Draft EURACHEM/CITAC Guide
Quantifying Uncertainty in Analytical Measurement

Second Edition

Draft: June 1999

Prepared by the EURACHEM Measurement Uncertainty Working Group
in collaboration with members of CITAC and AOAC International

Contents

Foreword to the Second 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 made on the basis of 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: establishing traceability of the measurements, the use of validated methods of analysis, the use of defined internal quality control procedures, participation in proficiency testing schemes, and becoming accredited to an International Standard, normally the ISO/IEC Guide 25 [G.1]. In analytical chemistry there has been in the past greater emphasis on precision of results obtained using a specified method rather than traceability to a defined standard or SI unit. Consequently this has led the use of "official methods" to fulfil legislative and trading requirements. However as there is now a formal requirement to establish the confidence of results it is essential that a measurement is traceable to defined standard such as a SI unit, reference material or where applicable a defined, or empirical**,** (sec. 5.2.) method. Internal quality control procedures, proficiency testing and accreditation can be an aid in establishing traceability to a given standard.

As a consequence of these requirements, chemists are, for their part, coming under increasing pressure to demonstrate the quality of their results, *i.e.* to demonstrate their fitness for purpose by giving a measure of the confidence that can be placed on the result, including the degree to which a result would be expected to agree with other results, normally irrespective of the 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" [G.2] by ISO in collaboration with BIPM, IEC, IFCC, IUPAC, IUPAP and OIML, which formally established general rules for evaluating and expressing uncertainty in measurement across a broad spectrum of measurements. This document shows how the concepts in the ISO Guide may be applied in chemical measurement. It first gives an introduction to 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.

This Guide assumes that the evaluation of uncertainty requires the analyst to look closely at all the possible sources of uncertainty. It recognises that, although a detailed study of this kind may require a considerable effort, it is essential that the effort expended should not be disproportionate. It suggests that in practice a preliminary study will quickly identify the most significant sources of uncertainty, and as the examples showed, the value obtained for the total uncertainty is almost entirely controlled by the major contributions. It recommends that a good estimate can be made by concentrating effort on the largest contributions and that once evaluated for a given method applied in a particular laboratory, 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 method itself or the equipment used is changed, in which case the estimate would be reviewed as part of the normal re-validation.

The first edition of the EURACHEM Guide for "Quantifying Uncertainty in Analytical Measurement [G.3] was published in 1995 based on the ISO Guide.

The first edition of the EURACHEM Guide has now been revised in the light of experiences gained in its practical application in chemistry laboratories and the even greater awareness of the need to introduce formal quality assurance procedures by laboratories. The second edition stresses that the procedures introduced by a laboratory to estimate its measurement uncertainty must be integrated with its existing quality assurance measures, with these measures themselves frequently providing much of the information required to evaluate the measurement uncertainty. It attempts to correct the impression gained within the

wider Analytical Community that it is only the so-called component-by-component approach to the estimation of measurement uncertainty that is acceptable to the customers of providers of analytical data.

NOTE Worked examples are given in Appendix A. A numbered list of definitions is given at Appendix B. Terms are defined, upon their first occurrence in the main body of the text, via a reference to one of these lists. The convention is adopted of printing defined terms in bold face upon their first occurrence: a reference to the definition immediately follows, enclosed in square brackets. The definitions are, in the main, taken from the International vocabulary of basic and general standard terms in Metrology (VIM) [G.4**]**, the Guide [G.2] and ISO 3534 (Statistics - Vocabulary and symbols) [G.5] 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, and Appendix F lists many common uncertainty sources and methods of estimating the value of the uncertainties. A bibliography is provided at Appendix G.

1. Scope

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". It is applicable at all levels of accuracy and in all fields - from routine analysis to basic research and to empirical and rational methods [see section **5.3.**]. 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.
- Development and certification of reference materials.
- Research and development.

As formal quality assurance measures have to be introduced by laboratories in a number of sectors this second EURACHEM Protocol is now able to illustrate how data from the following procedures may be used for the estimation of measurement uncertainty:

- Evaluation of the effect on the analytical result of the identified sources of uncertainty for a single method in a single laboratory.
- 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.

2. Uncertainty

2.1. Definition of Uncertainty

2.1.1. The word *uncertainty* means *doubt*, and thus in its broadest sense *uncertainty of measurement* means doubt about the validity of the result of a measurement as well as doubt as to the exactness of the result.

