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ข้อแนะนำในการหาค่าความไม่แน่นอน ในการวัดเชิงวิเคราะห์

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สำนักงานมาตรฐานผลิตภัณฑ์อุตสาหกรรม
กระทรวงอุตสาหกรรม ถนนพระรามที่ 6 กรุงเทพมหานคร 10400
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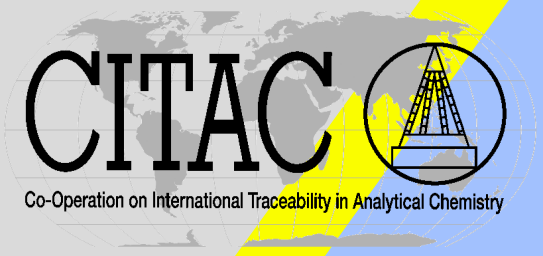
บทนำ

มอก.17025-2548 : ข้อกำหนดทั่วไปว่าด้วยความสามารถของห้องปฏิบัติการทดสอบและห้องปฏิบัติการสอบเทียบ ได้กำหนดขึ้นเพื่อใช้เป็นเกณฑ์สำหรับห้องปฏิบัติการที่ต้องการแสดงให้เห็นว่ามีการดำเนินงานด้านคุณภาพและความสามารถทางวิชาการ และใช้เป็นเกณฑ์สำหรับการประเมินเพื่อให้การรับรองห้องปฏิบัติการ โดยข้อกำหนดทางวิชาการ ข้อ 5.4.6 ได้กล่าวถึงการประมาณค่าความไม่แน่นอนของการวัดที่ห้องปฏิบัติการต้องจัดทำ ดังนั้น สมอ.ในฐานะหน่วยงานผู้ให้การรับรองห้องปฏิบัติการทดสอบและสอบเทียบ จึงเห็นสมควรจัดทำเอกสารข้อแนะนำในการหาค่าความไม่แน่นอนในการวัดเชิงวิเคราะห์ สำหรับเป็นแนวทางการดำเนินการของห้องปฏิบัติการ รวมทั้งใช้เป็นแนวทางสำหรับผู้ประเมินในการตรวจประเมินการวัดเชิงวิเคราะห์ ซึ่งในการนี้ สมอ.เห็นควรรับเอกสารข้อแนะนำ “ Quantifying Uncertainty in Analytical Measurement : 2012 (Third Edition) ” ที่จัดทำขึ้นโดย EURACHEM/CITAC มาใช้เป็นแนวทางในการหาค่าความไม่แน่นอนในการวัดเชิงวิเคราะห์ ที่เกี่ยวข้องกับการทดสอบทางเคมี หรือสามารถประยุกต์ใช้กับการทดสอบที่เกี่ยวข้องได้ ซึ่งหวังว่าคงมีประโยชน์กับผู้นำไปประยุกต์ใช้ตามสมควร

Eurachem



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Quantifying Uncertainty in Analytical Measurement

Third Edition

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Cooperation on International Traceability in Analytical Chemistry

EURACHEM/CITAC Guide

Quantifying Uncertainty in Analytical Measurement

Third Edition

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CITAC Reference
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Foreword to the Third Edition

Many important decisions are based on the results of chemical quantitative analysis; the results are used, for example, to estimate yields, to check materials against specifications or statutory limits, or to estimate monetary value. Whenever decisions are based on analytical results, it is important to have some indication of the quality of the results, that is, the extent to which they can be relied on for the purpose in hand. Users of the results of chemical analysis, particularly in those areas concerned with international trade, are coming under increasing pressure to eliminate the replication of effort frequently expended in obtaining them. Confidence in data obtained outside the user's own organisation is a prerequisite to meeting this objective. In some sectors of analytical chemistry it is now a formal (frequently legislative) requirement for laboratories to introduce quality assurance measures to ensure that they are capable of and are providing data of the required quality. Such measures include: the use of validated methods of analysis; the use of defined internal quality control (QC) procedures; participation in proficiency testing (PT) schemes; accreditation based on ISO/IEC 17025 [H.1], and establishing traceability of the results of the measurements.

In analytical chemistry, there has been great emphasis on the precision of results obtained using a specified method, rather than on their traceability to a defined standard or SI unit. This has led the use of "official methods" to fulfil legislative and trading requirements. However as there is a formal requirement to establish the confidence of results it is essential that a measurement result is traceable to defined references such as SI units or reference materials even when using an operationally defined or empirical (sec. 5.4.) method. The Eurachem/CITAC Guide "Traceability in Chemical Measurement" [H.9] explains how metrological traceability is established in the case of operationally defined procedures.

As a consequence of these requirements, chemists are, for their part, coming under increasing pressure to demonstrate the quality of their results, and in particular to demonstrate their fitness for purpose by giving a measure of the confidence that can be placed on the result. This is expected to include the degree to which a result would be expected to agree with other results, normally irrespective of the analytical methods used. One useful measure of this is measurement uncertainty.

Although the concept of measurement uncertainty has been recognised by chemists for many years, it was the publication in 1993 of the "Guide to the Expression of Uncertainty in Measurement" (the GUM) [H.2] by ISO in collaboration with BIPM, IEC, IFCC, ILAC, IUPAC, IUPAP and OIML, which formally established general rules for evaluating and expressing uncertainty in measurement across a broad spectrum of measurements. This EURACHEM/CITAC document shows how the concepts in the ISO Guide may be applied in chemical measurement. It first introduces the concept of uncertainty and the distinction between uncertainty and error. This is followed by a description of the steps involved in the evaluation of uncertainty with the processes illustrated by worked examples in Appendix A.

The evaluation of uncertainty requires the analyst to look closely at all the possible sources of uncertainty. However, although a detailed study of this kind may require a considerable effort, it is essential that the effort expended should not be disproportionate. In practice a preliminary study will quickly identify the most significant sources of uncertainty and, as the examples show, the value obtained for the combined uncertainty is almost entirely controlled by the major contributions. A good estimate of uncertainty can be made by concentrating effort on the largest contributions. Further, once evaluated for a given method applied in a particular laboratory (i.e. a particular measurement procedure), the uncertainty estimate obtained may be reliably applied to subsequent results obtained by the method in the same laboratory, provided that this is justified by the relevant quality control data. No further effort should be necessary unless the procedure itself or the equipment used is changed, in which case the uncertainty estimate would be reviewed as part of the normal re-validation.

Method development involves a similar process to the evaluation of uncertainty arising from each individual source; potential sources of uncertainty are investigated and method adjusted to reduce the uncertainty to an acceptable level where possible. (Where specified as a numerical upper limit for uncertainty, the acceptable

level of measurement uncertainty is called the 'target measurement uncertainty' [H.7]). The performance of the method is then quantified in terms of precision and trueness. Method validation is carried out to ensure that the performance obtained during development can be achieved for a particular application and if necessary the performance figures adjusted. In some cases the method is subjected to a collaborative study and further performance data obtained. Participation in proficiency testing schemes and internal quality control measurements primarily check that the performance of the method is maintained, but also provides additional information. All of these activities provide information that is relevant to the evaluation of uncertainty. This Guide presents a unified approach to the use of different kinds of information in uncertainty evaluation.

The first edition of the EURACHEM Guide for "Quantifying Uncertainty in Analytical Measurement" [H.3] was published in 1995 based on the ISO Guide. The second edition [H.4] was prepared in collaboration with CITAC in 2000 in the light of practical experience of uncertainty estimation in chemistry laboratories and the even greater awareness of the need to introduce formal quality assurance procedures by laboratories. The second edition stressed that the procedures introduced by a laboratory to estimate its measurement uncertainty should be integrated with existing quality assurance measures, since these measures frequently provide much of the information required to evaluate the measurement uncertainty.

This third edition retains the features of the second edition and adds information based on developments in uncertainty estimation and use since 2000. The additional material provides improved guidance on the expression of uncertainty near zero, new guidance on the use of Monte Carlo methods for uncertainty evaluation, improved guidance on the use of proficiency testing data and improved guidance on the assessment of compliance of results with measurement uncertainty. The guide therefore provides explicitly for the use of validation and related data in the construction of uncertainty estimates in full compliance with the formal ISO Guide principles set out in the ISO Guide to the Expression of Uncertainty in measurement [H.2]. The approach is also consistent with the requirements of ISO/IEC 17025:2005 [H.1].

This third edition implements the 1995 edition of the ISO Guide to the Expression of Uncertainty in Measurement as re-issued in 2008 [H.2]. Terminology therefore follows the GUM. Statistical terminology follows ISO 3534 Part 2 [H.8]. Later terminology introduced in the International vocabulary of metrology - Basic and general concepts and associated terms (VIM) [H.7] is used otherwise. Where GUM and VIM terms differ significantly, the VIM terminology is additionally discussed in the text. Additional guidance on the concepts and definitions used in the VIM is provided in the Eurachem Guide "Terminology in Analytical Measurement - Introduction to VIM 3" [H.5]. Finally, it is so common to give values for mass fraction as a percentage that a compact nomenclature is necessary; mass fraction quoted as a percentage is given the units of g/100 g for the purposes of this Guide.

NOTE Worked examples are given in Appendix A. A numbered list of definitions is given at Appendix B. The convention is adopted of printing defined terms in bold face upon their first occurrence in the text, with a reference to Appendix B enclosed in square brackets. The definitions are, in the main, taken from the International vocabulary of basic and general standard terms in Metrology (VIM) [H.7], the Guide [H.2] and ISO 3534-2 (Statistics - Vocabulary and symbols - Part 2: Applied Statistics) [H.8]. Appendix C shows, in general terms, the overall structure of a chemical analysis leading to a measurement result. Appendix D describes a general procedure which can be used to identify uncertainty components and plan further experiments as required; Appendix E describes some statistical operations used in uncertainty estimation in analytical chemistry, including a numerical spreadsheet method and the use of Monte Carlo simulation. Appendix F discusses measurement uncertainty near detection limits. Appendix G lists many common uncertainty sources and methods of estimating the value of the uncertainties. A bibliography is provided at Appendix H.

1. Scope and Field of Application

1.1. This Guide gives detailed guidance for the evaluation and expression of uncertainty in quantitative chemical analysis, based on the approach taken in the ISO "Guide to the Expression of Uncertainty in Measurement" [H.2]. It is applicable at all levels of accuracy and in all fields - from routine analysis to basic research and to empirical and rational methods (sees section 5.5.). Some common areas in which chemical measurements are needed, and in which the principles of this Guide may be applied, are:

- Quality control and quality assurance in manufacturing industries.
- Testing for regulatory compliance.
- Testing utilising an agreed method.
- Calibration of standards and equipment.
- Measurements associated with the development and certification of reference materials.
- Research and development.

1.2. Note that additional guidance will be required in some cases. In particular, reference material value assignment using consensus methods (including multiple measurement methods) is not covered, and the use of uncertainty estimates in compliance statements and the expression and use of uncertainty at low levels may require additional guidance. Uncertainties associated with sampling operations are not explicitly treated, since they are treated in detail in the EURACHEM guide "Measurement uncertainty arising from sampling: A guide to methods and approaches" [H.6].

1.3. Since formal quality assurance measures have been introduced by laboratories in a number of sectors this EURACHEM Guide illustrates how the following may be used for the estimation of measurement uncertainty:

- Evaluation of the effect of the identified sources of uncertainty on the analytical result for a single method implemented as a defined **measurement procedure [B.6]** in a single laboratory.
- Information from method development and validation.
- Results from defined internal quality control procedures in a single laboratory.
- Results from collaborative trials used to validate methods of analysis in a number of competent laboratories.
- Results from proficiency test schemes used to assess the analytical competency of laboratories.

1.4. It is assumed throughout this Guide that, whether carrying out measurements or assessing the performance of the measurement procedure, effective quality assurance and control measures are in place to ensure that the measurement process is stable and in control. Such measures normally include, for example, appropriately qualified staff, proper maintenance and calibration of equipment and reagents, use of appropriate reference standards, documented measurement procedures and use of appropriate check standards and control charts. Reference [H.10] provides further information on analytical QA procedures.

NOTE: This paragraph implies that all analytical methods are assumed in this guide to be implemented via fully documented procedures. Any general reference to analytical methods accordingly implies the presence of such a procedure. Strictly, measurement uncertainty can only be applied to the results of such a procedure and not to a more general **method of measurement [B.7]**.

2. Uncertainty

2.1. Definition of uncertainty

2.1.1. The definition of the term uncertainty (of measurement) used in this protocol and taken from the current Guide to the Expression of uncertainty in measurement [H.2] is:

"A parameter associated with the result of a measurement, that characterises the dispersion of the values that could reasonably be attributed to the measurand**"**

NOTE 1 The parameter may be, for example, a **standard deviation [B.20]** (or a given multiple of it), or the width of a confidence interval.

NOTE 2 Uncertainty of measurement comprises, in general, many components. Some of these components may be evaluated from the statistical distribution of the results of series of measurements and can be characterised by standard deviations. The other components, which also can be characterised by standard deviations, are evaluated from assumed probability distributions based on experience or other information.

NOTE 3 It is understood that the result of the measurement is the best estimate of the value of the measurand, and that all components of uncertainty, including those arising from systematic effects, such as components associated with corrections and reference standards, contribute to the dispersion.

The following paragraphs elaborate on the definition; the more recent VIM definition is also discussed in section 2.5.

2.1.2. In many cases in chemical analysis, the **measurand [B.4]** will be the concentration* of an analyte. However chemical analysis is used to measure other quantities, *e.g.* colour, pH, *etc.,* and therefore the general term "measurand" will be used.

2.1.3. The definition of uncertainty given above focuses on the range of values that the analyst believes could reasonably be attributed to the measurand.

2.1.4. In general use, the word *uncertainty* relates to the general concept of *doubt*. In this guide, the word *uncertainty,* without adjectives, refers either to a parameter associated with the definition above, or to the limited knowledge about a particular value. *Uncertainty of measurement* does not imply doubt about the validity of a measurement; on the contrary, knowledge of the uncertainty implies increased confidence in the validity of a measurement result.

2.2. Uncertainty sources

2.2.1. In practice the uncertainty on the result may arise from many possible sources, including examples such as incomplete definition of the measurand, sampling, matrix effects and interferences, environmental conditions, uncertainties of masses and volumetric equipment, reference values, approximations and assumptions incorporated in the measurement method and procedure, and random variation (a fuller description of uncertainty sources is given in section 6.7.)

2.3. Uncertainty components

2.3.1. In estimating the overall uncertainty, it may be necessary to take each source of uncertainty and treat it separately to obtain the contribution from that source. Each of the separate contributions to uncertainty is referred to as an uncertainty component. When expressed as a standard deviation, an uncertainty component is known as a **standard uncertainty [B.10].** If there is correlation between any components then this has to be taken into account by determining the covariance. However, it is often possible to evaluate the combined effect of several components. This may reduce the overall effort involved and, where components whose contribution is evaluated together are correlated, there may be no additional need to take account of the correlation.

* In this guide, the unqualified term "concentration" applies to any of the particular quantities *mass* concentration, *amount* concentration, *number* concentration or *volume* concentration unless units are quoted (*e.g.* a concentration quoted in mg L^{-1} is evidently a mass concentration). Note also that many other quantities used to express composition, such as mass fraction, substance content and mole fraction, can be directly related to concentration.

2.3.2. For a measurement result y, the total uncertainty, termed **combined standard uncertainty [B.11]** and denoted by $u_c(y)$, is an estimated standard deviation equal to the positive square root of the total variance obtained by combining all the uncertainty components, however evaluated, using the law of propagation of uncertainty (see section 8.) or by alternative methods (Appendix E describes two useful numerical methods: the use of a spreadsheet and Monte Carlo simulation).

2.3.3. For most purposes in analytical chemistry, an **expanded uncertainty [B.12]** U, should be used. The expanded uncertainty provides an interval within which the value of the measurand is believed to lie with a higher level of confidence. U is obtained by multiplying $u_c(y)$, the combined standard uncertainty, by a **coverage factor [B.13]** k. The choice of the factor k is based on the level of confidence desired. For an approximate level of confidence of 95 %, k is usually set to 2.

NOTE The coverage factor k should always be stated so that the combined standard uncertainty of the measured quantity can be recovered for use in calculating the combined standard uncertainty of other measurement results that may depend on that quantity.

2.4. Error and uncertainty

2.4.1. It is important to distinguish between error and uncertainty. **Error [B.16]** is defined as the difference between an individual result and the **true value [B.2]** of the measurand. In practice, an observed measurement error is the difference between the observed value and a reference value. As such, error – whether theoretical or observed – is a single value. In principle, the value of a known error can be applied as a correction to the result.

NOTE Error is an idealised concept and errors cannot be known exactly.

2.4.2. Uncertainty, on the other hand, takes the form of a range or interval, and, if estimated for an analytical procedure and defined sample type, may apply to all determinations so described. In general, the value of the uncertainty cannot be used to correct a measurement result.

2.4.3. To illustrate further the difference, the result of an analysis after correction may by chance be very close to the value of the measurand, and hence have a negligible error. However, the uncertainty may still be very large, simply because the analyst is very unsure of how close that result is to the value of the measurand.

2.4.4. The uncertainty of the result of a measurement should never be interpreted as representing the error itself, nor the error remaining after correction.

2.4.5. An error is regarded as having two components, namely, a random component and a systematic component.

2.4.6. Random error [B.17] typically arises from unpredictable variations of **influence quantities [B.3]**. These random effects give rise to variations in repeated observations of the measurand. The random error of an analytical result cannot be compensated for, but it can usually be reduced by increasing the number of observations.

NOTE 1 The experimental standard deviation of the **arithmetic mean [B.19]** or average of a series of observations is *not* the random error of the mean, although it is so referred to in some publications on uncertainty. It is instead a measure of the uncertainty of the mean due to some random effects. The exact value of the random error in the mean arising from these effects cannot be known.

2.4.7. Systematic error [B.18] is defined as a component of error which, in the course of a number of analyses of the same measurand, remains constant or varies in a predictable way. It is independent of the number of measurements made and cannot therefore be reduced by increasing the number of analyses under constant measurement conditions.

2.4.8. Constant systematic errors, such as failing to make an allowance for a reagent blank in an assay, or inaccuracies in a multi-point instrument calibration, are constant for a given level of the measurement value but may vary with the level of the measurement value.

2.4.9. Effects which change systematically in magnitude during a series of analyses, caused, for example by inadequate control of experimental conditions, give rise to systematic errors that are not constant.

EXAMPLES:

1. A gradual increase in the temperature of a set of samples during a chemical analysis can lead to progressive changes in the result.

2. Sensors and probes that exhibit ageing effects over the time-scale of an experiment can also introduce non-constant systematic errors.

2.4.10. The result of a measurement should be corrected for all recognised significant systematic effects.

NOTE Measuring instruments and systems are often adjusted or calibrated using measurement standards and reference materials to correct for systematic effects. The uncertainties associated with these standards and materials and the uncertainty in the correction must still be taken into account.

2.4.11. A further type of error is a spurious error, or blunder. Errors of this type invalidate a measurement and typically arise through human failure or instrument malfunction. Transposing digits in a number while recording data, an air bubble lodged in a spectrophotometer flow-through cell, or accidental cross-contamination of test items are common examples of this type of error.

2.4.12. Measurements for which errors such as these have been detected should be rejected and no attempt should be made to incorporate the errors into any statistical analysis. However, errors such as digit transposition can be corrected (exactly), particularly if they occur in the leading digits.

2.4.13. Spurious errors are not always obvious and, where a sufficient number of replicate measurements is available, it is usually appropriate to apply an outlier test to check for the presence of suspect members in the data set. Any positive result obtained from such a test should be considered with care and, where possible, referred back to the originator for confirmation. It is generally not wise to reject a value on purely statistical grounds.

2.4.14. Uncertainties estimated using this guide are not intended to allow for the possibility of spurious errors/blunders.

2.5. The VIM 3 definition of uncertainty

2.5.1. The revised VIM [H.7] introduces the following definition of measurement uncertainty:

measurement uncertainty
uncertainty of measurement
uncertainty

"non-negative parameter characterizing the dispersion of the quantity values being attributed to a measurand, based on the information used"

NOTE 1: Measurement uncertainty includes components arising from systematic effects, such as components associated with corrections and the assigned quantity values of measurement standards, as well as the definitional uncertainty. Sometimes estimated systematic effects are not corrected for but, instead, associated measurement uncertainty components are incorporated.

NOTE 2: The parameter may be, for example, a standard deviation called standard measurement uncertainty (or a specified multiple of it), or the half-width of an interval, having a stated coverage probability.

NOTE 3: Measurement uncertainty comprises, in general, many components. Some of these may be evaluated by Type A evaluation of measurement uncertainty from the statistical distribution of the quantity values from series of measurements and can be characterized by standard deviations. The other components, which may be evaluated by Type B evaluation of measurement uncertainty, can also be characterized by standard deviations, evaluated from probability density functions based on experience or other information.

NOTE 4: In general, for a given set of information, it is understood that the measurement uncertainty is associated with a stated quantity value attributed to the measurand. A modification of this value results in a modification of the associated uncertainty.

2.5.2. The changes to the definition do not materially affect the meaning for the purposes of analytical measurement. Note 1, however, adds the possibility that additional terms may be incorporated in the uncertainty budget to allow for uncorrected systematic effects. Chapter 7 provides further details of treatment of uncertainties associated with systematic effects.

3. Analytical Measurement and Uncertainty

3.1. Method validation

3.1.1. In practice, the fitness for purpose of analytical methods applied for routine testing is most commonly assessed through method validation studies [H.11]. Such studies produce data on overall performance and on individual influence factors which can be applied to the estimation of uncertainty associated with the results of the method in normal use.

3.1.2. Method validation studies rely on the determination of overall method performance parameters. These are obtained during method development and interlaboratory study or following in-house validation protocols. Individual sources of error or uncertainty are typically investigated only when significant compared to the overall precision measures in use. The emphasis is primarily on identifying and removing (rather than correcting for) significant effects. This leads to a situation in which the majority of potentially significant influence factors have been identified, checked for significance compared to overall precision, and shown to be negligible. Under these circumstances, the data available to analysts consists primarily of overall performance figures, together with evidence of insignificance of most effects and some measurements of any remaining significant effects.

3.1.3. Validation studies for quantitative analytical methods typically determine some or all of the following parameters:

Precision. [B.1] The principal precision measures include repeatability standard deviation s_r, reproducibility standard deviation s_R, (ISO 3534-1) and intermediate precision, sometimes denoted s_{Zi}, with i denoting the number of factors varied (ISO 5725-3:1994). The repeatability s_r indicates the variability observed within a laboratory, over a short time, using a single operator, item of equipment *etc*. s_r may be estimated within a laboratory or by inter-laboratory study. Interlaboratory reproducibility standard deviation s_R for a particular method may only be estimated directly by interlaboratory study; it shows the variability obtained when different laboratories analyse the same sample. Intermediate precision relates to the variation in results observed when one or more factors, such as time, equipment and operator, are varied within a laboratory; different figures are obtained depending on which factors are held constant. Intermediate precision estimates are most commonly determined within laboratories but may also be determined by interlaboratory study. The observed precision of an analytical procedure is an essential component of overall uncertainty, whether determined by combination of individual variances or by study of the complete method in operation.

Bias. The bias of an analytical method is usually determined by study of relevant reference materials or by spiking studies. The determination of overall bias with respect to appropriate reference values is important in establishing **traceability [B.9]** to recognised standards (see section 3.2). Bias may be expressed as analytical recovery (value observed divided by value expected). Bias should be shown to be negligible or corrected for, but in either case the uncertainty associated with the determination of the bias remains an essential component of overall uncertainty.

Linearity. Linearity is an important property of methods used to make measurements at a range of concentrations. The linearity of the response to pure standards and to realistic samples may be determined. Linearity is not generally quantified, but is checked for by inspection or using significance tests for non-linearity. Significant non-linearity is usually corrected for by use of non-linear calibration functions or eliminated by choice of more restricted operating range. Any remaining deviations from linearity are normally sufficiently accounted for by overall precision estimates covering several concentrations, or within any uncertainties associated with calibration (Appendix E.3).

Detection limit. During method validation, the detection limit is normally determined only to establish the lower end of the practical operating range of a method. Though uncertainties near the detection limit may require careful consideration and special treatment (Appendix F), the detection limit, however determined, is not of direct relevance to uncertainty estimation.

Robustness or ruggedness. Many method development or validation protocols require that sensitivity to particular parameters be investigated directly. This is usually done by a preliminary 'ruggedness test', in which the effect of one or more parameter changes is observed. If significant (compared to the precision of the ruggedness test) a more detailed study is carried out to measure the size of the effect, and a permitted operating interval chosen accordingly. Ruggedness test data can therefore provide information on the effect of important parameters.

Selectivity. "Selectivity" relates to the degree to which a method responds uniquely to the required analyte. Typical selectivity studies investigate the effects of likely interferents, usually by adding the potential interferent to both blank and fortified samples and observing the response. The results are normally used to demonstrate that the practical effects are not significant. However, since the studies measure changes in response directly, it is possible to use the data to estimate the uncertainty associated with potential interferences, given knowledge of the range of interferent concentrations.

Note: The term "specificity" has historically been used for the same concept.

3.2. Conduct of experimental studies of method performance

3.2.1. The detailed design and execution of method validation and method performance studies is covered extensively elsewhere [H.11] and will not be repeated here. However, the main principles as they affect the relevance of a study applied to uncertainty estimation are pertinent and are considered below.

3.2.2. *Representativeness* is essential. That is, studies should, as far as possible, be conducted to provide a realistic survey of the number and range of effects operating during normal use of the method, as well as covering the concentration ranges and sample types within the scope of the method. Where a factor has been representatively varied during the course of a precision experiment, for example, the effects of that factor appear directly in the observed variance and need no additional study unless further method optimisation is desirable.

3.2.3. In this context, *representative variation* means that an influence parameter must take a distribution of values appropriate to the uncertainty in the parameter in question. For continuous parameters, this may be a permitted range or stated uncertainty; for discontinuous factors such as sample matrix, this range corresponds to the variety of types permitted or encountered in normal use of the method. Note that representativeness extends not only to the range of values, but to their distribution.

3.2.4. In selecting factors for variation, it is important to ensure that the larger effects are varied where possible. For example, where day to day variation (perhaps arising from recalibration effects) is substantial compared to repeatability, two determinations on each of five days will provide a better estimate of intermediate precision than five determinations on each of two days. Ten single determinations on separate days will be better still, subject to sufficient control, though this will provide no additional information on within-day repeatability.

3.2.5. It is generally simpler to treat data obtained from random selection than from systematic variation. For example, experiments performed at random times over a sufficient period will usually include representative ambient temperature effects, while experiments performed systematically at 24-hour intervals may be subject to bias due to regular ambient temperature variation during the working day. The former experiment needs only evaluate the overall standard deviation; in the latter, systematic variation of ambient temperature is required, followed by adjustment to allow for the actual distribution of temperatures. Random variation is, however, less efficient. A small number of systematic studies can quickly establish the size of an effect, whereas it will typically take well over 30 determinations to establish an uncertainty contribution to better than about 20 % relative accuracy. Where possible, therefore, it is often preferable to investigate small numbers of major effects systematically.

3.2.6. Where factors are known or suspected to interact, it is important to ensure that the effect of interaction is accounted for. This may be achieved either by ensuring random selection from different levels of interacting parameters, or by careful systematic design to obtain both variance and covariance information.

3.2.7. In carrying out studies of overall bias, it is important that the reference materials and values are relevant to the materials under routine test.

3.2.8. Any study undertaken to investigate and test for the significance of an effect should have

sufficient power to detect such effects before they become practically significant.

3.3. Traceability

3.3.1. It is important to be able to compare results from different laboratories, or from the same laboratory at different times, with confidence. This is achieved by ensuring that all laboratories are using the same measurement scale, or the same 'reference points'. In many cases this is achieved by establishing a chain of calibrations leading to primary national or international standards, ideally (for long-term consistency) the Systeme Internationale (SI) units of measurement. A familiar example is the case of analytical balances; each balance is calibrated using reference masses which are themselves checked (ultimately) against national standards and so on to the primary reference kilogram. This unbroken chain of comparisons leading to a known reference value provides 'traceability' to a common reference point, ensuring that different operators are using the same units of measurement. In routine measurement, the consistency of measurements between one laboratory (or time) and another is greatly aided by establishing traceability for all relevant intermediate measurements used to obtain or control a measurement result. Traceability is therefore an important concept in all branches of measurement.

3.3.2. Traceability is formally defined [H.7] as:

> "metrological traceability
>
> property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty."

The reference to uncertainty arises because the agreement between laboratories is limited, in part, by uncertainties incurred in each laboratory's traceability chain. Traceability is accordingly intimately linked to uncertainty. Traceability provides the means of placing all related measurements on a consistent measurement scale, while uncertainty characterises the 'strength' of the links in the chain and the agreement to be expected between laboratories making similar measurements.

3.3.3. In general, the uncertainty on a result which is traceable to a particular reference, will be the uncertainty on that reference together with the uncertainty on making the measurement relative to that reference.

3.3.4. The Eurachem/CITAC Guide "Traceability in Chemical Measurement" [H.9] identifies the essential activities in establishing traceability as:

i) Specifying the measurand, scope of measurements and the required uncertainty

ii) Choosing a suitable method of estimating the value, that is, a measurement procedure with associated calculation - an equation - and measurement conditions

iii) Demonstrating, through validation, that the calculation and measurement conditions include all the "influence quantities" that significantly affect the result, or the value assigned to a standard.

iv) Identifying the relative importance of each influence quantity

v) Choosing and applying appropriate reference standards

vi) Estimating the uncertainty

These activities are discussed in detail in the associated Guide [H.9] and will not be discussed further here. It is, however, noteworthy that most of these activities are also essential for the estimation of measurement uncertainty, which also requires an identified and properly validate procedure for measurement, a clearly stated measurand, and information on the calibration standards used (including the associated uncertainties).

4. The Process of Measurement Uncertainty Estimation

4.1. Uncertainty estimation is simple in principle. The following paragraphs summarise the tasks that need to be performed in order to obtain an estimate of the uncertainty associated with a measurement result. Subsequent chapters provide additional guidance applicable in different circumstances, particularly relating to the use of data from in house and collaborative method validation studies, QC data, data from proficiency testing (PT) and the use of formal uncertainty propagation principles. The steps involved are:

Step 1. Specify measurand

Write down a clear statement of what is being measured, including the relationship between the measurand and the input quantities (*e.g.* measured quantities, constants, calibration standard values *etc.*) upon which it depends. Where possible, include corrections for known systematic effects. The specification information should be given in the relevant Standard Operating Procedure (SOP) or other method description.

Step 2. Identify uncertainty sources

List the possible sources of uncertainty. This will include sources that contribute to the uncertainty on the parameters in the relationship specified in Step 1, but may include other sources and must include sources arising from chemical assumptions. A general procedure for forming a structured list is suggested at Appendix D.

Step 3. Quantify uncertainty components

Estimate the size of the uncertainty component associated with each potential source of uncertainty identified. It is often possible to estimate or determine a single contribution to uncertainty associated with a number of separate sources using data from validation studies, QC data etc. Using such data considerably reduces the effort required to evaluate the uncertainty and since it utilises actual experimental data can lead to reliable estimates of the uncertainty. This approach is described in Chapter 7. It is also important to consider whether available data accounts sufficiently for all sources of uncertainty, and plan additional experiments and studies carefully to ensure that all sources of uncertainty are adequately accounted for.

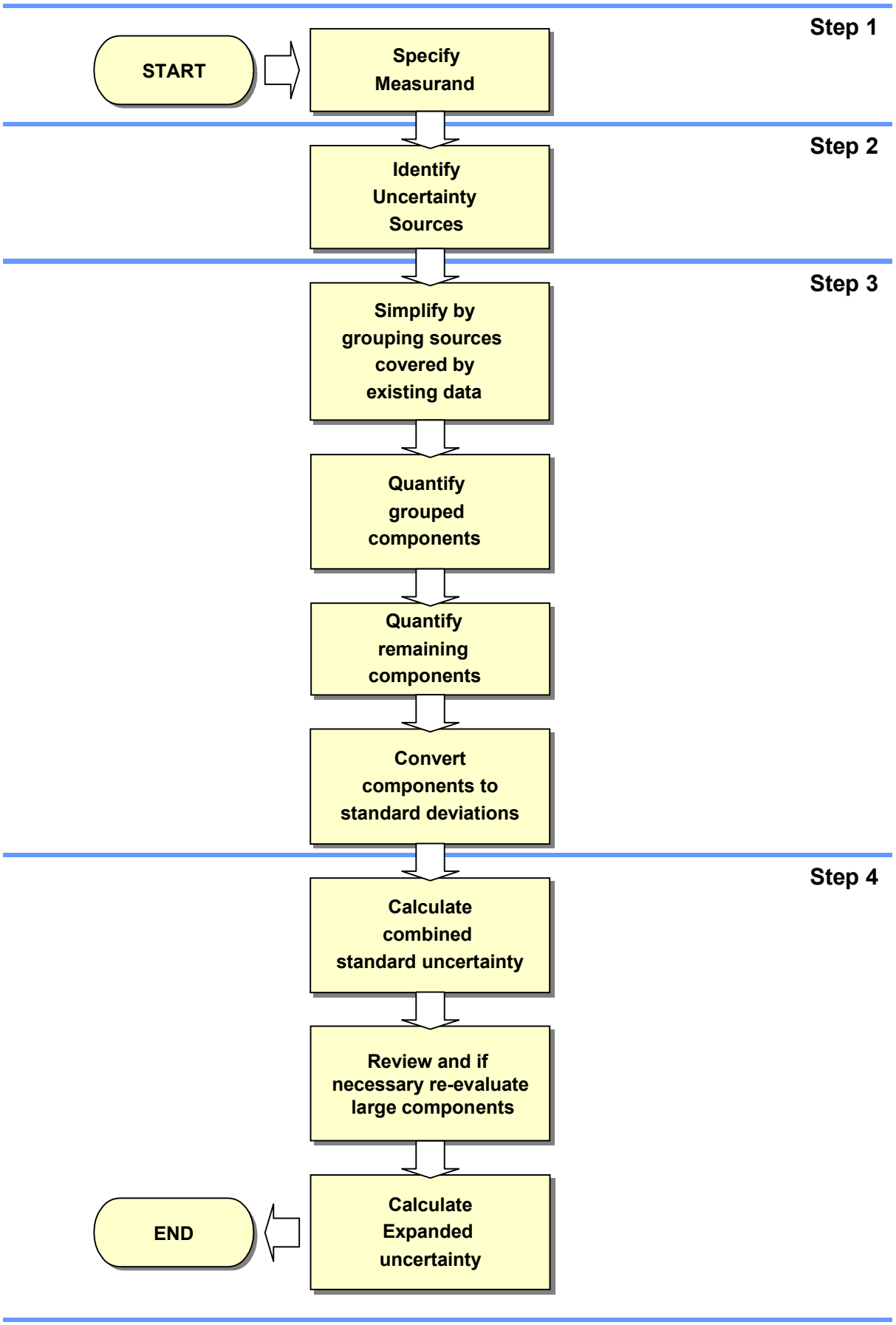
Step 4. Calculate combined uncertainty

The information obtained in step 3 will consist of a number of quantified contributions to overall uncertainty, whether associated with individual sources or with the combined effects of several sources. The contributions have to be expressed as standard deviations, and combined according to the appropriate rules, to give a combined standard uncertainty. The appropriate coverage factor should be applied to give an expanded uncertainty.

Figure 1 shows the process schematically.

4.2. The following chapters provide guidance on the execution of all the steps listed above and shows how the procedure may be simplified depending on the information that is available about the combined effect of a number of sources.

Figure 1: The Uncertainty Estimation Process



5. Step 1. Specification of the Measurand

5.1. In the context of uncertainty estimation, "specification of the measurand" requires both a clear and unambiguous statement of what is being measured, and a quantitative expression relating the value of the measurand to the parameters on which it depends. These parameters may be other measurands, quantities which are not directly measured, or constants. All of this information should be in the Standard Operating Procedure (SOP).

5.2. For most analytical measurements, a good definition of the measurand includes a statement of

a) the particular kind of quantity to be measured, usually the concentration or mass fraction of an analyte.

b) the item or material to be analysed and, if necessary, additional information on the location within the test item. For example, 'lead in patient blood' identifies a specific tissue within a test subject (the patient).

c) where necessary, the basis for calculation of the quantity reporting results. For example, the quantity of interest may be the amount extracted under specified conditions, or a mass fraction may be reported on a dry weight basis or after removal of some specified parts of a test material (such as inedible parts of foods).

NOTE 1: The term 'analyte' refers to the chemical species to be measured; the measurand is usually the concentration or mass fraction of the analyte.

NOTE 2: The term 'analyte level' is used in this document to refer generally to the value of quantities such as analyte concentration, analyte mass fraction etc. 'Level' is also used similarly for 'material', 'interferent' etc.

NOTE 3: The term 'measurand' is discussed in more detail in reference [H.5].

5.3. It should also be made clear whether a sampling step is included within the procedure or not. For example, is the measurand related just to the test item transmitted to the laboratory or to the bulk material from which the sample was taken? It is obvious that the uncertainty will be different in these two cases; where conclusions are to be drawn about the bulk material itself, primary sampling effects become important and are often much larger than the uncertainty associated with measurement of a laboratory test item. If sampling is part of the procedure used to obtain the measured result, estimation of uncertainties associated with the sampling procedure need to be considered. This is covered in considerable detail in reference [H.6].

5.4. In analytical measurement, it is particularly important to distinguish between measurements intended to produce results which are independent of the method used, and those which are not so intended. The latter are often referred to as *empirical* methods or *operationally defined* methods. The following examples may clarify the point further.

EXAMPLES:

1. Methods for the determination of the amount of nickel present in an alloy are normally expected to yield the same result, in the same units, usually expressed as a mass fraction or mole (amount) fraction. In principle, any systematic effect due to method bias or matrix would need to be corrected for, though it is more usual to ensure that any such effect is small. Results would not normally need to quote the particular method used, except for information. The method is not empirical.

2. Determinations of "extractable fat" may differ substantially, depending on the extraction conditions specified. Since "extractable fat" is entirely dependent on choice of conditions, the method used is *empirical*. It is not meaningful to consider correction for bias intrinsic to the method, since the measurand is defined by the method used. Results are generally reported with reference to the method, uncorrected for any bias intrinsic to the method. The method is considered empirical.

3. In circumstances where variations in the substrate, or matrix, have large and unpredictable effects, a procedure is often developed with the sole aim of achieving comparability between laboratories measuring the same material. The procedure may then be adopted as a local, national or international standard method on which trading or other decisions are taken, with no intent to obtain an absolute measure of the true amount of analyte present. Corrections for method bias or matrix effect are ignored by convention (whether or not they have been minimised in method

development). Results are normally reported uncorrected for matrix or method bias. The method is considered to be empirical.

5.5. The distinction between empirical and non-empirical (sometimes called *rational*) methods is important because it affects the estimation of uncertainty. In examples 2 and 3 above, because of the conventions employed, uncertainties associated with some quite large effects are not relevant in normal use. Due consideration should accordingly be given to whether the results are expected to be dependent upon, or independent of, the method in use and only those effects relevant to the result as reported should be included in the uncertainty estimate.

6. Step 2. Identifying Uncertainty Sources

6.1. A comprehensive list of relevant sources of uncertainty should be assembled. At this stage, it is not necessary to be concerned about the quantification of individual components; the aim is to be completely clear about what should be considered. In Step 3, the best way of treating each source will be considered.

6.2. In forming the required list of uncertainty sources it is usually convenient to start with the basic expression used to calculate the measurand from intermediate values. All the parameters in this expression may have an uncertainty associated with their value and are therefore potential uncertainty sources. In addition there may be other parameters that do not appear explicitly in the expression used to calculate the value of the measurand, but which nevertheless affect the measurement results, e.g. extraction time or temperature. These are also potential sources of uncertainty. All these different sources should be included. Additional information is given in Appendix C (Uncertainties in Analytical Processes).

6.3. The cause and effect diagram described in Appendix D is a very convenient way of listing the uncertainty sources, showing how they relate to each other and indicating their influence on the uncertainty of the result. It also helps to avoid double counting of sources. Although the list of uncertainty sources can be prepared in other ways, the cause and effect diagram is used in the following chapters and in all of the examples in Appendix A. Additional information is given in Appendix D (Analysing uncertainty sources).

6.4. Once the list of uncertainty sources is assembled, their effects on the result can, in principle, be represented by a formal measurement model, in which each effect is associated with a parameter or variable in an equation. The equation then forms a complete model of the measurement process in terms of all the individual factors affecting the result. This function may be very complicated and it may not be possible to write it down explicitly. Where possible, however, this should be done, as the form of the expression will generally determine the method of combining individual uncertainty contributions.

6.5. It may additionally be useful to consider a measurement procedure as a series of discrete operations (sometimes termed *unit operations*), each of which may be assessed separately to obtain estimates of uncertainty associated with them. This is a particularly useful approach where similar measurement procedures share common unit operations. The separate uncertainties for each operation then form contributions to the overall uncertainty.

6.6. In practice, it is more usual in analytical measurement to consider uncertainties associated with elements of overall method performance, such as observable precision and bias measured with respect to appropriate reference materials. These contributions generally form the dominant contributions to the uncertainty estimate, and are best modelled as separate effects on the result. It is then necessary to evaluate other possible contributions only to check their significance, quantifying only those that are significant. Further guidance on this approach, which applies particularly to the use of method validation data, is given in section 7.2.1.

6.7. Typical sources of uncertainty are

- Sampling

  Where in-house or field sampling form part of the specified procedure, effects such as random variations between different samples and any potential for bias in the sampling procedure form components of uncertainty affecting the final result.

- Storage Conditions

  Where test items are stored for any period prior to analysis, the storage conditions may affect the results. The duration of storage as well as conditions during storage should therefore be considered as uncertainty sources.

- Instrument effects

  Instrument effects may include, for example, the limits of accuracy on the calibration of an analytical balance; a temperature controller that may maintain a mean temperature which differs (within specification) from its

indicated set-point; an auto-analyser that could be subject to carry-over effects.

- Reagent purity

  The concentration of a volumetric solution will not be known exactly even if the parent material has been assayed, since some uncertainty related to the assaying procedure remains. Many organic dyestuffs, for instance, are not 100 % pure and can contain isomers and inorganic salts. The purity of such substances is usually stated by manufacturers as being *not less than* a specified level. Any assumptions about the degree of purity will introduce an element of uncertainty.

- Assumed stoichiometry

  Where an analytical process is assumed to follow a particular reaction stoichiometry, it may be necessary to allow for departures from the expected stoichiometry, or for incomplete reaction or side reactions.

- Measurement conditions

  For example, volumetric glassware may be used at an ambient temperature different from that at which it was calibrated. Gross temperature effects should be corrected for, but any uncertainty in the temperature of liquid and glass should be considered. Similarly, humidity may be important where materials are sensitive to possible changes in humidity.

- Sample effects

  The recovery of an analyte from a complex matrix, or an instrument response, may be affected by composition of the matrix. Analyte speciation may further compound this effect.

  The stability of a sample/analyte may change during analysis because of a changing thermal regime or photolytic effect.

  When a 'spike' is used to estimate recovery, the recovery of the analyte from the sample may differ from the recovery of the spike, introducing an uncertainty which needs to be evaluated.

- Computational effects

  Selection of the calibration model, *e.g.* using a straight line calibration on a curved response, leads to poorer fit and higher uncertainty.

  Truncation and round off can lead to inaccuracies in the final result. Since these are rarely predictable, an uncertainty allowance may be necessary.

- Blank Correction

  There will be an uncertainty on both the value and the appropriateness of the blank correction. This is particularly important in trace analysis.

- Operator effects

  Possibility of reading a meter or scale consistently high or low.

  Possibility of making a slightly different interpretation of the method.

- Random effects

  Random effects contribute to the uncertainty in all determinations. This entry should be included in the list as a matter of course.

NOTE: These sources are not necessarily independent.

7. Step 3. Quantifying Uncertainty

7.1. Introduction

7.1.1. Having identified the uncertainty sources as explained in Step 2 (Chapter 6), the next step is to quantify the uncertainty arising from these sources. This can be done by

- evaluating the uncertainty arising from each individual source and then combining them as described in Chapter 8. Examples A1 to A3 illustrate the use of this procedure.

or

- by determining directly the combined contribution to the uncertainty on the result from some or all of these sources using method performance data. Examples A4 to A6 represent applications of this procedure.

In practice, a combination of these is usually necessary and convenient.

7.1.2. Whichever of these approaches is used, most of the information needed to evaluate the uncertainty is likely to be already available from the results of validation studies, from QA/QC data and from other experimental work that has been carried out to check the performance of the method. However, data may not be available to evaluate the uncertainty from all of the sources and it may be necessary to carry out further work as described in sections 7.11. to 7.15.

7.2. Uncertainty evaluation procedure

7.2.1. The procedure used for estimating the overall uncertainty depends on the data available about the method performance. The stages involved in developing the procedure are

- **Reconcile the information requirements with the available data**

  First, the list of uncertainty sources should be examined to see which sources of uncertainty are accounted for by the available data, whether by explicit study of the particular contribution or by implicit variation within the course of whole-method experiments. These sources should be checked against the list prepared in Step 2 and any remaining sources should be listed to provide an auditable record of which contributions to the uncertainty have been included.

- **Plan to obtain the further data required**

  For sources of uncertainty not adequately covered by existing data, either seek additional information from the literature or standing data (certificates, equipment specifications *etc*.), or plan experiments to obtain the required additional data. Additional experiments may take the form of specific studies of a single contribution to uncertainty, or the usual method performance studies conducted to ensure representative variation of important factors.

7.2.2. It is important to recognise that not all of the components will make a significant contribution to the combined uncertainty; indeed, in practice it is likely that only a small number will. Unless there is a large number of them, components that are less than one third of the largest need not be evaluated in detail. A preliminary estimate of the contribution of each component or combination of components to the uncertainty should be made and those that are not significant eliminated.

7.2.3. The following sections provide guidance on the procedures to be adopted, depending on the data available and on the additional information required. Section 7.3. presents requirements for the use of prior experimental study data, including validation data. Section 7.4. briefly discusses evaluation of uncertainty solely from individual sources of uncertainty. This may be necessary for all, or for very few of the sources identified, depending on the data available, and is consequently also considered in later sections. Sections 7.5. to 7.10. describe the evaluation of uncertainty in a range of circumstances. Section 7.5. applies when using closely matched reference materials. Section 7.6. covers the use of collaborative study data and 7.7. the use of in-house validation data. 7.9. describes special considerations for empirical methods and 7.10. covers ad-hoc methods. Methods for quantifying individual components of uncertainty, including experimental studies, documentary and other data, modelling, and professional judgement are covered in more detail in sections 7.11. to 7.15. Section 7.16. covers the treatment of known bias in uncertainty estimation.

7.3. Relevance of prior studies

7.3.1. When uncertainty estimates are based at least partly on prior studies of method performance, it is necessary to demonstrate the validity of applying prior study results. Typically, this will consist of:

- Demonstration that a comparable precision to that obtained previously can be achieved.
- Demonstration that the use of the bias data obtained previously is justified, typically through determination of bias on relevant reference materials (see, for example, ISO Guide 33 [H.12]), by appropriate spiking studies, or by satisfactory performance on relevant proficiency schemes or other laboratory intercomparisons.
- Continued performance within statistical control as shown by regular QC sample results and the implementation of effective analytical quality assurance procedures.

7.3.2. Where the conditions above are met, and the method is operated within its scope and field of application, it is normally acceptable to apply the data from prior studies (including validation studies) directly to uncertainty estimates in the laboratory in question.

7.4. Evaluating uncertainty by quantification of individual components

7.4.1. In some cases, particularly when little or no method performance data is available, the most suitable procedure may be to evaluate each uncertainty component separately.

7.4.2. The general procedure used in combining individual components is to prepare a detailed quantitative model of the experimental procedure (cf. sections 5. and 6., especially 6.4.), assess the standard uncertainties associated with the individual input parameters, and combine them as described in Section 8.

7.4.3. In the interests of clarity, detailed guidance on the assessment of individual contributions by experimental and other means is deferred to sections 7.11. to 7.15. Examples A1 to A3 in Appendix A provide detailed illustrations of the procedure**.** Extensive guidance on the application of this procedure is also given in the ISO *Guide* [H.2].

7.5. Closely matched certified reference materials

- **7.5.1.** Measurements on certified reference materials are normally carried out as part of method validation or re-validation, effectively constituting a calibration of the whole measurement procedure against a traceable reference. Because this procedure provides information on the combined effect of many of the potential sources of uncertainty, it provides very good data for the assessment of uncertainty. Further details are given in section 7.7.4.

