abundance of ^{1}H and ^{13}C isotopes.

C.3.8 Characterization using indirect methods

Mass balance approach (confirmatory, not used for property value assignment):

— structurally related impurities: LC-HRMS; ^{1}H NMR and ^{1}H-^{13}C NMR;

— water: KFT;

— volatiles: thermogravimetric analysis;

— non-volatiles: ashing, ion chromatography with conductivity detector.

C.3.9 Treatment of non-detects in characterization

Non-observables were not considered.

Observed, below limit of quantification (LOQ): treated as uniform distribution along the interval 0 kg/kg to LOQ.

C.3.10 Combination of methods (to get one property value)

A probability mixture model (linear pool) was used to combine the results of coulometric titration and $q^{1}H\text{-}NMR_{IS}$ procedures.[21] This is a blending of the respective probability distributions for a full-structure inference of property values

C.3.11 Uncertainty estimation

Hierarchical Bayesian models were developed to evaluate the uncertainty of the $q^{1}H$-NMR purity result.[21] This approach is a hybrid of top-down and bottom-up approaches to the evaluation of measurement uncertainty and preserves statistical correlations associated with the use of different internal standards. This approach also observes the natural limit of 1 kg/kg for calculation of the measurement result.

For the coulometric titration results, a rigorous accounting of type A and type B uncertainties associated with the acidimetric assay, including values for physical constants, chemical and electrical interferences, and measurement repeatability, were combined according to a procedure consistent with the GUM. The combined standard uncertainty was determined and expanded (k = 1,97) with respect to the effective degrees of freedom.

For the mass balance approach, probability distributions were assigned for the measurement results for each impurity component and the purity was calculated using Monte Carlo procedures via the NIST Uncertainty Machine[46] consistent with the GUM, Supplement 1.[5]

C.3.12 Combination of data

The $qNMR_{IS}$ measurements consisted of four separate sets of samples, each prepared using a different internal standard and comprising at least 10 replicates. The qNMR result was calculated using a Monte Carlo method to fit a hierarchical model, based on the qNMR measurement equation, to all of the data collected from measurement of these sample sets.

The coulometric results were evaluated as the mean of replicate titrations, with uncertainty calculated according to a rigorous GUM-based approach.

The confirmatory mass balance approach result was evaluated using a Monte Carlo method via the NIST Uncertainty Machine[46] for the model in Formula (C.7):

$$w_{PC} = 1 - (w_{RS} + w_w + w_V + w_{NV}) \quad \text{(C.7)}$$

where

w_{PC} is the mass fraction of the PC;

w_{RS} is the mass fraction of structurally related impurities;

w_w is water content;

w_V is organic volatiles content;

w_{NV} is non-volatiles content.

The measured isotope ratios were used to calculate the average atomic weights of C, H and O in the candidate material and derive the respective molar mass of the benzoic acid CRM according to the chemical formula $C_7H_6O_2$.

C.3.13 Assignment of property values

Purity (kg/kg): determined as the mean and 95 % coverage interval of the pooled coulometry and $qNMR_{IS}$ result.

Specific amount of substance (mol/kg): this value was calculated from the same probability distribution as the purity result, converted using the determined molecular weight of benzoic acid.

The uncertainty of the certified property values are asymmetric intervals. Guidance for propagating these uncertainties and the associated probability distribution parameters are provided in the certificate of analysis.

C.3.14 Information in RM document

The following information as a minimum is included in the RM document:

— unit size (mass);

— intended use;

— certified property value and standard uncertainty;

— property value 95 % confidence interval;

— property value probability distribution;

— statement of metrological traceability;

— recommended storage;

— minimum sample size;

— expiration date;

— isotope atom fractions;

— measurement methods used for certification.

C.4 Purity assessment of Cystine

C.4.1 Information regarding the example

Target analyte (material): cystine; intended for use with microchemical procedures for the determination of carbon, hydrogen, nitrogen and sulfur in organic matter.

Methods used: q^1H-NMR with internal standard ($qNMR_{IS}$); elemental microanalysis, HPLC with UV detection (HPLC-UV), GC-MS, KFT.