2.1.2. In this guide, the word *uncertainty* without adjectives refers both to the general concept and to any or all measures of that concept. When a specific measure is intended, appropriate adjectives are used.

2.1.3. The definition of the term uncertainty (of measurement) used in this protocol and taken from the current version adopted for the International Vocabulary of Basic and General Terms in Metrology G.4 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.24 (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. The ISO Guide refers to these different cases as Type A and Type B estimations respectively.

2.1.4. In many cases in chemical analysis the measurand **[B.6]** 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.

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

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, sampling, matrix effects and interferences, environmental conditions, uncertainties of weights and volumetric equipment, reference values, approximations and assumptions incorporated in the measurement method and procedure, and random variation (a fuller description of uncertainty sources will be found at section **6.6.**)

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.14].** 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.

2.3.2. For a measurement result y, the total uncertainty, termed **combined standard uncertainty [B.15]** 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.).

2.3.3. For most purposes in analytical chemistry, an **expanded uncertainty [B.16]** U, should be used. The expanded uncertainty provides an interval within which the value of the measurand is believed to lie with a particular level of confidence. U is obtained by multiplying $u_c(y)$, the combined standard uncertainty, by a **coverage factor [B.17]** 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 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.20]** is defined as the difference between an individual result and the true value **[B.3]** of the measurand. As such, error is a single value.

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, and, if estimated for an analytical procedure and defined sample type, may apply to all determinations so described. No part of uncertainty can be corrected for.

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.

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.21] typically arises from unpredictable variations of influence quantities. These random effects, give rise to variations in repeated observations of the measurand. The random error of an analytical result cannot be compensated by correction but it can usually be reduced by increasing the number of observations.

NOTE 1 The experimental standard deviation of the **arithmetic mean [B.23]** 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.22] 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; however, 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.

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. 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. 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. Bias may be expressed as analytical recovery (value observed divided by value expected). Bias is expected to be negligible or otherwise accounted for, but the uncertainty associated with the determination of the bias remains an essential component of overall uncertainty.

Linearity. Linearity of response to an analyte is an important property where methods are used to quantify 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 non-linear calibration 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 (Section ###), 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/specificity. Though loosely defined, both terms relate 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.

Conduct of validation studies

3.1.4. The detailed design and execution of method validation studies is covered extensively elsewhere [G.6] 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.

3.1.5. *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.

3.1.6. 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.

3.1.7. 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.

3.1.8. 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.

3.1.9. 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.

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

3.1.11. 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, that is, significant compared to the largest component of uncertainty.

Relevance of prior studies

3.1.12. 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), 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.

3.1.13. Whether carrying out measurements or assessing the performance of the measurement procedure, effective quality assurance and control measures should be 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

3.1.14. 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 validation studies directly to uncertainty estimates in the laboratory in question.

3.2. Traceability

3.2.1. Traceability is intimately linked to uncertainty and is an important concept in all branches of measurement. It provides the basis for establishing the uncertainty on a particular result and for judging whether results agree or whether a result is above or below some prescribed limit. In order to compare results either with each other or with a limit, it is necessary for the results and the limit to be traceable to a common reference.

3.2.2. Traceability is formally defined **[**G.4**]** as:

> "The property of the result of a measurement or the value of a standard whereby it can be related to stated references, usually national or international standards, through an unbroken chain of comparisons all having stated uncertainties."

Thus it is a property of the result of a measurement. 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.

3.2.3. The stated references, as well as being national or international standards can, according to VIM [G.4], be a material measure, measuring instrument, reference material or measuring system, where a measuring system is further defined as:

> "The complete set of measuring instruments and other equipment assembled to carry out specific measurements"

with a note that the system may include material measures and chemical reagents.

3.2.4. Utilising these definitions it is possible to examine how traceability of the results might be established for the measurement procedures commonly used in analytical chemistry. For all such analytical measurement it is straightforward to ensure that the results from such operations as weighing, volume determination, temperature measurement are traceable to Systeme Internationale (SI) units. The evaluation of the uncertainty on the final result arising from such operations is described in example 1.

3.2.5. In all cases the calibration of the measuring equipment used must be traceable to appropriate standards. The quantification stage of the analytical procedure is often calibrated using either pure samples of the analyte or appropriate reference materials, whose values are traceable to the SI. This practice provides traceability of the results to SI for this part of the procedure. Because operations prior to the final quantification frequently introduce large effects, however, traceability of the results obtained from the complete measurement procedure is more difficult to establish.