NOTE: ISO Guide 33 [H.12] gives a useful account of the use of reference materials in checking method performance.

7.6. Uncertainty estimation using prior collaborative method development and validation study data

7.6.1. A collaborative study carried out to validate a published method, for example according to the AOAC/IUPAC protocol [H.13] or ISO 5725 standard [H.14], is a valuable source of data to support an uncertainty estimate. The data typically include estimates of reproducibility standard deviation, s_R, for several levels of response, a linear estimate of the dependence of s_R on level of response, and may include an estimate of bias based on CRM studies. How this data can be utilised depends on the factors taken into account when the study was carried out. During the 'reconciliation' stage indicated above (section 7.2.), it is necessary to identify any sources of uncertainty that are not covered by the collaborative study data. The sources which may need particular consideration are:

- Sampling. Collaborative studies rarely include a sampling step. If the method used in-house involves sub-sampling, or the measurand (see Specification) is estimating a bulk property from a small sample, then the effects of sampling should be investigated and their effects included.
- Pre-treatment. In most studies, samples are homogenised, and may additionally be stabilised, before distribution. It may be necessary to investigate and add the effects of the particular pre-treatment procedures applied in-house.
- Method bias. Method bias is often examined prior to or during interlaboratory study, where possible by comparison with reference

methods or materials. Where the bias itself, the uncertainty in the reference values used, and the precision associated with the bias check, are all small compared to s_R, no additional allowance need be made for bias uncertainty. Otherwise, it will be necessary to make additional allowances.

- Variation in conditions.
  Laboratories participating in a study may tend towards the means of allowed ranges of experimental conditions, resulting in an underestimate of the range of results possible within the method definition. Where such effects have been investigated and shown to be insignificant across their full permitted range, however, no further allowance is required.
- Changes in sample matrix. The uncertainty arising from matrix compositions or levels of interferents outside the range covered by the study will need to be considered.

7.6.2. Uncertainty estimation based on collaborative study data acquired in compliance with ISO 5725 is fully described in ISO 21748 "Guidance for the use of repeatability, reproducibility and trueness estimates in measurement uncertainty estimation". [H.15]. The general procedure recommended for evaluating measurement uncertainty using collaborative study data is as follows:

a) Obtain estimates of the repeatability, reproducibility and trueness of the method in use from published information about the method.

b) Establish whether the laboratory bias for the measurements is within that expected on the basis of the data obtained in a).

c) Establish whether the precision attained by current measurements is within that expected on the basis of the repeatability and reproducibility estimates obtained in a).

d) Identify any influences on the measurement that were not adequately covered in the studies referenced in a), and quantify the variance that could arise from these effects, taking into account the sensitivity coefficients and the uncertainties for each influence.

e) Where the bias and precision are under control, as demonstrated in steps b) and c), combine the reproducibility standard estimate at a) with the uncertainty associated with trueness (Steps a and b) and the effects of additional influences (step d) to form a combined uncertainty estimate.

This procedure is essentially identical to the general procedure set out in Section 7.2. Note, however, that it is important to check that the laboratory's performance is consistent with that expected for the measurement method in use.

The use of collaborative study data is illustrated in example A6 (Appendix A).

7.6.3. For methods operating within their defined scope, when the reconciliation stage shows that all the identified sources have been included in the validation study or when the contributions from any remaining sources such as those discussed in section 7.6.1. have been shown to be negligible, then the reproducibility standard deviation s_R, adjusted for concentration if necessary, may be used as the combined standard uncertainty.

7.6.4. The repeatability standard deviation s_r is not normally a suitable uncertainty estimate, since it excludes major uncertainty contributions.

7.7. Uncertainty estimation using in-house development and validation studies

7.7.1. In-house development and validation studies consist chiefly of the determination of the method performance parameters indicated in section 3.1.3. Uncertainty estimation from these parameters utilises:

- The best available estimate of overall precision.
- The best available estimate(s) of overall bias and its uncertainty.
- Quantification of any uncertainties associated with effects incompletely accounted for in the above overall performance studies.

Precision study

7.7.2. The precision should be estimated as far as possible over an extended time period, and chosen to allow natural variation of all factors affecting the result. This can be obtained from

- The standard deviation of results for a typical sample analysed several times over a period of time, using different analysts and equipment where possible (the results of measurements

on QC check samples can provide this information).

- The standard deviation obtained from replicate analyses performed on each of several samples.

  NOTE: Replicates should be performed at materially different times to obtain estimates of intermediate precision; within-batch replication provides estimates of repeatability only.

- From formal multi-factor experimental designs, analysed by ANOVA to provide separate variance estimates for each factor.

7.7.3. Note that precision frequently varies significantly with the level of response. For example, the observed standard deviation often increases significantly and systematically with analyte concentration. In such cases, the uncertainty estimate should be adjusted to allow for the precision applicable to the particular result. Appendix E.5 gives additional guidance on handling level-dependent contributions to uncertainty.

Bias study

7.7.4. Overall bias is best estimated by repeated analysis of a relevant CRM, using the complete measurement procedure. Where this is done, and the bias found to be insignificant, the uncertainty associated with the bias is simply the combination of the standard uncertainty on the CRM value with the standard deviation associated with the measurement of the bias.

NOTE: Bias estimated in this way combines bias in laboratory performance with any bias intrinsic to the method in use. Special considerations may apply where the method in use is empirical; see section 7.9.1.

- When the reference material is only approximately representative of the test materials, additional factors should be considered, including (as appropriate) differences in composition and homogeneity; reference materials are frequently more homogeneous that test samples. Estimates based on professional judgement should be used, if necessary, to assign these uncertainties (see section 7.15.).

- Any effects following from different concentrations of analyte; for example, it is not uncommon to find that extraction losses differ between high and low levels of analyte.

7.7.5. Bias for a method under study can also be determined by comparison of the results with those of a reference method. If the results show that the bias is not statistically significant, the standard uncertainty is that for the reference method (if applicable; see section 7.9.1.), combined with the standard uncertainty associated with the measured difference between methods. The latter contribution to uncertainty is given by the standard deviation term used in the significance test applied to decide whether the difference is statistically significant, as explained in the example below.

EXAMPLE

A method (method 1) for determining the concentration of selenium is compared with a reference method (method 2). The results (in mg kg^{-1}) from each method are as follows:

	$\bar{x}$	s	n
Method 1	5.40	1.47	5
Method 2	4.76	2.75	5

The standard deviations are pooled to give a pooled standard deviation s_c

$$s_c = \sqrt{\frac{1.47^2 \times (5-1) + 2.75^2 \times (5-1)}{5+5-2}} = 2.205$$

and a corresponding value of t:

$$t = \frac{(5.40 - 4.76)}{2.205\sqrt{\left(\frac{1}{5} + \frac{1}{5}\right)}} = \frac{0.64}{1.4} = 0.46$$

t_{crit} is 2.3 for 8 degrees of freedom, so there is no significant difference between the means of the results given by the two methods. However, the difference (0.64) is compared with a standard deviation term of 1.4 above. This value of 1.4 is the standard deviation associated with the difference, and accordingly represents the relevant contribution to uncertainty associated with the measured bias.

7.7.6. Overall bias can also be estimated by the addition of analyte to a previously studied material. The same considerations apply as for the study of reference materials (above). In addition, the differential behaviour of added material and material native to the sample should be considered and due allowance made. Such an allowance can be made on the basis of:

- Studies of the distribution of the bias observed for a range of matrices and levels of added analyte.

- Comparison of result observed in a reference material with the recovery of added analyte in the same reference material.
- Judgement on the basis of specific materials with known extreme behaviour. For example, oyster tissue, a common marine tissue reference, is well known for a tendency to c_0-precipitate some elements with calcium salts on digestion, and may provide an estimate of 'worst case' recovery on which an uncertainty estimate can be based (e.g. By treating the worst case as an extreme of a rectangular or triangular distribution).
- Judgement on the basis of prior experience.

7.7.7. Bias may also be estimated by comparison of the particular method with a value determined by the method of standard additions, in which known quantities of the analyte are added to the test material, and the correct analyte concentration inferred by extrapolation. The uncertainty associated with the bias is then normally dominated by the uncertainties associated with the extrapolation, combined (where appropriate) with any significant contributions from the preparation and addition of stock solution.

NOTE: To be directly relevant, the additions should be made to the original sample, rather than a prepared extract.

7.7.8. It is a general requirement of the ISO *Guide* that corrections should be applied for all recognised and significant systematic effects. Where a correction is applied to allow for a significant overall bias, the uncertainty associated with the bias is estimated as paragraph 7.7.5. described in the case of insignificant bias

7.7.9. Where the bias is significant, but is nonetheless neglected for practical purposes, additional action is necessary (see section 7.16.).

Additional factors

7.7.10. The effects of any remaining factors should be estimated separately, either by experimental variation or by prediction from established theory. The uncertainty associated with such factors should be estimated, recorded and combined with other contributions in the normal way.

7.7.11. Where the effect of these remaining factors is demonstrated to be negligible compared to the precision of the study (i.e. statistically insignificant), it is recommended that an uncertainty contribution equal to the standard deviation associated with the relevant significance test be associated with that factor.

EXAMPLE

The effect of a permitted 1-hour extraction time variation is investigated by a t-test on five determinations each on the same sample, for the normal extraction time and a time reduced by 1 hour. The means and standard deviations (in mg L^{-1}) were: Standard time: mean 1.8, standard deviation 0.21; alternate time: mean 1.7, standard deviation 0.17. A *t*-test uses the pooled variance of

$$\frac{(5-1)\times 0.21^2 + (5-1)\times 0.17^2}{(5-1)+(5-1)} = 0.037$$

to obtain

$$t = \frac{(1.8-1.7)}{\sqrt{0.037\times\left(\frac{1}{5}+\frac{1}{5}\right)}} = 0.82$$

This is not significant compared to t_{crit} = 2.3. But note that the difference (0.1) is compared with a calculated standard deviation term of $\sqrt{0.037\times(1/5+1/5)}$ =0.12. This value is the contribution to uncertainty associated with the effect of permitted variation in extraction time.

7.7.12. Where an effect is detected and is statistically significant, but remains sufficiently small to neglect in practice, the provisions of section 7.16. apply.

7.8. Using data from Proficiency Testing

7.8.1. Uses of PT data in uncertainty evaluation

Data from proficiency testing (PT) can also provide useful information for uncertainty evaluation. For methods already in use for a long time in the laboratory, data from proficiency testing (also called External Quality Assurance, EQA) can be used:

- for checking the estimated uncertainty with results from PT exercises for a single laboratory
- for estimating the laboratory's measurement uncertainty.

7.8.2. Validity of PT data for uncertainty evaluation

The advantage of using PT data is that, while principally a test of laboratories' performance, a single laboratory will, over time, test a range of well-characterised materials chosen for their relevance to the particular field of measurement.

Further, PT test items may be more similar to a routine test item than a CRM since the demands on stability and homogeneity are frequently less stringent.

The relative disadvantage of PT samples is the lack of traceable reference values similar to those for certified reference materials. Consensus values in particular are prone to occasional error. This demands due care in their use for uncertainty estimation, as indeed is recommended by IUPAC for interpretation of PT results in general. [H.16]. However, appreciable bias in consensus values is relatively infrequent as a proportion of all materials circulated, and substantial protection is provided by the extended timescale common in proficiency testing. PT assigned values, including those assigned by consensus of participants' results, may therefore be regarded as sufficiently reliable for most practical purposes.

The data obtained from a laboratory's participation in PT can be a good basis for uncertainty estimates provided the following conditions are fulfilled:

- The test items in PT should be reasonably representative of the routine test items. For example the type of material and range of values of the measurand should be appropriate.
- The assigned values have an appropriate uncertainty.
- The number of PT rounds is appropriate; a minimum of 6 different trials over an appropriate period of time is recommended in order to get a reliable estimate.
- Where consensus values are used, the number of laboratories participating should be sufficient for reliable characterisation of the material.

7.8.3. Use for checking uncertainty estimates

Proficiency tests (EQA) are intended to check periodically the overall performance of a laboratory. The laboratory's results from its participation in proficiency testing can accordingly be used to check the evaluated uncertainty, since that uncertainty should be compatible with the spread of results obtained by that laboratory over a number or proficiency test rounds.

7.8.4. Use for evaluating uncertainty

Over several rounds, the deviations of laboratory results from the assigned values can provide a preliminary evaluation of the measurement uncertainty for that laboratory

If the results for all the participants using the same method in the PT scheme are selected, the standard deviation obtained is equivalent to an estimate of interlaboratory reproducibility and can, in principle, be used in the same way as the reproducibility standard deviation obtained from collaborative study (section 7.6. above).

Eurolab Technical Reports 1/2002 "Measurement Uncertainty in Testing" [H.17], 1/2006 "Guide to the Evaluation of Measurement Uncertainty for Quantitative Test Results" [H.18] and "Measurement uncertainty revisited: Alternative approaches to uncertainty evaluation" [H.19] describe the use of PT data in more detail and provide worked examples, and a Nordtest guide [H.20] provides a general approach aimed at environmental laboratories.

7.9. Evaluation of uncertainty for empirical methods

7.9.1. An 'empirical method' is a method agreed upon for the purposes of comparative measurement within a particular field of application where the measurand characteristically depends upon the method in use. The method accordingly defines the measurand. Examples include methods for leachable metals in ceramics and dietary fibre in foodstuffs (see also section 5.4. and example A5)

7.9.2. Where such a method is in use within its defined field of application, the bias associated with the method is defined as zero. In such circumstances, bias estimation need relate only to the laboratory performance and should not additionally account for bias intrinsic to the method. This has the following implications.

7.9.3. Reference material investigations, whether to demonstrate negligible bias or to measure bias, should be conducted using reference materials certified using the particular method, or for which a value obtained with the particular method is available for comparison.

7.9.4. Where reference materials so characterised are unavailable, overall control of bias is associated with the control of method parameters

affecting the result; typically such factors as times, temperatures, masses, volumes *etc*. The uncertainty associated with these input factors must accordingly be assessed and either shown to be negligible or quantified (see example A6).

7.9.5. Empirical methods are normally subjected to collaborative studies and hence the uncertainty can be evaluated as described in section 7.6.

7.10. Evaluation of uncertainty for ad-hoc methods

7.10.1. Ad-hoc methods are methods established to carry out exploratory studies in the short term, or for a short run of test materials. Such methods are typically based on standard or well-established methods within the laboratory, but are adapted substantially (for example to study a different analyte) and will not generally justify formal validation studies for the particular material in question.

7.10.2. Since limited effort will be available to establish the relevant uncertainty contributions, it is necessary to rely largely on the known performance of related systems or blocks within these systems. Uncertainty estimation should accordingly be based on known performance on a related system or systems. This performance information should be supported by any study necessary to establish the relevance of the information. The following recommendations assume that such a related system is available and has been examined sufficiently to obtain a reliable uncertainty estimate, or that the method consists of blocks from other methods and that the uncertainty in these blocks has been established previously.

7.10.3. As a minimum, it is essential that an estimate of overall bias and an indication of precision be available for the method in question. Bias will ideally be measured against a reference material, but will in practice more commonly be assessed from spike recovery. The considerations of section 7.7.4. then apply, except that spike recoveries should be compared with those observed on the related system to establish the relevance of the prior studies to the ad-hoc method in question. The overall bias observed for the ad-hoc method, on the materials under test, should be comparable to that observed for the related system, within the requirements of the study.

7.10.4. A minimum precision experiment consists of a duplicate analysis. It is, however, recommended that as many replicates as practical are performed. The precision should be compared with that for the related system; the standard deviation for the ad-hoc method should be comparable.

NOTE: It recommended that the comparison be based on inspection. Statistical significance tests (e.g. an F-test) will generally be unreliable with small numbers of replicates and will tend to lead to the conclusion that there is 'no significant difference' simply because of the low power of the test.

7.10.5. Where the above conditions are met unequivocally, the uncertainty estimate for the related system may be applied directly to results obtained by the ad-hoc method, making any adjustments appropriate for concentration dependence and other known factors.

7.11. Quantification of individual components

7.11.1. It is nearly always necessary to consider some sources of uncertainty individually. In some cases, this is only necessary for a small number of sources; in others, particularly when little or no method performance data is available, every source may need separate study (see examples 1, 2 and 3 in Appendix A for illustrations). There are several general methods for establishing individual uncertainty components:

- Experimental variation of input variables
- From standing data such as measurement and calibration certificates
- By modelling from theoretical principles
- Using judgement based on experience or informed by modelling of assumptions

These different methods are discussed briefly below.

7.12. Experimental estimation of individual uncertainty contributions

7.12.1. It is often possible and practical to obtain estimates of uncertainty contributions from experimental studies specific to individual parameters.

7.12.2. The standard uncertainty arising from random effects is often measured from repeatability experiments and is quantified in terms of the standard deviation of the measured values. In practice, no more than about fifteen

replicates need normally be considered, unless a high precision is required.

7.12.3. Other typical experiments include:

- Study of the effect of a variation of a single parameter on the result. This is particularly appropriate in the case of continuous, controllable parameters, independent of other effects, such as time or temperature. The rate of change of the result with the change in the parameter can be obtained from the experimental data. This is then combined directly with the uncertainty in the parameter to obtain the relevant uncertainty contribution.

NOTE: The change in parameter should be sufficient to change the result substantially compared to the precision available in the study (e.g. by five times the standard deviation of replicate measurements)

- Robustness studies, systematically examining the significance of moderate changes in parameters. This is particularly appropriate for rapid identification of significant effects, and commonly used for method optimisation. The method can be applied in the case of discrete effects, such as change of matrix, or small equipment configuration changes, which have unpredictable effects on the result. Where a factor is found to be significant, it is normally necessary to investigate further. Where insignificant, the associated uncertainty is (at least for initial estimation) that obtained from the robustness study.

- Systematic multifactor experimental designs intended to estimate factor effects and interactions. Such studies are particularly useful where a categorical variable is involved. A categorical variable is one in which the value of the variable is unrelated to the size of the effect; laboratory numbers in a study, analyst names, or sample types are examples of categorical variables. For example, the effect of changes in matrix type (within a stated method scope) could be estimated from recovery studies carried out in a replicated multiple-matrix study. An analysis of variance would then provide within- and between-matrix components of variance for observed analytical recovery. The between-matrix component of variance would provide a standard uncertainty associated with matrix variation.

7.13. Estimation based on other results or data

7.13.1. It is often possible to estimate some of the standard uncertainties using whatever relevant information is available about the uncertainty on the quantity concerned. The following paragraphs suggest some sources of information.

7.13.2. Quality Control (QC) data. As noted previously it is necessary to ensure that the quality criteria set out in standard operating procedures are achieved, and that measurements on QC samples show that the criteria continue to be met. Where reference materials are used in QC checks, section 7.5. shows how the data can be used to evaluate uncertainty. Where any other stable material is used, the QC data provides an estimate of intermediate precision (Section 7.7.2.). When stable QC samples are not available, quality control can use duplicate determinations or similar methods for monitoring repeatability; over the long term, the pooled repeatability data can be used to form an estimate of the repeatability standard deviation, which can form a part of the combined uncertainty.

7.13.3. QC data also provides a continuing check on the value quoted for the uncertainty. Clearly, the combined uncertainty arising from random effects cannot be less than the standard deviation of the QC measurements.

7.13.4. Further detail on the use of QC data in uncertainty evaluation can be found in recent NORDTEST and EUROLAB guides [H.19, H.20].

7.13.5. Suppliers' information. For many sources of uncertainty, calibration certificates or suppliers catalogues provide information. For example, the tolerance of volumetric glassware may be obtained from the manufacturer's catalogue or a calibration certificate relating to a particular item in advance of its use.

7.14. Modelling from theoretical principles

7.14.1. In many cases, well-established physical theory provides good models for effects on the result. For example, temperature effects on volumes and densities are well understood. In such cases, uncertainties can be calculated or estimated from the form of the relationship using the uncertainty propagation methods described in section 8.

7.14.2. In other circumstances, it may be necessary to use approximate theoretical models combined with experimental data. For example, where an analytical measurement depends on a timed derivatisation reaction, it may be necessary to assess uncertainties associated with timing. This might be done by simple variation of elapsed time. However, it may be better to establish an approximate rate model from brief experimental studies of the derivatisation kinetics near the concentrations of interest, and assess the uncertainty from the predicted rate of change at a given time.

7.15. Estimation based on judgement

7.15.1. The evaluation of uncertainty is neither a routine task nor a purely mathematical one; it depends on detailed knowledge of the nature of the measurand and of the measurement method and procedure used. The quality and utility of the uncertainty quoted for the result of a measurement therefore ultimately depends on the understanding, critical analysis, and integrity of those who contribute to the assignment of its value.

7.15.2. Most distributions of data can be interpreted in the sense that it is less likely to observe data in the margins of the distribution than in the centre. The quantification of these distributions and their associated standard deviations is done through repeated measurements.

7.15.3. However, other assessments of intervals may be required in cases when repeated measurements cannot be performed or do not provide a meaningful measure of a particular uncertainty component.

7.15.4. There are numerous instances in analytical chemistry when the latter prevails, and judgement is required. For example:

- An assessment of recovery and its associated uncertainty cannot be made for every single sample. Instead, an assessment is made for classes of samples (*e.g.* grouped by type of matrix), and the results applied to all samples of similar type. The degree of similarity is itself an unknown, thus this inference (from type of matrix to a specific sample) is associated with an extra element of uncertainty that has no frequentist interpretation.
- The model of the measurement as defined by the specification of the analytical procedure is used for converting the measured quantity to the value of the measurand (analytical result). This model is - like all models in science - subject to uncertainty. It is only assumed that nature behaves according to the specific model, but this can never be known with ultimate certainty.
- The use of reference materials is highly encouraged, but there remains uncertainty regarding not only the true value, but also regarding the relevance of a particular reference material for the analysis of a specific sample. A judgement is required of the extent to which a proclaimed standard substance reasonably resembles the nature of the samples in a particular situation.
- Another source of uncertainty arises when the measurand is insufficiently defined by the procedure. Consider the determination of "permanganate oxidizable substances" that are undoubtedly different whether one analyses ground water or municipal waste water. Not only factors such as oxidation temperature, but also chemical effects such as matrix composition or interference, may have an influence on this specification.
- A common practice in analytical chemistry calls for spiking with a single substance, such as a close structural analogue or isotopomer, from which either the recovery of the respective native substance or even that of a whole class of compounds is judged. Clearly, the associated uncertainty is experimentally assessable provided the analyst is prepared to study the recovery at all concentration levels and ratios of measurands to the spike, and all "relevant" matrices. But frequently this experimentation is avoided and substituted by judgements on
  - the concentration dependence of recoveries of measurand,
  - the concentration dependence of recoveries of spike,
  - the dependence of recoveries on (sub)type of matrix,
  - the identity of binding modes of native and spiked substances.

7.15.5. Judgement of this type is not based on immediate experimental results, but rather on a subjective (personal) probability, an expression which here can be used synonymously with "degree of belief", "intuitive probability" and

"credibility" [H.21]. It is also assumed that a degree of belief is not based on a snap judgement, but on a well considered mature judgement of probability.

7.15.6. Although it is recognised that subjective probabilities vary from one person to another, and even from time to time for a single person, they are not arbitrary as they are influenced by common sense, expert knowledge, and by earlier experiments and observations.

7.15.7. This may appear to be a disadvantage, but need not lead in practice to worse estimates than those from repeated measurements. This applies particularly if the true, real-life, variability in experimental conditions cannot be simulated and the resulting variability in data thus does not give a realistic picture.

7.15.8. A typical problem of this nature arises if long-term variability needs to be assessed when no collaborative study data are available. A scientist who dismisses the option of substituting subjective probability for an actually measured one (when the latter is not available) is likely to ignore important contributions to combined uncertainty, thus being ultimately less objective than one who relies on subjective probabilities.

7.15.9. For the purpose of estimation of combined uncertainties two features of degree of belief estimations are essential:

- degree of belief is regarded as interval valued which is to say that a lower and an upper bound similar to a classical probability distribution is provided,
- the same computational rules apply in combining 'degree of belief' contributions of uncertainty to a combined uncertainty as for standard deviations derived by other methods.

7.16. Significance of bias

7.16.1. It is a general requirement of the ISO *Guide* that corrections should be applied for all recognised and significant systematic effects.

7.16.2. In deciding whether a known bias can reasonably be neglected, the following approach is recommended:

i) Estimate the combined uncertainty without considering the relevant bias.

ii) Compare the bias with the combined uncertainty.

iii) Where the bias is not significant compared to the combined uncertainty, the bias may be neglected.

iv) Where the bias is significant compared to the combined uncertainty, additional action is required. Appropriate actions might:

- Eliminate or correct for the bias, making due allowance for the uncertainty of the correction.
- Report the observed bias and its uncertainty in addition to the result.

NOTE: Where a known bias is uncorrected by convention, the method should be considered empirical (see section 7.8).

8. Step 4. Calculating the Combined Uncertainty

8.1. Standard uncertainties

8.1.1. Before combination, all uncertainty contributions must be expressed as standard uncertainties, that is, as standard deviations. This may involve conversion from some other measure of dispersion. The following rules give some guidance for converting an uncertainty component to a standard deviation.

8.1.2. Where the uncertainty component was evaluated experimentally from the dispersion of repeated measurements, it can readily be expressed as a standard deviation. For the contribution to uncertainty in single measurements, the standard uncertainty is simply the observed standard deviation; for results subjected to averaging, the **standard deviation of the mean [B.21]** is used.

8.1.3. Where an uncertainty estimate is derived from previous results and data, it may already be expressed as a standard deviation. However where a confidence interval is given with a level of confidence p%, (in the form $\pm a$ at p%) then divide the value a by the appropriate percentage point of the Normal distribution for the level of confidence given to calculate the standard deviation.

EXAMPLE

> A specification states that a balance reading is within ±0.2 mg with 95 % confidence. From standard tables of percentage points on the normal distribution, a 95 % confidence interval is calculated using a value of 1.96. Using this figure gives a standard uncertainty of (0.2/1.96) ≈ 0.1.

8.1.4. If limits of $\pm a$ are given without a confidence level and there is reason to expect that extreme values are likely, it is normally appropriate to assume a rectangular distribution, with a standard deviation of $a/\sqrt{3}$ (see Appendix E).

EXAMPLE

> A 10 mL Grade A volumetric flask is certified to within ±0.2 mL. The standard uncertainty is $0.2/\sqrt{3} \approx 0.12$ mL.

8.1.5. If limits of $\pm a$ are given without a confidence level, but there is reason to expect that extreme values are unlikely, it is normally appropriate to assume a triangular distribution, with a standard deviation of $a/\sqrt{6}$ (see Appendix E).

EXAMPLE

> A 10 mL Grade A volumetric flask is certified to within ±0.2 mL, but routine in-house checks show that extreme values are rare. The standard uncertainty is $0.2/\sqrt{6} \approx 0.08$ mL.

8.1.6. Where an estimate is to be made on the basis of judgement, it may be possible to estimate the component directly as a standard deviation. If this is not possible then an estimate should be made of the maximum deviation which could reasonably occur in practice (excluding simple mistakes). If a smaller value is considered substantially more likely, this estimate should be treated as descriptive of a triangular distribution. If there are no grounds for believing that a small error is more likely than a large error, the estimate should be treated as characterising a rectangular distribution.

8.1.7. Conversion factors for the most commonly used distribution functions are given in Appendix E.1.

8.2. Combined standard uncertainty

8.2.1. Following the estimation of individual or groups of components of uncertainty and expressing them as standard uncertainties, the next stage is to calculate the combined standard uncertainty using one of the procedures described below.

8.2.2. The general relationship between the combined standard uncertainty $u_c(y)$ of a value y and the uncertainty of the independent parameters $x_1, x_2, \ldots x_n$ on which it depends is

$$u_c(y(x_1,x_2,\ldots)) = \sqrt{\sum_{i=1,n} c_i^2 u(x_i)^2} = \sqrt{\sum_{i=1,n} u(y,x_i)^2}$$ *

where $y(x_1,x_2,..)$ is a function of several parameters $x_1,x_2...$, c_i is a sensitivity coefficient evaluated as $c_i=\partial y/\partial x_i$, the partial differential of y with respect to x_i and $u(y,x_i)$ denotes the uncertainty in y arising from the uncertainty in x_i. Each variable's contribution $u(y,x_i)$ is just the

* The ISO *Guide* uses the shorter form $u_i(y)$ instead of $u(y,x_i)$

square of the associated uncertainty expressed as a standard deviation multiplied by the square of the relevant sensitivity coefficient. These sensitivity coefficients describe how the value of y varies with changes in the parameters x_1, x_2 *etc*.

NOTE: Sensitivity coefficients may also be evaluated directly by experiment; this is particularly valuable where no reliable mathematical description of the relationship exists.

8.2.3. Where variables are not independent, the relationship is more complex:

$$u(y(x_{i,j\ldots})) = \sqrt{\sum_{i=1,n} c_i^2 u(x_i)^2 + \sum_{\substack{i,k=1,n \\ i \neq k}} c_i c_k \cdot u(x_i, x_k)}$$

where $u(x_i,x_k)$ is the covariance between x_i and x_k and c_i and c_k are the sensitivity coefficients as described and evaluated in 8.2.2. The covariance is related to the correlation coefficient r_{ik} by

$$u(x_i,x_k) = u(x_i) \cdot u(x_k) \cdot r_{ik}$$

where $-1 \leq r_{ik} \leq 1$.

8.2.4. These general procedures apply whether the uncertainties are related to single parameters, grouped parameters or to the method as a whole. However, when an uncertainty contribution is associated with the whole procedure, it is usually expressed as an effect on the final result. In such cases, or when the uncertainty on a parameter is expressed directly in terms of its effect on y, the sensitivity coefficient $\partial y/\partial x_i$ is equal to 1.0.

EXAMPLE

A result of 22 mg L^{-1} shows an observed standard deviation of 4.1 mg L^{-1}. The standard uncertainty $u(y)$ associated with precision under these conditions is 4.1 mg L^{-1}. The implicit model for the measurement, neglecting other factors for clarity, is

$$y = (\text{Calculated result}) + \varepsilon$$

where ε represents the effect of random variation under the conditions of measurement. $\partial y/\partial \varepsilon$ is accordingly 1.0

8.2.5. Except for the case above, when the sensitivity coefficient is equal to one, and for the special cases given in Rule 1 and Rule 2 below, the general procedure requiring the generation of partial differentials, or an alternative numerical method, should be employed. Appendix E gives details of a numerical method, suggested by Kragten [H.22], which makes effective use of spreadsheet software to provide a combined standard uncertainty from input standard uncertainties and a known measurement model. Appendix E also describes the use of Monte Carlo simulation, an alternative numerical approach. It is recommended that these, or other appropriate computer-based methods, be used for all but the simplest cases.

8.2.6. In some cases, the expressions for combining uncertainties reduce to much simpler forms. Two simple rules for combining standard uncertainties are given here.

Rule 1

For models involving only a sum or difference of quantities, *e.g.* $y=(p+q+r+...)$, the combined standard uncertainty $u_c(y)$ is given by

$$u_c(y(p,q..)) = \sqrt{u(p)^2 + u(q)^2 + ...}$$

Rule 2

For models involving only a product or quotient, *e.g.* $y=(p \times q \times r \times ...)$ or $y= p / (q \times r \times ...)$, the combined standard uncertainty $u_c(y)$ is given by

$$u_c(y) = y\sqrt{\left(\frac{u(p)}{p}\right)^2 + \left(\frac{u(q)}{q}\right)^2 + ...}$$

where $(u(p)/p)$ *etc*. are the uncertainties in the parameters, expressed as relative standard deviations.

NOTE Subtraction is treated in the same manner as addition, and division in the same way as multiplication.

8.2.7. For the purposes of combining uncertainty components, it is most convenient to break the original mathematical model down to expressions which consist solely of operations covered by one of the rules above. For example, the expression

$$(o + p) \Big/ (q + r)$$

should be broken down to the two elements $(o+p)$ and $(q+r)$. The interim uncertainties for each of these can then be calculated using rule 1 above; these interim uncertainties can then be combined using rule 2 to give the combined standard uncertainty.

8.2.8. The following examples illustrate the use of the above rules:

EXAMPLE 1

$y = (p-q+r)$ The values are p=5.02, q=6.45 and r=9.04 with standard uncertainties $u(p)$=0.13, $u(q)$=0.05 and $u(r)$= 0.22.

$$y = 5.02 - 6.45 + 9.04 = 7.61$$

$$u(y) = \sqrt{0.13^2 + 0.05^2 + 0.22^2} = 0.26$$

EXAMPLE 2

$y = (op/qr)$. The values are o=2.46, p=4.32, q=6.38 and r=2.99, with standard uncertainties of $u(o)$=0.02, $u(p)$=0.13, $u(q)$=0.11 and $u(r)$= 0.07.

$$y = (2.46 \times 4.32) / (6.38 \times 2.99) = 0.56$$

$$u(y) = 0.56 \times \sqrt{\left(\frac{0.02}{2.46}\right)^2 + \left(\frac{0.13}{4.32}\right)^2 + \left(\frac{0.11}{6.38}\right)^2 + \left(\frac{0.07}{2.99}\right)^2}$$

$$\Rightarrow u(y) = 0.56 \times 0.043 = 0.024$$

8.2.9. There are many instances in which the magnitudes of components of uncertainty vary with the level of analyte. For example, uncertainties in recovery may be smaller for high levels of material, or spectroscopic signals may vary randomly on a scale approximately proportional to intensity (constant coefficient of variation). In such cases, it is important to take account of the changes in the combined standard uncertainty with level of analyte. Approaches include:

- Restricting the specified procedure or uncertainty estimate to a small range of analyte concentrations.
- Providing an uncertainty estimate in the form of a relative standard deviation.
- Explicitly calculating the dependence and recalculating the uncertainty for a given result.

Appendix E.5 gives additional information on these approaches.

8.3. Expanded uncertainty

8.3.1. The final stage is to multiply the combined standard uncertainty by the chosen coverage factor in order to obtain an expanded uncertainty. The expanded uncertainty is required to provide an interval which may be expected to encompass a large fraction of the distribution of values which could reasonably be attributed to the measurand.

8.3.2. In choosing a value for the coverage factor k, a number of issues should be considered. These include:

- The level of confidence required
- Any knowledge of the underlying distributions
- Any knowledge of the number of values used to estimate random effects (see 8.3.3 below).

8.3.3. For most purposes it is recommended that k is set to 2. However, this value of k may be insufficient where the combined uncertainty is based on statistical observations with relatively few degrees of freedom (less than about six). The choice of k then depends on the effective number of degrees of freedom.

8.3.4. Where the combined standard uncertainty is dominated by a single contribution with fewer than six degrees of freedom, it is recommended that k be set equal to the two-tailed value of Student's t for the number of degrees of freedom associated with that contribution, and for the level of confidence required (normally 95 %). Table 1 (page 29) gives a short list of values for t, including degrees of freedom above six for critical applications.

EXAMPLE:

A combined standard uncertainty for a weighing operation is formed from contributions u_{cal}=0.01 mg arising from calibration uncertainty and s_{obs}=0.08 mg based on the standard deviation of five repeated observations. The combined standard uncertainty u_c is equal to $\sqrt{0.01^2 + 0.08^2} = 0.081$ mg. This is clearly dominated by the repeatability contribution s_{obs}, which is based on five observations, giving 5-1=4 degrees of freedom. k is accordingly based on Student's t. The two-tailed value of t for four degrees of freedom and 95 % confidence is, from tables, 2.8; k is accordingly set to 2.8 and the expanded uncertainty U=2.8×0.081=0.23 mg.

8.3.5. The *Guide* [H.2] gives additional guidance on choosing k where a small number of measurements is used to estimate large random effects, and should be referred to when estimating degrees of freedom where several contributions are significant.

8.3.6. Where the distributions concerned are normal, a coverage factor of 2 (or chosen according to paragraphs 8.3.3.-8.3.5. Using a level of confidence of 95 %) gives an interval containing approximately 95 % of the distribution of values. It is not recommended that this interval is taken to imply a 95 % confidence interval without a knowledge of the distribution concerned.

Table 1: Student's *t* for 95 % confidence (2-tailed)

Degrees of freedom ν	*t*
1	12.7
2	4.3
3	3.2
4	2.8
5	2.6
6	2.4
8	2.3
10	2.2
14	2.1
28	2.0

Values of *t* are rounded to one decimal place. For intermediate degrees of freedom *v*, either use the next lower value of *v* or refer to tables or software.

9. Reporting Uncertainty

9.1. General

9.1.1. The information necessary to report the result of a measurement depends on its intended use. The guiding principles are:

- present sufficient information to allow the result to be re-evaluated if new information or data become available
- it is preferable to err on the side of providing too much information rather than too little.

9.1.2. When the details of a measurement, including how the uncertainty was determined, depend on references to published documentation, it is imperative that the documentation to hand is kept up to date and consistent with the methods in use.

9.2. Information required

9.2.1. A complete report of a measurement result should include or refer to documentation containing,

- a description of the methods used to calculate the measurement result and its uncertainty from the experimental observations and input data
- the values and sources of all corrections and constants used in both the calculation and the uncertainty analysis
- a list of all the components of uncertainty with full documentation on how each was evaluated

9.2.2. The data and analysis should be presented in such a way that its important steps can be readily followed and the calculation of the result repeated if necessary.

9.2.3. Where a detailed report including intermediate input values is required, the report should

- give the value of each input value, its standard uncertainty and a description of how each was obtained
- give the relationship between the result and the input values and any partial derivatives, covariances or correlation coefficients used to account for correlation effects
- state the estimated number of degrees of freedom for the standard uncertainty of each input value (methods for estimating degrees of freedom are given in the ISO Guide [H.2]).

NOTE: Where the functional relationship is extremely complex or does not exist explicitly (for example, it may only exist as a computer program), the relationship may be described in general terms or by citation of appropriate references. In such cases, it must be clear how the result and its uncertainty were obtained.

9.2.4. When reporting the results of routine analysis, it may be sufficient to state only the value of the expanded uncertainty and the value of k.

9.3. Reporting standard uncertainty

9.3.1. When uncertainty is expressed as the combined standard uncertainty u_c (that is, as a single standard deviation), the following form is recommended:

"(Result): x (units) [with a] standard uncertainty of u_c (units) [where standard uncertainty is as defined in the ISO/IEC Guide to the Expression of Uncertainty in Measurement and corresponds to one standard deviation.]"

NOTE The use of the symbol ± is not recommended when using standard uncertainty as the symbol is commonly associated with intervals corresponding to high levels of confidence.

Terms in parentheses [] may be omitted or abbreviated as appropriate.

EXAMPLE:

Total nitrogen: 3.52 g/100 g

Standard uncertainty: 0.07 g/100 g *

*Standard uncertainty corresponds to one standard deviation.

9.4. Reporting expanded uncertainty

9.4.1. Unless otherwise required, the result x should be stated together with the expanded uncertainty U calculated using a coverage factor

k=2 (or as described in section 8.3.3.). The following form is recommended:

"(Result): $(x \pm U)$ (units)

[where] the reported uncertainty is [an expanded uncertainty as defined in the International Vocabulary of Basic and General terms in metrology, 2nd ed., ISO 1993,] calculated using a coverage factor of 2, [which gives a level of confidence of approximately 95 %]"

Terms in parentheses [] may be omitted or abbreviated as appropriate. The coverage factor should, of course, be adjusted to show the value actually used.

EXAMPLE:

Total nitrogen: (3.52 ± 0.14) g/100 g *

*The reported uncertainty is an expanded uncertainty calculated using a coverage factor of 2 which gives a level of confidence of approximately 95 %.

9.5. Numerical expression of results

9.5.1. The numerical values of the result and its uncertainty should not be given with an excessive number of digits. Whether expanded uncertainty U or a standard uncertainty u is given, it is seldom necessary to give more than two significant digits for the uncertainty. Results should be rounded to be consistent with the uncertainty given.

9.6. Asymmetric intervals

9.6.1. In some circumstances, particularly relating to uncertainties in results near zero (Appendix F) or following Monte Carlo estimation (Appendix E.3), the distribution associated with the result may be strongly asymmetric. It may then be inappropriate to quote a single value for the uncertainty. Instead, the limits of the estimated coverage interval should be given. If it is likely that the result and its uncertainty will be used in further calculations, the standard uncertainty should also be given.

EXAMPLE:

Purity (as a mass fraction) might be reported as:

Purity: 0.995 with approximate 95 % confidence interval 0.983 to 1.000 based on a standard uncertainty of 0.005 and 11 degrees of freedom

9.7. Compliance against limits

9.7.1. Regulatory compliance often requires that a measurand, such as the concentration of a toxic substance, be shown to be within particular limits. Measurement uncertainty clearly has implications for interpretation of analytical results in this context. In particular:

- The uncertainty in the analytical result may need to be taken into account when assessing compliance.
- The limits may have been set with some allowance for measurement uncertainties.

Consideration should be given to both factors in any assessment.

9.7.2. Detailed guidance on how to take uncertainty into account when assessing compliance is given in the EURACHEM Guide "Use of uncertainty information in compliance assessment" [H.24]. The following paragraphs summarise the principles of reference [H.24].

9.7.3. The basic requirements for deciding whether or not to accept the test item are:

- A specification giving upper and/or lower permitted limits of the characteristics (measurands) being controlled.
- A decision rule that describes how the measurement uncertainty will be taken into account with regard to accepting or rejecting a product according to its specification and the result of a measurement.
- The limit(s) of the acceptance or rejection zone (i.e. the range of results), derived from the decision rule, which leads to acceptance or rejection when the measurement result is within the appropriate zone.

EXAMPLE:

A decision rule that is currently widely used is that a result implies non compliance with an upper limit if the measured value exceeds the limit by the expanded uncertainty. With this decision rule, then only case (i) in Figure 2 would imply non compliance. Similarly, for a decision rule that a result implies compliance only if it is below the limit by the expanded uncertainty, only case (iv) would imply compliance.

9.7.4. In general the decision rules may be more complicated than these. Further discussion may be found in reference H.24.

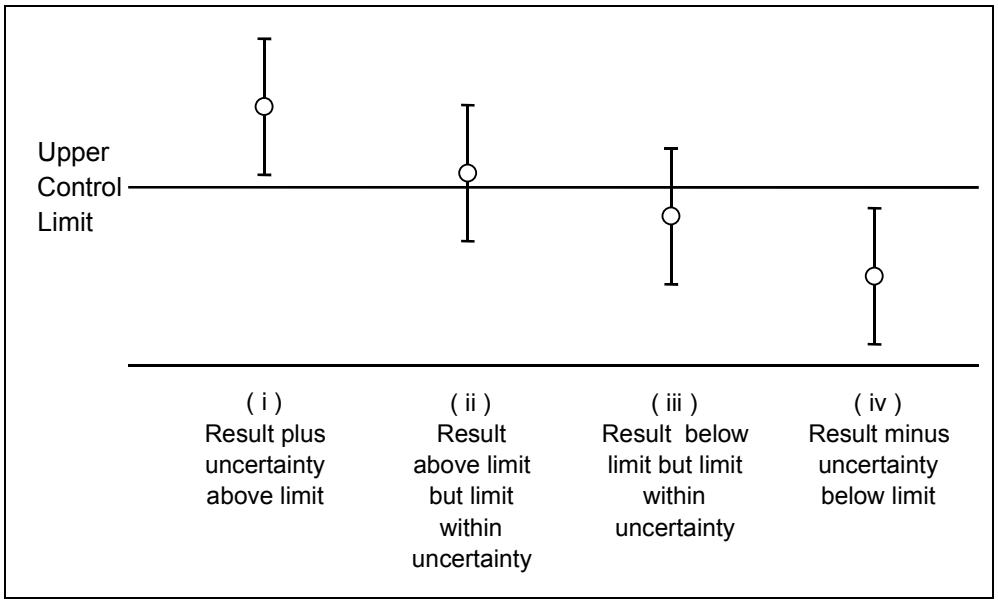


Figure 2: Uncertainty and compliance limits

Appendix A. Examples

Introduction

General introduction

These examples illustrate how the techniques for evaluating uncertainty, described in sections 5-7, can be applied to some typical chemical analyses. They all follow the procedure shown in the flow diagram (Figure 1 on page 11). The uncertainty sources are identified and set out in a cause and effect diagram (see appendix D). This helps to avoid double counting of sources and also assists in the grouping together of components whose combined effect can be evaluated. Examples 1-6 illustrate the use of the spreadsheet method of Appendix E.2 for calculating the combined uncertainties from the calculated contributions $u(y,x_i)$.*

Each of examples 1-6 has an introductory summary. This gives an outline of the analytical method, a table of the uncertainty sources and their respective contributions, a graphical comparison of the different contributions, and the combined uncertainty.

Examples 1-3 and 5 illustrate the evaluation of the uncertainty by the quantification of the uncertainty arising from each source separately. Each gives a detailed analysis of the uncertainty associated with the measurement of volumes using volumetric glassware and masses from difference weighings. The detail is for illustrative purposes, and should not be taken as a general recommendation as to the level of detail required or the approach taken. For many analyses, the uncertainty associated with these operations will not be significant and such a detailed evaluation will not be necessary. It would be sufficient to use typical values for these operations with due allowance being made for the actual values of the masses and volumes involved.

Example A1

Example A1 deals with the very simple case of the preparation of a calibration standard of cadmium in HNO_3 for atomic absorption spectrometry (AAS). Its purpose is to show how to evaluate the components of uncertainty arising from the basic operations of volume measurement and weighing and how these components are combined to determine the overall uncertainty.

Example A2

This deals with the preparation of a standardised solution of sodium hydroxide (NaOH) which is standardised against the titrimetric standard potassium hydrogen phthalate (KHP). It includes the evaluation of uncertainty on simple volume measurements and weighings, as described in example A1, but also examines the uncertainty associated with the titrimetric determination.

Example A3

Example A3 expands on example A2 by including the titration of an HCl against the prepared NaOH solution.

Example A4

This illustrates the use of in house validation data, as described in section 7.7., and shows how the data can be used to evaluate the uncertainty arising from combined effect of a number of sources. It also shows how to evaluate the uncertainty associated with method bias.

Example A5

This shows how to evaluate the uncertainty on results obtained using a standard or "empirical" method to measure the amount of heavy metals leached from ceramic ware using a defined procedure, as described in section 7.2.-7.9. Its purpose is to show how, in the absence of collaborative trial data or ruggedness testing results, it is necessary to consider the uncertainty arising from the range of the parameters (e.g. temperature, etching time and acid strength) allowed in the method definition. This process is considerably simplified when collaborative study data is available, as is shown in the next example.

* Section 8.2.2. explains the theory behind the calculated contributions $u(y,x_i)$.

Example A6

The sixth example is based on an uncertainty estimate for a crude (dietary) fibre determination. Since the analyte is defined only in terms of the standard method, the method is operationally defined, or empirical. In this case, collaborative study data, in-house QA checks and literature study data were available, permitting the approach described in section 7.6. The in-house studies verify that the method is performing as expected on the basis of the collaborative study. The example shows how the use of collaborative study data backed up by in-house method performance checks can substantially reduce the number of different contributions required to form an uncertainty estimate under these circumstances.

Example A7

This gives a detailed description of the evaluation of uncertainty on the measurement of the lead content of a water sample using isotope dilution mass spectrometry (IDMS). In addition to identifying the possible sources of uncertainty and quantifying them by statistical means the examples shows how it is also necessary to include the evaluation of components based on judgement as described in section 7.15. Use of judgement is a special case of Type B evaluation as described in the ISO Guide [H.2].

Example A1: Preparation of a Calibration Standard

Summary

Goal

A calibration standard is prepared from a high purity metal (cadmium) with a concentration of ca.1000 mg L^{-1}.

Measurement procedure

The surface of the high purity metal is cleaned to remove any metal-oxide contamination. Afterwards the metal is weighed and then dissolved in nitric acid in a volumetric flask. The stages in the procedure are shown in the following flow chart.