C.4.2 Sourcing

High-purity cystine was sourced from a commercial chemical provider. Suitability tests of chemical purity were performed via q^1H-NMR prior to acceptance and bottling of the material.

C.4.3 Identity

Identity of cystine was verified through determination of chemical structure and mass purity via q^1H-NMR_{IS}. Measured values of elemental composition (mass fractions of C, H, S and N) via elemental microanalysis were consistent with the theoretical average elemental composition of cystine.

C.4.4 Assessment of homogeneity

Measurement of purity via q^1H-NMR_{IS} was conducted using 22 units selected uniformly from across the production lot of 651 units. No significant heterogeneity was observed with respect to the uncertainty of certified purity value, nor was there a trend in purity with respect to the filling order.

C.4.5 Assessment and monitoring of stability

This material has undergone stability assessment with respect to purity and elemental composition every 10 years since sourcing. Stored under recommended conditions, this material is adequately stable for many years.

C.4.6 Consideration of metrological traceability

Metrological traceability of the certified purity value is to the SI through practical realization of measurement units for chemical mass fraction (expressed as a percentage), established through an unbroken chain of comparisons to the Benzoic Acid CRM.[25]

C.4.7 Characterization using direct methods

The following two direct methods were performed:

— $qNMR_{IS}$: a dimethyl malonic acid internal standard was used for calibration. The purity value of the internal standard is metrologically traceable to the SI through relation to the certified value of a bezoic acid CRM utilized as a primary standard for qNMR.[25]

— Elemental analysis for C, H, S and N was performed on 50 bottles of the original production lot by three independent outside laboratories.

C.4.8 Characterization using indirect methods

Impurity components were assayed via HPLC-UV, GC-MS and KFT.

C.4.9 Treatment of non-detects in characterization

Non-detects were not considered in the assessment of uncertainty of the certified purity value determined by direct measurement procedures.

C.4.10 Combination of methods (to get one property value)

Certified chemical identity: determinations of chemical structure and purity are both required to evaluate this property without ambiguity. Primary evidence for this characterization is through $q^1H\text{-}NMR_{IS}$, supported by elemental analysis, GC-MS and KFT analyses to confirm the certified purity value.

C.4.11 Uncertainty estimation or assignment of property values

Certified chemical identity, theoretical elemental composition:

The theoretical elemental composition of cystine was calculated for $C_6H_{12}N_2O_4S_2$ using IUPAC standard atomic weights of the elements, the IUPAC Molecular Weight Calculator shiny app[47] and Monte Carlo procedures via the NIST Uncertainty Machine.[46] Cystine molecular weight, required to calculate theoretical average mass fractions of the elements in cystine, was calculated without additional knowledge of provenance or natural variations in isotopic abundance for the molecular formula.

A Monte Carlo method based on the measurement function for $q^1H\text{-}NMR_{IS}$ and qNMR experiment data inputs was used to calculate the purity result. This statistical model was constrained by the mass fraction limit, 1 kg/kg,[21] to calculate the certified purity value and uncertainty, which specify the range of values attributable to the measurand with a confidence level of approximately 95 %.

C.4.12 Combination of data

Data were combined as follows:

— Certified chemical identity: unambiguous determination of chemical structure using NMR, elemental composition by microanalysis and assessment of cystine purity substantiate confidence in this property evaluation.

— Certified chemical purity: direct measurement of cystine via $q^1H\text{-}NMR_{IS}$ was implemented to evaluate the certified property value and associated uncertainty. Direct elemental analysis and determination of impurities confirms the accuracy of the certified properties.

Assignment of property values:

— Certified chemical identity: established through unambiguous determination of chemical structure and high purity between 0,997 kg/kg and 1,000 kg/kg.

— Certified chemical purity: determined as the mean value and 95 % coverage interval of values calculated using the Monte Carlo method based for $q^1H\text{-}NMR_{IS}$ measurements.

C.4.13 Information in RM document

The following information as a minimum is included in the RM document:

— unit size (mass);

— intended use;

— certified properties;

— property value standard uncertainty;

— property value 95 % confidence interval;

— property value probability distribution;

— statement of metrological traceability;

— recommended storage;

— minimum sample size;