3.2.6. Typical ways in which the traceability of the result of the complete analytical procedure might be established are

1. By using a primary method
2. By using the analytical procedure to make measurements on a quantified pure sample of the analyte
3. By using the analytical procedure to make measurements on an appropriate Certified Reference Material (CRM)
4. By making measurements using a defined procedure.

It may also be necessary to use a combination of these methods. Each is discussed in turn below.

3.2.7. Measurements using Primary Methods

A primary method is currently described as follows:

> "A primary method of measurement is a method having the highest metrological qualities, whose operation is completely described and understood in terms of SI units and whose results are accepted without reference to a standard of the same quantity."
>
> with notes that
>
> "…a primary direct method results in a value of an unknown quantity without reference to a standard of the same quantity."
>
> ".. a primary ratio method results in a value of the ratio of two values of the samew quantity without reference to a standard of the same quantity."

It is normally understood that a Primary method is traceable directly to the SI, and is of the smallest achievable uncertainty. It is usually a method used to define the base units. It is accodingly traceable to the SI by definition, but rarely available to routine testing or calibration laboratories.

3.2.8. Measurements on a pure sample of the analyte.

In principle traceability can be achieved by measurement of a quantified sample of the pure analyte, for example by spiking or by standard additions. However it may be difficult to establish the relative response of the measurement system to the quantified sample of the analyte and the sample being analysed. This is a particular example of a problem common to all areas of measurement; it is always necessary to evaluate the difference in response of the measurement system to the standard used and the sample under test. In many areas of measurement, particularly in the physical sciences, the causes of any potential difference have been investigated and well understood, any necessary corrections can be applied and the uncertainty on these corrections can be quantified. Unfortunately the same is not true for many chemical analyses and in the particular case of spiking or standard additions both the correction for the difference in response and its uncertainty may be large. Thus, although the traceability of the result to SI units can in principle be established, in practice, in all but the most simple cases, the uncertainty on the result may be unacceptably large or even unquantifiable. If the uncertainty is unquantifiable then traceability has not been established

3.2.9. Measurement on a Certified Reference Material (CRM)

Measurement on a CRM can reduce the uncertainty compared to the use of a pure sample, providing that there is a suitable CRM available. If the value of the CRM is traceable to SI, then these measurements could provide traceability to SI units and the evaluation of the uncertainty utilising reference materials is discussed in section 7.9.1. However, even in this case, the uncertainty on the result may be unacceptably large or even unquantifiable.

3.2.10. Measurement by means of defined procedure.

This is often used in combination with either of the ways 1 or 2 described above, since the uncertainty of the result obtained on the basis of the defined procedure will be less than if its traceability is back to SI units. This smaller uncertainty will only apply for comparison with results utilising the **same** procedure and for samples that are within the scope of the defined method, but this could be sufficient for example when carrying out analyses for production control or for certain regulatory purposes, if the regulations specify the measurement procedure to be used. This technique of utilising a defined procedure is not unique to analytical chemistry, for example for many years voltage measurements were made relative to standard cells prepared using a standard procedure because of the large uncertainty on realising the SI volt.

In addition the defined procedure is utilised, when the method defines the analyte, e.g. measurement of fat or fibre content of food. Measurements on CRMs or in house reference materials may also be carried out for QC purposes, but the traceability of the result is to the defined procedure. Again the results can only be compared with limits or other results obtained utilising the same procedure and for samples that are within the scope of the method. The evaluation of the uncertainty in this case is discussed in section **7.5.1.**

4. Principles 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 method validation studies and the use of formal uncertainty propagation principles. The steps involved are:

Step 1 Specification

Write down a clear statement of what is being measured, including the relationship between the measurand and the parameters (*e.g.* measured quantities, constants, calibration standards *etc.*) upon which it depends. Where possible, include corrections for known systematic effects. The specification information, if it exists, is normally 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 [CE].

Step 3 Quantify Uncertainty Components

Measure or 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. 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.