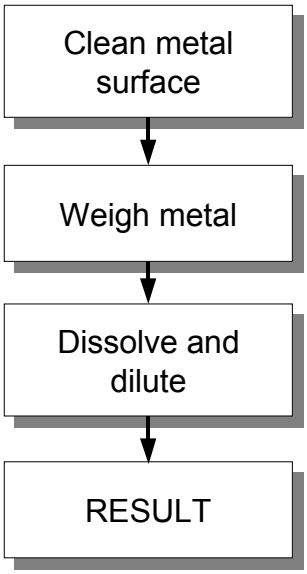


Figure A1. 1: Preparation of cadmium standard

Measurand

$$c_{Cd} = \frac{1000 \cdot m \cdot P}{V} \text{ [mg L}^{-1}\text{]}$$

where

c_{Cd} concentration of the calibration standard [mg L^{-1}]

1000 conversion factor from [mL] to [L]

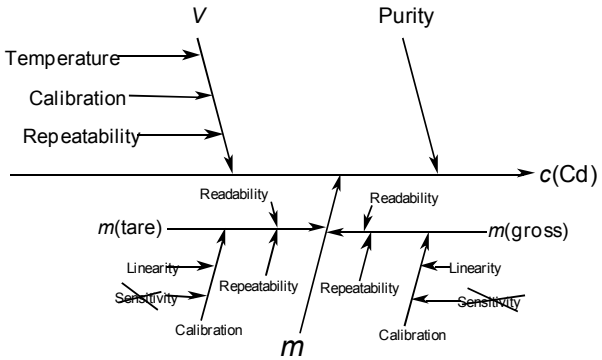
m mass of the high purity metal [mg]

P purity of the metal given as mass fraction

V volume of the liquid of the calibration standard [mL]

Identification of the uncertainty sources:

The relevant uncertainty sources are shown in the cause and effect diagram below:



Quantification of the uncertainty components

The values and their uncertainties are shown in the Table below.

Combined Standard Uncertainty

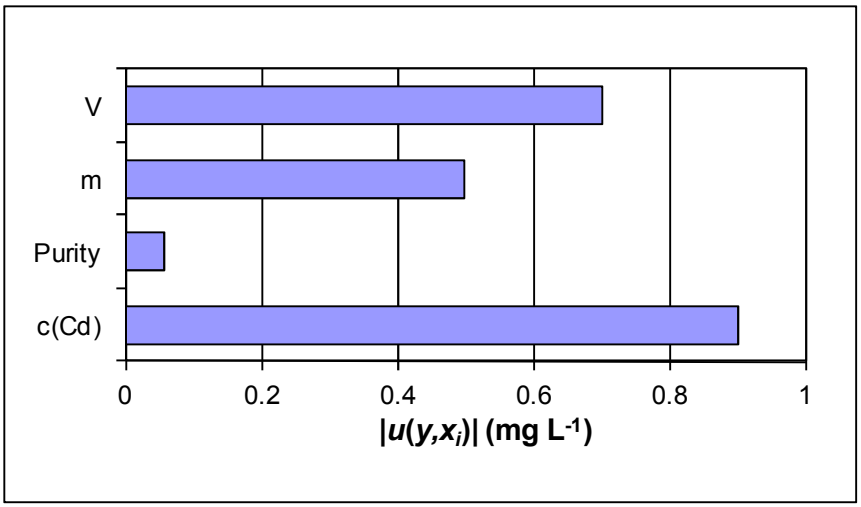
The combined standard uncertainty for the preparation of a 1002.7 mg L^{-1} Cd calibration standard is 0.9 mg L^{-1}

The different contributions are shown diagrammatically in Figure A1.2.

Table A1.1: Values and uncertainties

	Description	Value	Standard uncertainty	Relative standard uncertainty $u(x)/x$
P	Purity of the metal	0.9999	0.000058	0.000058
m	Mass of the metal	100.28 mg	0.05 mg	0.0005
V	Volume of the flask	100.0 mL	0.07 mL	0.0007
c_{Cd}	Concentration of the calibration standard	1002.7 mg L^{-1}	0.9 mg L^{-1}	0.0009

Figure A1.2: Uncertainty contributions in cadmium standard preparation



The values of $u(y,x_i) = (\partial y/\partial x_i).u(x_i)$ are taken from Table A1.3

Example A1: Preparation of a calibration standard. Detailed discussion

A1.1 Introduction

This first introductory example discusses the preparation of a calibration standard for atomic absorption spectroscopy (AAS) from the corresponding high purity metal (in this example $\approx$1000 mg L^{-1} Cd in dilute HNO_3). Even though the example does not represent an entire analytical measurement, the use of calibration standards is part of nearly every determination, because modern routine analytical measurements are relative measurements, which need a reference standard to provide traceability to the SI.

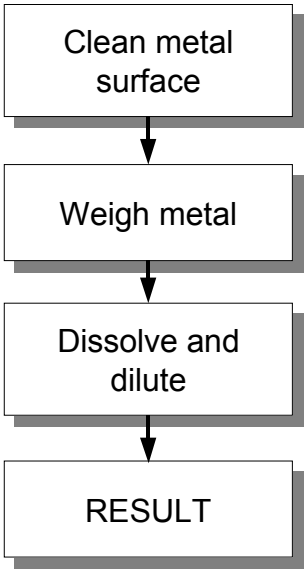
A1.2 Step 1: Specification

The goal of this first step is to write down a clear statement of what is being measured. This specification includes a description of the preparation of the calibration standard and the mathematical relationship between the measurand and the parameters upon which it depends.

Procedure

The specific information on how to prepare a calibration standard is normally given in a Standard Operating Procedure (SOP). The preparation consists of the following stages

Figure A1.3: Preparation of cadmium standard



The separate stages are:

i) The surface of the high purity metal is treated with an acid mixture to remove any metal-oxide contamination. The cleaning method is provided by the manufacturer of the metal and needs to be carried out to obtain the purity quoted on the certificate.

ii) The volumetric flask (100 mL) is weighed without and with the purified metal inside. The balance used has a resolution of 0.01 mg.

iii) 1 mL of nitric acid (65 g/100 g) and 3 mL of ion-free water are added to the flask to dissolve the cadmium (approximately 100 mg, weighed accurately). Afterwards the flask is filled with ion-free water up to the mark and mixed by inverting the flask at least thirty times.

Calculation:

The measurand in this example is the concentration of the calibration standard solution, which depends upon the weighing of the high purity metal (Cd), its purity and the volume of the liquid in which it is dissolved. The concentration is given by

$$c_{Cd} = \frac{1000 \cdot m \cdot P}{V} \text{ mg L}^{-1}$$

where

c_{Cd} concentration of the calibration standard [mg L^{-1}]

1000 conversion factor from [mL] to [L]

m mass of the high purity metal [mg]

P purity of the metal given as mass fraction

V volume of the liquid of the calibration standard [mL]

A1.3 Step 2: Identifying and analysing uncertainty sources

The aim of this second step is to list all the uncertainty sources for each of the parameters which affect the value of the measurand.

Purity

The purity of the metal (Cd) is quoted in the supplier's certificate as (99.99 ± 0.01) %. P is therefore 0.9999 ± 0.0001. These values depend on the effectiveness of the surface cleaning of the high purity metal. If the manufacturer's procedure is strictly followed, no additional uncertainty due to the contamination of the surface with metal-oxide needs to be added to the value given in the certificate.

Mass m

The second stage of the preparation involves weighing the high purity metal. A 100 mL quantity of a 1000 mg L^{-1} cadmium solution is to be prepared.

The relevant mass of cadmium is determined by a tared weighing, giving m= 0.10028 g

The manufacturer's literature identifies three uncertainty sources for the tared weighing: the repeatability; the readability (digital resolution) of the balance scale; and the contribution due to the uncertainty in the calibration function of the scale. This calibration function has two potential uncertainty sources, identified as the sensitivity of the balance and its linearity. The sensitivity can be neglected because the mass by difference is done on the same balance over a very narrow range.

NOTE: Buoyancy correction is not considered because all weighing results are quoted on the conventional basis for weighing in air [H.33] and the densities of Cd and steel are similar. Note 1 in Appendix G refers. The remaining uncertainties are too small to consider.

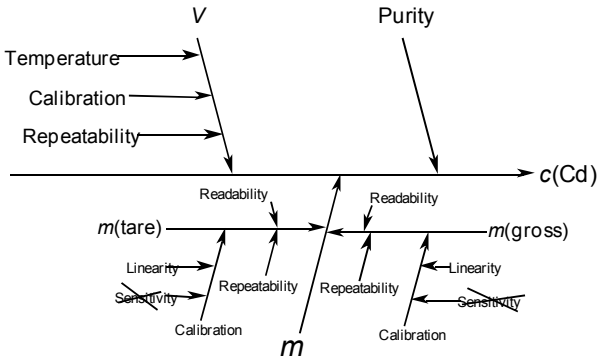
Volume V

The volume of the solution delivered by the volumetric flask is subject to three major sources of uncertainty:

- The uncertainty in the certified internal volume of the flask.
- Variation in filling the flask to the mark.
- The flask and solution temperatures differing from the temperature at which the volume of the flask was calibrated.

The different effects and their influences are shown as a cause and effect diagram in Figure A1.4 (see Appendix D for description).

Figure A1.4: Uncertainties in Cd Standard preparation



A1.4 Step 3: Quantifying the uncertainty components

In step 3 the size of each identified potential source of uncertainty is either directly measured, estimated using previous experimental results or derived from theoretical analysis.

Purity

The purity of the cadmium is given on the certificate as 0.9999 ± 0.0001. Because there is no additional information about the uncertainty value, a rectangular distribution is assumed. To obtain the standard uncertainty $u(P)$ the value of 0.0001 has to be divided by $\sqrt{3}$ (see Appendix E1.1)

$$u(P) = \frac{0.0001}{\sqrt{3}} = 0.000058$$

Mass m

The uncertainty associated with the mass of the cadmium is estimated, using the data from the calibration certificate and the manufacturer's recommendations on uncertainty estimation, as 0.05 mg. This estimate takes into account the three contributions identified earlier (Section A1.3).

NOTE: Detailed calculations for uncertainties in mass can be very intricate, and it is important to refer to manufacturer's literature where mass uncertainties are dominant. In this example, the calculations are omitted for clarity.

Volume V

The volume has three major influences; calibration, repeatability and temperature effects.

i) *Calibration*: The manufacturer quotes a volume for the flask of 100 mL ± 0.1 mL

measured at a temperature of 20 °C. The value of the uncertainty is given without a confidence level or distribution information, so an assumption is necessary. Here, the standard uncertainty is calculated assuming a triangular distribution.

$$\frac{0.1\,\text{mL}}{\sqrt{6}} = 0.04\,\text{mL}$$

NOTE: A triangular distribution was chosen, because in an effective production process, the nominal value is more likely than extremes. The resulting distribution is better represented by a triangular distribution than a rectangular one.

ii) *Repeatability*: The uncertainty due to variations in filling can be estimated from a repeatability experiment on a typical example of the flask used. A series of ten fill and weigh experiments on a typical 100 mL flask gave a standard deviation of 0.02 mL. This can be used directly as a standard uncertainty.

iii) *Temperature*: According to the manufacturer the flask has been calibrated at a temperature of 20 °C, whereas the laboratory temperature varies between the limits of ±4 °C. The uncertainty from this effect can be calculated from the estimate of the temperature range and the coefficient of the volume expansion. The volume expansion of the liquid is considerably larger than that of the flask, so only the former needs to be considered. The coefficient of volume expansion for water is 2.1×10^{-4} °C^{-1}, which leads to a volume variation of

$$\pm(100\times4\times2.1\times10^{-4}) = \pm0.084\,\text{mL}$$

The standard uncertainty is calculated using the assumption of a rectangular distribution for the temperature variation i.e.

Table A1.2: Values and Uncertainties

Description	Value x	$u(x)$	$u(x)/x$
Purity of the metal P	0.9999	0.000058	0.000058
Mass of the metal m (mg)	100.28	0.05 mg	0.0005
Volume of the flask V (mL)	100.0	0.07 mL	0.0007

$$\frac{0.084\,\text{mL}}{\sqrt{3}} = 0.05\,\text{mL}$$

The three contributions are combined to give the standard uncertainty $u(V)$ of the volume V

$$u(V) = \sqrt{0.04^2 + 0.02^2 + 0.05^2} = 0.07\,\text{mL}$$

A1.5 Step 4: Calculating the combined standard uncertainty

c_{Cd} is given by

$$c_{\text{Cd}} = \frac{1000\cdot m\cdot P}{V} \qquad [\text{mg L}^{-1}]$$

The intermediate values, their standard uncertainties and their relative standard uncertainties are summarised overleaf (Table A1.2)

Using those values, the concentration of the calibration standard is

$$c_{\text{Cd}} = \frac{1000\times100.28\times0.9999}{100.0} = 1002.7\ \text{mg L}^{-1}$$

For this simple multiplicative expression, the uncertainties associated with each component are combined as follows.

$$\frac{u_{\text{c}}(c_{\text{Cd}})}{c_{\text{Cd}}} = \sqrt{\left(\frac{u(P)}{P}\right)^2 + \left(\frac{u(m)}{m}\right)^2 + \left(\frac{u(V)}{V}\right)^2}$$

$$= \sqrt{0.000058^2 + 0.0005^2 + 0.0007^2}$$

$$= 0.0009$$

$$u_{\text{c}}(c_{\text{Cd}}) = c_{\text{Cd}}\times0.0009 = 1002.7\,\text{mg L}^{-1}\times0.0009$$

$$= 0.9\,\text{mg L}^{-1}$$

It is preferable to derive the combined standard uncertainty ($u_{\text{c}}(c_{\text{Cd}})$) using the spreadsheet method given in Appendix E, since this can be utilised even for complex expressions. The completed spreadsheet is shown in Table A1.3. The values of the parameters are entered in the second row from C2 to E2. Their standard uncertainties are in the row below (C3-E3). The spreadsheet copies the values from C2-E2 into the second column from B5 to B7. The result (c(Cd)) using these values is given in B9. The C5 shows the value of P from C2 plus its uncertainty given in C3. The result of the calculation using the values C5-C7 is given in C9. The columns D and E follow a similar procedure. The values shown in the row 10 (C10-E10) are the differences of the row (C9-

E9) minus the value given in B9. In row 11 (C11-E11) the values of row 10 (C10-E10) are squared and summed to give the value shown in B11. B13 gives the combined standard uncertainty, which is the square root of B11.

The contributions of the different parameters are shown in Figure A1.5. The contribution of the uncertainty on the volume of the flask is the largest and that from the weighing procedure is similar. The uncertainty on the purity of the cadmium has virtually no influence on the overall uncertainty.

The expanded uncertainty $U(c_{Cd})$ is obtained by multiplying the combined standard uncertainty with a coverage factor of 2, giving

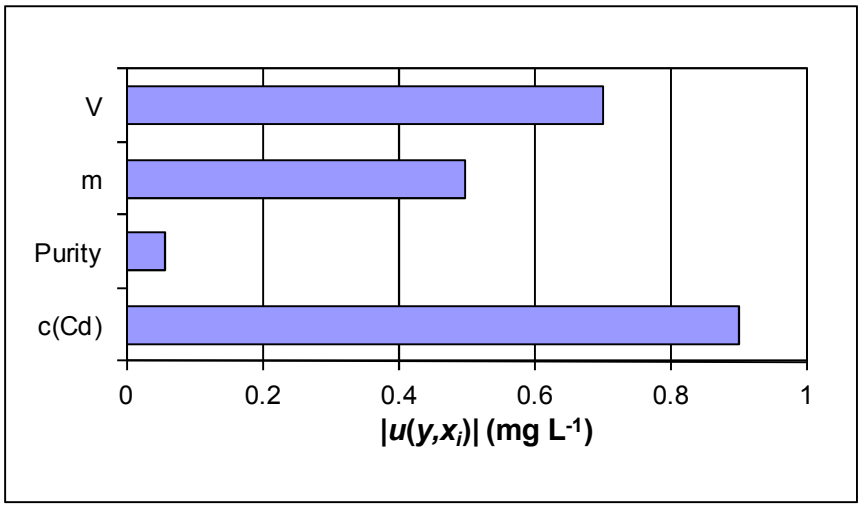
$$U(c_{Cd}) = 2 \times 0.9\,\text{mg l}^{-1} = 1.8\,\text{mg L}^{-1}$$

Table A1.3: Spreadsheet calculation of uncertainty

	A	B	C	D	E
1			P	m	V
2		Value	0.9999	100.28	100.00
3		Uncertainty	0.000058	0.05	0.07
4					
5	P	0.9999	0.999958	0.9999	0.9999
6	m	100.28	100.28	100.33	100.28
7	V	100.0	100.00	100.00	100.07
8					
9	c(Cd)	1002.69972	1002.75788	1003.19966	1001.99832
10	$u(y,x_i)$*		0.05816	0.49995	-0.70140
11	$u(y)^2$, $u(y,x_i)^2$	0.74529	0.00338	0.24995	0.49196
12					
13	u(c(Cd))	0.9			

*The sign of the difference has been retained

Figure A1.5: Uncertainty contributions in cadmium standard preparation



The values of $u(y,x_i) = (\partial y/\partial x_i).u(x_i)$ are taken from Table A1.3

Example A2: Standardising a Sodium Hydroxide Solution

Summary

Goal

A solution of sodium hydroxide (NaOH) is standardised against the titrimetric standard potassium hydrogen phthalate (KHP).

Measurement procedure

The titrimetric standard (KHP) is dried and weighed. After the preparation of the NaOH solution the sample of the titrimetric standard (KHP) is dissolved and then titrated using the NaOH solution. The stages in the procedure are shown in the flow chart Figure A2.1.

Measurand:

$$c_{\mathrm{NaOH}} = \frac{1000 \cdot m_{\mathrm{KHP}} \cdot P_{\mathrm{KHP}}}{M_{\mathrm{KHP}} \cdot V_{\mathrm{T}}} \qquad [\mathrm{mol\,L^{-1}}]$$

where

c_{NaOH} concentration of the NaOH solution [mol L^{-1}]

1000 conversion factor [mL] to [L]

m_{KHP} mass of the titrimetric standard KHP [g]

P_{KHP} purity of the titrimetric standard given as mass fraction

M_{KHP} molar mass of KHP [g mol^{-1}]

V_{T} titration volume of NaOH solution [mL]

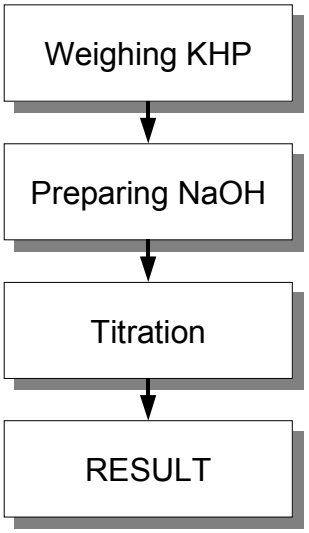


Figure A2.1: Standardising NaOH

Identification of the uncertainty sources:

The relevant uncertainty sources are shown as a cause and effect diagram in Figure A2.2.

Quantification of the uncertainty components

The different uncertainty contributions are given in Table A2.1, and shown diagrammatically in Figure A2.3. The combined standard uncertainty for the 0.10214 mol L^{-1} NaOH solution is 0.00010 mol L^{-1}

Table A2.1: Values and uncertainties in NaOH standardisation

	Description	Value x	Standard uncertainty u	Relative standard uncertainty $u(x)/x$
rep	Repeatability	1.0	0.0005	0.0005
m_{KHP}	Mass of KHP	0.3888 g	0.00013 g	0.00033
P_{KHP}	Purity of KHP	1.0	0.00029	0.00029
M_{KHP}	Molar mass of KHP	204.2212 g mol^{-1}	0.0038 g mol^{-1}	0.000019
V_{T}	Volume of NaOH for KHP titration	18.64 mL	0.013 mL	0.0007
c_{NaOH}	NaOH solution	0.10214 mol L^{-1}	0.00010 mol L^{-1}	0.00097

Figure A2.2: Cause and effect diagram for titration

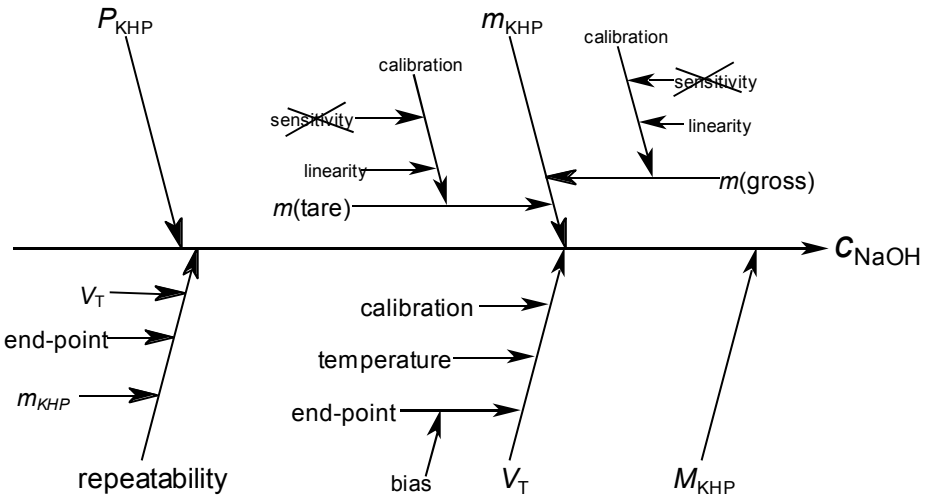
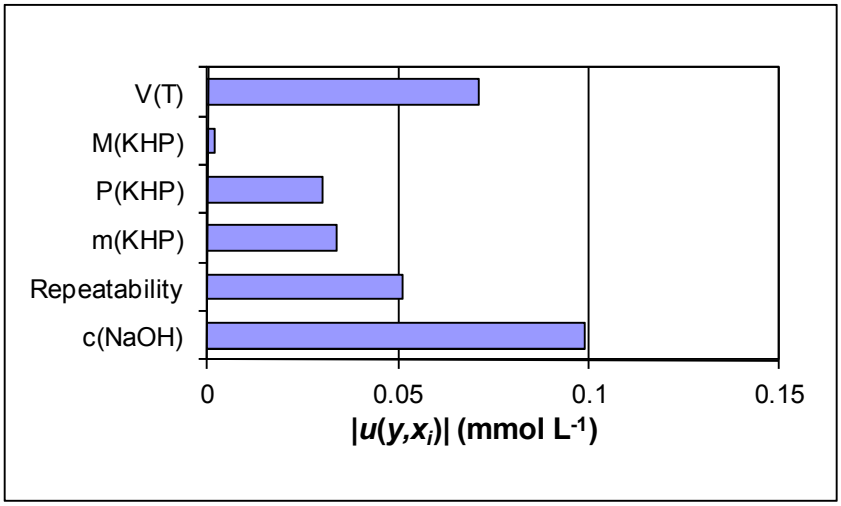


Figure A2.3: Contributions to Titration uncertainty



The values of $u(y,x_i) = (\partial y/\partial x_i).u(x_i)$ are taken from Table A2.3

Example A2: Standardising a sodium hydroxide solution. Detailed discussion

A2.1 Introduction

This second introductory example discusses an experiment to determine the concentration of a solution of sodium hydroxide (NaOH). The NaOH is titrated against the titrimetric standard potassium hydrogen phthalate (KHP). It is assumed that the NaOH concentration is known to be of the order of 0.1 mol L^{-1}. The end-point of the titration is determined by an automatic titration system using a combined pH-electrode to measure the shape of the pH-curve. The functional composition of the titrimetric standard potassium hydrogen phthalate (KHP), which is the number of free protons in relation to the overall number of molecules, provides traceability of the concentration of the NaOH solution to the SI system.

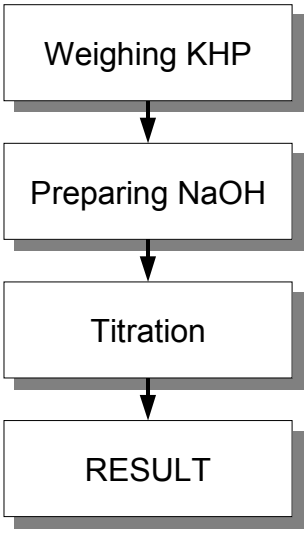
A2.2 Step 1: Specification

The aim of the first step is to describe the measurement procedure. This description consists of a listing of the measurement steps and a mathematical statement of the measurand and the parameters upon which it depends.

Procedure:

The measurement sequence to standardise the NaOH solution has the following stages.

Figure A2.4: Standardisation of a solution of sodium hydroxide



The separate stages are:

i) The primary standard potassium hydrogen phthalate (KHP) is dried according to the supplier's instructions. The instructions are given in the supplier's certificate, which also states the purity of the titrimetric standard and its uncertainty. A titration volume of approximately 19 mL of 0.1 mol L^{-1} solution of NaOH entails weighing out an amount as close as possible to

$$\frac{204.2212 \times 0.1 \times 19}{1000 \times 1.0} = 0.388\,\text{g}$$

The weighing is carried out on a balance with a last digit of 0.1 mg.

ii) A 0.1 mol L^{-1} solution of sodium hydroxide is prepared. In order to prepare 1 L of solution, it is necessary to weigh out ≈4 g NaOH. However, since the concentration of the NaOH solution is to be determined by assay against the primary standard KHP and not by direct calculation, no information on the uncertainty sources connected with the molecular weight or the mass of NaOH taken is required.

iii) The weighed quantity of the titrimetric standard KHP is dissolved with ≈50 mL of ion-free water and then titrated using the NaOH solution. An automatic titration system controls the addition of NaOH and records the pH-curve. It also determines the end-point of the titration from the shape of the recorded curve.

Calculation:

The measurand is the concentration of the NaOH solution, which depends on the mass of KHP, its purity, its molecular weight and the volume of NaOH at the end-point of the titration

$$c_{\text{NaOH}} = \frac{1000 \cdot m_{\text{KHP}} \cdot P_{\text{KHP}}}{M_{\text{KHP}} \cdot V_{\text{T}}} \qquad [\text{mol L}^{-1}]$$

where

c_{NaOH} concentration of the NaOH solution [mol L^{-1}]

1000 conversion factor [mL] to [L]

m_{KHP} mass of the titrimetric standard KHP [g]

P_{KHP} purity of the titrimetric standard given as mass fraction

M_{KHP} molar mass of KHP [g mol^{-1}]

V_T titration volume of NaOH solution [mL]

A2.3 Step 2: Identifying and analysing uncertainty sources

The aim of this step is to identify all major uncertainty sources and to understand their effect on the measurand and its uncertainty. This has been shown to be one of the most difficult step in evaluating the uncertainty of analytical measurements, because there is a risk of

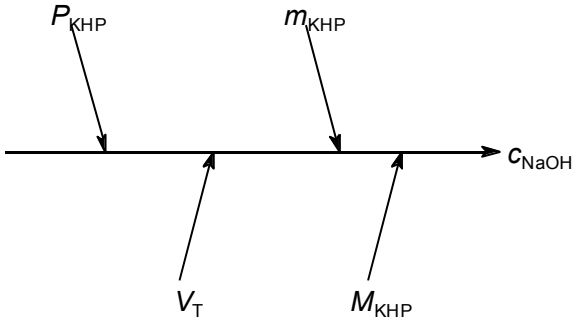


Figure A2.5: First step in setting up a cause and effect diagram

neglecting uncertainty sources on the one hand and an the other of double-counting them. The use of a cause and effect diagram (Appendix D) is one possible way to help prevent this happening. The first step in preparing the diagram is to draw the four parameters of the equation of the measurand as the main branches.

Afterwards, each step of the method is considered and any further influence quantity is added as a factor to the diagram working outwards from the main effect. This is carried out for each branch until effects become sufficiently remote, that is, until effects on the result are negligible.

Mass m_{KHP}

Approximately 388 mg of KHP are weighed to standardise the NaOH solution. The weighing procedure is a weight by difference. This means that a branch for the determination of the tare (m_{tare}) and another branch for the gross weight (m_{gross}) have to be drawn in the cause and effect diagram. Each of the two weighings is subject to run to run variability and the uncertainty of the calibration of the balance. The calibration itself has two possible uncertainty sources: the sensitivity and the linearity of the calibration function. If the weighing is done on the same scale and over a small range of weight then the sensitivity contribution can be neglected.

All these uncertainty sources are added into the cause and effect diagram (see Figure A2.6).

Purity P_{KHP}

The purity of KHP is quoted in the supplier's catalogue to be within the limits of 99.95 % and 100.05 %. P_{KHP} is therefore 1.0000 ±0.0005. There is no other uncertainty source if the drying procedure was performed according to the supplier's specification.

Molar mass M_{KHP}

Potassium hydrogen phthalate (KHP) has the

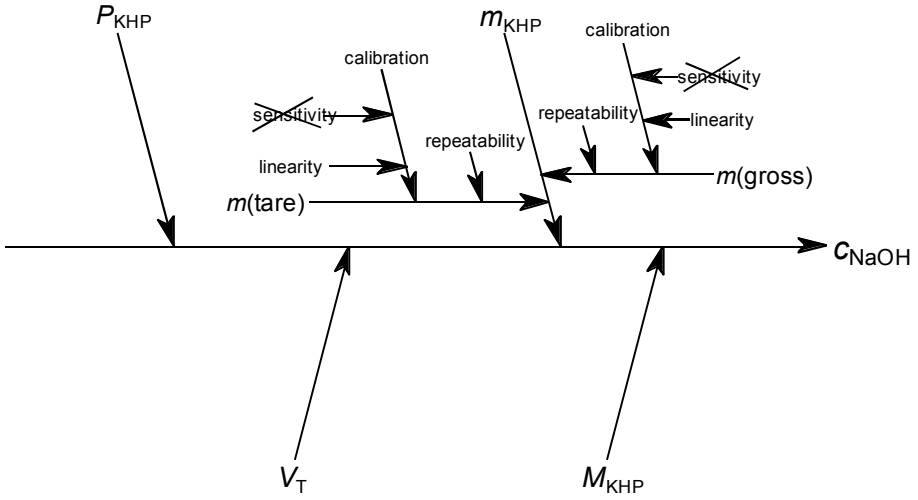


Figure A2.6:Cause and effect diagram with added uncertainty sources for the weighing procedure

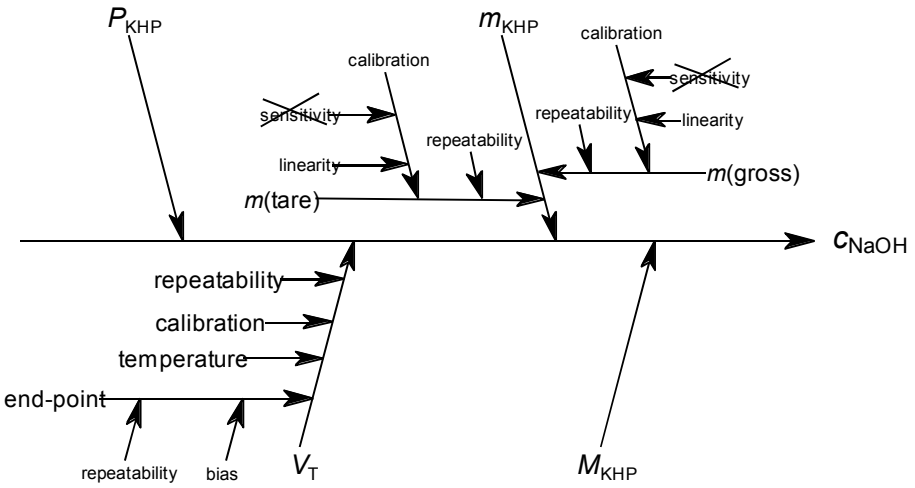


Figure A2.7: Cause and effect diagram (all sources)

empirical formula

$$C_8H_5O_4K$$

The uncertainty in the molar mass of the compound can be determined by combining the uncertainty in the atomic weights of its constituent elements. A table of atomic weights including uncertainty estimates is published biennially by IUPAC in the Journal of Pure and Applied Chemistry. The molar mass can be calculated directly from these; the cause and effect diagram (Figure A2.7) omits the individual atomic masses for clarity

Volume V_T

The titration is accomplished using a 20 mL piston burette. The delivered volume of NaOH from the piston burette is subject to the same three uncertainty sources as the filling of the volumetric flask in the previous example. These uncertainty sources are the repeatability of the delivered volume, the uncertainty of the calibration of that volume and the uncertainty resulting from the difference between the temperature in the laboratory and that of the calibration of the piston burette. In addition there is the contribution of the end-point detection, which has two uncertainty sources.

1. The repeatability of the end-point detection, which is independent of the repeatability of the volume delivery.
2. The possibility of a systematic difference between the determined end-point and the equivalence point (bias), due to carbonate absorption during the titration and inaccuracy in the mathematical evaluation of the end-point from the titration curve.

These items are included in the cause and effect diagram shown in Figure A2.7.

A2.4 Step 3: Quantifying uncertainty components

In step 3, the uncertainty from each source identified in step 2 has to be quantified and then converted to a standard uncertainty. All experiments always include at least the repeatability of the volume delivery of the piston burette and the repeatability of the weighing operation. Therefore it is reasonable to combine all the repeatability contributions into one contribution for the overall experiment and to use the values from the method validation to quantify its size, leading to the revised cause and effect diagram in Figure A2.8.

The method validation shows a repeatability for the titration experiment of 0.05 %. This value can be directly used for the calculation of the combined standard uncertainty.

Mass m_{KHP}

The relevant weighings are:

container and KHP:	60.5450 g (observed)
container less KHP:	60.1562 g (observed)
KHP	0.3888 g (calculated)

Because of the combined repeatability term identified above, there is no need to take into account the weighing repeatability. Any systematic offset across the scale will also cancel.

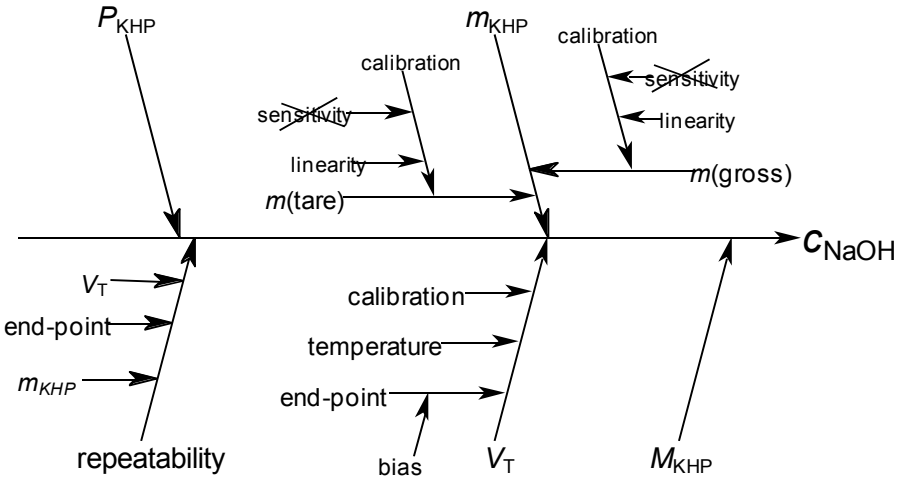


Figure A2.8: Cause and effect diagram (repeatabilities combined)

The uncertainty therefore arises solely from the balance linearity uncertainty.

Linearity: The calibration certificate of the balance quotes ±0.15 mg for the linearity. This value is the maximum difference between the actual mass on the pan and the reading of the scale. The balance manufacturer's own uncertainty evaluation recommends the use of a rectangular distribution to convert the linearity contribution to a standard uncertainty.

The balance linearity contribution is accordingly

$$\frac{0.15\,\text{mg}}{\sqrt{3}} = 0.09\,\text{mg}$$

This contribution has to be counted twice, once for the tare and once for the gross weight, because each is an independent observation and the linearity effects are not correlated.

This gives for the standard uncertainty $u(m_{KHP})$ of the mass m_{KHP}, a value of

$$u(m_{KHP}) = \sqrt{2 \times (0.09^2)}$$
$$\Rightarrow u(m_{KHP}) = 0.13\,\text{mg}$$

NOTE 1: Buoyancy correction is not considered because all weighing results are quoted on the conventional basis for weighing in air [H.33]. The remaining uncertainties are too small to consider. Note 1 in Appendix G refers.

NOTE 2: There are other difficulties when weighing a titrimetric standard. A temperature difference of only 1 °C between the standard and the balance causes a drift in the same order of magnitude as the repeatability contribution. The titrimetric standard has been completely dried, but the weighing procedure is carried out at a humidity of around 50 % relative humidity, so adsorption of some moisture is expected.

Purity P_{KHP}

P_{KHP} is 1.0000±0.0005. The supplier gives no further information concerning the uncertainty in the catalogue. Therefore this uncertainty is taken as having a rectangular distribution, so the standard uncertainty $u(P_{KHP})$ is $0.0005/\sqrt{3} = 0.00029$.

Molar mass M_{KHP}

From the IUPAC table current at the time of measurement, the atomic weights and listed uncertainties for the constituent elements of KHP ($C_8H_5O_4K$) were:

Element	Atomic weight	Quoted uncertainty	Standard uncertainty
C	12.0107	±0.0008	0.00046
H	1.00794	±0.00007	0.000040
O	15.9994	±0.0003	0.00017
K	39.0983	±0.0001	0.000058

For each element, the standard uncertainty is found by treating the IUPAC quoted uncertainty as forming the bounds of a rectangular distribution. The corresponding standard uncertainty is therefore obtained by dividing those values by $\sqrt{3}$.

The separate element contributions to the molar mass, together with the uncertainty contribution for each, are:

	Calculation	Result	Standard uncertainty
C_8	8×12.0107	96.0856	0.0037
H_5	5×1.00794	5.0397	0.00020
O_4	4×15.9994	63.9976	0.00068
K	1×39.0983	39.0983	0.000058

The uncertainty in each of these values is calculated by multiplying the standard uncertainty in the previous table by the number of atoms.

This gives a molar mass for KHP of

$$M_{\mathrm{KHP}} = 96.0856 + 5.0397 + 63.9976 + 39.0983$$
$$= 204.2212\ \mathrm{g\ mol^{-1}}$$

As this expression is a sum of independent values, the standard uncertainty $u(M_{\mathrm{KHP}})$ is a simple square root of the sum of the squares of the contributions:

$$u(M_{\mathrm{KHP}}) = \sqrt{0.0037^2 + 0.0002^2 + 0.00068^2 + 0.000058^2}$$
$$\Rightarrow u(M_{\mathrm{KHP}}) = 0.0038\ \mathrm{g\ mol^{-1}}$$

NOTE: Since the element contributions to M_{KHP} are simply the sum of the single atom contributions, it might be expected from the general rule for combining uncertainty contributions that the uncertainty for each element contribution would be calculated from the sum of squares of the single atom contributions, that is, for carbon, $u(M_{\mathrm{C}}) = \sqrt{8 \times 0.00037^2} = 0.001\,\mathrm{g\,mol^{-1}}$.

Recall, however, that this rule applies only to independent contributions, that is, contributions from separate determinations of the value. In this case, the total is obtained by multiplying a single value by 8. Notice that the contributions from different elements are independent, and will therefore combine in the usual way.

Volume V_{T}

1. *Repeatability of the volume delivery*: As before, the repeatability has already been taken into account via the combined repeatability term for the experiment.

2. *Calibration*: The limits of accuracy of the delivered volume are indicated by the manufacturer as a ± figure. For a 20 mL piston burette this number is typically ±0.03 mL. Assuming a triangular distribution gives a standard uncertainty of $0.03/\sqrt{6} = 0.012$ ml.

   Note: The ISO Guide (F.2.3.3) recommends adoption of a triangular distribution if there are reasons to expect values in the centre of the range being more likely than those near the bounds. For the glassware in examples A1 and A2, a triangular distribution has been assumed (see the discussion under Volume uncertainties in example A1).

3. *Temperature*: The uncertainty due to the lack of temperature control is calculated in the same way as in the previous example, but this time taking a possible temperature variation of ±3 °C (with a 95 % confidence). Again using the coefficient of volume expansion for water as 2.1×10^{-4} °C^{-1} gives a value of

   $$\frac{19 \times 2.1\times10^{-4} \times 3}{1.96} = 0.006\,\mathrm{mL}$$

   Thus the standard uncertainty due to incomplete temperature control is 0.006 mL.

NOTE: When dealing with uncertainties arising from incomplete control of environmental factors such as temperature, it is essential to take account of any correlation in the effects on different intermediate values. In this example, the dominant effect on the solution temperature is taken as the differential heating effects of different solutes, that is, the solutions are not equilibrated to ambient temperature. Temperature effects on each solution concentration at STP are therefore uncorrelated in this example, and are consequently treated as independent uncertainty contributions.

4. *Bias of the end-point detection*: The titration is performed under a layer of Argon to exclude any bias due to the absorption of CO_2 in the titration solution. This approach follows the principle that it is better to prevent any bias than to correct for it. There are no other indications that the end-point determined from the shape of the pH-curve does not correspond to the equivalence-point, because a strong acid is titrated with a strong base. Therefore it is

Table A2.2: Values and uncertainties for titration

	Description	Value x	Standard uncertainty $u(x)$	Relative standard uncertainty $u(x)/x$
rep	Repeatability	1.0	0.0005	0.0005
m_{KHP}	Weight of KHP	0.3888 g	0.00013 g	0.00033
P_{KHP}	Purity of KHP	1.0	0.00029	0.00029
M_{KHP}	Molar mass of KHP	204.2212 g mol^{-1}	0.0038 g mol^{-1}	0.000019
V_T	Volume of NaOH for KHP titration	18.64 mL	0.013 mL	0.0007

assumed that the bias of the end-point detection and its uncertainty are negligible.

V_T is found to be 18.64 mL and combining the remaining contributions to the uncertainty $u(V_T)$ of the volume V_T gives a value of

$$u(V_T) = \sqrt{0.012^2 + 0.006^2}$$
$$\Rightarrow u(V_T) = 0.013 \text{ mL}$$

A2.5 Step 4: Calculating the combined standard uncertainty

c_{NaOH} is given by

$$c_{NaOH} = \frac{1000 \cdot m_{KHP} \cdot P_{KHP}}{M_{KHP} \cdot V_T} \qquad [\text{mol L}^{-1}]$$

The values of the parameters in this equation, their standard uncertainties and their relative standard uncertainties are collected in Table A2.2

Using the values given above:

$$c_{NaOH} = \frac{1000 \times 0.3888 \times 1.0}{204.2212 \times 18.64} = 0.10214 \text{ mol L}^{-1}$$

For a multiplicative expression (as above) the standard uncertainties are used as follows:

$$\frac{u_c(c_{NaOH})}{c_{NaOH}} = \sqrt{\left(\frac{u(rep)}{rep}\right)^2 + \left(\frac{u(m_{KHP})}{m_{KHP}}\right)^2 + \left(\frac{u(P_{KHP})}{P_{KHP}}\right)^2 + \left(\frac{u(M_{KHP})}{M_{KHP}}\right)^2 + \left(\frac{u(V_T)}{V_T}\right)^2}$$

$$\Rightarrow \frac{u_c(c_{NaOH})}{c_{NaOH}} = \sqrt{0.0005^2 + 0.00033^2 + 0.00029^2 + 0.000019^2 + 0.00070^2}$$
$$= 0.00097$$
$$\Rightarrow u_c(c_{NaOH}) = c_{NaOH} \times 0.00097 = 0.000099 \text{ mol L}^{-1}$$

Spreadsheet software is used to simplify the above calculation of the combined standard uncertainty (see Appendix E.2). The spreadsheet filled in with the appropriate values is shown as Table A2.3, which appears with additional explanation.

It is instructive to examine the relative contributions of the different parameters. The contributions can easily be visualised using a histogram. Figure A2.9 shows the calculated values $|u(y,x_i)|$ from Table A2.3.

The contribution of the uncertainty of the titration volume V_T is by far the largest followed by the repeatability. The weighing procedure and the purity of the titrimetric standard show the same order of magnitude, whereas the uncertainty in the molar mass is again nearly an order of magnitude smaller.

Figure A2.9: Uncertainty contributions in NaOH standardisation

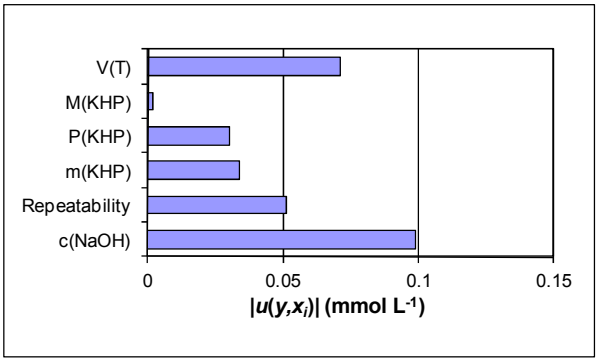


Table A2.3: Spreadsheet calculation of titration uncertainty

	A	B	C	D	E	F	G
1			Rep	m(KHP)	P(KHP)	M(KHP)	V(T)
2		Value	1.0	0.3888	1.0	204.2212	18.64
3		Uncertainty	0.0005	0.00013	0.00029	0.0038	0.013
4							
5	rep	1.0	1.0005	1.0	1.0	1.0	1.0
6	m(KHP)	0.3888	0.3888	0.38893	0.3888	0.3888	0.3888
7	P(KHP)	1.0	1.0	1.0	1.00029	1.0	1.0
8	M(KHP)	204.2212	204.2212	204.2212	204.2212	204.2250	204.2212
9	V(T)	18.64	18.64	18.64	18.64	18.64	18.653
10							
11	c(NaOH)	0.102136	0.102187	0.102170	0.102166	0.102134	0.102065
12	$u(y,x_i)$		0.000051	0.000034	0.000030	-0.000002	-0.000071
13	$u(y)^2$, $u(y,x_i)^2$	9.72E-9	2.62E-9	1.16E-9	9E-10	4E-12	5.041E-9
14							
15	u(c(NaOH))	0.000099					

The values of the parameters are given in the second row from C2 to G2. Their standard uncertainties are entered in the row below (C3-G3). The spreadsheet copies the values from C2-G2 into the second column from B5 to B9. The result (c(NaOH)) using these values is given in B11. C5 shows the value of the repeatability from C2 plus its uncertainty given in C3. The result of the calculation using the values C5-C9 is given in C11. The columns D and G follow a similar procedure. The values shown in the row 12 (C12-G12) are the (signed) differences of the row (C11-G11) minus the value given in B11. In row 13 (C13-G13) the values of row 12 (C12-G12) are squared and summed to give the value shown in B13. B15 gives the combined standard uncertainty, which is the square root of B13.

A2.6 Step 5: Re-evaluate the significant components

The contribution of $V(T)$ is the largest one. The volume of NaOH for titration of KHP ($V(T)$) itself is affected by four influence quantities: the repeatability of the volume delivery, the calibration of the piston burette, the difference between the operation and calibration temperature of the burette and the repeatability of the end-point detection. Checking the size of each contribution, the calibration is by far the largest. Therefore this contribution needs to be investigated more thoroughly.

The standard uncertainty of the calibration of $V(T)$ was calculated from the data given by the manufacturer assuming a triangular distribution. The influence of the choice of the shape of the distribution is shown in Table A2.4.

According to the ISO Guide 4.3.9 Note 1:

> "For a normal distribution with expectation μ and standard deviation σ, the interval μ ±3σ encompasses approximately 99.73 percent of the distribution. Thus, if the upper and lower bounds a_+ and a_- define 99.73 percent limits rather than 100 percent limits, and X_i can be assumed to be approximately normally distributed rather than there being no specific knowledge about X_i [between the bounds], then $u^2(x_i) = a^2/9$. By comparison, the variance of a symmetric rectangular distribution of the half-width a is $a^2/3$ ... and that of a symmetric triangular distribution of the half-width a is $a^2/6$ ... The magnitudes of the variances of the three distributions are surprisingly similar in view of the differences in the assumptions upon which they are based."

Thus the choice of the distribution function of this influence quantity has little effect on the value of the combined standard uncertainty ($u_c(c_{NaOH})$) and it is adequate to assume that it is triangular.

The expanded uncertainty $U(c_{NaOH})$ is obtained by multiplying the combined standard uncertainty by a coverage factor of 2.

$$U(c_{NaOH}) = 0.00010 \times 2 = 0.0002 \text{ mol L}^{-1}$$

Thus the concentration of the NaOH solution is **(0.1021 ±0.0002) mol L^{-1}**.

Table A2.4: Effect of different distribution assumptions

Distribution	factor	$u(V(T;cal))$ (mL)	$u(V(T))$ (mL)	$u_c(c_{NaOH})$ (mol L^{-1})
Rectangular	$\sqrt{3}$	0.017	0.019	0.00011
Triangular	$\sqrt{6}$	0.012	0.015	0.000099
Normal[Note 1]	$\sqrt{9}$	0.010	0.013	0.000085

Note 1: The factor of $\sqrt{9}$ arises from the factor of 3 in Note 1 of ISO Guide 4.3.9 (see page 49 for details).

Example A3: An Acid/Base Titration

Summary

Goal

A solution of hydrochloric acid (HCl) is standardised against a solution of sodium hydroxide (NaOH) with known content.

Measurement procedure

A solution of hydrochloric acid (HCl) is titrated against a solution of sodium hydroxide (NaOH), which has been standardised against the titrimetric standard potassium hydrogen phthalate (KHP), to determine its concentration. The stages of the procedure are shown in Figure A3.1.

Measurand:

$$c_{HCl} = \frac{1000 \cdot m_{KHP} \cdot P_{KHP} \cdot V_{T2}}{V_{T1} \cdot M_{KHP} \cdot V_{HCl}} \quad [\text{mol L}^{-1}]$$

where the symbols are as given in Table A3.1 and the value of 1000 is a conversion factor from mL to litres.

Identification of the uncertainty sources:

The relevant uncertainty sources are shown in Figure A3.2.

Quantification of the uncertainty components

The final uncertainty is estimated as 0.00016 mol L^{-1}. Table A3.1 summarises the values and their uncertainties; Figure A3.3 shows the values diagrammatically.

Figure A3.1: Titration procedure

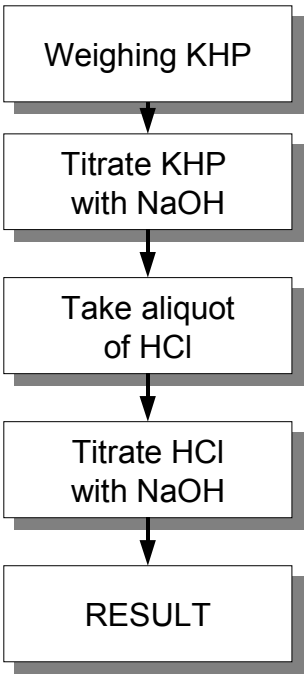


Figure A3.2: Cause and Effect diagram for acid-base titration

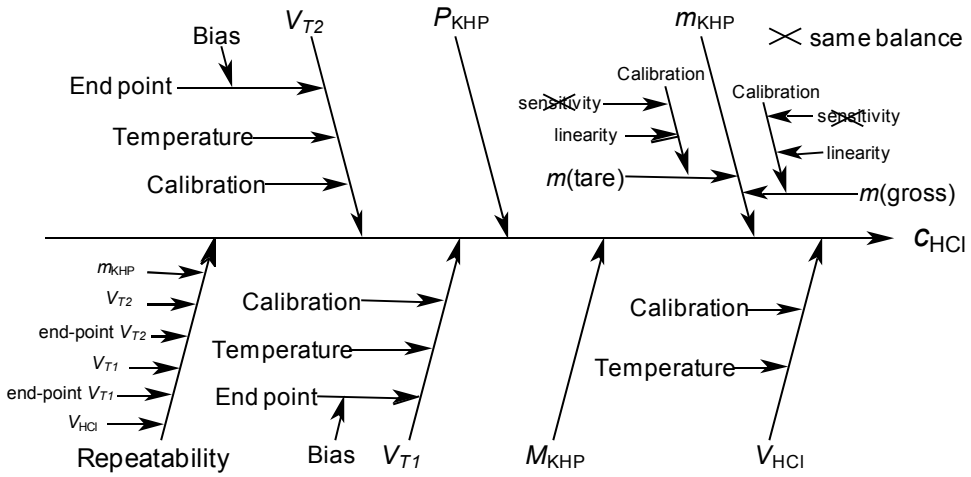
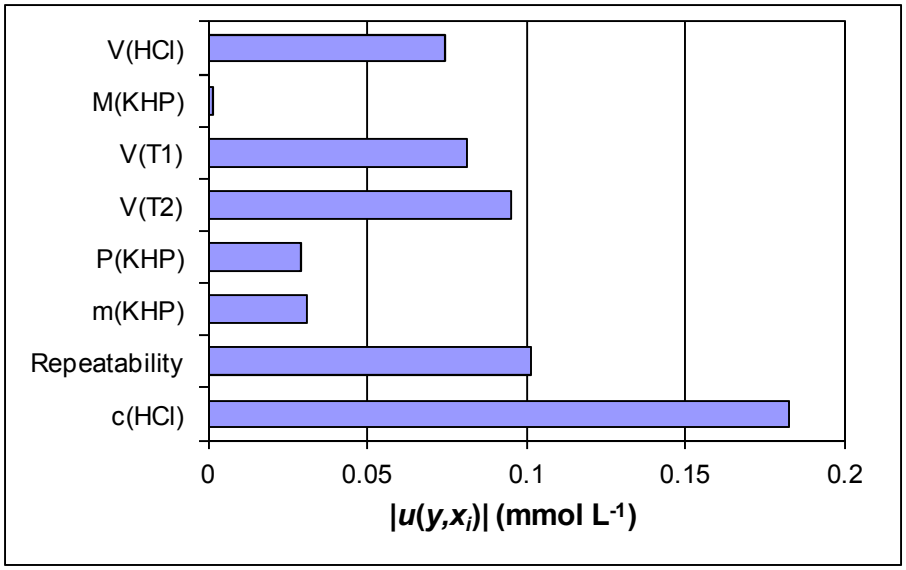


Table A3.1: Acid-base Titration values and uncertainties

	Description	Value x	Standard uncertainty $u(x)$	Relative standard uncertainty $u(x)/x$
rep	Repeatability	1	0.001	0.001
m_{KHP}	Weight of KHP	0.3888 g	0.00013 g	0.00033
P_{KHP}	Purity of KHP	1.0	0.00029	0.00029
V_{T2}	Volume of NaOH for HCl titration	14.89 mL	0.015 mL	0.0010
V_{T1}	Volume of NaOH for KHP titration	18.64 mL	0.016 mL	0.00086
M_{KHP}	Molar mass of KHP	204.2212 g mol^{-1}	0.0038 g mol^{-1}	0.000019
V_{HCl}	HCl aliquot for NaOH titration	15 mL	0.011 mL	0.00073
c_{HCl}	HCl solution concentration	0.10139 mol L^{-1}	0.00016 mol L^{-1}	0.0016

Figure A3.3: Uncertainty contributions in acid-base titration



The values of $u(y,x_i) = (\partial y/\partial x_i).u(x_i)$ are taken from Table A3.3.

Example A3: An acid/base titration. Detailed discussion

A3.1 Introduction

This example discusses a sequence of experiments to determine the concentration of a solution of hydrochloric acid (HCl). In addition, a number of special aspects of the titration technique are highlighted. The HCl is titrated against solution of sodium hydroxide (NaOH), which was freshly standardised with potassium hydrogen phthalate (KHP). As in the previous example (A2) it is assumed that the HCl concentration is known to be of the order of 0.1 mol L^{-1} and that the end-point of the titration is determined by an automatic titration system using the shape of the pH-curve. This evaluation gives the measurement uncertainty in terms of the SI units of measurement.

A3.2 Step 1: Specification

A detailed description of the measurement procedure is given in the first step. It compromises a listing of the measurement steps and a mathematical statement of the measurand.

Procedure

The determination of the concentration of the HCl solution consists of the following stages (See also Figure A3.4):

i) The titrimetric standard potassium hydrogen phthalate (KHP) is dried to ensure the purity quoted in the supplier's certificate. Approximately 0.388 g of the dried standard is then weighed to achieve a titration volume of 19 mL NaOH.

ii) The KHP titrimetric standard is dissolved with ≈50 mL of ion free water and then titrated using the NaOH solution. A titration system controls automatically the addition of NaOH and samples the pH-curve. The end-point is evaluated from the shape of the recorded curve.

iii) 15 mL of the HCl solution is transferred by means of a volumetric pipette. The HCl solution is diluted with de-ionised water to give ≈50 mL solution in the titration vessel.

iv) The same automatic titrator performs the measurement of HCl solution.

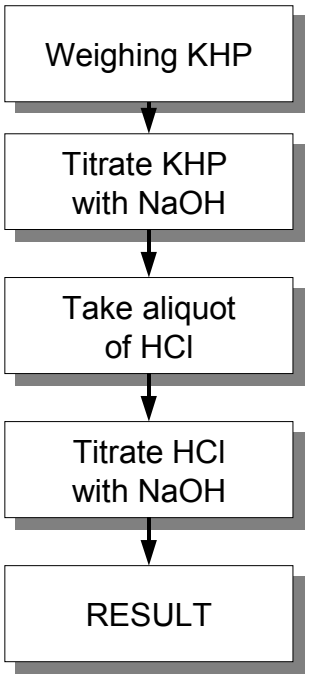


Figure A3.4: Determination of the concentration of a HCl solution

Calculation

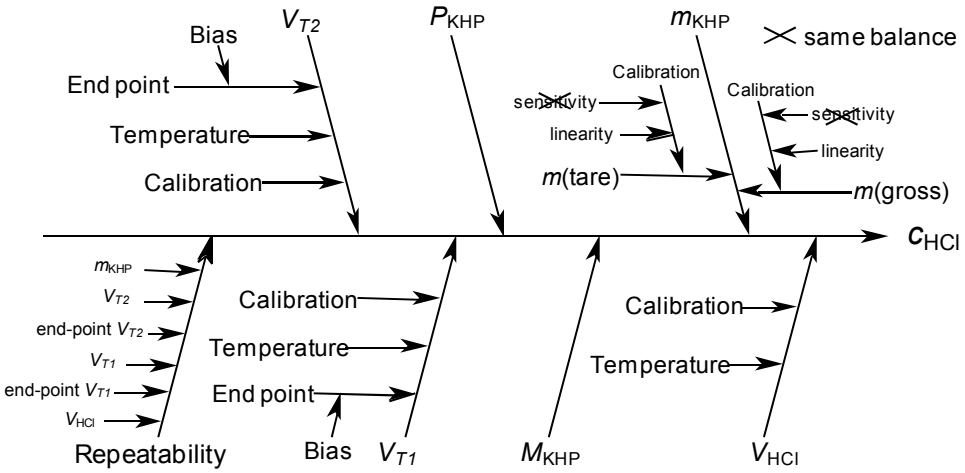
The measurand is the concentration of the HCl solution, c_{HCl}. It depends on the mass of KHP, its purity, its molecular weight, the volumes of NaOH at the end-point of the two titrations and the aliquot of HCl.:

$$c_{HCl} = \frac{1000 \cdot m_{KHP} \cdot P_{KHP} \cdot V_{T2}}{V_{T1} \cdot M_{KHP} \cdot V_{HCl}} \qquad [\text{mol L}^{-1}]$$

where

c_{HCl}	concentration of the HCl solution [mol L^{-1}]
1000	conversion factor [mL] to [L]
m_{KHP}	mass of KHP taken [g]
P_{KHP}	purity of KHP given as mass fraction
V_{T2}	volume of NaOH solution to titrate HCl [mL]
V_{T1}	volume of NaOH solution to titrate KHP [mL]
M_{KHP}	molar mass of KHP [g mol^{-1}]
V_{HCl}	volume of HCl titrated with NaOH solution [mL]

Figure A3.5: Final cause and effect diagram



A3.3 Step 2: Identifying and analysing uncertainty sources

The different uncertainty sources and their influence on the measurand are best analysed by visualising them first in a cause and effect diagram (Figure A3.5).

Because a repeatability estimate is available from validation studies for the procedure as a whole, there is no need to consider all the repeatability contributions individually. They are therefore grouped into one contribution (shown in the revised cause and effect diagram in Figure A3.5).

The influences on the parameters V_{T2}, V_{T1}, m_{KHP}, P_{KHP} and M_{KHP} have been discussed extensively in the previous example, therefore only the new influence quantities of V_{HCl} will be dealt with in more detail in this section.

Volume V_{HCl}

15 mL of the investigated HCl solution is to be transferred by means of a volumetric pipette. The delivered volume of the HCl from the pipette is subject to the same three sources of uncertainty as all the volumetric measuring devices.

1. The variability or repeatability of the delivered volume
2. The uncertainty in the stated volume of the pipette
3. The solution temperature differing from the calibration temperature of the pipette.

A3.4 Step 3: Quantifying uncertainty components

The goal of this step is to quantify each uncertainty source analysed in step 2. The quantification of the branches or rather of the different components was described in detail in the previous two examples. Therefore only a summary for each of the different contributions will be given.

Repeatability

The method validation shows a repeatability for the determination of 0.001 (as RSD). This value can be used directly for the calculation of the combined standard uncertainty associated with the different repeatability terms.

Mass m_{KHP}

Calibration/linearity: The balance manufacturer quotes ±0.15 mg for the linearity contribution. This value represents the maximum difference between the actual mass on the pan and the reading of the scale. The linearity contribution is assumed to show a rectangular distribution and is converted to a standard uncertainty:

$$\frac{0.15}{\sqrt{3}} = 0.087\,\text{mg}$$

The contribution for the linearity has to be accounted for twice, once for the tare and once for the gross mass, leading to an uncertainty $u(m_{KHP})$ of

$$u(m_{KHP}) = \sqrt{2 \times (0.087)^2}$$
$$\Rightarrow u(m_{KHP}) = 0.12\,\text{mg}$$

NOTE 1: The contribution is applied twice because no assumptions are made about the form of the non-linearity. The non-linearity is accordingly treated as a systematic effect on each weighing, which varies randomly in magnitude across the measurement range.

NOTE 2: Buoyancy correction is not considered because all weighing results are quoted on the conventional basis for weighing in air [H.33]. The remaining uncertainties are too small to consider. Note 1 in Appendix G refers.

P(KHP)

P(KHP) is given in the supplier's certificate as 100 % with uncertainty quoted as ±0.05 % (or ±0.0005). This is taken as a rectangular distribution, so the standard uncertainty $u(P_{KHP})$ is

$$u(P_{KHP}) = \frac{0.0005}{\sqrt{3}} = 0.00029 .$$

V(T2)

i) *Calibration*: Figure given by the manufacturer (±0.03 mL) and treated as a triangular distribution: $u = 0.03/\sqrt{6} = 0.012$ mL.

ii) *Temperature*: The possible temperature variation is within the limits of ±4 °C and treated as a rectangular distribution: $u = 15 \times 2.1 \times 10^{-4} \times 4/\sqrt{3} = 0.007$ mL.

iii) *Bias of the end-point detection*: A bias between the determined end-point and the equivalence-point due to atmospheric CO_2 can be prevented by performing the titration under argon. No uncertainty allowance is made.

V_{T2} is found to be 14.89 mL and combining the two contributions to the uncertainty $u(V_{T2})$ of the volume V_{T2} gives a value of

$$u(V_{T2}) = \sqrt{0.012^2 + 0.007^2}$$
$$\Rightarrow u(V_{T2}) = 0.014 \text{ mL}$$

Volume V_{T1}

All contributions except the one for the temperature are the same as for V_{T2}

i) *Calibration*: $0.03/\sqrt{6} = 0.012$ mL

ii) *Temperature*: The approximate volume for the titration of 0.3888 g KHP is 19 mL NaOH, therefore its uncertainty contribution is $19 \times 2.1 \times 10^{-4} \times 4/\sqrt{3} = 0.009$ mL.

iii) *Bias*: Negligible

V_{T1} is found to be 18.64 mL with a standard uncertainty $u(V_{T1})$ of

$$u(V_{T1}) = \sqrt{0.012^2 + 0.009^2}$$
$$\Rightarrow u(V_{T1}) = 0.015 \text{ mL}$$

Molar mass M_{KHP}

Atomic weights and listed uncertainties (from current IUPAC tables) for the constituent elements of KHP ($C_8H_5O_4K$) are:

Element	Atomic weight	Quoted uncertainty	Standard uncertainty
C	12.0107	±0.0008	0.00046
H	1.00794	±0.00007	0.000040
O	15.9994	±0.0003	0.00017
K	39.0983	±0.0001	0.000058

For each element, the standard uncertainty is found by treating the IUPAC quoted uncertainty as forming the bounds of a rectangular distribution. The corresponding standard uncertainty is therefore obtained by dividing those values by $\sqrt{3}$.

The molar mass M_{KHP} for KHP and its uncertainty $u(M_{KHP})$are, respectively:

$$M_{KHP} = 8 \times 12.0107 + 5 \times 1.00794 + 4 \times 15.9994 + 39.0983$$
$$= 204.2212 \text{ g mol}^{-1}$$
$$u(M_{KHP}) = \sqrt{(8 \times 0.00046)^2 + (5 \times 0.00004)^2 + (4 \times 0.00017)^2 + 0.000058^2}$$
$$\Rightarrow u(F_{KHP}) = 0.0038 \text{ g mol}^{-1}$$

NOTE: The single atom contributions are not independent. The uncertainty for the atom contribution is therefore calculated by multiplying the standard uncertainty of the atomic weight by the number of atoms.

Volume V_{HCl}

i) *Calibration*: Uncertainty stated by the manufacturer for a 15 mL pipette as ±0.02 mL and treated as a triangular distribution: $0.02/\sqrt{6} = 0.008$ mL.

ii) *Temperature*: The temperature of the laboratory is within the limits of ±4 °C. Use of a rectangular temperature distribution gives a standard uncertainty of $15 \times 2.1 \times 10^{-4} \times 4/\sqrt{3}$ = 0.007 mL.

Combining these contributions gives

$$u(V_{HCl}) = \sqrt{0.0037^2 + 0.008^2 + 0.007^2}$$
$$\Rightarrow u(V_{HCl}) = 0.011 \text{ mL}$$

Table A3.2: Acid-base Titration values and uncertainties (2-step procedure)

	Description	Value x	Standard Uncertainty $u(x)$	Relative standard uncertainty $u(x)/x$
rep	Repeatability	1	0.001	0.001
m_{KHP}	Mass of KHP	0.3888 g	0.00012 g	0.00031
P_{KHP}	Purity of KHP	1.0	0.00029	0.00029
V_{T2}	Volume of NaOH for HCl titration	14.89 mL	0.014 mL	0.00094
V_{T1}	Volume of NaOH for KHP titration	18.64 mL	0.015 mL	0.00080
M_{KHP}	Molar mass of KHP	204.2212 g mol^{-1}	0.0038 g mol^{-1}	0.000019
V_{HCl}	HCl aliquot for NaOH titration	15 mL	0.011 mL	0.00073

A3.5 Step 4: Calculating the combined standard uncertainty

c_{HCl} is given by

$$c_{HCl} = \frac{1000 \cdot m_{KHP} \cdot P_{KHP} \cdot V_{T2}}{V_{T1} \cdot M_{KHP} \cdot V_{HCl}}$$

NOTE: The repeatability estimate is, in this example, treated as a relative effect; the complete model equation is therefore

$$c_{HCl} = \frac{1000 \cdot m_{KHP} \cdot P_{KHP} \cdot V_{T2}}{V_{T1} \cdot M_{KHP} \cdot V_{HCl}} \times rep$$

All the intermediate values of the two step experiment and their standard uncertainties are collected in Table A3.2. Using these values:

$$c_{HCl} = \frac{1000 \times 0.3888 \times 1.0 \times 14.89}{18.64 \times 204.2212 \times 15} \times 1 = 0.10139 \text{ mol L}^{-1}$$

The uncertainties associated with each component are combined accordingly:

$$\frac{u_c(c_{HCl})}{c_{HCl}} = \sqrt{\begin{array}{l}\left(\frac{u(m_{KHP})}{m_{KHP}}\right)^2 + \left(\frac{u(P_{KHP})}{P_{KHP}}\right)^2 + \left(\frac{u(V_{T2})}{V_{T2}}\right)^2 \\ + \left(\frac{u(V_{T1})}{V_{T1}}\right)^2 + \left(\frac{u(M_{KHP})}{M_{KHP}}\right)^2 + \left(\frac{u(V_{HCl})}{V_{HCl}}\right)^2 \\ + u(rep)^2\end{array}}$$

$$= \sqrt{\begin{array}{l}0.00031^2 + 0.00029^2 + 0.00094^2 + \\ 0.00080^2 + 0.000019^2 + 0.00073^2 + 0.001^2\end{array}}$$

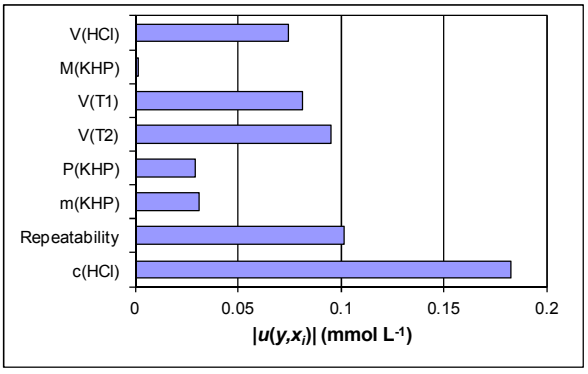
$$= 0.0018$$

$$\Rightarrow u_c(c_{HCl}) = c_{HCl} \times 0.0018 = 0.00018 \text{ mol L}^{-1}$$

A spreadsheet method (see Appendix E) can be used to simplify the above calculation of the combined standard uncertainty. The spreadsheet filled in with the appropriate values is shown in Table A3.3, with an explanation.

The sizes of the different contributions can be compared using a histogram. Figure A3.6 shows the values of the contributions $|u(y,x_i)|$ from Table A3.3.

Figure A3.6: Uncertainties in acid-base titration



The expanded uncertainty $U(c_{HCl})$ is calculated by multiplying the combined standard uncertainty by a coverage factor of 2:

$$U(c_{HCl}) = 0.00018 \times 2 = 0.0004 \text{ mol L}^{-1}$$

The concentration of the HCl solution is

$$(0.1014 \pm 0.0004) \text{ mol L}^{-1}$$

Table A3.3: Acid-base Titration – spreadsheet calculation of uncertainty

	A	B	C	D	E	F	G	H	I
1			rep	m(KHP)	P(KHP)	V(T2)	V(T1)	M(KHP)	V(HCl)
2		value	1.0	0.3888	1.0	14.89	18.64	204.2212	15
3		uncertainty	0.001	0.00012	0.00029	0.014	0.015	0.0038	0.011
4									
5	rep	1.0	1.001	1.0	1.0	1.0	1.0	1.0	1.0
6	m(KHP)	0.3888	0.3888	0.38892	0.3888	0.3888	0.3888	0.3888	0.3888
7	P(KHP)	1.0	1.0	1.0	1.00029	1.0	1.0	1.0	1.0
8	V(T2)	14.89	14.89	14.89	14.89	14.904	14.89	14.89	14.89
9	V(T1)	18.64	18.64	18.64	18.64	18.64	18.655	18.64	18.64
10	M(KHP)	204.2212	204.2212	204.2212	204.2212	204.2212	204.2212	204.2250	204.2212
11	V(HCl)	15	15	15	15	15	15	15	15.011
12									
13	c(HCl)	0.101387	0.101489	0.101418	0.101417	0.101482	0.101306	0.101385	0.101313
14	u(y, x_i)		0.000101	0.000031	0.000029	0.000095	-0.000082	-0.0000019	-0.000074
15	$u(y)^2$, $u(y, x_i)^2$	3.34E-8	1.03E-8	9.79E-10	8.64E-10	9.09E-9	6.65E-9	3.56E-12	5.52E-9
16									
17	u(c(HCl))	0.00018							

The values of the parameters are given in the second row from C2 to I2. Their standard uncertainties are entered in the row below (C3-I3). The spreadsheet copies the values from C2-I2 into the second column from B5 to B11. The result (c(HCl)) using these values is given in B13. The C5 shows the value of the repeatability from C2 plus its uncertainty given in C3. The result of the calculation using the values C5-C11 is given in C13. The columns D to I follow a similar procedure. The values shown in the row 14 (C14-I14) are the (signed) differences of the row (C13-H13) minus the value given in B13. In row 15 (C15-I15) the values of row 14 (C14-I14) are squared and summed to give the value shown in B15. B17 gives the combined standard uncertainty, which is the square root of B15.

A3.6 Special aspects of the titration example

Three special aspects of the titration experiment will be dealt with in this second part of the example. It is interesting to see what effect changes in the experimental set up or in the implementation of the titration would have on the final result and its combined standard uncertainty.

Influence of a mean room temperature of 25°C

For routine analysis, analytical chemists rarely correct for the systematic effect of the temperature in the laboratory on the volume. This question considers the uncertainty introduced by the corrections required.

The volumetric measuring devices are calibrated at a temperature of 20°C. But rarely does any analytical laboratory have a temperature controller to keep the room temperature that level. For illustration, consider correction for a mean room temperature of 25°C.

The final analytical result is calculated using the corrected volumes and not the calibrated volumes at 20°C. A volume is corrected for the temperature effect according to

$$V' = V[1 - \alpha(T - 20)]$$

where

V' volume at 20°C

V volume at the mean temperature T

α expansion coefficient of an aqueous solution [$°C^{-1}$]

T observed temperature in the laboratory [°C]

The equation of the measurand has to be rewritten:

$$c_{HCl} = \frac{1000 \cdot m_{KHP} \cdot P_{KHP}}{M_{KHP}} \cdot \frac{V'_{T2}}{V'_{T1} \cdot V'_{HCl}}$$

Including the temperature correction terms gives:

$$c_{\mathrm{HCl}} = \frac{1000 \cdot m_{\mathrm{KHP}} \cdot P_{\mathrm{KHP}}}{M_{\mathrm{KHP}}} \cdot \frac{V'_{\mathrm{T2}}}{V'_{\mathrm{T1}} \cdot V'_{\mathrm{HCl}}}$$
$$= \left(\frac{1000 \cdot m_{\mathrm{KHP}} \cdot P_{\mathrm{KHP}}}{M_{\mathrm{KHP}}} \right) \times \left(\frac{V_{\mathrm{T2}}[1-\alpha(T-20)]}{V_{\mathrm{T1}}[1-\alpha(T-20)] \cdot V_{\mathrm{HCl}}[1-\alpha(T-20)]} \right)$$

This expression can be simplified by assuming that the mean temperature T and the expansion coefficient of an aqueous solution α are the same for all three volumes

$$c_{\mathrm{HCl}} = \left(\frac{1000 \cdot m_{\mathrm{KHP}} \cdot P_{\mathrm{KHP}}}{M_{\mathrm{KHP}}} \right) \times \left(\frac{V_{\mathrm{T2}}}{V_{\mathrm{T1}} \cdot V_{\mathrm{HCl}} \cdot [1-\alpha(T-20)]} \right)$$

This gives a slightly different result for the HCl concentration at 20°C:

$$c_{\mathrm{HCl}} = \frac{1000 \times 0.3888 \times 1.0 \times 14.89}{204.2236 \times 18.64 \times 15 \times [1 - 2.1 \times 10^{-4}(25-20)]} = 0.10149 \ \mathrm{mol\,L^{-1}}$$

The figure is still within the range given by the combined standard uncertainty of the result at a mean temperature of 20°C, so the result is not significantly affected. Nor does the change affect the evaluation of the combined standard uncertainty, because a temperature variation of ±4°C at the mean room temperature of 25°C is still assumed.

Visual end-point detection

A bias is introduced if the indicator phenolphthalein is used for visual end-point detection, instead of an automatic titration system extracting the equivalence-point from the pH curve. The change of colour from transparent to red/purple occurs between pH 8.2 and 9.8 leading to an excess volume, introducing a bias compared to the end-point detection employing a pH meter. Investigations have shown that the excess volume is around 0.05 mL with a standard uncertainty for the visual detection of the end-point of approximately 0.03 mL. The bias arising from the excess volume has to be considered in the calculation of the final result. The actual volume for the visual end-point detection is given by

$$V_{\mathrm{T1;Ind}} = V_{\mathrm{T1}} + V_{\mathrm{Excess}}$$

where

$V_{\mathrm{T1;Ind}}$:volume from a visual end-point detection

V_{T1} volume at the equivalence-point

V_{Excess} excess volume needed to change the colour of phenolphthalein

The volume correction quoted above leads to the following changes in the equation of the measurand

$$c_{\mathrm{HCl}} = \frac{1000 \cdot m_{\mathrm{KHP}} \cdot P_{\mathrm{KHP}} \cdot (V_{\mathrm{T2;Ind}} - V_{\mathrm{Excess}})}{M_{\mathrm{KHP}} \cdot (V_{\mathrm{T1;Ind}} - V_{\mathrm{Excess}}) \cdot V_{\mathrm{HCl}}}$$

The standard uncertainties $u(V_{\mathrm{T2}})$ and $u(V_{\mathrm{T1}})$ have to be recalculated using the standard uncertainty of the visual end-point detection as the uncertainty component of the repeatability of the end-point detection.

$$u(V_{\mathrm{T1}}) = u(V_{\mathrm{T1;Ind}} - V_{\mathrm{Excess}}) = \sqrt{0.012^2 + 0.009^2 + 0.03^2} = 0.034 \ \mathrm{mL}$$

$$u(V_{\mathrm{T2}}) = u(V_{\mathrm{T2;Ind}} - V_{\mathrm{Excess}}) = \sqrt{0.012^2 + 0.007^2 + 0.03^2} = 0.033 \ \mathrm{mL}$$

The combined standard uncertainty

$$u_{\mathrm{c}}(c_{\mathrm{HCl}}) = 0.0003 \ \mathrm{mol\ L^{-1}}$$

is considerable larger than before.

Triple determination to obtain the final result

The two step experiment is performed three times to obtain the final result. The triple determination is expected to reduce the contribution from repeatability, and hence reduce the overall uncertainty.

As shown in the first part of this example, all the run to run variations are combined to one single component, which represents the overall experimental repeatability as shown in the in the cause and effect diagram (Figure A3.5).

The uncertainty components are quantified in the following way:

Mass m_{KHP}

Linearity: $0.15/\sqrt{3} = 0.087$ mg

$\Rightarrow u(m_{\mathrm{KHP}}) = \sqrt{2 \times 0.87^2} = 0.12$ mg

Purity P_{KHP}

Purity: $0.0005/\sqrt{3} = 0.00029$

Volume V_{T2}

calibration: $0.03/\sqrt{6} = 0.012$ mL

temperature:

$$15 \times 2.1 \times 10^{-4} \times 4/\sqrt{3} = 0.007 \text{ mL}$$

$$\Rightarrow u(V_{T2}) = \sqrt{0.012^2 + 0.007^2} = 0.014 \text{ mL}$$

Repeatability

The quality log of the triple determination shows a mean long term standard deviation of the experiment of 0.001 (as RSD). It is not recommended to use the actual standard deviation obtained from the three determinations because this value has itself an uncertainty of 52 %. The standard deviation of 0.001 is divided by the square root of $\sqrt{3}$ to obtain the standard uncertainty of the triple determination (three independent measurements)

$$Rep = 0.001/\sqrt{3} = 0.00058 \text{ (as RSD)}$$

Volume V_{HCl}

calibration: $0.02/\sqrt{6} = 0.008$ mL

temperature: $15 \times 2.1 \times 10^{-4} \times 4/\sqrt{3} = 0.007$ mL

$$\Rightarrow u(V_{HCl}) = \sqrt{0.008^2 + 0.007^2} = 0.01 \text{ mL}$$

Molar mass M_{KHP}

$$u(M_{KHP}) = 0.0038 \text{ g mol}^{-1}$$

Volume V_{T1}

calibration: $0.03/\sqrt{6} = 0.12$ mL

temperature:

$$19 \times 2.1 \times 10^{-4} \times 4/\sqrt{3} = 0.009 \text{ mL}$$

$$\Rightarrow u(V_{T1}) = \sqrt{0.012^2 + 0.009^2} = 0.015 \text{ mL}$$

All the values of the uncertainty components are summarised in Table A3.4. The combined standard uncertainty is 0.00016 mol L^{-1}, which is a very modest reduction due to the triple determination. The comparison of the uncertainty contributions in the histogram, shown in Figure A3.7, highlights some of the reasons for that result. Though the repeatability contribution is much reduced, the volumetric uncertainty contributions remain, limiting the improvement.

Figure A3.7: Replicated Acid-base Titration values and uncertainties

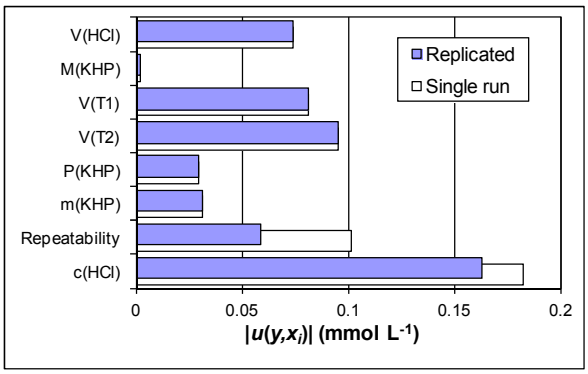


Table A3.4: Replicated Acid-base Titration values and uncertainties

	Description	Value x	Standard Uncertainty $u(x)$	Relative Standard Uncertainty $u(x)/x$
Rep	Repeatability of the determination	1.0	0.00058	0.00058
m_{KHP}	Mass of KHP	0.3888 g	0.00013 g	0.00033
P_{KHP}	Purity of KHP	1.0	0.00029	0.00029
V_{T2}	Volume of NaOH for HCl titration	14.90 mL	0.014 mL	0.00094
V_{T1}	Volume of NaOH for KHP titration	18.65 mL	0.015 mL	0.0008
M_{KHP}	Molar mass of KHP	204.2212 g mol^{-1}	0.0038 g mol^{-1}	0.000019
V_{HCl}	HCl aliquot for NaOH titration	15 mL	0.01 mL	0.00067

Example A4: Uncertainty Estimation from In-House Validation Studies. Determination of Organophosphorus Pesticides in Bread.

Summary

Goal

The amount of an organophosphorus pesticide residue in bread is determined employing an extraction and a GC procedure.

Measurement procedure

The stages needed to determine the amount of organophosphorus pesticide residue are shown in Figure A4.1

Measurand:

$$P_{\text{op}} = \frac{I_{\text{op}} \cdot c_{ref} \cdot V_{\text{op}}}{I_{\text{ref}} \cdot Rec \cdot m_{\text{sample}}} \cdot F_{\text{hom}} \cdot F_{\text{I}} \text{ mg kg}^{-1}$$

where

P_{op}	Mass fraction of pesticide in the sample [mg kg^{-1}]
I_{op}	Peak intensity of the sample extract
c_{ref}	Mass concentration of the reference standard [μg mL^{-1}]
V_{op}	Final volume of the extract [mL]
I_{ref}	Peak intensity of the reference standard
Rec	Recovery
m_{sample}	Mass of the investigated sub-sample [g]
F_{I}	Correction factor representing the effect of intermediate precision under intermediate conditions
F_{hom}	Correction factor for sample inhomogeneity

Identification of the uncertainty sources:

The relevant uncertainty sources are shown in the cause and effect diagram in Figure A4.2.

Quantification of the uncertainty components:

Based on in-house validation data, the three major contributions are listed in Table A4.1 and shown diagrammatically in Figure A4.3 (values are from Table A4.5).

Figure A4.1: Organophosphorus pesticides analysis

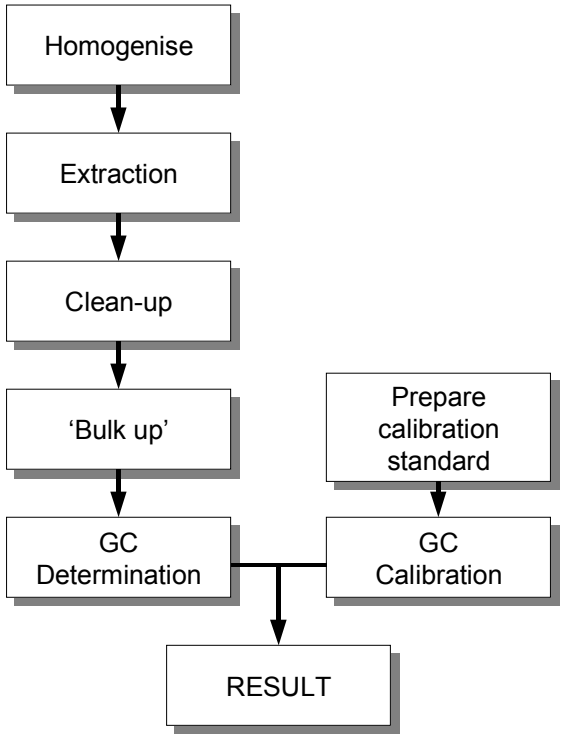


Table A4.1: Uncertainties in pesticide analysis

Description	Value x	Standard uncertainty $u(x)$	Relative standard uncertainty $u(x)/x$	Comments
Precision (1)	1.0	0.27	0.27	Based on duplicate tests of different types of samples
Bias (Rec) (2)	0.9	0.043	0.048	Spiked samples
Other sources (3) (Homogeneity)	1.0	0.2	0.2	Estimation based on model assumptions
P_{op}	- -	- -	0.34	Relative standard uncertainty

Figure A4.2: Uncertainty sources in pesticide analysis

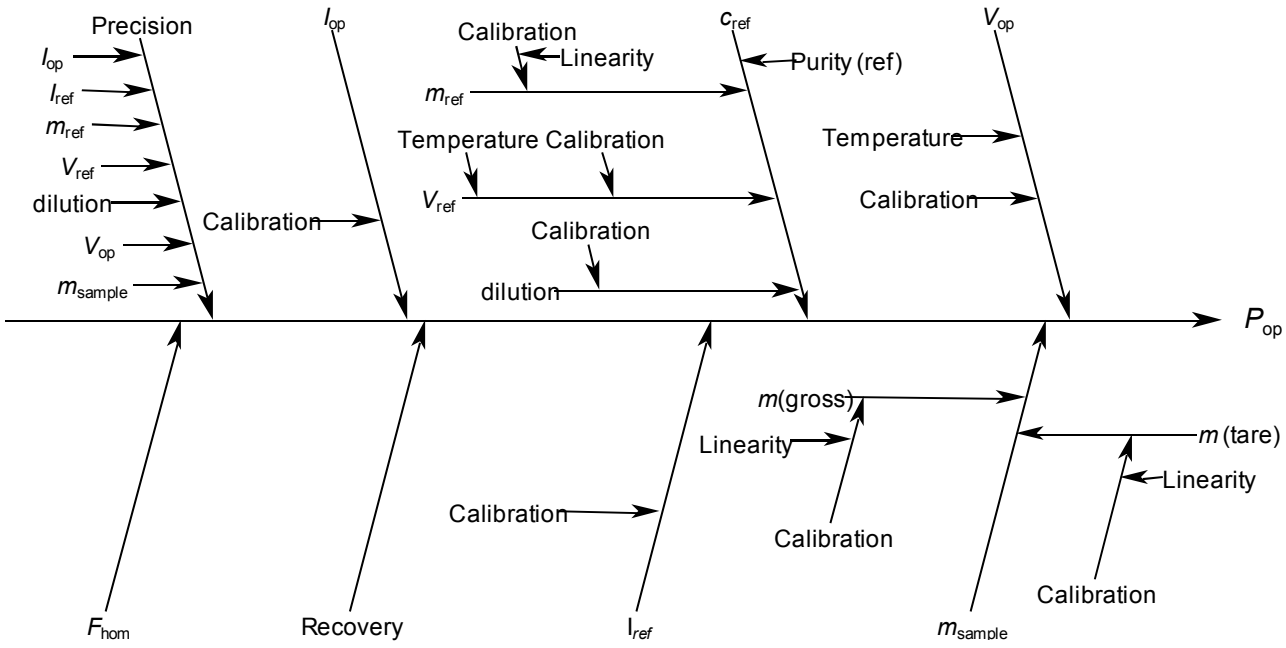
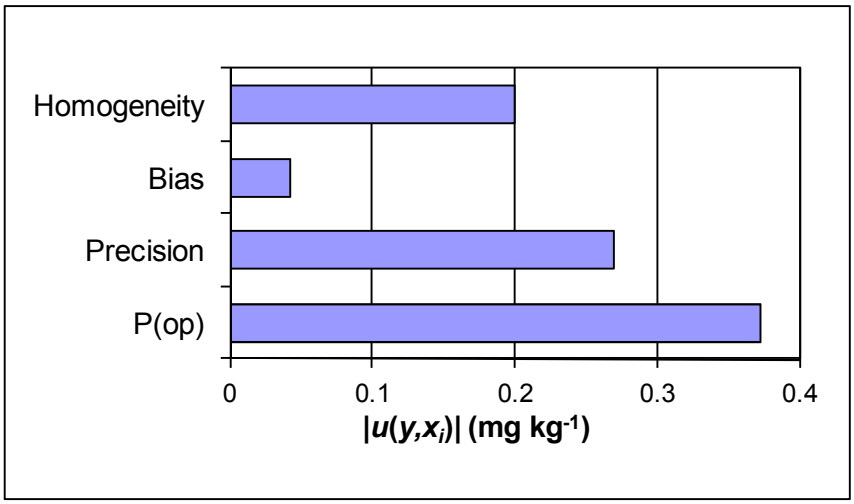


Figure A4.3: Uncertainties in pesticide analysis



The values of $u(y,x_i) = (\partial y/\partial x_i).u(x_i)$ are taken from Table A4.5

Example A4: Determination of organophosphorus pesticides in bread. Detailed discussion.

A4.1 Introduction

This example illustrates the way in which in-house validation data can be used to quantify the measurement uncertainty. The aim of the measurement is to determine the amount of an organophosphorus pesticides residue in bread. The validation scheme and experiments establish performance by measurements on spiked samples. It is assumed the uncertainty due to any difference in response of the measurement to the spike and the analyte in the sample is small compared with the total uncertainty on the result.

A4.2 Step 1: Specification

The measurand is the mass fraction of pesticide in a bread sample. The detailed specification of the measurand for more extensive analytical methods is best done by a comprehensive description of the different stages of the analytical method and by providing the equation of the measurand.

Procedure

The measurement procedure is illustrated schematically in Figure A4.4. The separate stages are:

i) Homogenisation: The complete sample is divided into small (approx. 2 cm) fragments, a random selection is made of about 15 of these, and the sub-sample homogenised. Where extreme inhomogeneity is suspected proportional sampling is used before blending.

ii) Weighing of sub-sample for analysis gives mass m_{sample}

iii) Extraction: Quantitative extraction of the analyte with organic solvent, decanting and drying through a sodium sulphate columns, and concentration of the extract using a Kuderna-Danish apparatus.

iv) Liquid-liquid extraction:

v) Acetonitrile/hexane liquid partition, washing the acetonitrile extract with hexane, drying the hexane layer through sodium sulphate column.

vi) Concentration of the washed extract by gas blown-down of extract to near dryness.

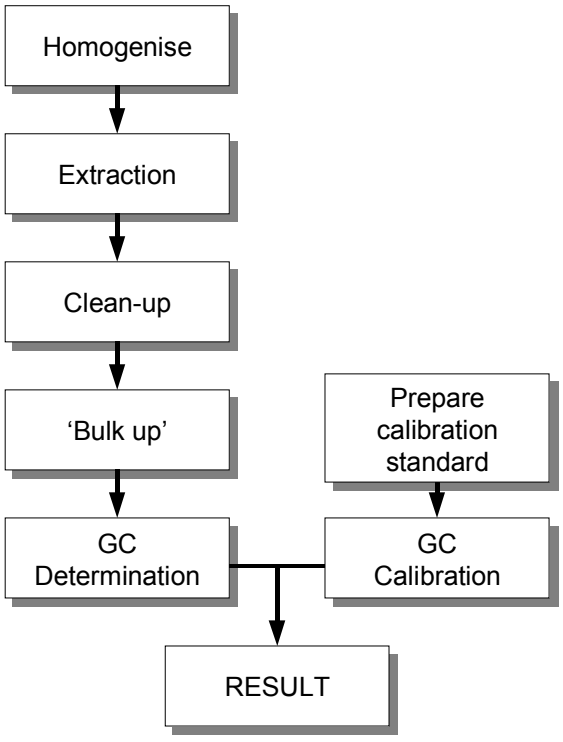
vii) Dilution to standard volume V_{op} (approximately 2 mL) in a 10 mL graduated tube.

viii) Measurement: Injection and GC measurement of 5 µL of sample extract to give the peak intensity I_{op}.

ix) Preparation of an approximately 5 µg mL^{-1} standard (actual mass concentration c_{ref}).

x) GC calibration using the prepared standard and injection and GC measurement of 5 µL of the standard to give a reference peak intensity I_{ref}.

Figure A4.4: Organophosphorus pesticides analysis



Calculation

The mass concentration c_{op} in the final sample extract is given by

$$c_{\mathrm{op}} = c_{\mathrm{ref}} \cdot \frac{I_{\mathrm{op}}}{I_{\mathrm{ref}}} \qquad \mu\mathrm{g\,mL^{-1}}$$

and the estimate P_{op} of the level of pesticide in the bulk sample (in mg kg^{-1}) is given by

$$P_{\mathrm{op}} = \frac{c_{\mathrm{op}} \cdot V_{\mathrm{op}}}{Rec \cdot m_{\mathrm{sample}}} \qquad \mathrm{mg\,kg^{-1}}$$

or, substituting for c_{op},

$$P_{\mathrm{op}} = \frac{I_{\mathrm{op}} \cdot c_{\mathrm{ref}} \cdot V_{\mathrm{op}}}{I_{\mathrm{ref}} \cdot Rec \cdot m_{\mathrm{sample}}} \qquad \mathrm{mg\,kg^{-1}}$$

where

P_{op} Mass fraction of pesticide in the sample [mg kg^{-1}]

I_{op} Peak intensity of the sample extract

c_{ref} Mass concentration of the reference standard [µg mL^{-1}]

V_{op} Final volume of the extract [mL]

I_{ref} Peak intensity of the reference standard

Rec Recovery

m_{sample} Mass of the investigated sub-sample [g]

Scope

The analytical method is applicable to a small range of chemically similar pesticides at levels between 0.01 and 2 mg kg^{-1} with different kinds of bread as matrix.

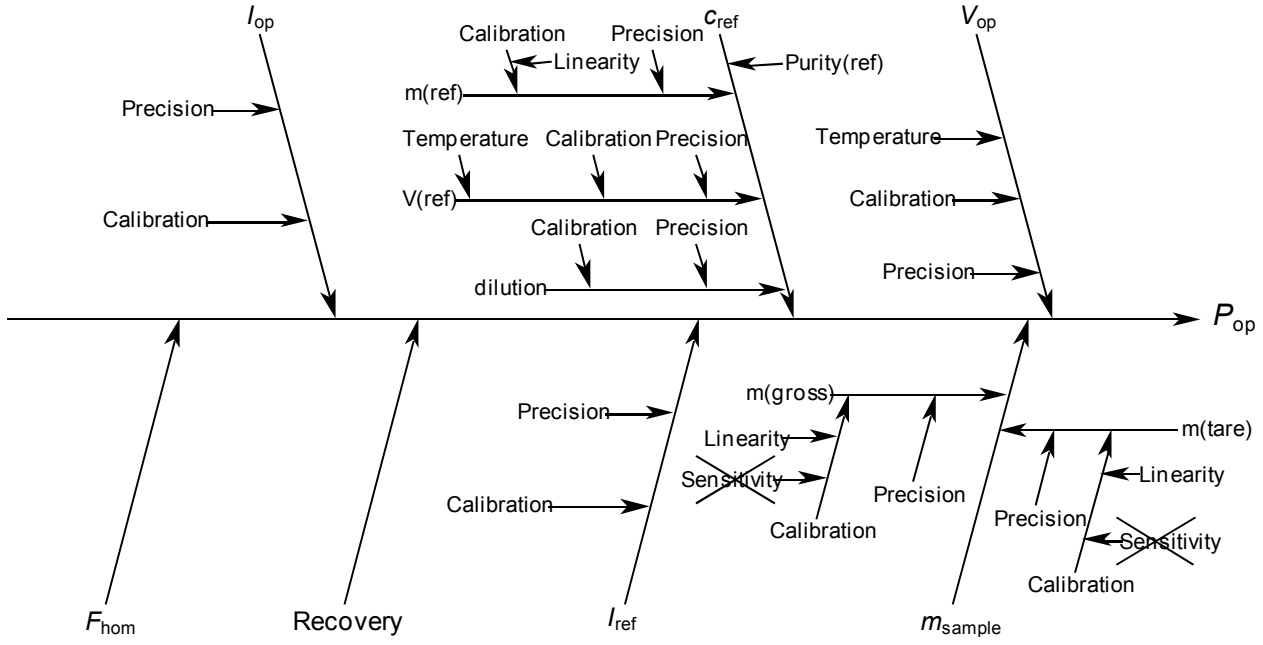
A4.3 Step 2: Identifying and analysing uncertainty sources

The identification of all relevant uncertainty sources for such a complex analytical procedure is best done by drafting a cause and effect diagram. The parameters in the equation of the measurand are represented by the main branches of the diagram. Further factors are added to the diagram, considering each step in the analytical procedure (A4.2), until the contributory factors become sufficiently remote.