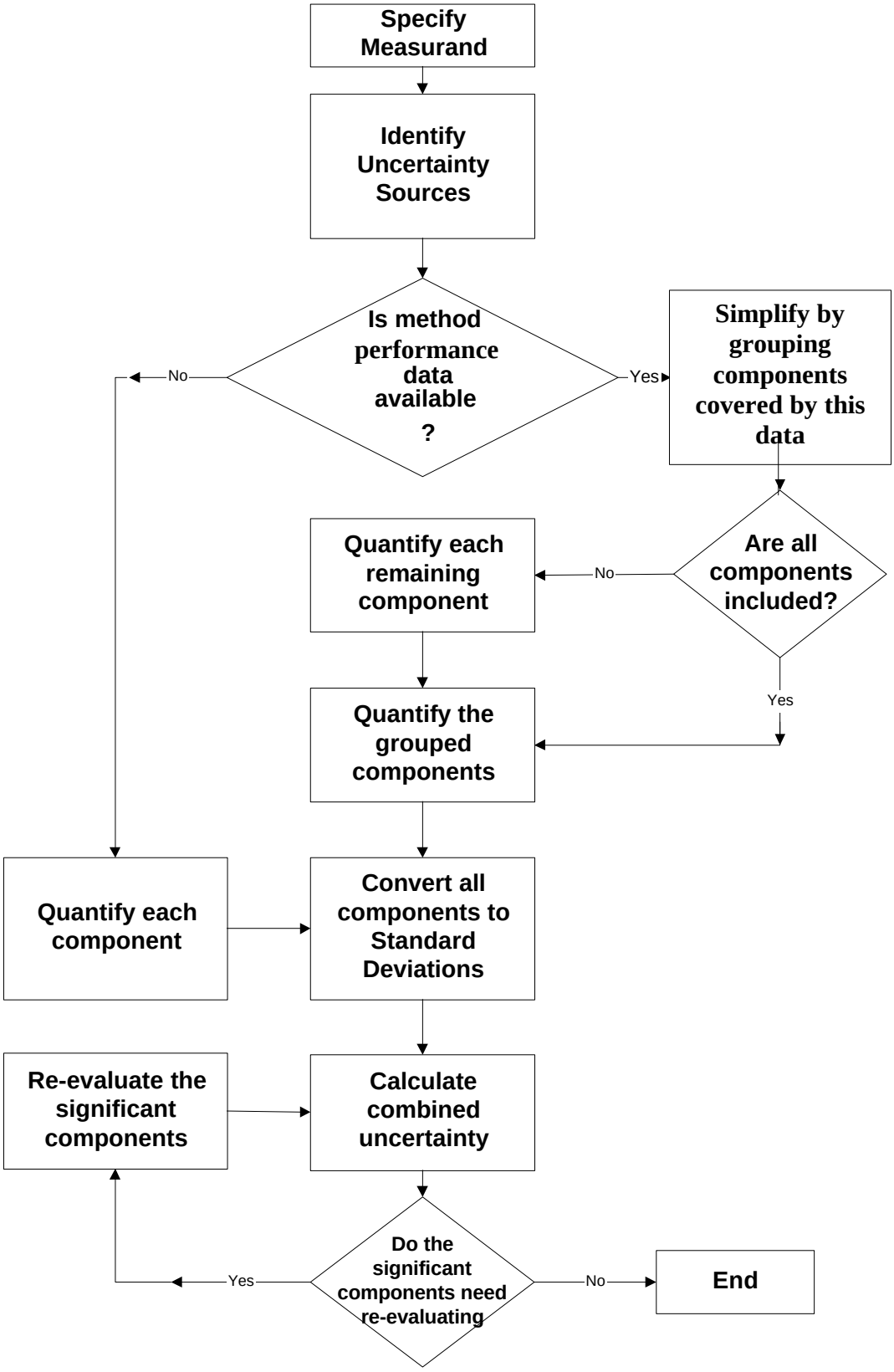
Step 4 Calculate Total Uncertainty

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 combined 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

5.1. In the context of uncertainty estimation, "specification" 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 SOP.

5.2. 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*. 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 or mole 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.

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.

3. In circumstances where variations in the substrate, or matrix, have large and unpredictable effects, a systematic procedure is often developed with the sole aim of achieving comparability between laboratories measuring the same material. The method may then be adopted as a local, national or international standard 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.

5.3. 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 possible 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 nevertheless effect part or the whole measurement process and contribute to the uncertainty on the value of the measurand, 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 (Structure of Analytical Procedures) and in Appendix D (Analysing uncertainty sources).