The sample inhomogeneity is not a parameter in the original equation of the measurand, but it appears to be a significant effect in the analytical procedure. A new branch, F(hom), representing the sample inhomogeneity is accordingly added to the cause and effect diagram (Figure A4.5).

Finally, the uncertainty branch due to the inhomogeneity of the sample has to be included in the calculation of the measurand. To show the effect of uncertainties arising from that source clearly, it is useful to write

Figure A4.5: Cause and effect diagram with added main branch for sample inhomogeneity



$$P_{\text{op}} = \frac{I_{\text{op}} \cdot c_{\text{ref}} \cdot V_{\text{op}}}{I_{\text{ref}} \cdot Rec \cdot m_{\text{sample}}} \cdot F_{\text{hom}} \qquad [\text{mg kg}^{-1}]$$

where F_{hom} is a correction factor assumed to be unity in the original calculation. This makes it clear that the uncertainties in the correction factor must be included in the estimation of the overall uncertainty. The final expression also shows how the uncertainty will apply.

NOTE: Correction factors: This approach is quite general, and may be very valuable in highlighting hidden assumptions. In principle, every measurement has associated with it such correction factors, which are normally assumed unity. For example, the uncertainty in c_{op} can be expressed as a standard uncertainty for c_{op}, or as the standard uncertainty which represents the uncertainty in a correction factor. In the latter case, the value is identically the uncertainty for c_{op} expressed as a relative standard deviation.

A4.4 Step 3: Quantifying uncertainty components

In accordance with section 7.7., the quantification of the different uncertainty components utilises data from the in-house development and validation studies:

- The best available estimate of the overall run to run variation of the analytical process.
- The best possible estimation of the overall bias (*Rec*) and its uncertainty.
- Quantification of any uncertainties associated with effects incompletely accounted for the overall performance studies.

Some rearrangement the cause and effect diagram is useful to make the relationship and coverage of these input data clearer (Figure A4.6). A new 'Precision' branch is added to represent all the effects covered by the intermediate precision study. This does not include the Purity contribution to c_{ref} as the same pure reference material was used for both measurements in each pair of duplicates.

NOTE: In normal use, samples are run in small batches, each batch including a calibration set, a recovery check sample to control bias and random duplicate to check within-run precision. Corrective action is taken if these checks show significant departures from the performance found during validation. This basic QC fulfils the main requirements for use of the validation data in uncertainty estimation for routine testing.

Having inserted the extra effect 'Precision' into the cause and effect diagram, the implied model for calculating P_{op} becomes

$$P_{\text{op}} = \frac{I_{\text{op}} \cdot c_{\text{ref}} \cdot V_{\text{op}}}{I_{\text{ref}} \cdot Rec \cdot m_{\text{sample}}} \cdot F_{\text{hom}} \cdot F_{\text{I}} \ \text{mg kg}^{-1} \quad \text{Eq. A4.1}$$

where F_{I} is a factor representing the effect of

Figure A4.6: Cause and effect diagram after rearrangement to accommodate the data of the validation study

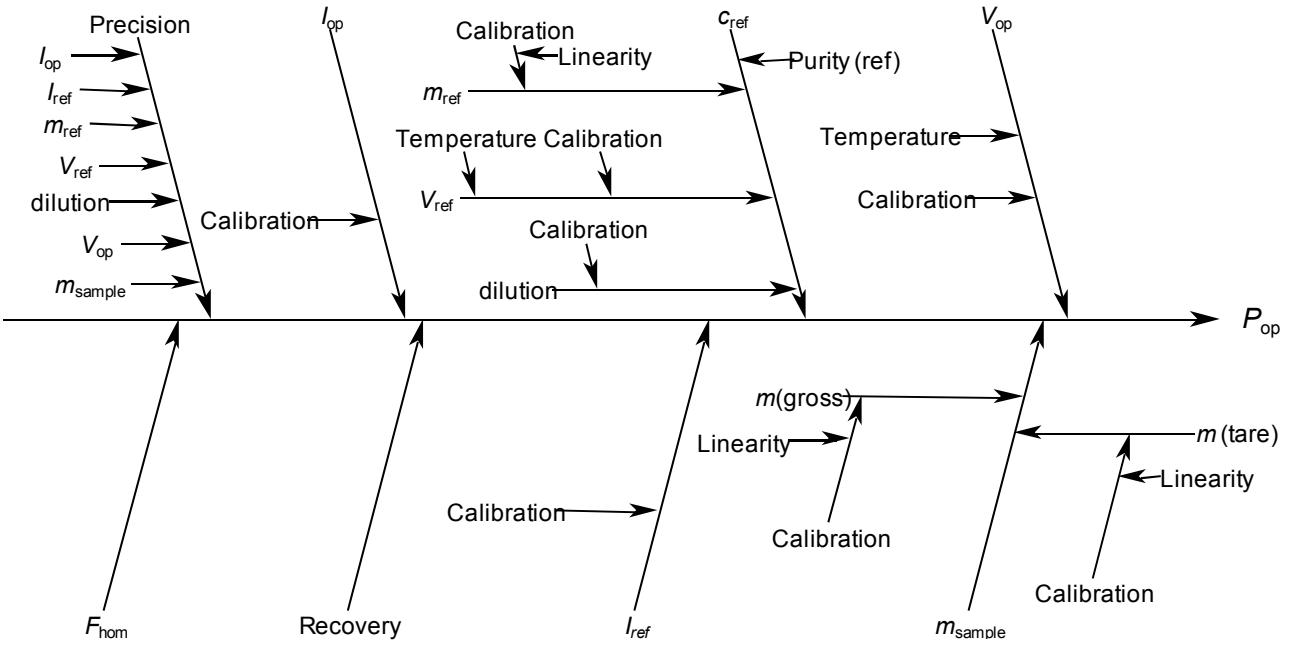


Table A4.2: Results of duplicate pesticide analysis[Note 1]

Residue	D1 [mg kg^{-1}]	D2 [mg kg^{-1}]	Mean [mg kg^{-1}]	Difference D1-D2	Difference/ mean
Malathion	1.30	1.30	1.30	0.00	0.000
Malathion	1.30	0.90	1.10	0.40	0.364
Malathion	0.57	0.53	0.55	0.04	0.073
Malathion	0.16	0.26	0.21	-0.10	-0.476
Malathion	0.65	0.58	0.62	0.07	0.114
Pirimiphos Methyl	0.04	0.04	0.04	0.00	0.000
Chlorpyrifos Methyl	0.08	0.09	0.085	-0.01	-0.118
Pirimiphos Methyl	0.02	0.02	0.02	0.00	0.000
Chlorpyrifos Methyl	0.01	0.02	0.015	-0.01	-0.667
Pirimiphos Methyl	0.02	0.01	0.015	0.01	0.667
Chlorpyrifos Methyl	0.03	0.02	0.025	0.01	0.400
Chlorpyrifos Methyl	0.04	0.06	0.05	-0.02	-0.400
Pirimiphos Methyl	0.07	0.08	0.75	-0.10	-0.133
Chlorpyrifos Methyl	0.01	0.01	0.10	0.00	0.000
Pirimiphos Methyl	0.06	0.03	0.045	0.03	0.667

Note 1: Duplicates were taken over different runs

variation under intermediate precision conditions. That is, the precision is treated as a multiplicative factor F_I like the homogeneity. This form is chosen for convenience in calculation, as will be seen below.

The evaluation of the different effects is now considered.

1. Precision study

The overall run to run variation (precision) of the analytical procedure was performed with a number of duplicate tests (same homogenised sample, complete extraction/determination procedure repeated in two different runs) for typical organophosphorus pesticides found in different bread samples. The results are collected in Table A4.2.

The normalised difference data (the difference divided by the mean) provides a measure of the overall run to run variability (intermediate precision). To obtain the estimated relative standard uncertainty for single determinations, the standard deviation of the normalised differences is taken and divided by $\sqrt{2}$ to correct from a standard deviation for pairwise differences to the standard uncertainty for the single values. This gives a value for the standard uncertainty due to run to run variation of the overall analytical process, including run to run recovery variation but excluding homogeneity effects, of $0.382/\sqrt{2} = 0.27$

NOTE: At first sight, it may seem that duplicate tests provide insufficient degrees of freedom. But it is not the goal to obtain very accurate numbers for the precision of the analytical process for one specific pesticide in one special kind of bread. It is more important in this study to test a wide variety of different materials (different bread types in this case) and analyte levels, giving a representative selection of typical organophosphorus pesticides. This is done in the most efficient way by duplicate tests on many materials, providing (for the precision estimate) approximately one degree of freedom for each material studied in duplicate. This gives a total of 15 degrees of freedom.

2. Bias study

The bias of the analytical procedure was investigated during the in-house validation study using spiked samples (homogenised samples were

Table A4.3: Results of pesticide recovery studies

Substrate	Residue Type	Conc. [mg kg^{-1}]	N[1)]	Mean [2)] [%]	s [2)][%]
Waste Oil	PCB	10.0	8	84	9
Butter	OC	0.65	33	109	12
Compound Animal Feed I	OC	0.325	100	90	9
Animal & Vegetable Fats I	OC	0.33	34	102	24
Brassicas 1987	OC	0.32	32	104	18
Bread	OP	0.13	42	90	28
Rusks	OP	0.13	30	84	27
Meat & Bone Feeds	OC	0.325	8	95	12
Maize Gluten Feeds	OC	0.325	9	92	9
Rape Feed I	OC	0.325	11	89	13
Wheat Feed I	OC	0.325	25	88	9
Soya Feed I	OC	0.325	13	85	19
Barley Feed I	OC	0.325	9	84	22

(1) The number of experiments carried out

(2) The mean and sample standard deviation s are given as percentage recoveries.

split and one portion spiked). Table A4.3 collects the results of a long term study of spiked samples of various types.

The relevant line (marked with grey colour) is the "bread" entry line, which shows a mean recovery for forty-two samples of 90 %, with a standard deviation (s) of 28 %. The standard uncertainty was calculated as the standard deviation of the mean $u(\overline{Rec}) = 0.28/\sqrt{42} = 0.0432$.

A Student's t test is used to determine whether the mean recovery is significantly different from 1.0. The test statistic t is calculated using the following equation

$$t = \frac{\left|1 - \overline{Rec}\right|}{u(\overline{Rec})} = \frac{(1 - 0.9)}{0.0432} = 2.31$$

This value is compared with the 2-tailed critical value t_{crit}, for n–1 degrees of freedom at 95 % confidence (where n is the number of results used to estimate $\overline{Rec}$). If t is greater or equal than the critical value t_{crit} than $\overline{Rec}$ is significantly different from 1.

$$t = 2.31 \geq t_{crit;41} \cong 2.021$$

In this example a correction factor ($1/\overline{Rec}$) is being applied and therefore $\overline{Rec}$ is explicitly included in the calculation of the result.

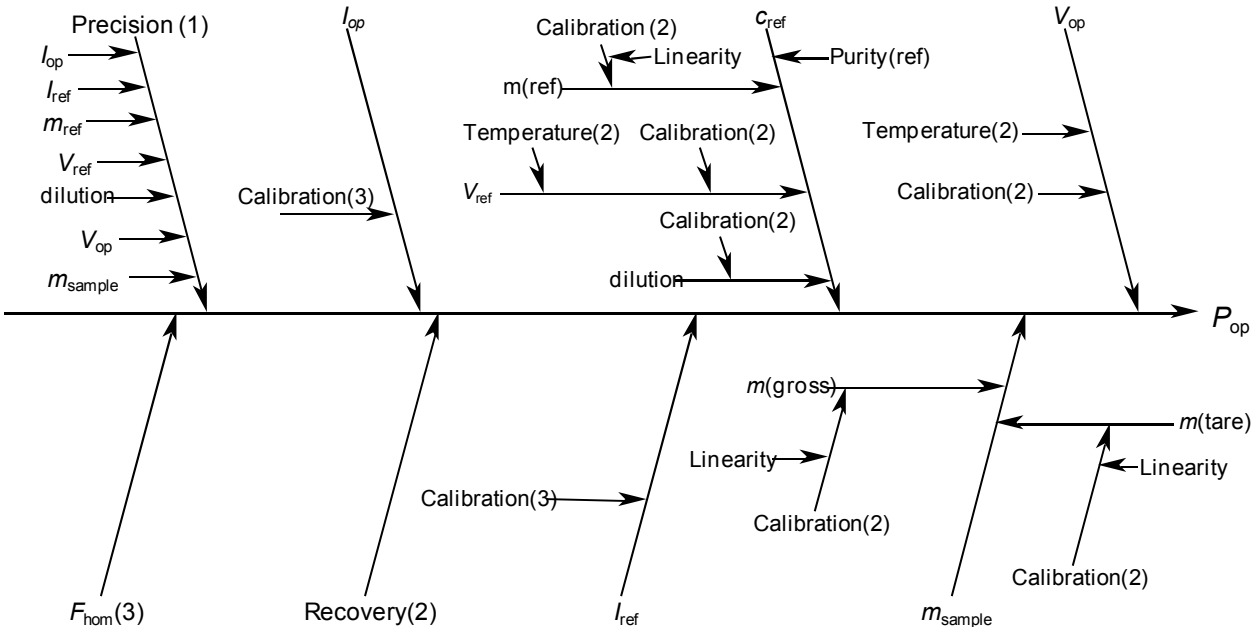
3. Other sources of uncertainty

The cause and effect diagram in Figure A4.7 shows which other sources of uncertainty are (1) adequately covered by the precision data, (2) covered by the recovery data or (3) have to be further examined and eventually considered in the calculation of the measurement uncertainty.

All balances and the important volumetric measuring devices are under regular control. Precision and recovery studies take into account the influence of the calibration of the different volumetric measuring devices because during the investigation various volumetric flasks and pipettes have been used. The extensive variability studies, which lasted for more than half a year, also cover influences of the environmental temperature on the result. This leaves only the reference material purity, possible nonlinearity in GC response (represented by the 'calibration' terms for I_{ref} and I_{op} in the diagram), and the sample homogeneity as additional components requiring study.

The purity of the reference standard is given by the manufacturer as 99.53 % ±0.06 %. The purity

Figure A4.7: Evaluation of other sources of uncertainty



(1) Contribution (F_1 in equation A4.1) included in the relative standard deviation calculated from the intermediate precision study of the analytical procedure.

(2) Considered during the bias study of the analytical procedure.

(3) To be considered during the evaluation of the other sources of uncertainty.

is potential an additional uncertainty source with a standard uncertainty of $0.0006/\sqrt{3} = 0.00035$ (rectangular distribution). But the contribution is so small (compared, for example, to the precision estimate) that it is clearly safe to neglect this contribution.

Linearity of response to the relevant organophosphorus pesticides within the given concentration range is established during validation studies. In addition, with multi-level studies of the kind indicated in Table A4.2 and Table A4.3, nonlinearity would contribute to the observed precision. No additional allowance is required. The in-house validation study has proven that this is not the case.

The homogeneity of the bread sub-sample is the last remaining other uncertainty source. No literature data were available on the distribution of trace organic components in bread products, despite an extensive literature search (at first sight this is surprising, but most food analysts attempt homogenisation rather than evaluate inhomogeneity separately). Nor was it practical to measure homogeneity directly. The contribution has therefore been estimated on the basis of the sampling method used.

To aid the estimation, a number of feasible pesticide residue distribution scenarios were considered, and a simple binomial distribution used to calculate the standard uncertainty for the total included in the analysed sample (see section A4.6). The scenarios, and the calculated relative standard uncertainties in the amount of pesticide in the final sample, were:

- Scenario (a) Residue distributed on the top surface only: 0.58.
- Scenario (b) Residue distributed evenly over the surface only: 0.20.
- Scenario (c) Residue distributed evenly through the sample, but reduced in concentration by evaporative loss or decomposition close to the surface: 0.05-0.10 (depending on the "surface layer" thickness).

Scenario (a) is specifically catered for by proportional sampling or complete homogenisation (see section A4.2, Procedure paragraph i). This would only arise in the case of decorative additions (whole grains) added to one

Table A4.4: Uncertainties in pesticide analysis

Description	Value x	Standard uncertainty $u(x)$	Relative standard uncertainty $u(x)/x$	Remark
Precision (1)	1.0	0.27	0.27	Duplicate tests of different types of samples
Bias (*Rec*) (2)	0.9	0.043	0.048	Spiked samples
Other sources (3) (Homogeneity)	1.0	0.2	0.2	Estimations founded on model assumptions
P_{op}	- -	- -	0.34	Relative standard uncertainty

surface. Scenario (b) is therefore considered the likely worst case. Scenario (c) is considered the most probable, but cannot be readily distinguished from (b). On this basis, the value of 0.20 was chosen.

NOTE: For more details on modelling inhomogeneity see the last section of this example.

A4.5 Step 4: Calculating the combined standard uncertainty

During the in-house validation study of the analytical procedure the intermediate precision, the bias and all other feasible uncertainty sources had been thoroughly investigated. Their values and uncertainties are collected in Table A4.4.

The relative values are combined because the model (equation A4.1) is entirely multiplicative:

$$\frac{u_c(P_{op})}{P_{op}} = \sqrt{0.27^2 + 0.048^2 + 0.2^2} = 0.34$$

$$\Rightarrow u_c(P_{op}) = 0.34 \times P_{op}$$

The spreadsheet for this case (Table A4.5) takes the form shown in Table A4.5. Note that the spreadsheet calculates an absolute value uncertainty (0.377) for a nominal corrected result of 1.1111, giving a value of 0.373/1.1111=0.34.

The relative sizes of the three different contributions can be compared by employing a histogram. Figure A4.8 shows the values $|u(y,x_i)|$ taken from Table A4.5.

The precision is the largest contribution to the measurement uncertainty. Since this component is derived from the overall variability in the method, further experiments would be needed to show where improvements could be made. For example, the uncertainty could be reduced significantly by homogenising the whole loaf before taking a sample.

The expanded uncertainty $U(P_{op})$ is calculated by multiplying the combined standard uncertainty with a coverage factor of 2 to give:

Table A4.5: Uncertainties in pesticide analysis

	A	B	C	D	E
1			Precision	Bias	Homogeneity
2		value	1.0	0.9	1.0
3		uncertainty	0.27	0.043	0.2
4					
5	Precision	1.0	1.27	1.0	1.0
6	Bias	0.9	0.9	0.943	0.9
7	Homogeneity	1.0	1.0	1.0	1.2
8					
9	P_{op}	1.1111	1.4111	1.0604	1.333
10	$u(y, x_i)$		0.30	-0.0507	0.222
11	$u(y)^2$, $u(y, x_i)^2$	0.1420	0.09	0.00257	0.04938
12					
13	$u(P_{op})$	0.377	(0.377/1.111 = 0.34 as a relative standard uncertainty)		

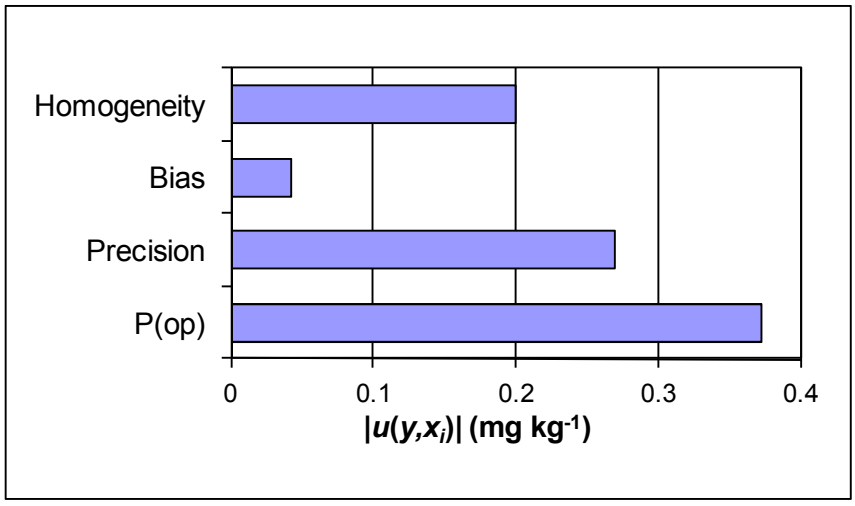
The values of the parameters are entered in the second row from C2 to E2. Their standard uncertainties are in the row below (C3:E3). The spreadsheet copies the values from C2-E2 into the second column from B5 to B7. The result using these values is given in B9 (=B5×B7/B6, based on equation A4.1). C5 shows the precision term from C2 plus its uncertainty given in C3. The result of the calculation using the values C5:C7 is given in C9. The columns D and E follow a similar procedure. The values shown in the row 10 (C10:E10) are the (signed) differences of the row (C9:E9) minus the value given in B9. In row 11 (C11:E11) the values of row 10 (C10:E10) are squared and summed to give the value shown in B11. B13 gives the combined standard uncertainty, which is the square root of B11.

$$U(P_{op}) = 0.34 \times P_{op} \times 2 = 0.68 \times P_{op}$$

A4.6 Special aspect: Modelling inhomogeneity

Assuming that all of the analyte in a sample can be extracted for analysis irrespective of its state, the worst case for inhomogeneity is the situation where some part or parts of a sample contain all

Figure A4.8: Uncertainties in pesticide analysis



The values of $u(y,x_i) = (\partial y/\partial x_i).u(x_i)$ are taken from Table A4.5

of the analyte. A more general, but closely related, case is that in which two levels, say L_1 and L_2 of analyte concentration are present in different parts of the whole sample. The effect of such inhomogeneity in the case of random sub-sampling can be estimated using binomial statistics. The values required are the mean μ and the standard deviation σ of the amount of material in n equal portions selected randomly after separation.

These values are given by

$$\mu = n \cdot (p_1 l_1 + p_2 l_2) \Rightarrow$$

$$\mu = np_1 \cdot (l_1 - l_2) + nl_2 \text{ [1]}$$

$$\sigma^2 = np_1 \cdot (1 - p_1) \cdot (l_1 - l_2)^2 \text{ [2]}$$

where l_1 and l_2 are the amount of substance in portions from regions in the sample containing total fraction L_1 and L_2 respectively, of the total amount X, and p_1 and p_2 are the probabilities of selecting portions from those regions (n must be small compared to the total number of portions from which the selection is made).

The figures shown above were calculated as follows, assuming that a typical sample loaf is approximately $12 \times 12 \times 24$ cm, using a portion size of $2 \times 2 \times 2$ cm (total of 432 portions) and assuming 15 such portions are selected at random and homogenised.

Scenario (a)

The material is confined to a single large face (the top) of the sample. L_2 is therefore zero as is l_2; and L_1=1. Each portion including part of the top surface will contain an amount l_1 of the material. For the dimensions given, clearly one in six (2/12) of the portions meets this criterion, p_1 is therefore 1/6, or 0.167, and l_1 is X/72 (*i.e.* there are 72 "top" portions).

This gives

$$\mu = 15 \times 0.167 \times l_1 = 2.5 l_1$$

$$\sigma^2 = 15 \times 0.167 \times (1 - 0.17) \times l_1^2 = 2.08 l_1^2$$

$$\Rightarrow \sigma = \sqrt{2.08 l_1^2} = 1.44 l_1$$

$$\Rightarrow RSD = \frac{\sigma}{\mu} = 0.58$$

NOTE: To calculate the level X in the entire sample, μ is multiplied back up by 432/15, giving a mean estimate of X of

$$X = \frac{432}{15} \times 2.5 \times l_1 = 72 \times \frac{X}{72} = X$$

This result is typical of random sampling; the expectation value of the mean is exactly the mean value of the population. For random sampling, there is thus no contribution to overall uncertainty other that the run to run variability, expressed as σ or RSD here.

Scenario (b)

The material is distributed evenly over the whole surface. Following similar arguments and assuming that all surface portions contain the same amount l_1 of material, l_2 is again zero, and p_1 is, using the dimensions above, given by

$$p_1 = \frac{(12 \times 12 \times 24) - (8 \times 8 \times 20)}{(12 \times 12 \times 24)} = 0.63$$

i.e. p_1 is that fraction of sample in the "outer" 2 cm. Using the same assumptions then $l_1 = X/272$.

NOTE: The change in value from scenario (a)

This gives:

$$\mu = 15 \times 0.63 \times l_1 = 9.5 l_1$$

$$\sigma^2 = 15 \times 0.63 \times (1 - 0.63) \times l_1^2 = 3.5 l_1^2$$

$$\Rightarrow \sigma = \sqrt{3.5 l_1^2} = 1.87 l_1$$

$$\Rightarrow RSD = \frac{\sigma}{\mu} = 0.2$$

Scenario (c)

The amount of material near the surface is reduced to zero by evaporative or other loss. This case can be examined most simply by considering it as the inverse of scenario (b), with p_1=0.37 and l_1 equal to X/160. This gives

$$\mu = 15 \times 0.37 \times l_1 = 5.6 l_1$$

$$\sigma^2 = 15 \times 0.37 \times (1 - 0.37) \times l_1^2 = 3.5 l_1^2$$

$$\Rightarrow \sigma = \sqrt{3.5 \times l_1^2} = 1.87 l_1$$

$$\Rightarrow RSD = \frac{\sigma}{\mu} = 0.33$$

However, if the loss extends to a depth less than the size of the portion removed, as would be expected, each portion contains some material l_1 and l_2 would therefore both be non-zero. Taking the case where all outer portions contain 50 %

"centre" and 50 % "outer" parts of the sample

$$l_1 = 2 \times l_2 \Rightarrow l_1 = X/296$$

$$\mu = 15 \times 0.37 \times (l_1 - l_2) + 15 \times l_2$$
$$= 15 \times 0.37 \times l_2 + 15 \times l_2 = 20.6\, l_2$$

$$\sigma^2 = 15 \times 0.37 \times (1 - 0.37) \times (l_1 - l_2)^2 = 3.5\, l_2^2$$

giving an *RSD* of $1.87/20.6 = 0.09$

In the current model, this corresponds to a depth of 1 cm through which material is lost. Examination of typical bread samples shows crust thickness typically of 1 cm or less, and taking this to be the depth to which the material of interest is lost (crust formation itself inhibits lost below this depth), it follows that realistic variants on scenario (c) will give values of σ/μ not above 0.09.

NOTE: In this case, the reduction in uncertainty arises because the inhomogeneity is on a smaller scale than the portion taken for homogenisation. In general, this will lead to a reduced contribution to uncertainty. It follows that no additional modelling need be done for cases where larger numbers of small inclusions (such as grains incorporated in the bulk of a loaf) contain disproportionate amounts of the material of interest. Provided that the probability of such an inclusion being incorporated into the portions taken for homogenisation is large enough, the contribution to uncertainty will not exceed any already calculated in the scenarios above.

Example A5: Determination of Cadmium Release from Ceramic Ware by Atomic Absorption Spectrometry

Summary

Goal

The amount of cadmium released from ceramic ware is determined using atomic absorption spectrometry. The procedure employed is the operationally defined standard method BS 6748, implementing Council Directive 84/500/EEC.

Measurement procedure

The different stages in determining the amount of cadmium released from ceramic ware are given in the flow chart (Figure A5.1).

Measurand:

The measurand is the mass of cadmium released per unit area according to BS 6748 and calculated for a particular test item from

$$r = \frac{c_0 \cdot V_L}{a_V} \cdot d \cdot f_{acid} \cdot f_{time} \cdot f_{temp} \qquad \text{mg dm}^{-2}$$

The variables are described in Table A5.1.

Identification of the uncertainty sources:

The relevant uncertainty sources are shown in the cause and effect diagram at Figure A5.2.

Quantification of the uncertainty sources:

The sizes of the different contributions are given in Table A5.1 and shown diagrammatically in Figure A5.2

Figure A5.1: Extractable metal procedure

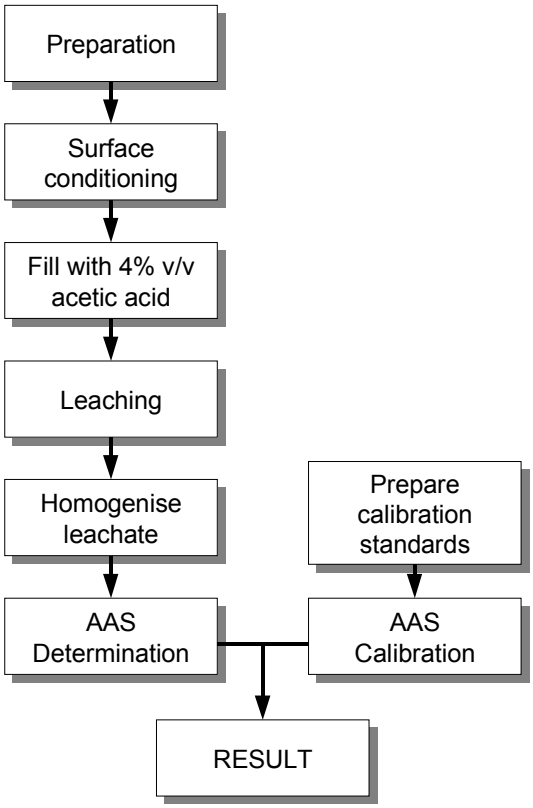


Table A5.1: Uncertainties in extractable cadmium determination

	Description	Value x	Standard uncertainty $u(x)$	Relative standard uncertainty $u(x)/x$
c_0	Content of cadmium in the extraction solution	0.26 mg L^{-1}	0.018 mg L^{-1}	0.069
d	Dilution factor (if used)	1.0 Note 1	0 Note 1	0 Note 1
V_L	Volume of the leachate	0.332 L	0.0018 L	0.0054
a_V	Surface area of the liquid	5.73 dm^2	0.19 dm^2	0.033
f_{acid}	Influence of the acid concentration	1.0	0.0008	0.0008
f_{time}	Influence of the duration	1.0	0.001	0.001
f_{temp}	Influence of temperature	1.0	0.06	0.06
r	Mass of cadmium leached per unit area	0.015 mg dm^{-2}	0.0014 mg dm^{-2}	0.092

Note 1: No dilution was applied in the present example; d is accordingly exactly 1.0

Figure A5.2: Uncertainty sources in leachable cadmium determination

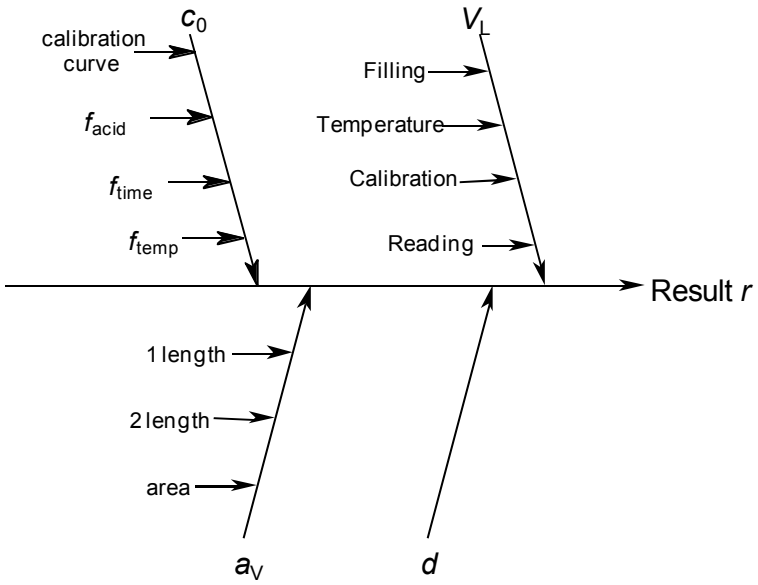
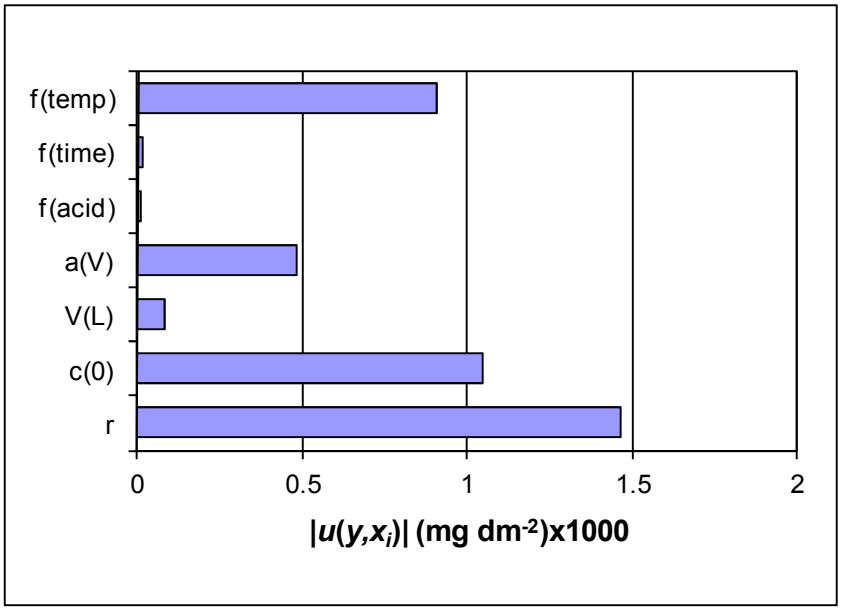


Figure A5.3: Uncertainties in leachable Cd determination



The values of $u(y,x_i) = (\partial y/\partial x_i).u(x_i)$ are taken from Table A5.4

Example A5: Determination of cadmium release from ceramic ware by atomic absorption spectrometry. Detailed discussion.

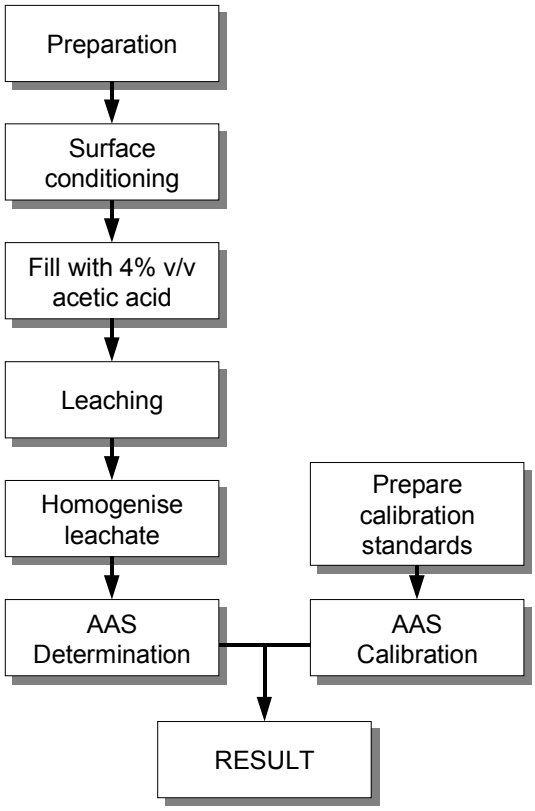
A5.1 Introduction

This example demonstrates the uncertainty evaluation of an operationally defined ('empirical') method; in this case, the determination of metal release from a 'category 1' item of ceramic ware according to BS 6748, which follows Council Directive 84/500/EEC. The test is used to determine by atomic absorption spectroscopy (AAS) the amount of lead or cadmium leached from the surface of ceramic ware by a 4 % (*v*/*v*) aqueous solution of acetic acid. The results obtained with this analytical method are only expected to be compared with other results obtained by the same method.

A5.2 Step 1: Specification

The complete procedure is given in British Standard BS 6748:1986 "Limits of metal release from ceramic ware, glass ware, glass ceramic ware and vitreous enamel ware" and this forms the specification for the measurand. Only a general description is given here.

Figure A5.4: Extractable metal procedure



A5.2.1 Apparatus and Reagent specifications

The reagent specifications affecting the uncertainty study are:

- A freshly prepared solution of 4 % *v*/*v* glacial acetic acid in water, made up by dilution of 40 mL glacial acetic to 1 L.
- A (1000 ±1) mg L^{-1} standard lead solution in 4 % (*v*/*v*) acetic acid.
- A (500 ±0.5) mg L^{-1} standard cadmium solution in 4 % (*v*/*v*) acetic acid.

Laboratory glassware is required to be of at least class B and incapable of releasing detectable levels of lead or cadmium in 4 % *v*/*v* acetic acid during the test procedure. The atomic absorption spectrophotometer is required to have detection limits of at most 0.2 mg L^{-1} for lead and 0.02 mg L^{-1} for cadmium.

A5.2.2 Procedure

The general procedure is illustrated schematically in Figure A5.4. The specifications affecting the uncertainty estimation are:

i) The sample is conditioned to (22±2) °C. Where appropriate ('category 1' articles, as in this example), the surface area is determined. For this example, a surface area of 5.73 dm^2 was obtained (Table A5.1 and Table A5.3 include the experimental values for the example).

ii) The conditioned sample is filled with 4 % *v*/*v* acid solution at (22±2) °C to within 1 mm from the overflow point, measured from the upper rim of the sample, or to within 6 mm from the extreme edge of a sample with a flat or sloping rim.

iii) The quantity of 4 % *v*/*v* acetic acid required or used is recorded to an accuracy of ±2 % (in this example, 332 mL acetic acid was used).

iv) The sample is allowed to stand at (22 ±2) °C for 24 hours (in darkness if cadmium is determined) with due precaution to prevent evaporation loss.

v) After standing, the solution is stirred sufficiently for homogenisation, and a test portion removed, diluted by a factor *d* if necessary, and analysed by AA, using

appropriate wavelengths and, in this example, a least squares calibration curve.

vi) The result is calculated (see below) and reported as the amount of lead and/or cadmium in the total volume of the extracting solution, expressed in milligrams of lead or cadmium per square decimetre of surface area for category 1 articles or milligrams of lead or cadmium per litre of the volume for category 2 and 3 articles.

NOTE: Complete copies of BS 6748:1986 can be obtained by post from BSI customer services, 389 Chiswick High Road, London W4 4AL England ☎ +44 (0) 208 996 9001

A5.3 Step 2: Identity and analysing uncertainty sources

Step 1 describes an 'empirical method'. If such a method is used within its defined field of application, the bias of the method is defined as zero. Therefore bias estimation relates to the laboratory performance and not to the bias intrinsic to the method. Because no reference material certified for this standardised method is available, overall control of bias is related to the control of method parameters influencing the result. Such influence quantities are time, temperature, mass and volumes, *etc*.

The concentration c_0 of lead or cadmium in the acetic acid after dilution is determined by atomic absorption spectrometry and calculated using

$$c_0 = \frac{(A_0 - B_0)}{B_1} \qquad \text{mg l}^{-1}$$

where

c_0 concentration of lead or cadmium in the extraction solution [mg L^{-1}]

A_0 absorbance of the metal in the sample extract

B_0 intercept of the calibration curve

B_1 slope of the calibration curve

For category 1 vessels the empirical method calls for the result to be expressed as mass r of lead or cadmium leached per unit area. r is given by

$$r = \frac{c_0 \cdot V_L}{a_V} \cdot d = \frac{V_L \cdot (A_0 - B_0)}{a_V \cdot B_1} \cdot d \quad \text{mg dm}^{-2}$$

where the additional parameters are

r mass of Cd or Pb leached per unit area [mg dm^{-2}]

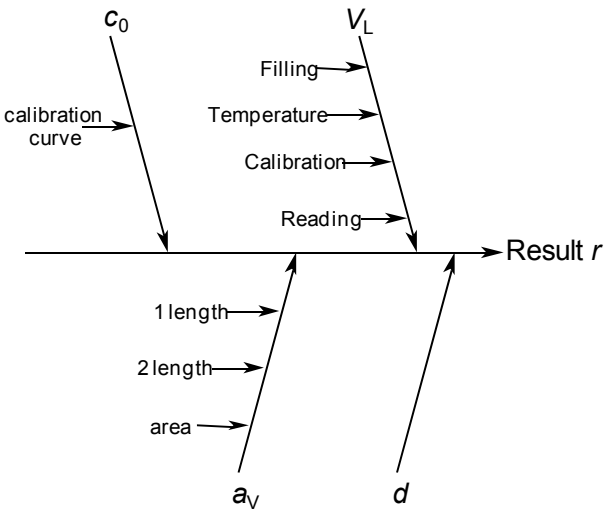
V_L the volume of the leachate [L]

a_V the surface area of the liquid meniscus [dm^2]

d factor by which the sample was diluted

The first part of the above equation of the measurand is used to draft the basic cause and effect diagram (Figure A5.5).

Figure A5.5:Initial cause and effect diagram



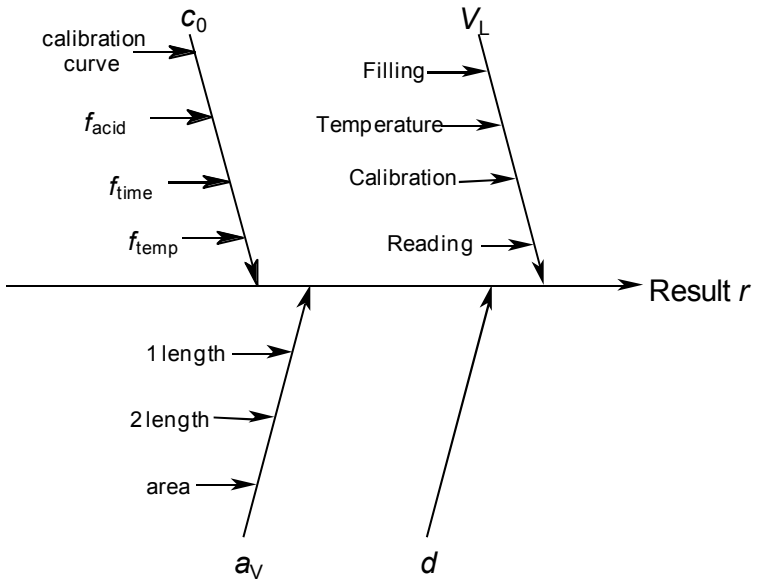
There is no reference material certified for this empirical method with which to assess the laboratory performance. All the feasible influence quantities, such as temperature, time of the leaching process and acid concentration therefore have to be considered. To accommodate the additional influence quantities the equation is expanded by the respective correction factors leading to

$$r = \frac{c_0 \cdot V_L}{a_V} \cdot d \cdot f_{acid} \cdot f_{time} \cdot f_{temp}$$

These additional factors are also included in the revised cause and effect diagram (Figure A5.6). They are shown there as effects on c_0.

NOTE: The latitude in temperature permitted by the standard is a case of an uncertainty arising as a result of incomplete specification of the measurand. Taking the effect of temperature into account allows estimation of the range of results which could be reported whilst complying with the empirical method as well as is practically possible. Note particularly that variations in the result caused by different operating temperatures within the range cannot reasonably be described as bias as they represent results obtained in accordance with the specification.

Figure A5.6:Cause and effect diagram with added hidden assumptions (correction factors)



A5.4 Step 3: Quantifying uncertainty sources

The aim of this step is to quantify the uncertainty arising from each of the previously identified sources. This can be done either by using experimental data or from well based assumptions.

Dilution factor d

For the current example, no dilution of the leaching solution is necessary, therefore no uncertainty contribution has to be accounted for.

Volume V_L

Filling: The empirical method requires the vessel to be filled 'to within 1 mm from the brim' or, for a shallow article with sloping rim, within 6 mm from the edge. For a typical, approximately cylindrical, drinking or kitchen utensil, 1 mm will represent about 1 % of the height of the vessel. The vessel will therefore be 99.5 ±0.5 % filled (i.e. V_L will be approximately 0.995 ±0.005 of the vessel's volume).

Temperature: The temperature of the acetic acid has to be 22 ±2ºC. This temperature range leads to an uncertainty in the determined volume, due to a considerable larger volume expansion of the liquid compared with the vessel. The standard uncertainty of a volume of 332 mL, assuming a rectangular temperature distribution, is

$$\frac{2.1\times10^{-4}\times332\times2}{\sqrt{3}} = 0.08\,\text{mL}$$

Reading: The volume V_L used is to be recorded to within 2 %, in practice, use of a measuring cylinder allows an inaccuracy of about 1 % (i.e. $0.01V_L$). The standard uncertainty is calculated assuming a triangular distribution.

Calibration: The volume is calibrated according to the manufacturer's specification within the range of ±2.5 mL for a 500 mL measuring cylinder. The standard uncertainty is obtained assuming a triangular distribution.

For this example a volume of 332 mL is found and the four uncertainty components are combined accordingly

$$u(V_L) = \sqrt{\left(\frac{0.005\times332}{\sqrt{6}}\right)^2 + (0.08)^2 + \left(\frac{0.01\times332}{\sqrt{6}}\right)^2 + \left(\frac{2.5}{\sqrt{6}}\right)^2}$$
$$= 1.83\,\text{mL}$$

Cadmium concentration c_0

The amount of leached cadmium is calculated using a manually prepared calibration curve. For this purpose five calibration standards, with a concentration 0.1 mg L^{-1}, 0.3 mg L^{-1}, 0.5 mg L^{-1}, 0.7 mg L^{-1} and 0.9 mg L^{-1}, were prepared from a 500 ±0.5 mg L^{-1} cadmium reference standard. The linear least squares fitting procedure used assumes that the uncertainties of the values of the abscissa are considerably smaller than the uncertainty on the values of the ordinate.

Therefore the usual uncertainty calculation procedures for c_0 only reflect the uncertainty due to random variation in the absorbance and not the uncertainty of the calibration standards, nor the inevitable correlations induced by successive dilution from the same stock. Appendix E.3 provides guidance on treating uncertainties in the reference values if required; in this case, however, the uncertainty of the calibration standards is sufficiently small to be neglected.

The five calibration standards were measured three times each, providing the results in Table A5.2. The calibration curve is given by

$$A_i = c_i \cdot B_1 + B_0 + e_i$$

where

A_j i^{th} measurement of absorbance

c_i concentration of the calibration standard corresponding to the i^{th} absorbance measurement

B_1 slope

B_0 intercept

e_i the residual error

and the results of the linear least square fit are

	Value	Standard deviation
B_1	0.2410	0.0050
B_0	0.0087	0.0029

with a correlation coefficient r of 0.997. The fitted line is shown in Figure A5.7. The residual standard deviation S is 0.005486. Although there is evidence of slight curvature, the linear model and residual standard deviation were considered sufficient for the purpose.

Table A5.2: Calibration results

Concentration [mg L^{-1}]	Absorbance (replicates)		
	1	2	3
0.1	0.028	0.029	0.029
0.3	0.084	0.083	0.081
0.5	0.135	0.131	0.133
0.7	0.180	0.181	0.183
0.9	0.215	0.230	0.216

The actual leach solution was measured twice, leading to a concentration c_0 of 0.26 mg L^{-1}. The calculation of the uncertainty $u(c_0)$ associated with the linear least square fitting procedure is described in detail in Appendix E.4. Therefore only a short description of the different calculation steps is given here.

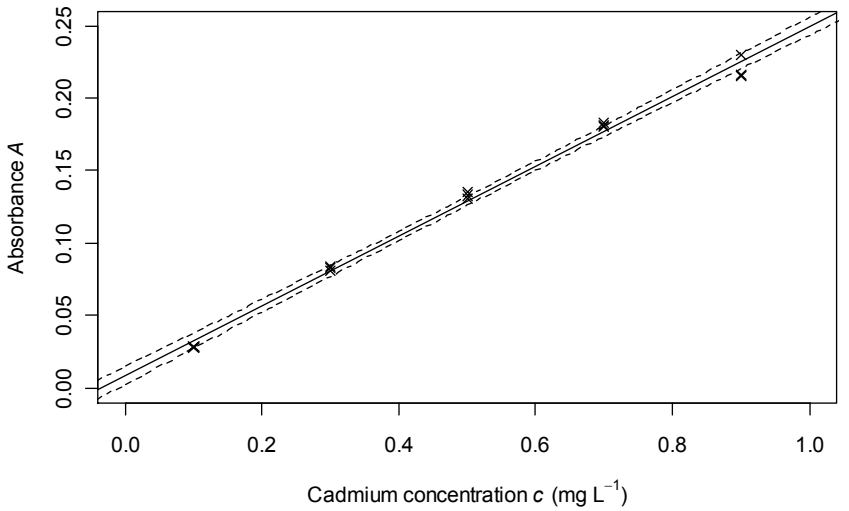
$u(c_0)$ is given by

$$u(c_0) = \frac{S}{B_1}\sqrt{\frac{1}{p} + \frac{1}{n} + \frac{(c_0 - \bar{c})^2}{S_{xx}}}$$

$$= \frac{0.005486}{0.241}\sqrt{\frac{1}{2} + \frac{1}{15} + \frac{(0.26 - 0.5)^2}{1.2}}$$

$$\Rightarrow u(c_0) = 0.018 \text{ mg L}^{-1}$$

Figure A5.7:Linear least square fit and uncertainty interval for duplicate determinations



The dashed lines show the 95 % confidence interval for the line.

with the residual standard deviation S given by

$$S = \sqrt{\frac{\sum_{j=1}^{n}[A_j - (B_0 + B_1 \cdot c_j)]^2}{n-2}} = 0.005486$$

(which has units of mg L^{-1}) and

$$S_{xx} = \sum_{j=1}^{n}(c_j - \bar{c})^2 = 1.2$$

(which has units of $(\text{mg L}^{-1})^2$) where

B_1	slope
p	number of measurements to determine c_0
n	number of measurements for the calibration
c_0	determined cadmium concentration of the leached solution
$\bar{c}$	mean value of the different calibration standards (n number of measurements)
i	index for the number of calibration standards
j	index for the number of measurements to obtain the calibration curve

<u>*Area* a_V</u>

Length measurement: Following Directive 84/500/EEC, the surface area of a category 1 item is taken as the area of the liquid meniscus formed when filled as directed above. The total surface area a_V for the sample vessel was calculated, from the measured diameter d=2.70 dm, to be $a_V = \pi d^2/4 = 3.142\times(2.77/2)^2 = 5.73$ dm^2. Since the item is approximately circular but not perfectly regular, measurements are estimated to be within 2 mm at 95 % confidence. This gives an estimated dimensional measurement uncertainty of 1 mm (0.01 dm) after dividing the 95 % figure by 1.96. The area calculation involves the square of the diameter d, so the combined uncertainty cannot be obtained by applying the simple rules of section 8.2.6. Instead, it is necessary to obtain the standard uncertainty in total area arising from uncertainty in d by applying the method of paragraph 8.2.2. or by using numerical methods. Using Kragten's method (Appendix E.2) gives a standard uncertainty in a_V arising from uncertainty in d of 0.042 dm^2.

Effect of shape on area estimation: Since the item has not a perfect geometric shape, there is also an uncertainty in any area calculation; in this example, this is estimated to contribute an additional 5 % at 95 % confidence, that is, a standard uncertainty in area of 5.73×0.05/1.96 = 0.146 dm^2.

These two uncertainty contributions are combined to give

$$u(a_V) = \sqrt{0.042^2 + 0.146^2} = 0.19 \text{ dm}^2$$

<u>*Temperature effect* f_{temp}</u>

A number of studies of the effect of temperature on metal release from ceramic ware have been undertaken[1-5]. In general, the temperature effect is substantial and a near-exponential increase in metal release with temperature is observed until limiting values are reached. Only one study[1] has given an indication of effects in the range of 20-25°C. From the graphical information presented the change in metal release with temperature near 25°C is approximately linear, with a gradient of approximately 5 % °C^{-1}. For the ±2°C range allowed by the empirical method this leads to a factor f_{temp} of 1±0.1. Converting this to a standard uncertainty gives, assuming a rectangular distribution:

$$u(f_{temp}) = 0.1/\sqrt{3} = 0.06$$

<u>*Time effect* f_{time}</u>

For a relatively slow process such as leaching, the amount leached will be approximately proportional to time for small changes in the time. Krinitz and Franco[1] found a mean change in concentration over the last six hours of leaching of approximately 1.8 mg L^{-1} in 86 mg L^{-1}, that is, about 0.3 %/h. For a time of (24±0.5)h c_0 will therefore need correction by a factor f_{time} of 1±(0.5×0.003) =1±0.0015. This is a rectangular distribution leading to the standard uncertainty

$$u(f_{time}) = 0.0015/\sqrt{3} \cong 0.001.$$

<u>*Acid concentration* f_{acid}</u>

One study of the effect of acid concentration on lead release showed that changing concentration from 4 to 5 % *v*/*v* increased the lead released from a particular ceramic batch from 92.9 to 101.9 mg L^{-1}, i.e. a change in f_{acid} of $(101.9 - 92.9)/92.9 = 0.097$ or close to 0.1. Another study, using a hot leach method, showed a comparable change (50 % change in lead extracted on a change of from 2 to 6 % *v*/*v*)[3]. Assuming this effect as approximately linear with acid concentration gives an estimated change in f_{acid} of approximately 0.1 per % *v*/*v* change in acid concentration. In a separate experiment the

concentration and its standard uncertainty have been established using titration with a standardised NaOH titre (3.996 % *v*/*v* u = 0.008 % *v*/*v*). Taking the uncertainty of 0.008 % *v*/*v* on the acid concentration suggests an uncertainty for f_{acid} of 0.008×0.1 = 0.0008. As the uncertainty on the acid concentration is already expressed as a standard uncertainty, this value can be used directly as the uncertainty associated with f_{acid}.

NOTE: In principle, the uncertainty value would need correcting for the assumption that the single study above is sufficiently representative of all ceramics. The present value does, however, give a reasonable estimate of the magnitude of the uncertainty.

A5.5 Step 4: Calculating the combined standard uncertainty

The amount of leached cadmium per unit area, assuming no dilution, is given by

$$r = \frac{c_0 \cdot V_L}{a_V} \cdot f_{acid} \cdot f_{time} \cdot f_{temp} \qquad \text{mg dm}^{-2}$$

The intermediate values and their standard uncertainties are collected in Table A5.3. Employing those values

$$r = \frac{0.26 \times 0.332}{5.73} \times 1.0 \times 1.0 \times 1.0 = 0.015 \text{ mg dm}^{-2}$$

In order to calculate the combined standard uncertainty of a multiplicative expression (as above) the standard uncertainties of each component are used as follows:

$$\frac{u_c(r)}{r} = \sqrt{\left(\frac{u(c_0)}{c_0}\right)^2 + \left(\frac{u(V_L)}{V_L}\right)^2 + \left(\frac{u(a_V)}{a_V}\right)^2 + \left(\frac{u(f_{acid})}{f_{acid}}\right)^2 + \left(\frac{u(f_{time})}{f_{time}}\right)^2 + \left(\frac{u(f_{temp})}{f_{temp}}\right)^2}$$

$$= \sqrt{0.069^2 + 0.0054^2 + 0.033^2 + 0.0008^2 + 0.001^2 + 0.06^2} = 0.097$$

$$\Rightarrow u_c(r) = 0.097r = 0.0015 \text{ mg dm}^{-2}$$

The simpler spreadsheet approach to calculate the combined standard uncertainty is shown in Table A5.4. A description of the method is given in Appendix E.

The contributions of the different parameters and influence quantities to the measurement uncertainty are illustrated in Figure A5.8, comparing the size of each of the contributions (C13:H13 in Table A5.4) with the combined uncertainty (B16).

The expanded uncertainty $U(r)$ is obtained by applying a coverage factor of 2

$$U_r = 0.0015 \times 2 = 0.003 \text{ mg dm}^{-2}$$

Thus the amount of released cadmium measured according to BS 6748:1986

$$(0.015 \pm 0.003) \text{ mg dm}^{-2}$$

where the stated uncertainty is calculated using a coverage factor of 2.

A5.6 References for Example 5

1. B. Krinitz, V. Franco, J. AOAC **56** 869-875 (1973)
2. B. Krinitz, J. AOAC **61**, 1124-1129 (1978)
3. J. H. Gould, S. W. Butler, K. W. Boyer, E. A. Stelle, J. AOAC **66**, 610-619 (1983)
4. T. D. Seht, S. Sircar, M. Z. Hasan, Bull. Environ. Contam. Toxicol. **10**, 51-56 (1973)
5. J. H. Gould, S. W. Butler, E. A. Steele, J. AOAC **66**, 1112-1116 (1983)

Table A5.3: Intermediate values and uncertainties for leachable cadmium analysis

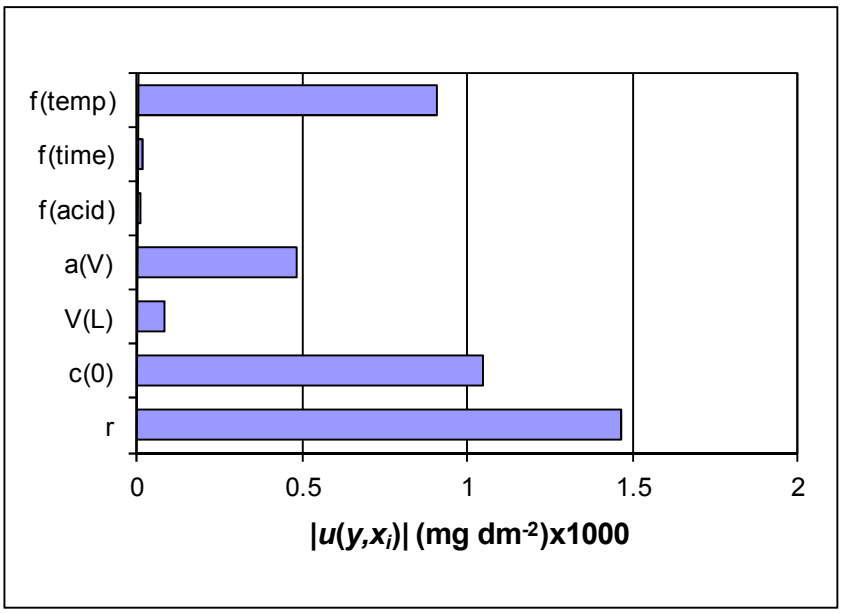
	Description	Value	Standard uncertainty $u(x)$	Relative standard uncertainty $u(x)/x$
c_0	Content of cadmium in the extraction solution	0.26 mg L^{-1}	0.018 mg L^{-1}	0.069
V_L	Volume of the leachate	0.332 L	0.0018 L	0.0054
a_V	Surface area of the liquid	5.73 dm^2	0.19 dm^2	0.033
f_{acid}	Influence of the acid concentration	1.0	0.0008	0.0008
f_{time}	Influence of the duration	1.0	0.001	0.001
f_{temp}	Influence of temperature	1.0	0.06	0.06

Table A5.4: Spreadsheet calculation of uncertainty for leachable cadmium analysis

	A	B	C	D	E	F	G	H
1			c_0	V_L	a_V	f_{acid}	f_{time}	f_{temp}
2		value	0.26	0.332	10.01	1	1	1
3		uncertainty	0.018	0.0018	0.27	0.0008	0.001	0.06
4								
5	c_0	0.26	0.278	0.26	0.26	0.26	0.26	0.26
6	V_L	0.332	0.332	0.3338	0.332	0.332	0.332	0.332
7	a_V	5.73	5.73	5.73	5.92	5.73	5.73	5.73
8	f_{acid}	1	1	1	1	1.0008	1	1
9	f_{time}	1	1	1	1	1	1.001	1
10	f_{temp}	1	1	1	1	1	1	1.06
11								
12	r	0.015065	0.016108	0.015146	0.014581	0.015077	0.015080	0.015968
13	$u(y,x_i)$		0.001043	0.000082	-0.000483	0.000012	0.000015	0.000904
14	$u(y)^2$, $u(y,x_i)^2$	2.15E-06	1.09E-06	6.67E-09	2.34E-07	1.45E-10	2.27E-10	8.17E-07
15								
16	$u_c(r)$	0.001465						

The values of the parameters are entered in the second row from C2 to H2, and their standard uncertainties in the row below (C3:H3). The spreadsheet copies the values from C2:H2 into the second column (B5:B10). The result (r) using these values is given in B12. C5 shows the value of c_0 from C2 plus its uncertainty given in C3. The result of the calculation using the values C5:C10 is given in C12. The columns D and H follow a similar procedure. Row 13 (C13:H13) shows the (signed) differences of the row (C12:H12) minus the value given in B12. In row 14 (C14:H14) the values of row 13 (C13:H13) are squared and summed to give the value shown in B14. B16 gives the combined standard uncertainty, which is the square root of B14.

Figure A5.8: Uncertainties in leachable Cd determination



The values of $u(y,x_i) = (\partial y/\partial x_i).u(x_i)$ are taken from Table A5.4

Example A6: The Determination of Crude Fibre in Animal Feeding Stuffs

Summary

Goal

The determination of crude fibre by a regulatory standard method.

Measurement procedure

The measurement procedure is a standardised procedure involving the general steps outlined in Figure A6.1. These are repeated for a blank sample to obtain a blank correction.

Measurand

The fibre content as a percentage of the sample by weight, C_{fibre}, is given by:

$$C_{\text{fibre}} = \frac{(b-c)\times 100}{a}$$

Where:

- a is the mass (g) of the sample. (Approximately 1 g)
- b is the loss of mass (g) after ashing during the determination;
- c is the loss of mass (g) after ashing during the blank test.

Identification of uncertainty sources

A full cause and effect diagram is provided as Figure A6.9.

Quantification of uncertainty components

Laboratory experiments showed that the method was performing in house in a manner that fully justified adoption of collaborative study reproducibility data. No other contributions were significant in general. At low levels it was necessary to add an allowance for the specific drying procedure used. Typical resulting uncertainty estimates are tabulated below (as standard uncertainties) (Table A6.1).

Figure A6.1: Fibre determination.

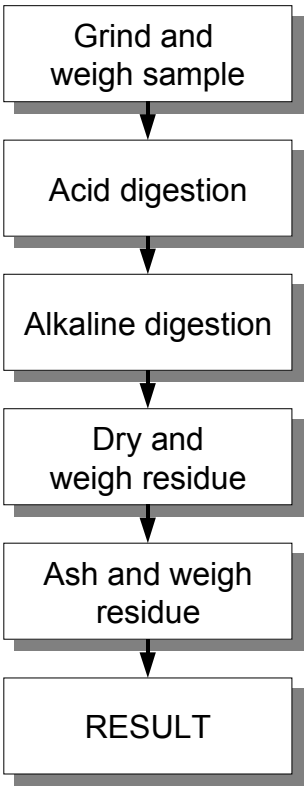


Table A6.1: Combined standard uncertainties

Fibre content (% m/m)	Standard uncertainty $u_c(C_{\text{fibre}})$ (% m/m)	Relative Standard uncertainty $u_c(C_{\text{fibre}}) / C_{\text{fibre}}$
2.5	$\sqrt{0.29^2 + 0.115^2} = 0.31$	0.12
5	0.4	0.08
10	0.6	0.06

Example A6: The determination of crude fibre in animal feeding stuffs. Detailed discussion

A6.1 Introduction

Crude fibre is defined in the method scope as the amount of fat-free organic substances which are insoluble in acid and alkaline media. The procedure is standardised and its results used directly. Changes in the procedure change the measurand; this is accordingly an example of an operationally defined (empirical) method.

Collaborative trial data (repeatability and reproducibility) were available for this statutory method. The precision experiments described were planned as part of the in-house evaluation of the method performance. There is no suitable reference material (i.e. certified by the same method) available for this method.

A6.2 Step 1: Specification

The specification of the measurand for more extensive analytical methods is best done by a comprehensive description of the different stages of the analytical method and by providing the equation of the measurand.

Procedure

The procedure, a complex digestion, filtration, drying, ashing and weighing procedure, which is also repeated for a blank crucible, is summarised in Figure A6.2. The aim is to digest most components, leaving behind all the undigested material. The organic material is ashed, leaving an inorganic residue. The difference between the dry organic/inorganic residue weight and the ashed residue weight is the "fibre content". The main stages are:

i) Grind the sample to pass through a 1mm sieve
ii) Weigh 1g of the sample into a weighed crucible
iii) Add a set of acid digestion reagents at stated concentrations and volumes. Boil for a stated, standardised time, filter and wash the residue.
iv) Add standard alkali digestion reagents and boil for the required time, filter, wash and rinse with acetone.
v) Dry to constant weight at a standardised temperature ("constant weight" is not defined within the published method; nor are other drying conditions such as air circulation or dispersion of the residue).
vi) Record the dry residue weight.
vii) Ash at a stated temperature to "constant weight" (in practice realised by ashing for a set time decided after in house studies).
viii) Weigh the ashed residue and calculate the fibre content by difference, after subtracting the residue weight found for the blank crucible.

Measurand

The fibre content as a percentage of the sample by weight, C_{fibre}, is given by:

$$C_{\text{fibre}} = \frac{(b-c)\times 100}{a}$$

Where:

a is the mass (g) of the sample. Approximately 1 g of sample is taken for analysis.

b is the loss of mass (g) after ashing during the determination.

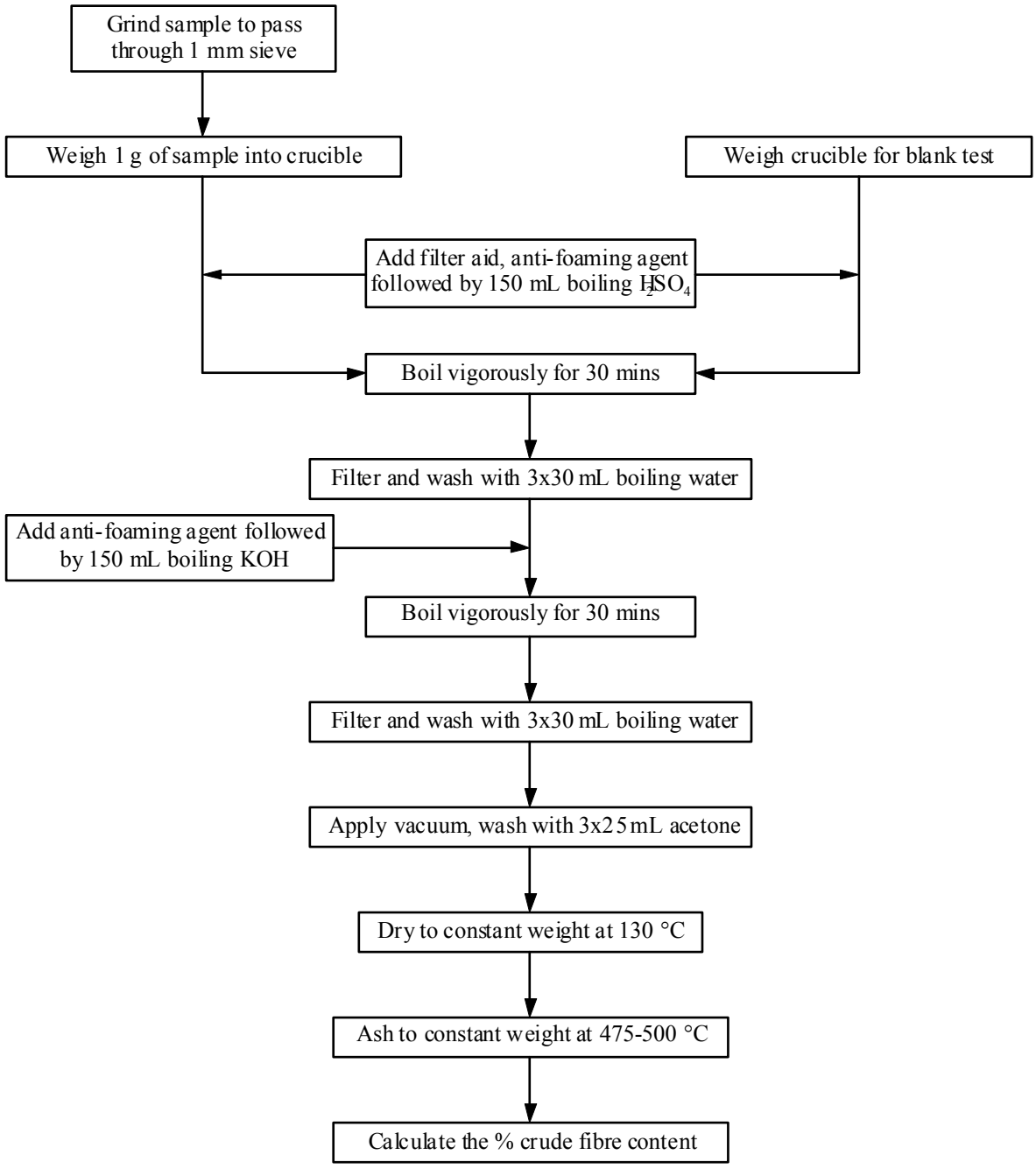
c is the loss of mass (g) after ashing during the blank test.

A6.3 Step 2: Identifying and analysing uncertainty sources

A range of sources of uncertainty was identified. These are shown in the cause and effect diagram for the method (see Figure A6.9). This diagram was simplified to remove duplication following the procedures in Appendix D; this, together with removal of insignificant components (particularly the balance calibration and linearity), leads to the simplified cause and effect diagram in Figure A6.10.

Since prior collaborative and in-house study data were available for the method, the use of these data is closely related to the evaluation of different contributions to uncertainty and is accordingly discussed further below.

Figure A6.2: Flow diagram illustrating the stages in the regulatory method for the determination of fibre in animal feeding stuffs



A6.4 Step 3: Quantifying uncertainty components

Collaborative trial results

The method has been the subject of a collaborative trial. Five different feeding stuffs representing typical fibre and fat concentrations were analysed in the trial. Participants in the trial carried out all stages of the method, including grinding of the samples. The repeatability and reproducibility estimates obtained from the trial are presented in Table A6.2.

As part of the in-house evaluation of the method, experiments were planned to evaluate the repeatability (within batch precision) for feeding stuffs with fibre concentrations similar to those of the samples analysed in the collaborative trial. The results are summarised in Table A6.2. Each estimate of in-house repeatability is based on 5 replicates.

Table A6.2: Summary of results from collaborative trial of the method and in-house repeatability check

Sample	Fibre content (% m/m)			
	Collaborative trial results			In-house repeatability standard deviation
	Mean	Reproducibility standard deviation (s_R)	Repeatability standard deviation (s_r)	
A	2.3	0.293	0.198	0.193
B	12.1	0.563	0.358	0.312
C	5.4	0.390	0.264	0.259
D	3.4	0.347	0.232	0.213
E	10.1	0.575	0.391	0.327

The estimates of repeatability obtained in-house were comparable to those obtained from the collaborative trial. This indicates that the method precision in this particular laboratory is similar to that of the laboratories which took part in the collaborative trial. It is therefore acceptable to use the reproducibility standard deviation from the collaborative trial in the uncertainty budget for the method. To complete the uncertainty budget we need to consider whether there are any other effects not covered by the collaborative trial which need to be addressed. The collaborative trial covered different sample matrices and the pre-treatment of samples, as the participants were supplied with samples which required grinding prior to analysis. The uncertainties associated with matrix effects and sample pre-treatment do not therefore require any additional consideration. Other parameters which affect the result relate to the extraction and drying conditions used in the method. Although the reproducibility standard deviation will normally include the effect of variation in these parameters, they were investigated separately to ensure the laboratory bias was under control (i.e., small compared to the reproducibility standard deviation). The parameters considered are discussed below.

Loss of mass on ashing

As there is no appropriate reference material for this method, in-house bias has to be assessed by considering the uncertainties associated with individual stages of the method. Several factors will contribute to the uncertainty associated with the loss of mass after ashing:

- acid concentration;
- alkali concentration;
- acid digestion time;
- alkali digestion time;
- drying temperature and time;
- ashing temperature and time.

Reagent concentrations and digestion times

The effects of acid concentration, alkali concentration, acid digestion time and alkali digestion time have been studied in previously published papers. In these studies, the effect of changes in the parameter on the result of the analysis was evaluated. For each parameter the sensitivity coefficient (*i.e.,* the rate of change in the final result with changes in the parameter) and the uncertainty in the parameter were calculated.

The uncertainties given in Table A6.3 are small compared to the reproducibility figures presented in Table A6.2. For example, the reproducibility standard deviation for a sample containing 2.3 % m/m fibre is 0.293 % m/m. The uncertainty associated with variations in the acid digestion time is estimated as 0.021 % m/m (i.e., 2.3×0.009). We can therefore safely neglect the uncertainties associated with variations in these method parameters.

Drying temperature and time

No prior data were available. The method states that the sample should be dried at 130 °C to

Table A6.3: Uncertainties associated with method parameters

Parameter	Sensitivity coefficient[Note 1]	Uncertainty in parameter	Uncertainty in final result as RSD [Note 4]
acid concentration	0.23 $(\text{mol L}^{-1})^{-1}$	0.0013 mol L^{-1} [Note 2]	0.00030
alkali concentration	0.21 $(\text{mol L}^{-1})^{-1}$	0.0023 mol L^{-1} [Note 2]	0.00048
acid digestion time	0.0031 min^{-1}	2.89 mins [Note 3]	0.0090
alkali digestion time	0.0025 min^{-1}	2.89 mins [Note 3]	0.0072

Note 1. The sensitivity coefficients were estimated by plotting the normalised change in fibre content against reagent strength or digestion time. Linear regression was then used to calculate the rate of change of the result of the analysis with changes in the parameter.

Note 2. The standard uncertainties in the concentrations of the acid and alkali solutions were calculated from estimates of the precision and trueness of the volumetric glassware used in their preparation, temperature effects etc. See examples A1-A3 for further examples of calculating uncertainties for the concentrations of solutions.

Note 3. The method specifies a digestion time of 30 minutes. The digestion time is controlled to within ±5 minutes. This is a rectangular distribution which is converted to a standard uncertainty by dividing by √3.

Note 4. The uncertainty in the final result, as a relative standard deviation, is calculated by multiplying the sensitivity coefficient by the uncertainty in the parameter.

"constant weight". In this case the sample is dried for 3 hours at 130 °C and then weighed. It is then dried for a further hour and re-weighed. Constant weight is defined in this laboratory as a change of less than 2 mg between successive weighings. In an in-house study, replicate samples of four feeding stuffs were dried at 110, 130 and 150 °C and weighed after 3 and 4 hours drying time. In the majority of cases, the weight change between 3 and 4 hours at each drying temperature was less than 2 mg. This was therefore taken as the worst case estimate of the uncertainty in the weight change on drying. The range ±2 mg describes a rectangular distribution, which is converted to a standard uncertainty by dividing by √3. The uncertainty in the weight recorded after drying to constant weight is therefore 0.00115 g. The method specifies a sample weight of 1 g. For a 1 g sample, the uncertainty in drying to constant weight corresponds to a standard uncertainty of 0.115 % m/m in the fibre content. This source of uncertainty is independent of the fibre content of the sample. There will therefore be a fixed contribution of 0.115 % m/m to the uncertainty budget for each sample, regardless of the concentration of fibre in the sample. At all fibre concentrations, this uncertainty is smaller than the reproducibility standard deviation, and for all but the lowest fibre concentrations is less than 1/3 of the s_R value. Again, this source of uncertainty can usually be neglected. However for low fibre concentrations, this uncertainty is more than 1/3 of the s_R value so an additional term should be included in the uncertainty budget (see Table A6.4).

Ashing temperature and time

The method requires the sample to be ashed at 475 to 500 °C for at least 30 mins. A published study on the effect of ashing conditions involved determining fibre content at a number of different ashing temperature/time combinations, ranging from 450 °C for 30 minutes to 650 °C for 3 hours. No significant difference was observed between the fibre contents obtained under the different conditions. The effect on the final result of small variations in ashing temperature and time can therefore be assumed to be negligible.

Loss of mass after blank ashing

No experimental data were available for this parameter. However, the uncertainties arise primarily from weighing; the effects of variations in this parameter are therefore likely to be small and well represented in the collaborative study.

A6.5 Step 4: Calculating the combined standard uncertainty

This is an example of an empirical method for which collaborative trial data were available. The in-house repeatability was evaluated and found to be similar to that predicted by the collaborative

trial. It is therefore appropriate to use the s_R values from the collaborative trial provided that laboratory bias is controlled. The discussion presented in Step 3 leads to the conclusion that, with the exception of the effect of drying conditions at low fibre concentrations, the other sources of uncertainty identified in the cause and effect diagram are all small in comparison to s_R. In cases such as this, the uncertainty estimate can be based on the reproducibility standard deviation, s_R, obtained from the collaborative trial. For samples with a fibre content of 2.5 % m/m, an additional term has been included to take account of the uncertainty associated with the drying conditions.

Standard uncertainty

Typical standard uncertainties for a range of fibre concentrations are given in the Table A6.4 below.

Expanded uncertainty

Typical expanded uncertainties are given in Table A6.5 below. These were calculated using a coverage factor k of 2, which gives a level of confidence of approximately 95 %.

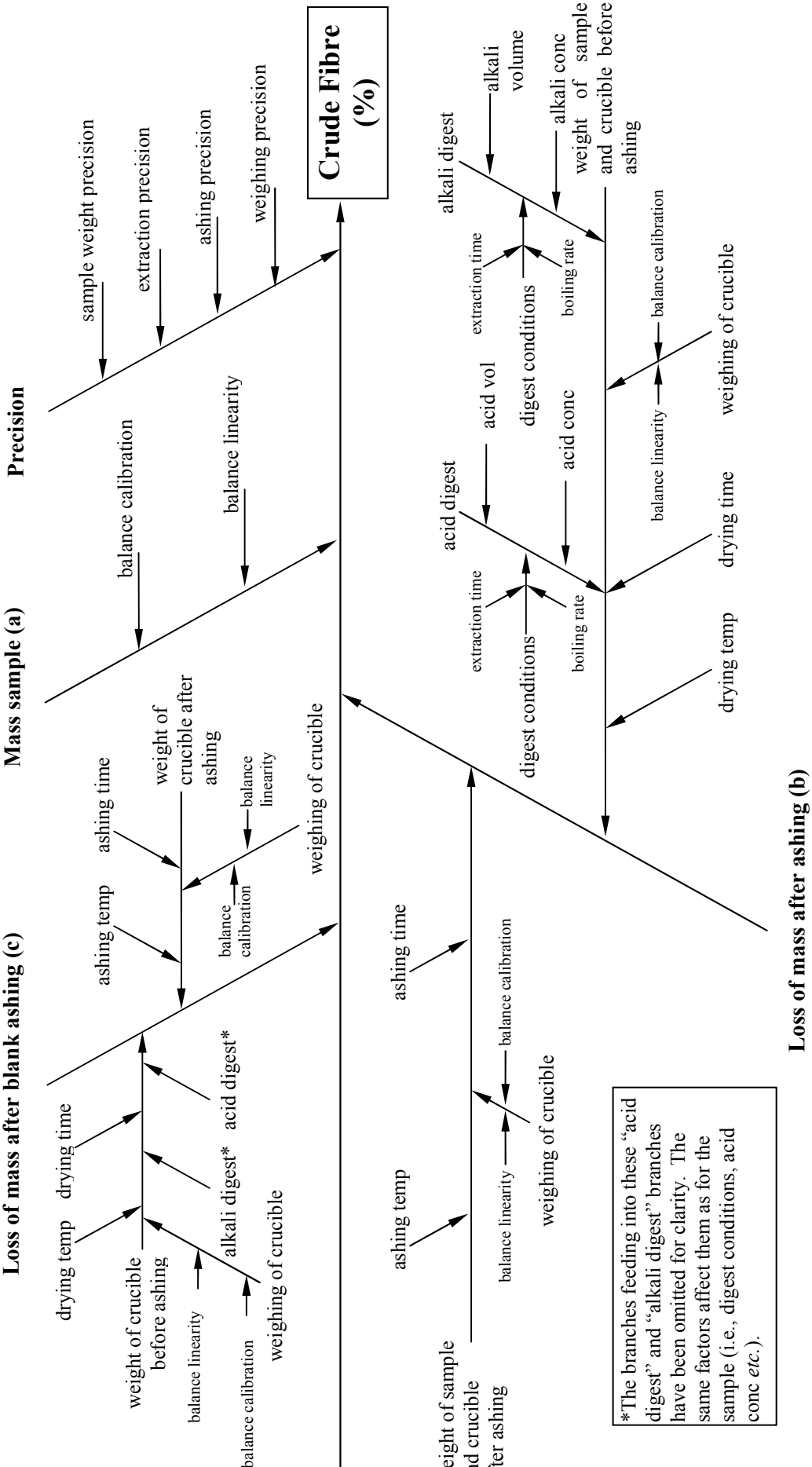
Table A6.4: Combined standard uncertainties

Fibre content (% m/m)	**Standard uncertainty $u_c(C_{fibre})$ (%m/m)**	**Relative standard uncertainty $u_c(C_{fibre}) / C_{fibre}$**
2.5	$\sqrt{0.29^2 + 0.115^2} = 0.31$	0.12
5	0.4	0.08
10	0.6	0.06

Table A6.5: Expanded uncertainties

Fibre content (% m/m)	**Expanded uncertainty $U(C_{fibre})$ (% m/m)**	**Expanded uncertainty (% of fibre content)**
2.5	0.62	25
5	0.8	16
10	0.12	12

Figure A6.9: Cause and effect diagram for the determination of fibre in animal feeding stuffs



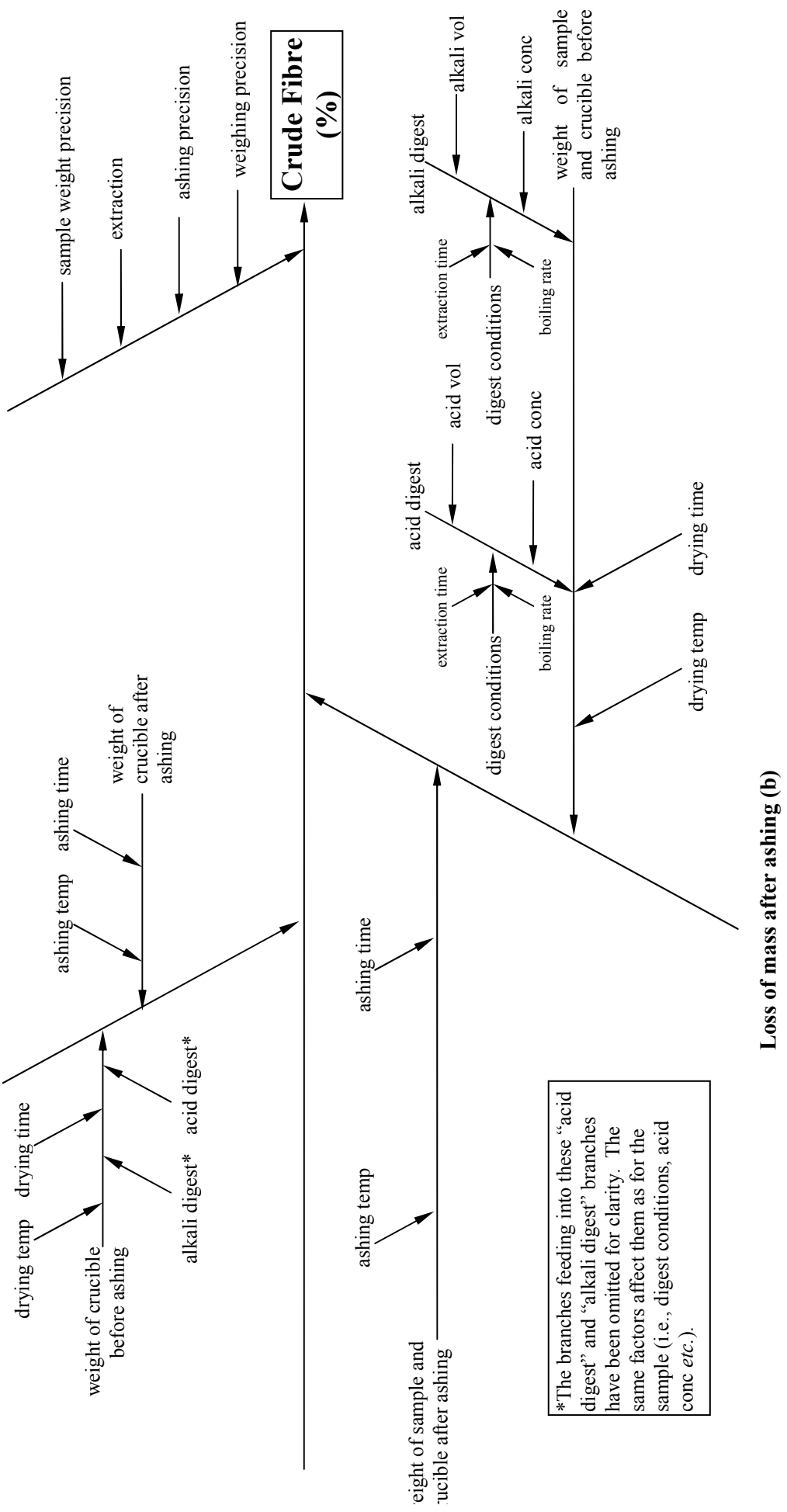


Figure A6.10: Simplified cause and effect diagram

Example A7: Determination of the Amount of Lead in Water Using Double Isotope Dilution and Inductively Coupled Plasma Mass Spectrometry

A7.1 Introduction

This example illustrates how the uncertainty concept can be applied to a measurement of the amount content of lead in a water sample using Isotope Dilution Mass Spectrometry (IDMS) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

General introduction to Double IDMS

IDMS is one of the techniques that is recognised by the Comité consultatif pour la quantité de matière (CCQM) to have the potential to be a primary method of measurement, and therefore a well defined expression which describes how the measurand is calculated is available. In the simplest case of isotope dilution using a certified spike, which is an enriched isotopic reference material, isotope ratios in the spike, the sample and a blend b of known masses of sample and spike are measured. The element amount content c_x in the sample is given by:

$$c_{\mathrm{x}} = c_{\mathrm{y}} \cdot \frac{m_{\mathrm{y}}}{m_{\mathrm{x}}} \cdot \frac{K_{\mathrm{y1}} \cdot R_{\mathrm{y1}} - K_{\mathrm{b}} \cdot R_{\mathrm{b}}}{K_{\mathrm{b}} \cdot R_{\mathrm{b}} - K_{\mathrm{x1}} \cdot R_{\mathrm{x1}}} \cdot \frac{\sum_i \left(K_{\mathrm{xi}} \cdot R_{\mathrm{xi}}\right)}{\sum_i \left(K_{\mathrm{yi}} \cdot R_{\mathrm{yi}}\right)} \qquad (1)$$

where c_{x} and c_{y} are element amount content in the sample and the spike respectively (the symbol c is used here instead of k for amount content[1] to avoid confusion with K-factors and coverage factors k). m_{x} and m_{y} are mass of sample and spike respectively. R_{x}, R_{y} and R_{b} are the isotope amount ratios. The indexes x, y and b represent the sample, the spike and the blend respectively. One isotope, usually the most abundant in the sample, is selected and all isotope amount ratios are expressed relative to it. A particular pair of isotopes, the reference isotope and preferably the most abundant isotope in the spike, is then selected as monitor ratio, e.g. $n(^{208}\mathrm{Pb})/n(^{206}\mathrm{Pb})$. R_{xi} and R_{yi} are all the possible isotope amount ratios in the sample and the spike respectively. For the reference isotope, this ratio is unity. K_{xi}, K_{yi} and K_{b} are the correction factors for mass discrimination, for a particular isotope amount ratio, in sample, spike and blend respectively. The K-factors are measured using a certified isotopic reference material according to equation (2).

$$K = K_0 + K_{\mathrm{bias}};\ \text{where}\, K_0 = \frac{R_{\mathrm{certified}}}{R_{\mathrm{observed}}} \qquad (2)$$

where K_0 is the mass discrimination correction factor at time 0, K_{bias} is a bias factor coming into effect as soon as the K-factor is applied to correct a ratio measured at a different time during the measurement. The K_{bias} also includes other possible sources of bias such as multiplier dead time correction, matrix effects *etc*. $R_{\mathrm{certified}}$ is the certified isotope amount ratio taken from the certificate of an isotopic reference material and R_{observed} is the observed value of this isotopic reference material. In IDMS experiments, using Inductively Coupled Plasma Mass Spectrometry (ICP-MS), mass fractionation will vary with time which requires that all isotope amount ratios in equation (1) need to be individually corrected for mass discrimination.

Certified material enriched in a specific isotope is often unavailable. To overcome this problem, 'double' IDMS is frequently used. The procedure uses a less well characterised, isotopically enriched spiking material in conjunction with a certified material (denoted z) of natural isotopic composition. The certified, natural composition material acts as the primary assay standard. Two blends are used; blend b is a blend between sample and enriched spike, as in equation (1). To perform double IDMS a second blend, b' is prepared from the primary assay standard with amount content c_z, and the enriched material y. This gives a similar expression to equation (1):

$$c_{\mathrm{z}} = c_{\mathrm{y}} \cdot \frac{m'_{\mathrm{y}}}{m_{\mathrm{z}}} \cdot \frac{K_{y1} \cdot R_{y1} - K'_{\mathrm{b}} \cdot R'_{\mathrm{b}}}{K'_{\mathrm{b}} \cdot R'_{\mathrm{b}} - K_{z1} \cdot R_{z1}} \cdot \frac{\sum_i \left(K_{\mathrm{zi}} \cdot R_{\mathrm{zi}}\right)}{\sum_i \left(K_{\mathrm{yi}} \cdot R_{\mathrm{yi}}\right)} \qquad (3)$$

where c_{z} is the element amount content of the primary assay standard solution and m_{z} the mass of the primary assay standard when preparing the new blend. m'_{y} is the mass of the enriched spike solution, K'_{b}, R'_{b}, K_{z1} and R_{z1} are the K-factor and the ratio for the new blend and the assay standard respectively. The index z represents the assay

Table A7.1. Summary of IDMS parameters

Parameter	Description	Parameter	Description
m_x	mass of sample in blend b [g]	m_y	mass of enriched spike in blend b [g]
m'_y	mass of enriched spike in blend b' [g]	m_z	mass of primary assay standard in blend b' [g]
c_x	amount content of the sample *x* [mol g^{-1} or μmol g^{-1}][Note 1]	c_z	amount content of the primary assay standard *z* [mol g^{-1} or μmol g^{-1}][Note 1]
c_y	amount content of the spike *y* [mol g^{-1} or μmol g^{-1}][Note 1]	c_{blank}	observed amount content in procedure blank [mol g^{-1} or μmol g^{-1}] [Note 1]
R_b	measured ratio of blend b, $n(^{208}Pb)/n(^{206}Pb)$	K_b	mass bias correction of R_b
R'_b	measured ratio of blend b', $n(^{208}Pb)/n(^{206}Pb)$	K'_b	mass bias correction of R'_b
R_{y1}	measured ratio of enriched isotope to reference isotope in the enriched spike	K_{y1}	mass bias correction of R_{y1}
R_{zi}	all ratios in the primary assay standard, R_{z1}, R_{z2} *etc*.	K_{zi}	mass bias correction factors for R_{zi}
R_{xi}	all ratios in the sample	K_{xi}	mass bias correction factors for R_{xi}
R_{x1}	measured ratio of enriched isotope to reference isotope in the sample *x*	R_{z1}	as R_{x1} but in the primary assay standard

Note 1: Units for amount content are always specified in the text.

standard. Dividing equation (1) with equation (3) gives

$$\frac{c_x}{c_z} = \frac{c_y \cdot \frac{m_y}{m_x} \cdot \frac{K_{y1} \cdot R_{y1} - K_b \cdot R_b}{K_b \cdot R_b - K_{x1} \cdot R_{x1}} \cdot \frac{\sum_i (K_{xi} \cdot R_{xi})}{\sum_i (K_{yi} \cdot R_{yi})}}{c_y \cdot \frac{m'_y}{m_z} \cdot \frac{K_{y1} \cdot R_{y1} - K'_b \cdot R'_b}{K'_b \cdot R'_b - K_{z1} \cdot R_{z1}} \cdot \frac{\sum_i (K_{zi} \cdot R_{zi})}{\sum_i (K_{yi} \cdot R_{yi})}} \quad (4)$$

Simplifying this equation and introducing a procedure blank, c_{blank}, we get:

$$c_x = c_z \cdot \frac{m_y}{m_x} \cdot \frac{m_z}{m'_y} \cdot \frac{K_{y1} \cdot R_{y1} - K_b \cdot R_b}{K_b \cdot R_b - K_{x1} \cdot R_{x1}} \times \frac{K'_b \cdot R'_b - K_{z1} \cdot R_{z1}}{K_{y1} \cdot R_{y1} - K'_b \cdot R'_b} \cdot \frac{\sum_i (K_{xi} \cdot R_{xi})}{\sum_i (K_{zi} \cdot R_{zi})} - c_{blank} \quad (5)$$

This is the final equation, from which c_y has been eliminated. In this measurement the number index on the amount ratios, *R*, represents the following actual isotope amount ratios:

$R_1 = n(^{208}Pb)/n(^{206}Pb)$ $R_2 = n(^{206}Pb)/n(^{206}Pb)$

$R_3 = n(^{207}Pb)/n(^{206}Pb)$ $R_4 = n(^{204}Pb)/n(^{206}Pb)$

For reference, the parameters are summarised in Table A7.1.

A7.2 Step 1: Specification

The general procedure for the measurements is shown in Table A7.2. The calculations and measurements involved are described below.

Calculation procedure for the amount content c_x

For this determination of lead in water, four blends each of b', (assay + spike), and b, (sample + spike), were prepared. This gives a total of 4 values for c_x. One of these determinations will be described in detail following Table A7.2, steps 1 to 4. The reported value for c_x will be the average of the four replicates.

Table A7.2. General procedure

Step	Description
1	Preparing the primary assay standard
2	Preparation of blends: b' and b
3	Measurement of isotope ratios
4	Calculation of the amount content of Pb in the sample, c_x
5	Estimating the uncertainty in c_x

Calculation of the Molar Mass

Due to natural variations in the isotopic composition of certain elements, e.g. Pb, the molar mass, M, for the primary assay standard has to be determined since this will affect the amount content c_z. Note that this is not the case when c_z is expressed in mol g^{-1}. The molar mass, M(E), for an element E, is numerically equal to the atomic weight of element E, A_r(E). The atomic weight can be calculated according to the general expression:

$$A_r(\mathrm{E}) = \frac{\sum_{i=1}^{p} R_i \cdot M\left({}^{i}\mathrm{E}\right)}{\sum_{i=1}^{p} R_i} \qquad (6)$$

where the values R_i are all true isotope amount ratios for the element E and $M({}^{i}\mathrm{E})$ are the tabulated nuclide masses.

Note that the isotope amount ratios in equation (6) have to be absolute ratios, that is, they have to be corrected for mass discrimination. With the use of proper indexes, this gives equation (7). For the calculation, nuclide masses, $M({}^{i}\mathrm{E})$, were taken from literature values[2], while Ratios, R_{zi}, and K_0-factors, K_0(zi), were measured (see Table A7.8). These values give

$$M(\mathrm{Pb,\ Assay\,1}) = \frac{\sum_{i=1}^{p} K_{zi} \cdot R_{zi} \cdot M_z\left({}^{i}\mathrm{E}\right)}{\sum_{i=1}^{p} K_{zi} \cdot R_{zi}} \qquad (7)$$
$$= 207.21034\ \mathrm{g\,mol^{-1}}$$

Measurement of K-factors and isotope amount ratios

To correct for mass discrimination, a correction factor, K, is used as specified in equation (2). The K_0-factor can be calculated using a reference material certified for isotopic composition. In this case, the isotopically certified reference material NIST SRM 981 was used to monitor a possible change in the K_0-factor. The K_0-factor is measured before and after the ratio it will correct. A typical sample sequence is: 1. (blank), 2. (NIST SRM 981), 3. (blank), 4. (blend 1), 5. (blank), 6. (NIST SRM 981), 7. (blank), 8. (sample), *etc*.

The blank measurements are not only used for blank correction, they are also used for monitoring the number of counts for the blank. No new measurement run was started until the blank count rate was stable and back to a normal level. Note that sample, blends, spike and assay standard were diluted to an appropriate amount content prior to the measurements. The results of ratio measurements, calculated K_0-factors and K_{bias} are summarised in Table A7.8.

Preparing the primary assay standard and calculating the amount content, c_z.

Two primary assay standards were produced, each from a different piece of metallic lead with a chemical purity of w=99.999 %. The two pieces came from the same batch of high purity lead. The pieces were dissolved in about 10 mL of 1:3 m/m HNO_3:water under gentle heating and then further diluted. Two blends were prepared from each of these two assay standards. The values from one of the assays is described hereafter.

0.36544 g lead, m_1, was dissolved and diluted in aqueous HNO_3 (0.5 mol L^{-1}) to a total of d_1=196.14 g. This solution is named *Assay 1*. A more diluted solution was needed and m_2=1.0292 g of *Assay 1*, was diluted in aqueous HNO_3 (0.5 mol L^{-1}) to a total mass of d_2=99.931g. This solution is named *Assay 2*. The amount content of Pb in *Assay 2*, c_z, is then calculated according to equation (8)

$$c_z = \frac{m_2}{d_2} \cdot \frac{m_1 \cdot w}{d_1} \cdot \frac{1}{M(\mathrm{Pb},\ Assay1)} \qquad (8)$$
$$= 9.2605 \times 10^{-8}\ \mathrm{mol\,g^{-1}} = 0.092605\ \mu\mathrm{mol\,g^{-1}}$$

Preparation of the blends

The mass fraction of the spike is known to be roughly 20µg Pb per g solution and the mass fraction of Pb in the sample is also known to be in this range. Table A7.3 shows the weighing data for the two blends used in this example.