6.3. The measurand has a relationship to the values *p,q,r*.. of these parameters which, in principle can be expressed algebraically as $y=f(p,q,r, ...)$,. The expression 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. It is introduced as a convenient way of indicating that the uncertainty on the value of y is dependent on the uncertainty in values of the parameters *p, q, r* etc.

6.4. 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..

6.5. 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 generally 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.**

6.6. Typical sources of uncertainty are

- Sampling

  Where sampling forms 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 molarity 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.

- 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 other elements of the matrix. Analyte speciation may further compound this effect.

  The stability of a sample/analyte may change during analysis as a result 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. The uncertainty on the value y of the measurand can be obtained

- by evaluating the uncertainty on the parameters *p, q, r* etc and calculating their contribution to the uncertainty on y
- by determining directly the combined contribution to the uncertainty on y from some or all of these parameters using, e.g. method performance data.
- or by using a combination of these

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 experimental work as described in section 7.8. or the uncertainty may be evaluated based on prior experience or judgement as described in section 7.10.

7.1.3. 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. Uncertainty evaluation using method performance data

7.2.1. The stages in estimating the overall uncertainty using existing data about the method performance 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 listed to provide an auditable record of the which contributions to the uncertainty have been included.

- **Obtain further data as required**

  For sources of uncertainty not adequately covered by existing data, either obtain 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. The following sections provide guidance on the coverage and limitations of data acquired in particular circumstances and on the additional information required for an estimate of overall uncertainty.

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

7.3.1. A collaborative study carried out, for example according to AOAC/IUPAC or ISO 5725 standards, to validate a published method, is a valuable source of data to support an uncertainty estimate. 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, the sources which need particular consideration are:

- Sampling. 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 mean 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.

7.3.2. 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 the sources such as those discussed in section 7.3.1. 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.

7.3.3. Where additional factors apply, these should be evaluated in the form of standard uncertainties and combined with the reproducibility standard deviation in the usual way (section 8.)

7.4. Uncertainty estimation during in-house development and validation studies

7.4.1. 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 requires:

- 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

7.4.2. The precision contribution should be estimated as far as possible over an extended time period, and chosen to allow natural variation of all factors affecting the result. Typical experiments include

- Distribution of results for a typical sample analysed several times over a period of time, using different analysts and equipment where possible (A QC check sample may provide sufficient information)
- The distribution of 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.

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

Bias study

7.4.3. 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 uncertainty in the CRM value and the standard deviation associated with the bias check (adjusted for number of determinations).

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 standardised; see section 7.5.1..

- When the reference material is only approximately representative of the test materials, additional factors should be considered, including (as appropriate) differences in homogeneity; reference materials are frequently more homogeneous that test samples
- 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

7.4.4. Bias for a method under study is frequently determined against a reference method, by comparison of the results of the two methods applied to the same samples. In such circumstances, given that the bias is not statistically significant, the uncertainty is that for the reference method (if applicable; see section **7.5.1.**), combined with the uncertainty associated with the measured difference between methods. The latter contribution to uncertainty commonly appears as the standard deviation term used in the significance test applied to decide whether the difference is statistically significant.

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

$$S_c = \sqrt{\left(\frac{1{\cdot}471^2 \times (5-1) + 2{\cdot}750^2 \times (5-1)}{(5+5-2)}\right)} = 2.205$$

and a corresponding value of t:

$$t = \frac{(5.40 - 4.76)}{2.205\sqrt{\left(\frac{1}{5}+\frac{1}{5}\right)}} = \frac{0.64}{1{\cdot}4} = 0.46$$

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. But 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.

7.4.5. Overall bias is also commonly studied by 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 error 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 co-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

7.4.6. 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.

7.4.7. 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 **7.4.4.** describe in the case of insignificant bias

7.4.8. Where the bias is not insignificant, but is nonetheless neglected for practical purposes, the uncertainty associated with bias should be increased by addition of a term equal to the measured bias.

NOTE The inclusion of a term equal to a measured bias is justifiable only to the extent that neglecting a significant bias is justifiable. It is better practice to report the bias and its uncertainty separately. Where this is not done, increasing the uncertainty estimate by including such a term simply avoids misleading any user of the reported result and uncertainty.

Additional factors

7.4.9. The effects of factors held constant during precision studies should be estimated separately, either by experimental variation or by prediction from established theory. Where practicable, the uncertainty associated with such factors should be estimated, recorded and combined with other contributions in the normal way.