Table A7.3

Blend	**b**		**b'**	
Solutions used	Spike	Sample	Spike	Assay 2
Parameter	m_y	m_x	m'_y	m_z
Mass (g)	1.1360	1.0440	1.0654	1.1029

Measurement of the procedure blank c_{Blank}

In this case, the procedure blank was measured using external calibration. A more exhaustive procedure would be to add an enriched spike to a blank and process it in the same way as the samples. In this example, only high purity reagents were used, which would lead to extreme ratios in the blends and consequent poor reliability for the enriched spiking procedure. The externally calibrated procedure blank was measured four times, and c_{Blank} found to be 4.5×10^{-7} µmol g^{-1}, with standard uncertainty 4.0×10^{-7} µmol g^{-1} evaluated as type A.

Calculation of the unknown amount content c_x

Inserting the measured and calculated data (Table A7.8) into equation (5) gives c_x=0.053738 µmol g^{-1}. The results from all four replicates are given in Table A7.4.

A7.3 Steps 2 and 3: Identifying and quantifying uncertainty sources

Strategy for the uncertainty calculation

If equations (2), (7) and (8) were to be included in the final IDMS equation (5), the sheer number of parameters would make the equation almost impossible to handle. To keep it simpler, K_0-factors and amount content of the standard assay solution and their associated uncertainties are treated separately and then introduced into the IDMS equation (5). In this case it will not affect the final combined uncertainty of c_x, and it is advisable to simplify for practical reasons.

For calculating the combined standard uncertainty, $u_c(c_x)$, the values from one of the measurements, as described in A7.2, will be used. The combined uncertainty of c_x will be calculated using the spreadsheet method described in Appendix E.

Table A7.4

	c_x (µmol g^{-1})
Replicate 1 (our example)	0.053738
Replicate 2	0.053621
Replicate 3	0.053610
Replicate 4	0.053822
Average	**0.05370**
Experimental standard deviation (s)	**0.0001**

Uncertainty on the K-factors

i) Uncertainty on K_0

K is calculated according to equation (2) and using the values of K_{x1} as an example gives for K_0:

$$K_0(x1) = \frac{R_{\text{certified}}}{R_{\text{observed}}} = \frac{2.1681}{2.1699} = 0.9992 \quad (9)$$

To calculate the uncertainty on K_0 we first look at the certificate where the certified ratio, 2.1681, has a stated uncertainty of 0.0008 based on a 95 % confidence interval. To convert an uncertainty based on a 95 % confidence interval to standard uncertainty we divide by 2. This gives a standard uncertainty of $u(R_{\text{certified}})$=0.0004. The observed amount ratio, $R_{\text{observed}}=n(^{208}\text{Pb})/n(^{206}\text{Pb})$, has a standard uncertainty of 0.0025 (as RSD). For the K-factor, the combined uncertainty can be calculated as:

$$\frac{u_c(K_0(x1))}{K_0(x1)} = \sqrt{\left(\frac{0.0004}{2.1681}\right)^2 + (0.0025)^2} = 0.002507 \quad (10)$$

This clearly points out that the uncertainty contributions from the certified ratios are negligible. Henceforth, the uncertainties on the measured ratios, R_{observed}, will be used for the uncertainties on K_0.

Uncertainty on K_{bias}

This bias factor is introduced to account for possible deviations in the value of the mass discrimination factor. As can be seen in equation (2) above, there is a bias associated with every K-factor. The values of these biases are in our case not known, and a value of 0 is applied. An uncertainty is, of course, associated with every

bias and this has to be taken into consideration when calculating the final uncertainty. In principle, a bias would be applied as in equation (11), using an excerpt from equation (5) and the parameters K_{y1} and R_{y1} to demonstrate this principle.

$$c_x = \ldots . \cdot \frac{\left(K_0(y1) + K_{bias}(y1)\right) \cdot R_{y1} - \ldots}{\ldots\ldots} \cdot \ldots . \quad (11)$$

The values of all biases, K_{bias}(yi, xi, zi), are (0 ± 0.001). This estimation is based on a long experience of lead IDMS measurements. All K_{bias}(yi, xi, zi) parameters are not included in detail in Table A7.5, Table A7.8 or in equation 5, but they are used in all uncertainty calculations.

Uncertainty of the weighed masses

In this case, a dedicated mass metrology lab performed the weighings. The procedure applied was a bracketing technique using calibrated weights and a comparator. The bracketing technique was repeated at least six times for every sample mass determination. Buoyancy correction was applied. Stoichiometry and impurity corrections were not applied in this case. The uncertainties from the weighing certificates were treated as standard uncertainties and are given in Table A7.8.

Table A7.5

	Value	Standard Uncertainty	Type[Note 1]
K_{bias}(zi)	0	0.001	B
R_{z1}	2.1429	0.0054	A
K_0(z1)	0.9989	0.0025	A
K_0(z3)	0.9993	0.0035	A
K_0(z4)	1.0002	0.0060	A
R_{z2}	1	0	A
R_{z3}	0.9147	0.0032	A
R_{z4}	0.05870	0.00035	A
M_1	207.976636	0.000003	B
M_2	205.974449	0.000003	B
M_3	206.975880	0.000003	B
M_4	203.973028	0.000003	B

Note 1. Type A (statistical evaluation) or Type B (other)

Uncertainty in the amount content of the Standard Assay Solution, c_z

i) Uncertainty in the atomic weight of Pb

First, the combined uncertainty of the molar mass of the assay solution, *Assay* 1, will be calculated. The values in Table A7.5 are known or have been measured:

According to equation (7), the calculation of the molar mass takes this form:

$$M(\mathrm{Pb}, Assay1) = \frac{K_{z1} \cdot R_{z1} \cdot M_1 + R_{z2} \cdot M_2 + K_{z3} \cdot R_{z3} \cdot M_3 + K_{z4} \cdot R_{z4} \cdot M_4}{K_{z1} \cdot R_{z1} + K_{z2} \cdot R_{z2} + K_{z3} \cdot R_{z3} + K_{z4} \cdot R_{z4}} \quad (12)$$

To calculate the combined standard uncertainty of the molar mass of Pb in the standard assay solution, the spreadsheet model described in Appendix E was used. There were eight measurements of every ratio and K_0. This gave a molar mass M(Pb, *Assay* 1)=207.2103 g mol^{-1}, with uncertainty 0.0010 g mol^{-1} calculated using the spreadsheet method.

ii) Calculation of the combined standard uncertainty in determining c_z

To calculate the uncertainty on the amount content of Pb in the standard assay solution, c_z the data from A7.2 and equation (8) are used. The uncertainties were taken from the weighing certificates, see A7.3. All parameters used in equation (8) are given with their uncertainties in Table A7.6.

The amount content, c_z, was calculated using equation (8). Following Appendix D.5 the combined standard uncertainty in c_z, is calculated to be $u_c(c_z)$=0.000028. This gives c_z=0.092606 µmol g^{-1} with a standard uncertainty of 0.000028 µmol g^{-1} (0.03 % as %RSD).

To calculate $u_c(c_x)$, for replicate 1, the spreadsheet model was applied (Appendix E). The uncertainty budget for replicate 1 will be representative for the measurement. Due to the number of parameters in equation (5), the spreadsheet will not be displayed. The value of the parameters and their uncertainties as well as the combined uncertainty of c_x can be seen in Table A7.8.

Table A7.6

	Value	Uncertainty
Mass of lead piece, m_1 (g)	0.36544	0.00005
Total mass first dilution, d_1 (g)	196.14	0.03
Aliquot of first dilution, m_2 (g)	1.0292	0.0002
Total mass of second dilution, d_2 (g)	99.931	0.01
Purity of the metallic lead piece, w (mass fraction)	0.99999	0.000005
Molar mass of Pb in the Assay Material, M (g mol^{-1})	207.2104	0.0010

A7.4 Step 4: Calculating the combined standard uncertainty

The average and the experimental standard deviation of the four replicates are displayed in Table A7.7. The numbers are taken from Table A7.4 and Table A7.8.

Table A7.7

Replicate 1		**Mean of replicates 1-4**		
c_x	0.05374	c_x	0.05370	µmol g^{-1}
$u_c(c_x)$	0.00018	s	0.00010 Note 1	µmol g^{-1}

Note 1.This is the experimental standard uncertainty and not the standard deviation of the mean.

In IDMS, and in many non-routine analyses, a complete statistical control of the measurement procedure would require limitless resources and time. A good way then to check if some source of uncertainty has been forgotten is to compare the uncertainties from the type A evaluations with the experimental standard deviation of the four replicates. If the experimental standard deviation is higher than the contributions from the uncertainty sources evaluated as type A, it could indicate that the measurement process is not fully understood. As an approximation, using data from Table A7.8, the sum of the type A evaluated experimental uncertainties can be calculated by taking 92.2 % of the total experimental uncertainty, which is 0.00041 µmol g^{-1}. This value is then clearly higher than the experimental standard deviation of 0.00010 µmol g^{-1}, see Table A7.7. This indicates that the experimental standard deviation is covered by the contributions from the type A evaluated uncertainties and that no further type A evaluated uncertainty contribution, due to the preparation of the blends, needs to be considered. There could however be a bias associated with the preparations of the blends. In this example, a possible bias in the preparation of the blends is judged to be insignificant in comparison to the major sources of uncertainty.

The amount content of lead in the water sample is then:

$$c_x = (0.05370 \pm 0.00036)\ \mu\text{mol g}^{-1}$$

The result is presented with an expanded uncertainty using a coverage factor of 2.

References for Example 7

1. T. Cvitaš, *Metrologia*, 1996, **33**, 35-39
2 G. Audi and A.H. Wapstra, Nuclear Physics, A565 (1993)

Table A7.8

parameter	uncertainty evaluation	value	experimental uncertainty (Note 1)	contribution to total u_c(%)	final uncertainty (Note 2)	contribution to total u_c(%)
ΣK_{bias}	B	0	0.001 Note 3	7.2	0.001 Note 3	37.6
c_z	B	0.092605	0.000028	0.2	0.000028	0.8
K_0(b)	A	0.9987	0.0025	14.4	0.00088	9.5
K_0(b')	A	0.9983	0.0025	18.3	0.00088	11.9
K_0(x1)	A	0.9992	0.0025	4.3	0.00088	2.8
K_0(x3)	A	1.0004	0.0035	1	0.0012	0.6
K_0(x4)	A	1.001	0.006	0	0.0021	0
K_0(y1)	A	0.9999	0.0025	0	0.00088	0
K_0(z1)	A	0.9989	0.0025	6.6	0.00088	4.3
K_0(z3)	A	0.9993	0.0035	1	0.0012	0.6
K_0(z4)	A	1.0002	0.006	0	0.0021	0
m_x	B	1.0440	0.0002	0.1	0.0002	0.3
m_{y1}	B	1.1360	0.0002	0.1	0.0002	0.3
m_{y2}	B	1.0654	0.0002	0.1	0.0002	0.3
m_z	B	1.1029	0.0002	0.1	0.0002	0.3
R_b	A	0.29360	0.00073	14.2	0.00026 Note 4	9.5
R'_b	A	0.5050	0.0013	19.3	0.00046	12.7
R_{x1}	A	2.1402	0.0054	4.4	0.0019	2.9
R_{x2}	Cons.	1	0		0	
R_{x3}	A	0.9142	0.0032	1	0.0011	0.6
R_{x4}	A	0.05901	0.00035	0	0.00012	0
R_{y1}	A	0.00064	0.00004	0	0.000014	0
R_{z1}	A	2.1429	0.0054	6.7	0.0019	4.4
R_{z2}	Cons.	1	0		0	
R_{z3}	A	0.9147	0.0032	1	0.0011	0.6
R_{z4}	A	0.05870	0.00035	0	0.00012	0
c_{Blank}	A	4.5×10^{-7}	4.0×10^{-7}	0	2.0×10^{-7}	0
c_x		**0.05374**	**0.00041**		**0.00018**	
			$\Sigma A_{contrib.}$=	92.2	$\Sigma A_{contrib.}$=	60.4
			$\Sigma B_{contrib.}$=	7.8	$\Sigma B_{contrib.}$=	39.6

Notes overleaf

Notes to Table A7.8

Note 1. The experimental uncertainty is calculated without taking the number of measurements on each parameter into account.

Note 2. In the final uncertainty the number of measurements has been taken into account. In this case all type A evaluated parameters have been measured 8 times. Their standard uncertainties have been divided by $\sqrt{8}$.

Note 3. This value is for one single K_{bias}. The parameter ΣK_{bias} is used instead of listing all K_{bias}(zi,xi,yi), which all have the same value (0 ± 0.001).

Note 4. R_b has been measured 8 times per blend giving a total of 32 observations. When there is no blend to blend variation, as in this example, all these 32 observations could be accounted for by implementing all four blend replicates in the model. This can be very time consuming and since, in this case, it does not affect the uncertainty noticeably, it is not done.

Appendix B. Definitions

General

B.1 Precision

Closeness of agreement between independent test results obtained under stipulated conditions [H.8].

NOTE 1 Precision depends only on the distribution of random errors and does not relate to the true value or the specified value.

NOTE 2 The measure of precision is usually expressed in terms of imprecision and computed as a standard deviation of the test results. Less precision is reflected by a larger standard deviation.

NOTE 3 "Independent test results" means results obtained in a manner not influenced by any previous result on the same or similar test object. Quantitative measures of precision depend critically on the stipulated conditions. Repeatability conditions and reproducibility conditions are particular sets of extreme stipulated conditions.

B.2 True value

value which characterizes a quantity or quantitative characteristic perfectly defined in the conditions which exist when that quantity or quantitative characteristic is considered [H.8].

NOTE 1 The true value of a quantity or quantitative characteristic is a theoretical concept and, in general, cannot be known exactly.

NOTE 2* For an explanation of the term "quantity", ISO 3534-2 refers to Note 1 of ISO 3534-2 paragraph 3.2.1., which states that "In this definition, a quantity can be either a "base quantity" such as mass, length, time, or a "derived quantity" such as velocity (length divided by time).

*This Note in the present Guide replaces Note 2 in ISO 3534-2.

B.3 Influence quantity

quantity that, in a direct measurement, does not affect the quantity that is actually measured, but affects the relation between the indication and the measurement result[H.7].

EXAMPLES

1. Frequency in the direct measurement with an ammeter of the constant amplitude of an alternating current.

2. Amount-of-substance concentration of bilirubin in a direct measurement of haemoglobin amount-of-substance concentration in human blood plasma

3. Temperature of a micrometer used for measuring the length of a rod, but not the temperature of the rod itself which can enter into the definition of the measurand.

4. Background pressure in the ion source of a mass spectrometer during a measurement of amount-of-substance fraction.

NOTE 1 An indirect measurement involves a combination of direct measurements, each of which may be affected by influence quantities.

NOTE 2 In the GUM, the concept 'influence quantity' is defined as in the second edition of the VIM, covering not only the quantities affecting the measuring system, as in the definition above, but also those quantities that affect the quantities actually measured. Also, in the GUM this concept is not restricted to direct measurements.

Measurement

B.4 Measurand

quantity intended to be measured [H.7].

NOTE: See reference H.5 for full Notes and a detailed discussion of "measurand" and its relation to amount or concentration of "analyte".

B.5 Measurement

process of experimentally obtaining one or more quantity values that can reasonably be attributed to a quantity [H.7].

NOTE: See reference H.5 for full Notes and a detailed discussion of "measurement" and "measurement result".

B.6 Measurement procedure

Set of operations, described specifically, used in the performance of measurements according to a given method [H.7].

NOTE A measurement procedure is usually recorded in a document that is sometimes itself called a "measurement procedure" (or a *measurement method*) and is usually in sufficient detail to enable an operator to carry out a measurement without additional information.

B.7 Method of measurement

A logical sequence of operations, described generically, used in the performance of measurements [H.7].

NOTE Methods of measurement may be qualified in various ways such as:

- substitution method
- differential method
- null method

Uncertainty

B.8 Uncertainty (of measurement)

Parameter associated with the result of a measurement, that characterises the dispersion of the values that could reasonably be attributed to the measurand [H.2]**.**

NOTE 1 The parameter may be, for example, a standard deviation (or a given multiple of it), or the width of a confidence interval.

NOTE 2 Uncertainty of measurement comprises, in general, many components. Some of these components may be evaluated from the statistical distribution of the results of a series of measurements and can be characterised by experimental standard deviations. The other components, which can also be characterised by standard deviations, are evaluated from assumed probability distributions based on experience or other information.

NOTE 3 It is understood that the result of the measurement is the best estimate of the value of the measurand and that all components of uncertainty, including those arising from systematic effects, such as components associated with corrections and reference standards, contribute to the dispersion.

B.9 Traceability

Property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty [H.7].

NOTE: See reference H.5 for full Notes and a detailed discussion of "metrological traceability" and section 3.3. for a discussion for the purpose of the present Guide.

B.10 Standard uncertainty

$u(x_i)$ Uncertainty of the result x_i of a measurement expressed as a standard deviation [H.2].

B.11 Combined standard uncertainty

$u_c(y)$ Standard uncertainty of the result y of a measurement when the result is obtained from the values of a number of other quantities, equal to the positive square root of a sum of terms, the terms being the variances or covariances of these other quantities weighted according to how the measurement result varies with these quantities [H.2].

B.12 Expanded uncertainty

U Quantity defining an interval about the result of a measurement that may be expected to encompass a large fraction of the distribution of values that could reasonably be attributed to the measurand [H.2].

NOTE 1 The fraction may be regarded as the coverage probability or level of confidence of the interval.

NOTE 2 To associate a specific level of confidence with the interval defined by the expanded uncertainty requires explicit or implicit assumptions regarding the probability distribution characterised by the measurement result and its combined standard uncertainty. The level of confidence that may be attributed to this interval can be known only to the extent to which such assumptions can be justified.

NOTE 3 An expanded uncertainty U is calculated from a combined standard uncertainty u_c and a coverage factor k using

$$U = k \times u_c$$

B.13 Coverage factor

k Numerical factor used as a multiplier of the combined standard uncertainty in order to obtain an expanded uncertainty [H.2].

NOTE A coverage factor is typically in the range 2 to 3.

B.14 Type A evaluation (of uncertainty)

Method of evaluation of uncertainty by the statistical analysis of series of observations [H.2].

B.15 Type B evaluation (of uncertainty)

Method of evaluation of uncertainty by means other than the statistical analysis of series of observations [H.2]

Error

B.16 Error (of measurement)

measured quantity value minus a reference quantity value [H.7]

NOTE: See reference H.5 for a detailed discussion of "measurement error" and related terms.

B.17 Random error

component of measurement error that in replicate measurements varies in an unpredictable manner [H.7].

NOTE: See reference H.5 for a detailed discussion of "measurement error" and related terms.

B.18 Systematic error

component of measurement error that in replicate measurements remains constant or varies in a predictable manner [H.7].

NOTE: See reference H.5 for a detailed discussion of "measurement error" and related terms.

Statistical terms

B.19 Arithmetic mean

$\bar{x}$ Arithmetic mean value of a sample of n results.

$$\bar{x} = \frac{\sum_{i=1,n} x_i}{n}$$

B.20 Sample Standard Deviation

s An estimate of the population standard deviation σ from a sample of n results.

$$s = \sqrt{\frac{\sum_{i=1}^{n} (x_i - \bar{x})^2}{n-1}}$$

B.21 Standard deviation of the mean

$s_{\bar{x}}$ The standard deviation of the mean $\bar{x}$ of n values taken from a population is given by

$$s_{\bar{x}} = \frac{s}{\sqrt{n}}$$

The terms "standard error" and "standard error of the mean" have also been used to describe the same quantity.

B.22 Relative Standard Deviation (RSD)

RSD An estimate of the standard deviation of a population from a (statistical) sample of *n* results divided by the mean of that sample.

Often known as coefficient of variation (CV). Also frequently stated as a percentage (denoted %RSD or %CV in this Guide).

$$\textbf{RSD} = \frac{s}{\bar{x}}$$

Appendix C. Uncertainties in Analytical Processes

C.1 In order to identify the possible sources of uncertainty in an analytical procedure it is helpful to break down the analysis into a set of generic steps:

1. **Sampling**
2. **Sample preparation**
3. **Presentation of Certified Reference Materials to the measuring system**
4. **Calibration of Instrument**
5. **Analysis (data acquisition)**
6. **Data processing**
7. **Presentation of results**
8. **Interpretation of results**

C.2 These steps can be further broken down by contributions to the uncertainty for each. The following list, though not necessarily comprehensive, provides guidance on factors which should be considered.

1. **Sampling**
   - Homogeneity.
   - Effects of specific sampling strategy (e.g. random, stratified random, proportional *etc*.)
   - Effects of movement of bulk medium (particularly density selection)
   - Physical state of bulk (solid, liquid, gas)
   - Temperature and pressure effects.
   - Does sampling process affect composition? E.g. differential adsorption in sampling system.
2. **Sample preparation**
   - Homogenisation and/or sub-sampling effects.
   - Drying.
   - Milling.
   - Dissolution.
   - Extraction.
   - Contamination.
   - Derivatisation (chemical effects)
   - Dilution errors.
   - (Pre-)Concentration.
   - Control of speciation effects.
3. **Presentation of Certified Reference Materials to the measuring system**
   - Uncertainty for CRM.
   - CRM match to sample
4. **Calibration of instrument**
   - Instrument calibration errors using a Certified Reference Material.
   - Reference material and its uncertainty.
   - Sample match to calibrant
   - Instrument precision
5. **Analysis**
   - Carry-over in auto analysers.
   - Operator effects, e.g. colour blindness, parallax, other systematic errors.
   - Interferences from the matrix, reagents or other analytes.
   - Reagent purity.
   - Instrument parameter settings, e.g. integration parameters
   - Run-to-run precision
6. **Data Processing**
   - Averaging.
   - Control of rounding and truncating.
   - Statistics.
   - Processing algorithms (model fitting, e.g. linear least squares).
7. **Presentation of Results**
   - Final result.
   - Estimate of uncertainty.
   - Confidence level.
8. **Interpretation of Results**
   - Against limits/bounds.
   - Regulatory compliance.
   - Fitness for purpose.

Appendix D. Analysing Uncertainty Sources

D.1 Introduction

It is commonly necessary to develop and record a list of sources of uncertainty relevant to an analytical method. It is often useful to structure this process, both to ensure comprehensive coverage and to avoid over-counting. The following procedure (based on a previously published method [H.26]), provides one possible means of developing a suitable, structured analysis of uncertainty contributions.

D.2 Principles of approach

D.2.1 The strategy has two stages:

- Identifying the effects on a result

  In practice, the necessary structured analysis is effected using a *cause and effect diagram* (sometimes known as an Ishikawa or 'fishbone' diagram) [H.27].

- Simplifying and resolving duplication

  The initial list is refined to simplify presentation and ensure that effects are not unnecessarily duplicated.

D.3 Cause and effect analysis

D.3.1 The principles of constructing a cause and effect diagram are described fully elsewhere. The procedure employed is as follows:

1. Write the complete equation for the result. The parameters in the equation form the main branches of the diagram. It is almost always necessary to add a main branch representing a nominal correction for overall bias, usually as recovery, and this is accordingly recommended at this stage if appropriate.
2. Consider each step of the method and add any further factors to the diagram, working outwards from the main effects. Examples include environmental and matrix effects.
3. For each branch, add contributory factors until effects become sufficiently remote, that is, until effects on the result are negligible.
4. Resolve duplications and re-arrange to clarify contributions and group related causes. It is convenient to group precision terms at this stage on a separate precision branch.

D.3.2 The final stage of the cause and effect analysis requires further elucidation. Duplications arise naturally in detailing contributions separately for every input parameter. For example, a run-to-run variability element is always present, at least nominally, for any influence factor; these effects contribute to any overall variance observed for the method as a whole and should not be added in separately if already so accounted for. Similarly, it is common to find the same instrument used to weigh materials, leading to over-counting of its calibration uncertainties. These considerations lead to the following additional rules for refinement of the diagram (though they apply equally well to any structured list of effects):

- Cancelling effects: remove both. For example, in a weight by difference, two weights are determined, both subject to the balance 'zero bias'. The zero bias will cancel out of the weight by difference, and can be removed from the branches corresponding to the separate weighings.
- Similar effect, same time: combine into a single input. For example, run-to-run variation on many inputs can be combined into an overall run-to-run precision 'branch'. Some caution is required; specifically, variability in operations carried out individually for every determination can be combined, whereas variability in operations carried out on complete batches (such as instrument calibration) will only be observable in between-batch measures of precision.
- Different instances: re-label. It is common to find similarly named effects which actually refer to different instances of similar measurements. These must be clearly distinguished before proceeding.

D.3.3 This form of analysis does not lead to uniquely structured lists. In the present example, temperature may be seen as either a direct effect on the density to be measured, or as an effect on the measured mass of material contained in a

density bottle; either could form the initial structure. In practice this does not affect the utility of the method. Provided that all significant effects appear once, somewhere in the list, the overall methodology remains effective.

D.3.4 Once the cause-and-effect analysis is complete, it may be appropriate to return to the original equation for the result and add any new terms (such as temperature) to the equation.

D.4 Example

D.4.1 The procedure is illustrated by reference to a simplified direct density measurement. Consider the case of direct determination of the density d(EtOH) of ethanol by weighing a known volume V in a suitable volumetric vessel of tare weight m_{tare} and gross weight including ethanol m_{gross}. The density is calculated from

$$d(\text{EtOH})=(m_{gross} - m_{tare})/V$$

For clarity, only three effects will be considered: Equipment calibration, Temperature, and the precision of each determination. Figures D1-D3 illustrate the process graphically.

D.4.2 A cause and effect diagram consists of a hierarchical structure culminating in a single outcome. For the present purpose, this outcome is a particular analytical result ('d(EtOH)' in Figure D1). The 'branches' leading to the outcome are the contributory effects, which include both the results of particular intermediate measurements and other factors, such as environmental or matrix effects. Each branch may in turn have further contributory effects. These 'effects' comprise all factors affecting the result, whether variable or constant; uncertainties in any of these effects will clearly contribute to uncertainty in the result.

D.4.3 Figure D1 shows a possible diagram obtained directly from application of steps 1-3. The main branches are the parameters in the equation, and effects on each are represented by subsidiary branches. Note that there are two 'temperature' effects, three 'precision' effects and three 'calibration' effects.

D.4.4 Figure D2 shows precision and temperature effects each grouped together following the second rule (same effect/time); temperature may be treated as a single effect on density, while the individual variations in each determination contribute to variation observed in replication of the entire method.

D.4.5 The calibration bias on the two weighings cancels, and can be removed (Figure D3) following the first refinement rule (cancellation).

D.4.6 Finally, the remaining 'calibration' branches would need to be distinguished as two (different) contributions owing to possible non-linearity of balance response, together with the calibration uncertainty associated with the volumetric determination.

Figure D1: Initial list

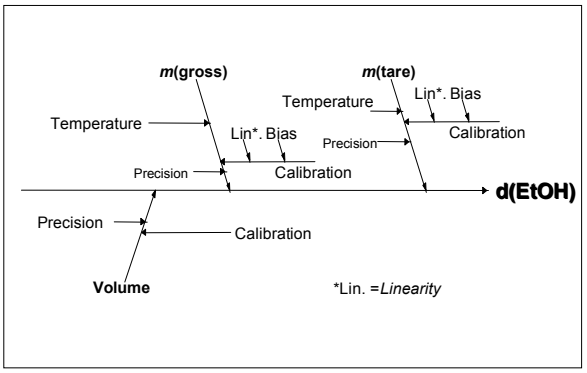


Figure D2: Combination of similar effects

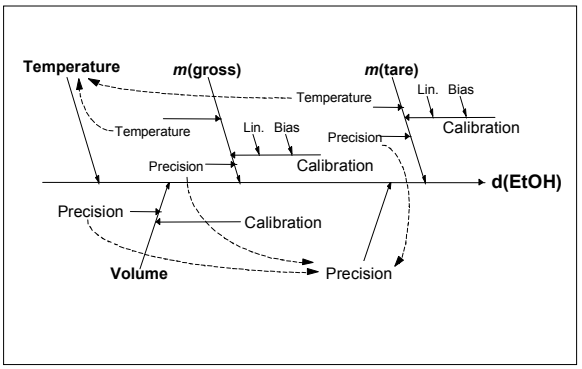
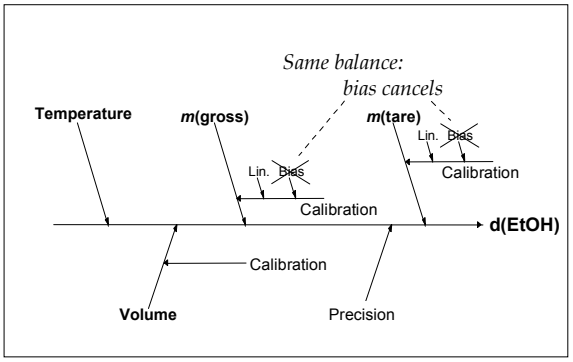


Figure D3: Cancellation



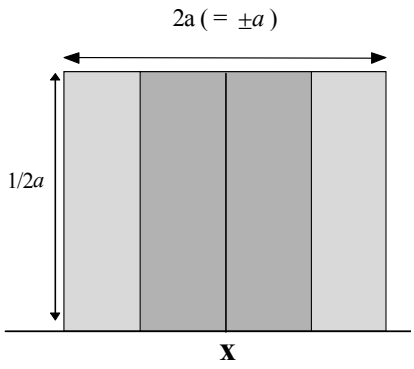
Appendix E. Useful Statistical Procedures

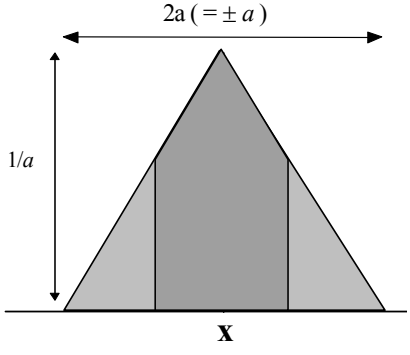
E.1 Distribution functions

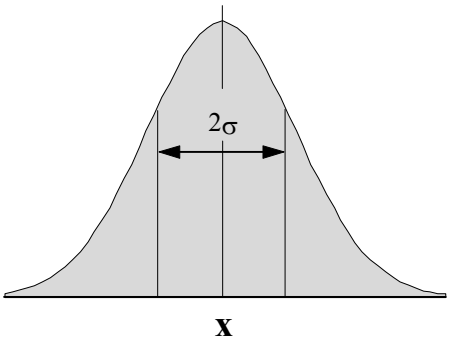
The following table shows how to calculate a standard uncertainty from the parameters of the two most important distribution functions, and gives an indication of the circumstances in which each should be used.

EXAMPLE

A chemist estimates a contributory factor as not less than 7 or more than 10, but feels that the value could be anywhere in between, with no idea of whether any part of the range is more likely than another. This is a description of a rectangular distribution function with a range $2a$=3 (semi range of a=1.5). Using the function below for a rectangular distribution, an estimate of the standard uncertainty can be calculated. Using the above range, a=1.5, results in a standard uncertainty of $(1.5/\sqrt{3}) = 0.87$.

Rectangular distribution		
Form	Use when:	Uncertainty
	• A certificate or other specification gives limits without specifying a level of confidence (e.g. 25 mL ± 0.05 mL) • An estimate is made in the form of a maximum range (±a) with no knowledge of the shape of the distribution.	$u(x)=\frac{a}{\sqrt{3}}$

Triangular distribution		
Form	Use when:	Uncertainty
	• The available information concerning x is less limited than for a rectangular distribution. Values close to x are more likely than near the bounds. • An estimate is made in the form of a maximum range (±a) described by a symmetric distribution.	$u(x)=\frac{a}{\sqrt{6}}$

Normal distribution		
Form	Use when:	Uncertainty
	▪ An estimate is made from repeated observations of a randomly varying process. • An uncertainty is given in the form of a standard deviation s, a relative standard deviation $s/\bar{x}$, or a percentage coefficient of variance %CV without specifying the distribution. • An uncertainty is given in the form of a 95 % (or other) confidence interval $x \pm c$ without specifying the distribution.	$u(x) = s$ $u(x) = s$ $u(x) = x \cdot (s / \bar{x})$ $u(x) = \frac{\%CV}{100} \cdot x$ $u(x) = c/2$ (for c at 95 %) $u(x) = c/3$ (for c at 99.7 %)

E.2 Spreadsheet method for uncertainty calculation

E.2.1 Spreadsheet software can be used to simplify the calculations shown in Section 8. The procedure takes advantage of an approximate numerical method of differentiation, and requires knowledge only of the calculation used to derive the final result (including any necessary correction factors or influences) and of the numerical values of the parameters and their uncertainties. The description here follows that of Kragten [H.22].

E.2.2 In the expression for $u(y(x_1, x_2...x_n))$

$$\sqrt{\sum_{i=1,n}\left(\frac{\partial y}{\partial x_i}\cdot u(x_i)\right)^2 + \sum_{i,k=1,n}\left(\frac{\partial y}{\partial x_i}\cdot\frac{\partial y}{\partial x_k}\cdot u(x_i,x_k)\right)}$$

provided that either $y(x_1, x_2...x_n)$ is linear in x_i or $u(x_i)$ is small compared to x_i, the partial differentials $(\partial y/\partial x_i)$ can be approximated by:

$$\frac{\partial y}{\partial x_i} \approx \frac{y(x_i + u(x_i)) - y(x_i)}{u(x_i)}$$

Multiplying by $u(x_i)$ to obtain the uncertainty $u(y,x_i)$ in y due to the uncertainty in x_i gives

$$u(y,x_i) \approx y(x_1,x_2,..(x_i+u(x_i))..x_n)-y(x_1,x_2,..x_i..x_n)$$

Thus $u(y,x_i)$ is just the difference between the values of y calculated for $[x_i+u(x_i)]$ and x_i respectively.

E.2.3 The assumption of linearity or small values of $u(x_i)/x_i$ will not be closely met in all cases. Nonetheless, the method does provide acceptable accuracy for practical purposes when considered against the necessary approximations made in estimating the values of $u(x_i)$. Reference H.22 discusses the point more fully and suggests methods of checking the validity of the assumption.

E.2.4 The basic spreadsheet is set up as follows, assuming that the result y is a function of the four parameters p, q, r, and s:

i) Enter the values of p, q, *etc*. and the formula for calculating y in column A of the spreadsheet. Copy column A across the following columns once for every variable in y (see Figure E2.1). It is convenient to place the values of the uncertainties $u(p)$, $u(q)$ and so on in row 1 as shown.

ii) Add $u(p)$ to p in cell B3, $u(q)$ to q in cell C4 *etc*., as in Figure E2.2. On recalculating the spreadsheet, cell B8 then becomes $f(p+u(p), q, r..)$ (denoted by f (p', q, r, ..) in Figures E2.2 and E2.3), cell C8 becomes $f(p, q+u(q), r,..)$ *etc*.

iii) In row 9 enter row 8 minus A8 (for example, cell B9 becomes B8-A8). This gives the values of $u(y,p)$ as

$$u(y,p)=f(p+u(p), q, r ..) - f(p,q,r ..) \text{ etc.}$$

NOTE: This gives a signed difference; the magnitude is the estimated standard uncertainty and the sign denotes the direction of change.

iv) To obtain the standard uncertainty on y, these individual contributions are squared, added together and then the square root taken, by entering $u(y,p)^2$ in row 10 (Figure E2.3) and putting the square root of their sum in A10. That is, cell A10 is set to the formula

SQRT(SUM(B10+C10+D10+E10))

which gives the standard uncertainty on y.

E.2.5 The contents of the cells B10, C10 *etc*. show the squared contributions $u(y,x_i)^2=(c_iu(x_i))^2$ of the individual uncertainty components to the uncertainty on y and hence it is easy to see which components are significant.

E.2.6 It is straightforward to allow updated calculations as individual parameter values change or uncertainties are refined. In step i) above, rather than copying column A directly to columns B-E, copy the values p to s by reference, that is, cells B3 to E3 all reference A3, B4 to E4 reference A4 *etc*. The horizontal arrows in Figure E2.1 show the referencing for row 3. Note that cells B8 to E8 should still reference the values in columns B to E respectively, as shown for column B by the vertical arrows in Figure E2.1. In step ii) above, add the references to row 1 by reference (as shown by the arrows in Figure E2.1). For example, cell B3 becomes A3+B1, cell C4 becomes A4+C1 *etc*. Changes to either parameters or uncertainties will then be reflected immediately in the overall result at A8 and the combined standard uncertainty at A10.

E.2.7 If any of the variables are correlated, the necessary additional term is added to the SUM in A10. For example, if p and q are correlated, with a correlation coefficient $r(p,q)$, then the extra term $2\times r(p,q) \times u(y,p) \times u(y,q)$ is added to the calculated sum before taking the square root. Correlation can therefore easily be included by adding suitable extra terms to the spreadsheet.

Figure E2.1

	A	B	C	D	E
1		u(p)	u(q)	u(r)	u(s)
2					
3	p	p	p	p	p
4	q	q	q	q	q
5	r	r	r	r	r
6	s	s	s	s	s
7					
8	y=f(p,q,..)	y=f(p,q,..)	y=f(p,q,..)	y=f(p,q,..)	y=f(p,q,..)
9					
10					
11					

Figure E2.2

	A	B	C	D	E
1		u(p)	u(q)	u(r)	u(s)
2					
3	p	p+u(p)	p	p	p
4	q	q	q+u(q)	q	q
5	r	r	r	r+u(r)	r
6	s	s	s	s	s+u(s)
7					
8	y=f(p,q,..)	y=f(p',...)	y=f(..q',..)	y=f(..r',..)	y=f(..s',..)
9		u(y,p)	u(y,q)	u(y,r)	u(y,s)
10					
11					

Figure E2.3

	A	B	C	D	E
1		u(p)	u(q)	u(r)	u(s)
2					
3	p	p+u(p)	p	p	p
4	q	q	q+u(q)	q	q
5	r	r	r	r+u(r)	r
6	s	s	s	s	s+u(s)
7					
8	y=f(p,q,..)	y=f(p',...)	y=f(..q',..)	y=f(..r',..)	y=f(..s',..)
9		u(y,p)	u(y,q)	u(y,r)	u(y,s)
10	u(y)	$u(y,p)^2$	$u(y,q)^2$	$u(y,r)^2$	$u(y,s)^2$
11					

E.3 Evaluation of Uncertainty using Monte Carlo Simulation

E.3.1 Introduction

Working group 1 (WG1) of the Joint Committee for Guides in Metrology (JCGM) published in 2008 a Supplement (GS1) to the GUM [H.23]. The Supplement describes a general approach termed "the propagation of distributions" for the evaluation of uncertainty of measurement. This approach is implemented numerically as a Monte Carlo Simulation (MCS) method. The method is simple in principle and easy to use given appropriate software. It is applicable in essentially all the circumstances in which the GUM and Kragten approach apply; in addition, it can be used when a measurement result is calculated by an iterative numerical procedure. This section gives a brief description of the method.

E.3.2 Principle

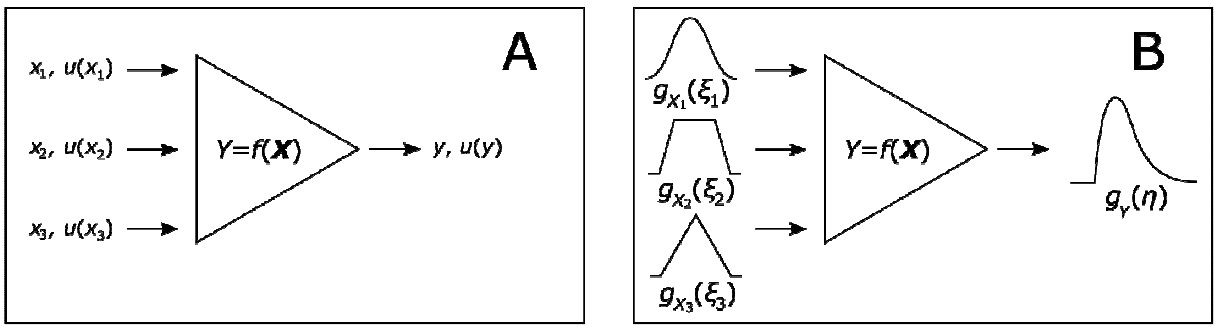
As in Appendix E.2, MCS requires a measurement model that describes the measurement process in terms of all the individual factors affecting the result. The measurement model can be in the form of an equation, as in Appendix E.2, or a computer programme or function that returns the measurement result. In addition, probability distributions (called the probability density functions or PDFs) for the input quantities, such as the normal, triangular or rectangular distributions described in Appendix E.1, are required. Section 8.1. describes how these PDFs can be obtained from commonly available information about the input quantities, such as lower or upper limits, or estimates and associated standard uncertainties; GS1 gives additional guidance for other cases.

Monte Carlo Simulation calculates the result corresponding to one value of each input quantity drawn at random from its PDF, and repeats this calculation a large number of times (trials), typically 10^5 to10^6. This process produces a set of simulated results which, under certain assumptions, forms an approximation to the PDF for the value of the measurand. From this set of simulated results, the mean value and standard deviation are calculated. In GUM Supplement 1, these are used respectively as the estimate of the measurand and the standard uncertainty associated with this estimate. This process is illustrated in Figure E.3.1B and compared with the usual GUM procedure in Figure E.3.1A. The GUM procedure combines the standard uncertainties associated with the estimates of the input quantities to give the standard uncertainty associated with the estimate of the measurand; the Supplement 1 procedure (Figure 1B) uses the input distributions to calculate an output distribution.

E.3.3 Relationship between MCS, GUM and Kragten approaches

In most cases the GUM, Kragten and MCS methods will give virtually the same value for the standard uncertainty associated with the estimate of the measurand. Differences become apparent when distributions are far from Normal and where the measurement result depends non-linearly on one or more input quantities. Where there is appreciable non-linearity, the basic GUM

Figure E.3.1



The Figure compares (A) the law of propagation of uncertainty and (B) the propagation of distributions for three independent input quantities. $g(\xi_i)$ is the probability density function (PDF) associated with x_i and $g(\eta)$ the density function for the result. .

approach given in section 8. applies poorly. Non-linearity is addressed in the GUM by extending the calculation to include higher-order terms (reference H.2 gives further detail). If that is the case, the Kragten approach (Appendix E2) is likely to give a more realistic estimate of the uncertainty than the first-order equation in section 8.2.2. because the Kragten approach calculates the actual changes in the result when the input quantities change by the standard uncertainty. MCS (for large enough simulations) gives a still better approximation because it additionally explores the extremes of the input and output distributions. Where distributions are substantially non-normal, the Kragten and basic GUM approaches provide estimated standard uncertainty, whereas MCS can give an indication of distribution and accordingly provides a better indication of the real 'coverage interval' than the interval $y \pm U$.

The principal disadvantages of MCS are

- greater computational complexity and computing time, especially if reliable intervals are to be obtained;
- calculated uncertainties vary from one run to the next because of the intentionally random nature of the simulation;
- it is difficult to identify the most important contributions to the combined uncertainty without repeating the simulation.

Using the basic GUM method, Kragten approach and MCS together, however, is nearly always useful in developing an appropriate strategy because the three give insight into different parts of the problem. Substantial differences between basic GUM and Kragten approaches will often indicate appreciable non-linearity, while large differences between the Kragten or basic GUM approach and MCS may signal important departures from normality. When the different methods give significantly different results, the reason for the difference should therefore be investigated.

E.3.4 Spreadsheet Implementation

MCS is best implemented in purpose-designed software. However, it is possible to use spreadsheet functions such as those listed in Table E3.1 to provide MCS estimates for modest simulation sizes. The procedure is illustrated using the following simple example, in which a value y is calculated from input values a, b and c according to

$$y = \frac{a}{b - c}$$

(This might, for example, be a mass fraction calculated from a measured analyte mass a and small gross and tare masses b and c respectively). The values, standard uncertainties and assigned distributions for a to c are listed in rows 3 and 4 of Table E3.2.

Table E3.2 also illustrates the procedure:

i) Input parameter values and their standard uncertainties (or, optionally for rectangular or triangular distributions, half-interval width) are entered at rows 3 and 4 of the spreadsheet.

Table E3.1: Spreadsheet formulae for Monte Carlo Simulation

Distribution	Formula for PDF[Note 1]
Normal	NORMINV(RAND(),*x*,*u*)
Rectangular	
given half-width *a*:	*x*+2**a**(RAND()-0.5)
given standard uncertainty *u*:	*x*+2**u**SQRT(3) *(RAND()-0.5)
Triangular	
given half-width *a*:	*x*+*a**(RAND()-RAND())
given standard uncertainty *u*:	*x*+*u**SQRT(6) *(RAND()-RAND())
t[Note 2]	*x*+*u**TINV(RAND(),ν_{eff})

Note 1. In these formulae, x should be replaced with the value of the input quantity x_i, u with the corresponding standard uncertainty, a with the half-width of the rectangular or triangular distribution concerned, and ν with the relevant degrees of freedom

Note 2. This formula is applicable when the standard uncertainty is given and known to be associated with a t distribution with ν degrees of freedom. This is typical of a reported standard uncertainty with reported effective degrees of freedom ν_{eff}.

Table E3.2: Spreadsheet implementation of Monte Carlo Simulation

	A	B	C	D	E	F	G
1							
2			a	b	c		y
3		Value	1.00	3.00	2.00		=C3/(D3-E3)
4		Standard uncertainty	0.05	0.15	0.10		=STDEV (G8:G507)
5		Distribution	Normal	Normal	Normal		
6							
7		Simulation	a	b	c		y
8			=NORMINV(RAND(), C$3,C$4)	=NORMINV(RAND(), D$3,D$4)	=NORMINV(RAND(), E$3,E$4)		=C8/(D8-E8)
9			1.024702	2.68585	1.949235		1.39110
10			1.080073	3.054451	1.925224		0.95647
11			0.943848	2.824335	2.067062		1.24638
12			0.970668	2.662181	1.926588		1.31957
⋮			⋮	⋮	⋮		⋮
506			1.004032	3.025418	1.861292		0.86248
507			0.949053	2.890523	2.082682		1.17480
508							

The values of the parameters are entered in the second row from C2 to E2, and their standard uncertainties in the row below (C3:E3). The calculation for the result y is entered in cell G3. Appropriate formulae for random number generation are entered in row 8, together with a copy of the calculation for the result (at G8, here). Note that G8 refers to the simulated values in row 8. Row 8 is copied down to give the desired number of Monte Carlo replicates; the figure shows the resulting random values from row 9 onward). The standard uncertainty in y is calculated as the standard deviation of the resulting simulated values of y.

ii) The calculation for the result y is entered at row 3, to the right of the list of input values.

iii) Starting from a suitable row below the values and uncertainties (row 8 is the starting row in Table E3.2), the appropriate formulae for each distribution are entered under each input parameter. Useful spreadsheet formulae for generating random samples from different PDFs are listed in Table E3.1. Notice that the formulae must include *fixed* references to the rows containing the parameter values and uncertainties (indicated by the $ in the formulae).

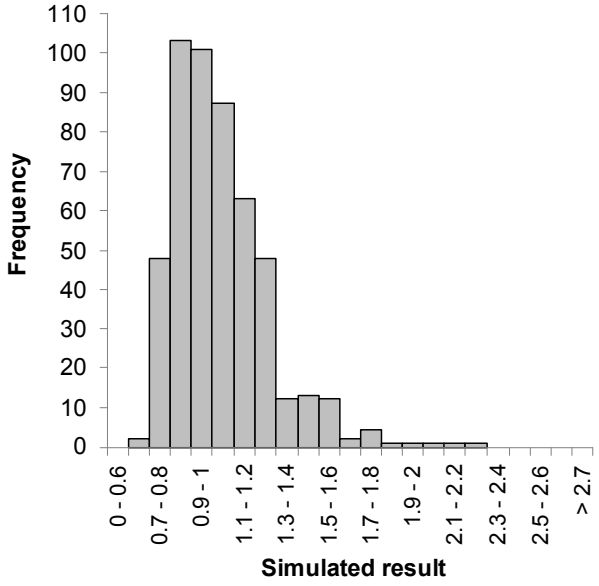
iv) The calculation for the result y is copied to the first row of random values, to the right of the list of input values.

v) The row containing random value formulae and the formula for the corresponding calculated result is copied down to give the desired number of replicates (500 in Table E3.2)

vi) The MCS estimate of the standard uncertainty in y is the standard deviation of all the simulated values of y; this is shown in cell G4 in Table E3.2.

The distribution can be inspected by generating a histogram using built-in spreadsheet functions. For the present example, using the values in Table E3.2, 500 replicates gave a standard uncertainty in y of 0.23. Repeating the simulation ten times (by recalculating the spreadsheet) gave values of standard uncertainty in the range 0.197 to 0.247. Comparison with the standard uncertainty of 0.187 calculated using the basic GUM approach shows that the simulation generally gives higher estimates of standard uncertainty. The reason for this can be seen on inspecting a histogram of the simulated results (Figure E3.1); although the input parameter distributions were normally

Figure E3.1: Example histogram of simulated results



distributed, the output shows appreciable positive skew, resulting in a higher standard uncertainty than expected. This arises from appreciable non-linearity; notice that the uncertainties in *b* and *c* are appreciable fractions of the denominator *b*-*c*, resulting in a proportion of very small values for the denominator and corresponding high estimates for *y*.

E.3.5 Practical considerations in using MCS for uncertainty evaluation

Number of MCS samples

MCS gives a good estimate of the standard uncertainty even for simulations with a few hundred trials; with as few as 200 trials, estimated standard uncertainties are expected to vary by about ±10 % from the best estimate, while for 1000 and 10 000 samples the expected ranges are about ±5 % and ±1.5 % (based on the 95 % interval for the chi-squared distribution). Bearing in mind that many input quantity uncertainties are derived from far fewer observations, comparatively small simulations of 500-5000 MCS samples are likely to be adequate at least for exploratory studies and often for reported standard uncertainties. For this purpose, spreadsheet MCS calculations are often sufficient.

Confidence intervals from MCS

It is also possible in principle to estimate confidence intervals from the MCS results without the use of effective degrees of freedom, for example by using the relevant quantiles. However, it is important not to be misled by the apparent detail in the PDF obtained for the result. The lack of detailed knowledge about the PDFs for the input quantities, because the information on which these PDFs are based is not always reliable, needs to be borne in mind. The tails of the PDFs are particularly sensitive to such information. Therefore, as is pointed out in GUM, section G 1.2, "it is normally unwise to try to distinguish between closely similar levels of confidence (say a 94 % and a 96 % level of confidence)". In addition, the GUM indicates that obtaining intervals with levels of confidence of 99 % or greater is especially difficult. Further, to obtain sufficient information about the tails of the PDF for the output quantity can require calculating the result for at least 10^6 trials . It then becomes important to ensure that the random number generator used by the software is capable of maintaining randomness for such large numbers of draws from the PDFs for the input quantities; this requires well characterised numerical software. GS1 recommends some reliable random number generators.

Bias due to asymmetry in the output distribution

When the measurement model is non-linear and the standard uncertainty associated with the estimate *y* is large compared to y (that is, u(y)/y is much greater than 10 %) the MCS PDF is likely to be asymmetric. In this case the mean value computed from the simulated results will be

different from the value of the measurand calculated using the estimates of the input quantities (as in GUM). For most practical purposes in chemical measurement, the result calculated from the original input values should be reported; the MCS estimate can, however, be used to provide the associated standard uncertainty.

E.3.6 Example of MCS evaluation of uncertainty

The following example is based on Example A2, determination of the concentration of sodium hydroxide using a potassium hydrogen phthalate (KHP) reference material.

The measurement function for the concentration c_{NaOH} of NaOH is

$$c_{\mathrm{NaOH}} = \frac{1\,000 m_{\mathrm{KHP}} P_{\mathrm{KHP}}}{M_{\mathrm{KHP}} V} \qquad [\mathrm{mol\ l^{-1}}],$$

where

m_{KHP} is the mass of the KHP,

P_{KHP} is the purity of KHP,

M_{KHP} is the molar mass of the KHP, and

V is the volume of NaOH for KHP titration.

Some of the quantities in this measurement function are themselves expressed in terms of further quantities. A representation of this function in terms of fundamental quantities is needed, because each of these quantities has to be described by a PDF as the basis of the Monte Carlo calculation.

m_{KHP} is obtained by difference weighings:

$$m_{\mathrm{KHP}} = m_{\mathrm{KHP},1} - m_{\mathrm{KHP},2}.$$

M_{KHP} Molar mass of KHP comprises four terms for the different elements in the molecular formula:

$$M_{\mathrm{KHP}} = M_{C_8} + M_{H_5} + M_{O_4} + M_K.$$

V depends on temperature and the calibration of the measuring system:

$$V = V_{\mathrm{T}}\left[1 + \alpha(T - T_0)\right]$$

Where α is the coefficient of volume expansion for water T is the laboratory temperature and T_0 the temperature at which the flask was calibrated

Further, a quantity R representing repeatability effects is included.

The resulting measurement function is

$$c_{\mathrm{NaOH}} = \frac{1000\left(m_{\mathrm{KHP},1} - m_{\mathrm{KHP},2}\right)}{\left(M_{\mathrm{C_8}} + M_{\mathrm{H_5}} + M_{\mathrm{O_4}} + M_{\mathrm{K}}\right) V_{\mathrm{T}}\left[1 - \alpha\left(T - T_0\right)\right]} \qquad [\mathrm{mol\ L^{-1}}]$$

These input quantities are each characterized by an appropriate PDF, depending on the information available about these quantities. Table A2.4 lists these quantities and gives the characterizing PDFs.

Since the contribution from V_{T} is dominant, two PDFs (Triangular, Normal) other than Rectangular are considered for this quantity to see the effect on the calculated results.

The standard uncertainty $u(c_{\mathrm{NaOH}})$ computed for the concentration c_{NaOH} with the three different PDFs for the uncertainty on V_{T} agree very well with those obtained conventionally using the GUM (Table E3.3) or Kragten approaches. Also the coverage factors k, obtained from the values of the results below and above which 2.5 % of the tails fell, correspond to those of a Normal distribution and support using $k = 2$ for the expanded uncertainty. However, the PDF for the concentration c_{NaOH} is discernibly influenced by using the rectangular distribution for the uncertainty on V_{T} . The calculations were carried out using a number of Monte Carlo trials ranging from 10^4 to 10^6; however a value of 10^4 gave sufficiently stable values for k and $u(c_{\mathrm{NaOH}})$. Larger numbers of trials give smoother approximations to the PDFs.

Table E3.3: Values, uncertainties and distributions for Example A2

Quantity	Description	Unit	Value	Standard uncertainty or half-width	Distribution
R	repeatability factor	1	1.0000	0.0005	Normal
$m_{\mathrm{KHP},1}$	container and KHP	g	60.5450	0.00015	Rectangular
$m_{\mathrm{KHP},2}$	container less KHP	g	60.1562	0.00015	Rectangular
P_{KHP}	purity of KHP	1	1.0000	0.0005	Rectangular
$M_{\mathrm{C_8}}$	molar mass of C_8	mol^{-1}	96.0856	0.0037	Rectangular
$M_{\mathrm{H_5}}$	molar mass of H_5	mol^{-1}	5.0397	0.00020	Rectangular
$M_{\mathrm{O_4}}$	molar mass of O_4	mol^{-1}	63.9976	0.00068	Rectangular
M_{K}	molar mass of K	mol^{-1}	39.0983	0.000058	Rectangular
V_{T}	volume of NaOH for KHP titration	mL	18.64	0.03	Rectangular
T-T_0	temperature calibration factor	K	0.0	1.53	Normal
α	Volume coefficient of expansion	$^{\circ}\mathrm{C}^{-1}$	2.1×10^{-4}	Negligible	

Table E3.3: Comparison of values for the uncertainty $u(c_{NaOH})$ obtained using GUM and MCS with different PDFs for the uncertainty on V_T

	V_T Triangular PDF	V_T Normal PDF	V_T Rectangular PDF
GUM*	**0 000099** mol L^{-1}	0.000085 mol L^{-1}	0.00011 mol L^{-1}
MCS	**0 000087** mol L^{-1}	**0 000087** mol L^{-1}	0.00011 mol L^{-1}

*The results from GUM and Kragten [E.2] approaches agree to at least two significant figures.

Figure E3.2: Concentration c_{NaOH} of based on V_T characterized by a triangular PDF k_{95}=1.94 u=0.000087 GUM value 0.00009

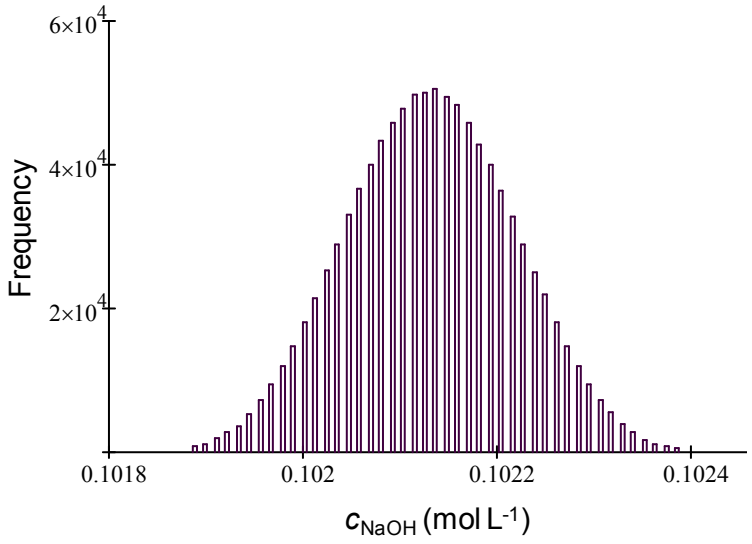
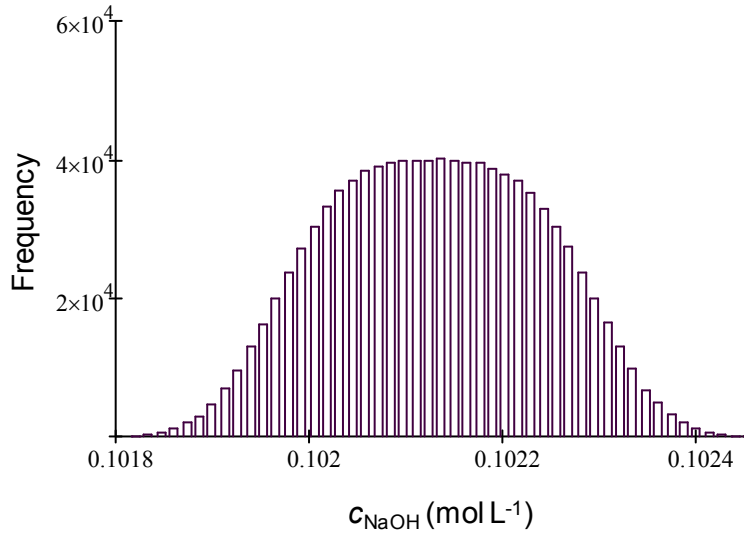


Figure E3.3: As Figure E3.2, but with V_T characterized by a Rectangular PDF k_{95}=1.83 u=0.00011



E.4 Uncertainties from linear least squares calibration

E.4.1 An analytical method or instrument is often calibrated by observing the responses, y, to different levels of the analyte, x. In most cases this relationship is taken to be linear viz:

$$y = b_0 + b_1 x \qquad \text{Eq. E3.1}$$

This calibration line is then used to obtain the concentration x_{pred} of the analyte from a sample which produces an observed response y_{obs} from

$$x_{pred} = (y_{obs} - b_0)/b_1 \qquad \text{Eq. E3.2}$$

It is usual to determine the constants b_1 and b_0 by weighted or un-weighted least squares regression on a set of n pairs of values (x_i, y_i).

E.4.2 There are four main sources of uncertainty to consider in arriving at an uncertainty on the estimated concentration x_{pred}:

- Random variations in measurement of y, affecting both the reference responses y_i and the measured response y_{obs}.
- Random effects resulting in errors in the assigned reference values x_i.
- Values of x_i and y_i may be subject to a constant unknown offset, for example arising when the values of x are obtained from serial dilution of a stock solution
- The assumption of linearity may not be valid

Of these, the most significant for normal practice are random variations in y, and methods of estimating uncertainty for this source are detailed here. The remaining sources are also considered briefly to give an indication of methods available.

E.4.3 The uncertainty $u(x_{pred}, y)$ in a predicted value x_{pred} due to variability in y can be estimated in several ways:

From calculated variance and covariance.

If the values of b_1 and b_0, their variances var(b_1), var(b_0) and their covariance, covar(b_1,b_0), are determined by the method of least squares, the variance on x, var(x), obtained using the formula in Chapter 8. and differentiating the normal equations, is given by

$$\text{var}(x_{pred}) = \frac{\text{var}(y_{obs}) + x_{pred}^2 \cdot \text{var}(b_1) + 2 \cdot x_{pred} \cdot \text{covar}(b_0, b_1) + \text{var}(b_0)}{b_1^2} \qquad \text{Eq. E3.3}$$

and the corresponding uncertainty $u(x_{pred}, y)$ is $\sqrt{\text{var}(x_{pred})}$.

From the calibration data.

The above formula for var(x_{pred}) can be written in terms of the set of n data points, (x_i, y_i), used to determine the calibration function:

$$\text{var}(x_{pred}) = \text{var}(y_{obs})/b_1^2 + \frac{S^2}{b_1^2} \cdot \left(\frac{1}{\sum w_i} + \frac{(x_{pred} - \bar{x})^2}{\cdot (\sum (w_i x_i^{\,2}) - (\sum w_i x_i)^2 / \sum w_i)} \right) \qquad \text{Eq. E3.4}$$

where $S^2 = \dfrac{\sum w_i (y_i - y_{fi})^2}{(n-2)}$, $(y_i - y_{fi})$ is the residual for the i^{th} point, n is the number of data points in the calibration, b_1 the calculated best fit gradient, w_i the weight assigned to y_i and $(x_{pred} - \bar{x})$ the difference between x_{pred} and the mean $\bar{x}$ of the n values x_1, x_2....

For unweighted data and where var(y_{obs}) is based on p measurements, equation E3.4 becomes

$$\text{var}(x_{pred}) = \frac{S^2}{b_1^2} \cdot \left(\frac{1}{p} + \frac{1}{n} + \frac{(x_{pred} - \bar{x})^2}{(\sum (x_i^2) - (\sum x_i)^2 / n)} \right) \qquad \text{Eq. E3.5}$$

This is the formula which is used in example 5 with $S_{xx} = \left[\sum (x_i^2) - \left(\sum x_i \right)^2 / n \right] = \sum (x_i - \bar{x})^2$.

From information given by software used to derive calibration curves.

Some software gives the value of S, variously described for example as RMS error or residual standard error. This can then be used in equation E3.4 or E3.5. However some software may also give the standard deviation $s(y_c)$ on a value of y calculated from the fitted line for some new value of x and this can be used to calculate var(x_{pred}) since, for p=1

$$s(y_c) = S \sqrt{1 + \frac{1}{n} + \frac{(x_{pred} - \bar{x})^2}{\left(\sum (x_i^2) - \left(\sum x_i \right)^2 / n \right)}}$$

giving, on comparison with equation E3.5,

$$\text{var}(x_{\text{pred}}) = [\, s(y_c) / b_1]^2 \qquad \text{Eq. E3.6}$$

E.4.4 The reference values x_i may each have uncertainties which propagate through to the final result. In practice, uncertainties in these values are usually small compared to uncertainties in the system responses y_i, and may be ignored. An approximate estimate of the uncertainty $u(x_{\text{pred}}, x_i)$ in a predicted value x_{pred} due to uncertainty in a particular reference value x_i is

$$u(x_{\text{pred}}, x_i) \approx u(x_i)/n \qquad \text{Eq. E3.7}$$

where n is the number of x_i values used in the calibration. This expression can be used to check the significance of $u(x_{\text{pred}}, x_i)$.

E.4.5 The uncertainty arising from the assumption of a linear relationship between y and x is not normally large enough to require an additional estimate. Providing the residuals show that there is no significant systematic deviation from this assumed relationship, the uncertainty arising from this assumption (in addition to that covered by the resulting increase in y variance) can be taken to be negligible. If the residuals show a systematic trend then it may be necessary to include higher terms in the calibration function. Methods of calculating var(x) in these cases are given in standard texts. It is also possible to make a judgement based on the size of the systematic trend.

E.4.6 The values of x and y may be subject to a constant unknown offset (e.g. arising when the values of x are obtained from serial dilution of a stock solution which has an uncertainty on its certified value). If the standard uncertainties on y and x from these effects are $u(y, \text{const})$ and $u(x, \text{const})$, then the uncertainty on the interpolated value x_{pred} is given by:

$$u(x_{\text{pred}})^2 = u(x, \text{const})^2 + (u(y, \text{const})/b_1)^2 + \text{var}(x) \qquad \text{Eq. E3.8}$$

E.4.7 The four uncertainty components described in E.4.2 can be calculated using equations Eq. E3.3 to Eq. E3.8. The overall uncertainty arising from calculation from a linear calibration can then be calculated by combining these four components in the normal way.

E.4.8 While the calculations above provide suitable approaches for the most common case of linear least squares regression, they do not apply to more general regression modelling methods that take account of uncertainties in x or correlations among x and/or y. A treatment of these more complex cases can be found in ISO TS 28037, *Determination and use of straight-line calibration functions* [H.28].

E.5 Documenting uncertainty dependent on analyte level

E.5.1 Introduction

E.5.1.1 It is often observed in chemical measurement that, over a large range of analyte concentrations (levels), dominant contributions to the overall uncertainty vary approximately proportionately to the level of analyte, that is $u(x) \propto x$. In such cases it is often sensible to quote uncertainties as relative standard deviations or coefficient of variation (for example, %CV).

E.5.1.2 Where the uncertainty is unaffected by level, for example at low levels, or where a relatively narrow range of analyte level is involved, it is generally most sensible to quote an absolute value for the uncertainty.

E.5.1.3 In some cases, both constant and proportional effects are important. This section sets out a general approach to recording uncertainty information where variation of uncertainty with analyte level is an issue and reporting as a simple coefficient of variation is inadequate.

E.5.2 Basis of approach

E.5.2.1 To allow for both proportionality of uncertainty and the possibility of an essentially constant value with level, the following general expression is used:

$$u(x)=\sqrt{s_0^2+(x \cdot s_1)^2} \qquad [1]$$

where

- $u(x)$ is the combined standard uncertainty in the result x (that is, the uncertainty expressed as a standard deviation)
- s_0 represents a constant contribution to the overall uncertainty
- s_1 is a proportionality constant.

The expression is based on the normal method of combining of two contributions to overall uncertainty, assuming one contribution (s_0) is constant and one (xs_1) proportional to the result. Figure E.5.1 shows the form of this expression.

NOTE: The approach above is practical only where it is possible to calculate a large number of values. Where experimental study is employed, it will not often be possible to establish the relevant parabolic relationship. In such circumstances, an adequate approximation can be obtained by simple linear regression through four or more combined uncertainties obtained at different analyte concentrations. This procedure is consistent with that employed in studies of reproducibility and repeatability according to ISO 5725:1994. The relevant expression is then $u(x) \approx s'_0 + x.s'_1$

E.5.2.2 The figure can be divided into approximate regions (**A** to **C** on the figure):

A: The uncertainty is dominated by the term s_0, and is approximately constant and close to s_0.

B: Both terms contribute significantly; the resulting uncertainty is significantly higher than either s_0 or xs_1, and some curvature is visible.

C: The term xs_1 dominates; the uncertainty rises approximately linearly with increasing x and is close to xs_1.

E.5.2.3 Note that in many experimental cases the complete form of the curve will not be apparent. Very often, the whole reporting range of analyte level permitted by the scope of the method falls within a single chart region; the result is a number of special cases dealt with in more detail below.

E.5.3 Documenting level-dependent uncertainty data

E.5.3.1 In general, uncertainties can be documented in the form of a value for each of s_0 and s_1. The values can be used to provide an uncertainty estimate across the scope of the method. This is particularly valuable when calculations for well characterised methods are implemented on computer systems, where the general form of the equation can be implemented independently of the values of the parameters (one of which may be zero - see below). It is accordingly recommended that, except in the special cases outlined below or where the dependence is strong but not linear*, uncertainties

* An important example of non-linear dependence is the effect of instrument noise on absorbance measurement at high absorbances near the upper limit of the instrument capability. This is particularly pronounced where absorbance is calculated from transmittance (as in infrared spectroscopy). Under these circumstances, baseline noise causes very large uncertainties in high absorbance figures, and the

are documented in the form of values for a constant term represented by s_0 and a variable term represented by s_1.

E.5.4. Special cases

E.5.4.1. Uncertainty not dependent on level of analyte (s_0 dominant)

The uncertainty will generally be effectively independent of observed analyte concentration when:

- The result is close to zero (for example, within the stated detection limit for the method). Region **A** in Figure E.5.1
- The possible range of results (stated in the method scope or in a statement of scope for the uncertainty estimate) is small compared to the observed level.

Under these circumstances, the value of s_1 can be recorded as zero. s_0 is normally the calculated standard uncertainty.

E.5.4.2. Uncertainty entirely dependent on analyte (s_1 dominant)

Where the result is far from zero (for example, above a 'limit of determination') and there is clear evidence that the uncertainty changes proportionally with the level of analyte permitted within the scope of the method, the term xs_1 dominates (see Region **C** in Figure E.5.1). Under these circumstances, and where the method scope does not include levels of analyte near zero, s_0 may reasonably be recorded as zero and s_1 is simply the uncertainty expressed as a relative standard deviation.

E.5.4.3. Intermediate dependence

In intermediate cases, and in particular where the situation corresponds to region **B** in Figure E.5.1, two approaches can be taken:

a) Applying variable dependence

The more general approach is to determine, record and use both s_0 and s_1. Uncertainty estimates, when required, can then be produced on the basis of the reported result. This remains the recommended approach where practical.

NOTE: See the note to section E.5.2.

b) Applying a fixed approximation

An alternative which may be used in general testing and where

- the dependence is not strong (that is, evidence for proportionality is weak)

or

- the range of results expected is moderate

leading in either case to uncertainties which do not vary by more than about 15 % from an average uncertainty estimate, it will often be reasonable to calculate and quote a fixed value of uncertainty for general use, based on the mean value of results expected. That is,

either

a mean or typical value for x is used to calculate a fixed uncertainty estimate, and this is used in place of individually calculated estimates

or

a single standard deviation has been obtained, based on studies of materials covering the full range of analyte levels permitted (within the scope of the uncertainty estimate), and there is little evidence to justify an assumption of proportionality. This should generally be treated as a case of zero dependence, and the relevant standard deviation recorded as s_0.

E.5.5. Determining s_0 and s_1

E.5.5.1. In the special cases in which one term dominates, it will normally be sufficient to use the uncertainty as standard deviation or relative standard deviation respectively as values of s_0 and s_1. Where the dependence is less obvious, however, it may be necessary to determine s_0 and s_1 indirectly from a series of estimates of uncertainty at different analyte levels.

E.5.5.2. Given a calculation of combined uncertainty from the various components, some of which depend on analyte level while others do not, it will normally be possible to investigate the dependence of overall uncertainty on analyte level by simulation. The procedure is as follows:

uncertainty rises much faster than a simple linear estimate would predict. The usual approach is to reduce the absorbance, typically by dilution, to bring the absorbance figures well within the working range; the linear model used here will then normally be adequate. Other examples include the 'sigmoidal' response of some immunoassay methods.

1: Calculate (or obtain experimentally) uncertainties $u(x_i)$ for at least ten levels x_i of analyte, covering the full range permitted.
2. Plot $u(x_i)^2$ against x_i^2
3. By linear regression, obtain estimates of m and c for the line $u(x)^2 = mx^2 + c$
4. Calculate s_0 and s_1 from $s_0 = \sqrt{c}$, $s_1 = \sqrt{m}$
5. Record s_0 and s_1

E.5.6. **Reporting**

E.5.6.1. The approach outlined here permits estimation of a standard uncertainty for any single result. In principle, where uncertainty information is to be reported, it will be in the form of

[result] ± [uncertainty]

where the uncertainty as standard deviation is calculated as above, and if necessary expanded (usually by a factor of two) to give increased confidence. Where a number of results are reported together, however, it may be possible, and is perfectly acceptable, to give an estimate of uncertainty applicable to all results reported.

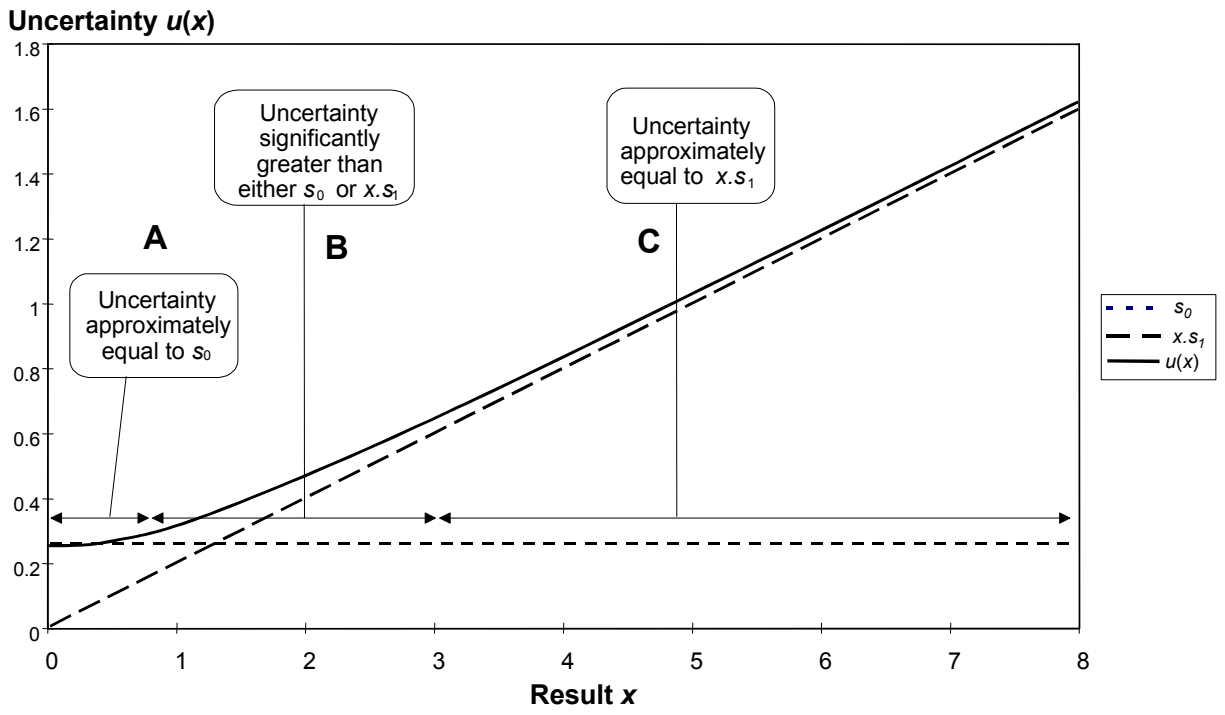
E.5.6.2. Table E.5.1 gives some examples. The uncertainty figures for a list of different analytes may usefully be tabulated following similar principles.

NOTE: Where a 'detection limit' or 'reporting limit' is used to give results in the form "<x" or "nd", it will normally be necessary to quote the limits used in addition to the uncertainties applicable to results above reporting limits.

Table E.5.1: Summarising uncertainty for several samples

Situation	Dominant term	Reporting example(s)
Uncertainty essentially constant across all results	s_0 or fixed approximation (sections E.5.4.1. or E.5.4.3.a)	Standard deviation: expanded uncertainty; 95 % confidence interval
Uncertainty generally proportional to level	xs_1 (see section E.5.4.2.)	relative standard deviation or coefficient of variance, optionally as percentage.
Mixture of proportionality and lower limiting value for uncertainty	Intermediate case (section E.5.4.3.)	quote CV or RSD, optionally as percentage, together with lower limit as standard deviation.

Figure E.5.1: Variation of uncertainty with observed result



Appendix F. Measurement Uncertainty at the Limit of Detection/Limit of Determination

F.1. Introduction

F.1.1. At low concentrations, an increasing number of effects become important, including, for example,

- the presence of noise or unstable baseline,
- the contribution of interferences to the (gross) signal,
- the influence of any analytical blank used, and
- losses during extraction, isolation or clean-up.

Because of such effects, as the analyte concentrations drop, the relative uncertainty associated with the result tends to increase, first to a substantial fraction of the result and finally to the point where the (symmetric) uncertainty interval includes zero. This region is typically associated with the practical limit of detection for a given method.

F.1.2. The terminology and conventions associated with measuring and reporting low levels of analyte have been widely discussed elsewhere (See Bibliography [H.29-H.32] for examples and definitions). Here, the term 'limit of detection' follows the IUPAC recommendation of reference H.31 which defines the limit of detection as a true amount of analyte which leads with high probability to the conclusion that the analyte is present, given a particular decision criterion. The decision criterion ('critical value') is usually set to ensure a low probability of declaring the analyte present when it is in fact absent. Following this convention, a analyte is declared present when the observed response is above the critical value. The limit of detection is usually approximately twice the critical value expressed in terms of analyte concentration.

F.1.3. It is widely accepted that the most important use of the 'limit of detection' is to show where method performance becomes insufficient for acceptable quantitation, so that improvements can be made. Ideally, therefore, quantitative measurements should not be made in this region. Nonetheless, so many analytes are important at very low levels that it is inevitable that measurements must be made, and results reported, in this region.

F.1.4. The ISO Guide on Measurement Uncertainty [H.2] does not give explicit instructions for the estimation of uncertainty when the results are small and the uncertainties large compared to the results. Indeed, the basic form of the 'law of propagation of uncertainties', described in chapter 8 of this guide, may cease to apply accurately in this region; one assumption on which the calculation is based is that the uncertainty is small relative to the value of the measurand. An additional, if philosophical, difficulty follows from the definition of uncertainty given by the ISO Guide: though negative observations are quite possible, and even common in this region, an implied dispersion including values below zero cannot be "... reasonably ascribed to the value of the measurand" when the measurand is a concentration, because concentrations themselves cannot be negative.

F.1.5. These difficulties do not preclude the application of the methods outlined in this guide, but some caution is required in interpretation and reporting the results of measurement uncertainty estimation in this region. The purpose of the present Appendix is to provide guidance to supplement that already available from other sources.

NOTE: Similar considerations may apply to other regions; for example, mole or mass fractions close to 100 % may lead to similar difficulties.

F.2. Observations and estimates

F.2.1. A fundamental principle of measurement science is that *results are estimates of true values*. Analytical results, for example, are available initially in units of the observed signal, e.g. mV, absorbance units *etc*. For communication to a wider audience, particularly to the customers of a

laboratory or to other authorities, the raw data need to be converted to a chemical quantity, such as concentration or amount of substance. This conversion typically requires a calibration procedure (which may include, for example, corrections for observed and well characterised losses). Whatever the conversion, however, the figure generated remains an observation, or signal. If the experiment is properly carried out, this observation remains the 'best estimate' of the value of the measurand.

F.2.2. Observations are not often constrained by the same fundamental limits that apply to real concentrations. For example, it is perfectly sensible to report an 'observed concentration', that is, an estimate, below zero. It is equally sensible to speak of a dispersion of possible *observations* which extends into the same region. For example, when performing an unbiased measurement on a sample with no analyte present, one *should* see about half of the observations falling below zero. In other words, reports like

observed concentration = 2.4±8 mg L^{-1}

observed concentration = -4.2±8 mg L^{-1}

are not only possible; they should be seen as valid statements about observations and their mean values.

F.2.3. While reporting observations and their associated uncertainties to an informed audience, there is no barrier to, or contradiction in, reporting the best estimate and its associated uncertainty even where the result implies an impossible physical situation. Indeed, in some circumstances (for example, when reporting a value for an analytical blank which will subsequently be used to correct other results) it is absolutely essential to report the observation and its uncertainty, however large.

F.2.4. This remains true wherever the end use of the result is in doubt. Since only the observation and its associated uncertainty can be used directly (for example, in further calculations, in trend analysis or for re-interpretation), the uncensored observation should always be available.

F.2.5. The ideal is accordingly to report valid observations and their associated uncertainty regardless of the values.

F.3. Interpreted results and compliance statements

F.3.1. Despite the foregoing, it must be accepted that many reports of analysis and statements of compliance include some interpretation for the end user's benefit. Typically, such an interpretation would include any relevant inference about the levels of analyte which could reasonably be present in a material. Such an interpretation is an inference about the real world, and consequently would be expected (by the end user) to conform to real limits. So, too, would any associated estimate of uncertainty in 'real' values. The following paragraphs summarise some accepted approaches. The first (use of 'less than' or 'greater than') is generally consistent with existing practice. Section F.5. describes an approach based on the properties of classical confidence intervals. This is very simple to use and will usually be adequate for most ordinary purposes. Where observations are particularly likely to fall below zero (or above 100 %), however, the classical approach may lead to unrealistically small intervals; for this situation, the Bayesian approach described in section F.6 is likely to be more appropriate.

F.4. Using 'less than' or 'greater than' in reporting

F.4.1. Where the end use of the reported results is well understood, and where the end user cannot realistically be informed of the nature of measurement observations, the use of 'less than', 'greater than' etc. should follow the general guidance provided elsewhere (for example in reference H.31) on the reporting of low level results.

F.4.2. One note of caution is pertinent. Much of the literature on capabilities of detection relies heavily on the statistics of repeated observations. It should be clear to readers of the current guide that observed variation is only rarely a good guide to the full uncertainty of results. Just as with results in any other region, careful consideration should accordingly be given to all the uncertainties affecting a given result before reporting the values.

F.5. Expanded uncertainty intervals near zero: Classical approach

F.5.1. The desired outcome is an expanded uncertainty interval which satisfies three requirements:

1. An interval that lies within the possible range (the 'possible range' is the concentration range from zero upwards).
2. A coverage close to the specified confidence level, so that an expanded uncertainty interval claimed to correspond to approximately 95 % confidence should be expected to contain the true value close to 95 % of the time.
3. Reported results that have minimal bias in the long term.

F.5.2. If the expanded uncertainty has been calculated using classical statistics, the interval – including any part lying below zero – will, by definition, have 95 % coverage. However, since the (true) value of the measurand cannot lie outside the possible range, it is possible to simply truncate this interval at the edge of the possible range and yet retain the required 95 % coverage. This truncated classical confidence interval therefore maintains exact 95 % coverage; it is also simple to implement using existing tools.

F.5.3. Where the mean observation is also outside the possible range, and the interval for the true concentration is required, the reported result should simply be shifted to zero. Shifting to this limit does, however, lead to a small long-term bias, which may well be unacceptable to customers (or PT providers) demanding raw data for their own statistical analysis. These customers will continue to require the raw observations regardless of natural limits. Nonetheless, simple truncation at zero can be shown to provide minimal bias among the range of options so far examined for this situation.

F.5.4. If this procedure is followed, the expanded uncertainty interval becomes progressively more asymmetric as the result approaches the limit. Figure 3 illustrates the situation near zero, where the measured mean is reported until the mean falls below zero, and the reported value is thereafter reported as zero.

F.5.5. Eventually, the classical interval falls entirely beyond the natural limit, implying an adjusted interval of [0, 0]. This may reasonably be taken as an indication that the results are inconsistent with any possible true concentration. The analyst should normally return to the original data and determine the cause, as for any other aberrant quality control observation.

F.5.6. If it is necessary to report the standard uncertainty as well as the (asymmetric) expanded uncertainty interval, it is recommended that the standard uncertainty used in constructing the confidence interval should be reported without change.

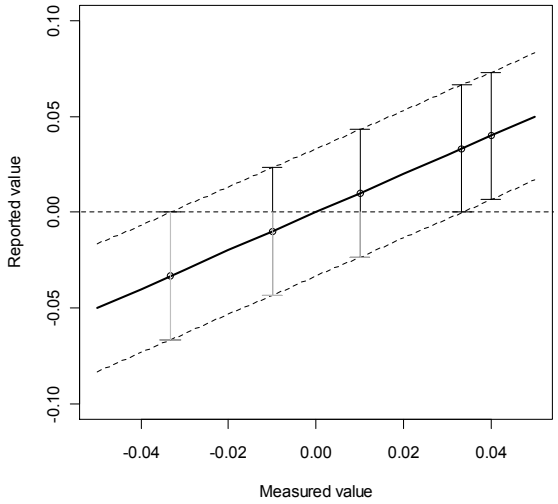


Figure 3. Truncating classical confidence intervals close to zero. The mean varies between -0.05 and 0.05, and the standard deviation is fixed at 0.01. The bold diagonal line shows how the reported value depends (before truncation) on the observed value; the diagonal dashed lines show the corresponding interval. The solid, partial bars show the reported uncertainty interval after truncation. Note that at observed mean values below zero, the simple truncated interval becomes unreasonably small; see paragraph F5.8.

F.6. Expanded uncertainty intervals near zero: Bayesian approach

F.6.1. Bayesian methods allow the combination of information from measurements with prior information about the possible (or likely) distribution of values of the measurand. The approach combines a 'prior' distribution with a likelihood (the distribution inferred from the

measurement results alone) to obtain a 'posterior distribution' which describes the distribution of values reasonably attributable to the measurand. The expanded uncertainty interval is then chosen to contain a suitable proportion of the distribution, while the reported value may be any point value that conveniently describes the location of the distribution. The mean, median and mode of the posterior distribution may all be used.

F.6.2. In the case of a quantity known to be limited to within a particular range (for example, above zero) and a measurement which provides information in the form of a *t*-distribution, it can be shown [H.32] that the resulting distribution of possible values is approximately a truncated *t*-distribution. To obtain a minimally biased result and an expanded uncertainty interval with appropriate coverage, it is recommended that

i) The mode of the posterior distribution be reported. For a truncated *t*-distribution, this is either the observed mean value or zero if the observed mean value is below zero.

ii) The expanded uncertainty interval is calculated as the maximum density interval containing the required fraction of the posterior distribution. The maximum density interval is also the shortest interval that contains the required fraction of the distribution.

F.6.3. For a *t* distribution based on an observed value $\bar{x}$, standard uncertainty u and (effective) degrees of freedom ν_{eff} the maximum density interval for the case of a lower bound at zero and level of confidence p may be obtained as follows:

i) Calculate

$$P_{\text{tot}} = 1 - P_{\text{t}}\left(-\bar{x}/u, \nu_{\text{eff}}\right)$$

where $P_{\text{t}}(q,\nu)$ is the cumulative probability for Student's *t*.

ii) Set

$$q_1 = q_{\text{t}}\left(1 - \left(1 - pP_{\text{tot}}\right)/2, \nu_{\text{eff}}\right)$$

where $q_{\text{t}}(P,\nu)$ is the quantile of student's *t* distribution for cumulative probability P and ν_{eff} degrees of freedom and p the desired level of confidence (usually 0.95).

iii) If $\left(\bar{x} - uq_1\right) \geq 0$, set the interval to $\bar{x} \pm uq_1$. If $\left(\bar{x} - uq_1\right) < 0$, the interval is set to

$$\left[0, \bar{x} + uq_{\text{t}}\left(P_{\text{t}}\left(-\bar{x}/s, \nu_{eff}\right) + pP_{\text{tot}}.\nu_{eff}\right)\right]$$

NOTE: Using the spreadsheets MS Excel or OpenOffice Calc, implementations of P_{t} and q_{t} are as follows:

$$P_{\text{t}}(q,\nu) = \begin{cases} \text{TDIST(ABS(}q\text{)},}\nu\text{,2)/2} & q < 0 \\ \text{1- TDIST(}q\text{,}\nu\text{,2)/2} & q \geq 0 \end{cases}$$

$q_{\text{t}}(P,\nu)$: =1-TINV(2*(1-*P*),*ν*),

where q and ν in the spreadsheet formulae are replaced with the required quantile $\left(-\bar{x}/u\right)$ and degrees of freedom ν_{eff}, and P is the desired cumulative probability (for example, 0.95).

The additional complexity follows from the fact that the TDIST function provides only the upper tail probabilities for P_{t} and TINV provides only a two-tailed value for q_{t}.

F.6.4. The Bayesian interval provides the same minimal bias as the classical approach described in section F.5., with the useful property that as the observed mean value falls further below zero, the reported uncertainty increases. This makes it particularly appropriate for reporting results which are expected to fall consistently very close to a limit such as zero or 100 %, such as in the estimation of purity for highly pure materials. The interval is, however, appreciably narrower than the classical interval in the range $0 < \bar{x} < 5u$, so does not have exactly 95 % success rate.

F.6.5. As for the classical approach, calculation of the reported value and of the uncertainty interval should take place only after all other calculations have been completed. For example, if combining several values near zero, *first* carry out the calculation and estimate the standard uncertainty in the value to be reported and *then* calculate the uncertainty interval.

F.6.6. If it is necessary to report the standard uncertainty as well as the (asymmetric) expanded uncertainty interval, it is recommended that, as for the classical approach above, the standard uncertainty used in constructing the confidence interval should be reported without change.

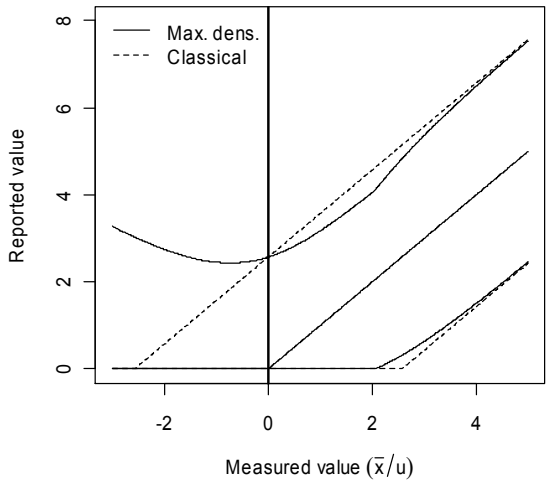


Figure 4. Bayesian maximum density interval (solid lines) for 5 degrees of freedom as a function of $\bar{x}$. The dashed line shows the corresponding classical interval.

Appendix G. Common Sources and Values of Uncertainty

The following tables summarise some typical examples of uncertainty components. The tables give:

- The particular measurand or experimental procedure (determining mass, volume *etc*)
- The main components and sources of uncertainty in each case
- A suggested method of determining the uncertainty arising from each source.
- An example of a typical case

The tables are intended only to indicate methods of estimating the value of some typical measurement uncertainty components in analytical measurement. They are not intended to be comprehensive, nor should the values given be used directly without independent justification. The values may, however, help in deciding whether a particular component is significant.

Determination	Uncertainty Components	Cause	Method of determination	Typical values	
				Example	Value
Mass	Balance calibration uncertainty	Limited accuracy in calibration	Stated on calibration certificate, converted to standard deviation	4-figure balance	0.5 mg
	Linearity		i) Experiment, with range of certified weights ii) Manufacturer's specification		ca. 0.5x last significant digit
	Readability	Limited resolution on display or scale	From last significant digit		0.5x last significant digit/√3
	Daily drift	Various, including temperature	Standard deviation of long term check weighings. Calculate as RSD if necessary.		ca. 0.5x last significant digit.
	Run to run variation	Various	Standard deviation of successive sample or check weighings		ca. 0.5x last significant digit.
	Density effects (*conventional* basis)[Note 1]	Calibration weight/sample density mismatch causes a difference in the effect of atmospheric buoyancy	Calculated from known or assumed densities and typical atmospheric conditions	Steel, Nickel Aluminium Organic solids Water Hydrocarbons	1 ppm 20 ppm 50-100 ppm 65 ppm 90 ppm
	Density effects (*in vacuo* basis)[Note 1]	As above.	Calculate atmospheric buoyancy effect and subtract buoyancy effect on calibration weight.	100 g water 10 g Nickel	+0.1g (effect) <1 mg (effect)

Note 1. For fundamental constants or SI unit definitions, mass determinations by weighing are usually corrected to the weight in vacuum. In most other practical situations, weight is quoted on a *conventional* basis as defined by OIML [H.32]. The convention is to quote weights at an air density of 1.2 kg m^{-3} and a sample density of 8000 kg m^{-3}, which corresponds to weighing steel at sea level in normal atmospheric conditions. The buoyancy correction to conventional mass is zero when the sample density is 8000 kg m^{-3} or the air density is 1.2 kg m^{-3}. Since the air density is usually very close to the latter value, correction to conventional weight can normally be neglected. The standard uncertainty values given for density-related effects on a conventional weight basis in the table above are sufficient for preliminary estimates for weighing on a conventional basis without buoyancy correction at sea level. Mass determined on the conventional basis may, however, differ from the 'true mass' (*in vacuo*) by 0.1 % or more (see the effects in the bottom line of the table above).

Determination	Uncertainty Components	Cause	Method of determination	Typical values	
				Example	Value
Volume (liquid)	Calibration uncertainty	Limited accuracy in calibration	Stated on manufacturer's specification, converted to standard deviation. For ASTM class A glassware of volume V, the limit is approximately $V^{0.6}/200$	10 mL (Grade A)	0.02 / √3 = 0.01 mL*
	Temperature	Temperature variation from the calibration temperature causes a difference in the volume at the standard temperature.	$\Delta T \cdot \alpha/(2\sqrt{3})$ gives the relative standard deviation, where ΔT is the possible temperature range and α the coefficient of volume expansion of the liquid. α is approximately 2×10^{-4} K^{-1} for water and 1×10^{-3} K^{-1} for organic liquids. For plastic volumetric equipment the coefficient of expansion for the vessel may also need to be considered	100 mL water	0.03 mL for operating within 3°C of the stated operating temperature α is typically 4×10^{-4} for polypropylene
	Run to run variation	Various	Standard deviation of successive check deliveries (found by weighing)	25 mL pipette	Replicate fill/weigh: **s** = 0.0092 mL

* Assuming rectangular distribution

Determination	Uncertainty Components	Cause	Method of determination	Typical values	
				Example	Value
Analyte concentration from a reference material certificate	Purity	Impurities reduce the amount of reference material present. Reactive impurities may interfere with the measurement.	Stated on manufacturer's certificate. Reference material certificates may give unqualified limits; these should accordingly be treated as rectangular distributions and divided by √3. Note: where the nature of the impurities is not stated, additional allowance or checks may need to be made to establish limits for interference etc.	Reference potassium hydrogen phthalate certified as 99.9 ±0.1 %	$0.1/\sqrt{3}$ = 0.06 %
	Concentration (certified)	Uncertainty in certified concentration in reference material.	Stated on manufacturer's certificate. Reference material certificates may give unqualified limits; these should accordingly be treated as rectangular distributions and divided by √3.	Cadmium acetate in 4 % acetic acid. Certified as (1000±2) mg L^{-1}.	$2/\sqrt{3}$ = 1.2 mg L^{-1} (0.0012 as RSD)*
	Concentration (made up from certified pure material)	Combination of uncertainties in reference values and intermediate steps	Combine values for prior steps as RSD throughout.	Cadmium acetate after three dilutions from 1000 mg L^{-1} to 0.5 mg L^{-1}	$\sqrt{0.0012^2 + 0.0017^2 + 0.0021^2 + 0.0017^2}$ = 0.0034 as RSD

*Assuming rectangular distribution

Determination	Uncertainty Components	Cause	Method of determination	Typical values	
				Example	Value
Absorbance	Instrument calibration Note: this component relates to absorbance reading versus reference absorbance, not to the calibration of concentration against absorbance reading	Limited accuracy in calibration.	Stated on calibration certificate as limits, converted to standard deviation		
	Run to run variation	Various	Standard deviation of replicate determinations, or QA performance.	Mean of 7 absorbance readings with s=1.63	$1.63/\sqrt{7} = 0.62$
Sampling	Homogeneity	Sub-sampling from inhomogeneous material will not generally represent the bulk exactly. Note: random sampling will generally result in zero bias. It may be necessary to check that sampling is actually random.	i) Standard deviation of separate sub-sample results (if the inhomogeneity is large relative to analytical accuracy). ii) Standard deviation estimated from known or assumed population parameters.	Sampling from bread of assumed two-valued inhomogeneity (See Example A4)	For 15 portions from 72 contaminated and 360 uncontaminated bulk portions: RSD = 0.58

Determination	Uncertainty Components	Cause	Method of determination	Typical values	
				Example	Value
Extraction recovery	Mean recovery	Extraction is rarely complete and may add or include interferents.	Recovery calculated as percentage recovery from matched reference material or representative spiking. Uncertainty obtained from standard deviation of mean of recovery experiments. Note: recovery may also be calculated directly from previously measured partition coefficients.	Recovery of pesticide from bread; 42 experiments, mean 90 %, s=28 % (See Example A4)	$28/\sqrt{42}$= 4.3 % (0.048 as RSD)
	Run to run variation in recovery	Various	Standard deviation of replicate experiments.	Recovery of pesticides from bread from paired replicate data. (See Example A4)	0.31 as RSD.

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