7.4.10. 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 then be 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 fond to be significant, it is normally necessary to investigate further. Where insignificant, the associated uncertainty is (at least for initial estimation) that associated with the robustness study.
- Systematic multifactor experimental designs intended to estimate factor effects and interactions.

7.4.11. Where the effect of an additional factor 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 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

$$((5-1)\times 0.21^2 + (5-1)\times 0.17^2) / ((5-1)+(5-1)) = 0.037$$

to obtain

$t = (1.8-1.7)/\sqrt{0.037\cdot(1/5+1/5)} = 0.82$; not significant compared to $t_{crit} = 2.3$. But note that the difference (0.1) is compared with a calculated standard deviation term, of $\sqrt{0.037\cdot(1/5+1/5)} = 0.3$. This value is the contribution to uncertainty associated with the effect of permitted variation in extraction time.

7.4.12. Where an effect is detected and is statistically significant, but remains sufficiently small to neglect in practice, it is recommended that an uncertainty contribution equal to the measured effect combined with its statistical uncertainty be associated with the effect.

NOTE: See the note to section **7.4.3.**

7.5. Empirical methods

7.5.1. 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.

7.5.2. 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.

7.5.3. 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.

7.5.4. Where reference materials so certified 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. Section **7.3.1.** then applies.

7.6. Ad-hoc methods

7.6.1. 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.

7.6.2. 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, combined with any specific study necessary to establish relevance of those studies. 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.

7.6.3. 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 **7.4.3.** 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.

7.6.4. 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.

7.6.5. 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.

7.6.6. Where these conditions are not met, it is not recommended that an uncertainty estimate be provided. Where an indication of reliability is nonetheless required, it is suggested that the measured bias and 95% confidence interval of the replicated results are reported, with the caveat that systematic effects on the result were not fully investigated.

7.7. Estimation based on other results or data

7.7.1. 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.

7.7.2. Proficiency Testing schemes A laboratory's results from participation in such schemes can be used as a check on the evaluated uncertainty, since the uncertainty should be compatible with the spread of results obtained by that laboratory over a number of proficiency test rounds. Further, in the special case where

- the compositions of samples used in the scheme cover the full range analysed routinely
- the assigned value is traceable, and
- the uncertainty on the assigned value is small compared to the observed spread of results

then the standard deviation of the results obtained from repeated rounds would provide a good estimate of the uncertainty arising from those parts of the measurement procedure within the scope of the scheme. Of course, systematic deviation from traceable assigned values and any other sources of uncertainty (such as those noted in section **7.3.1.**) must also be taken into account.

7.7.3. Quality Assurance (QA) 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 QA samples show that the criteria continue to be met. Where reference materials are used in QA checks, section **7.9.1.** shows how the data can be used to evaluate uncertainty. Where any other stable material is used, the QA data provides an estimate of intermediate precision (Section **7.4.2.**). QA data also forms 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 QA measurements.

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

7.8. Quantification from repeated observations

7.8.1. The standard uncertainty arising from random effects is typically 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.

7.8.2. By varying all parameters on which the result of a measurement is known to depend, its uncertainty could be evaluated by statistical means, but this is rarely possible in practice due to limited time and resources.

7.8.3. However, in many cases it will be found that just a few components of the uncertainty dominate. Where it is realistic to do so these parameters should be varied to the fullest practicable extent so that the evaluation of uncertainty is based as much as possible on observed data.

7.9. Using Reference Materials

7.9.1. Measurements on reference materials provide very good data for the assessment of uncertainty since they provide information on the combined effect of many of the potential sources of uncertainty. (ISO Guide 33 [G.7]gives a useful account of the use of reference materials in checking method performance). The sources that then need to be taken into account are:

- the uncertainty on the assigned value of the reference material.
- the reproducibility of the measurements made on the reference material.
- any difference between the measured value of the reference material and its assigned value.
- differences between the composition of the reference material and the sample.
- differences in the response of the measurement system to the reference material and the sample, *e.g.* due to interferences or matrix effects.
- operations that are carried out on the sample but not on the reference material *e.g.* taking of the original sample and its subdivision in the laboratory.

7.9.2. It is much easier to evaluate the above sources of uncertainty than to work systematically through an assessment of the effect of every potential source and therefore measurements on reference materials should always be carried out, even if only in house reference materials are available. This is discussed further in section **7.4.3.**

7.10. Estimation based on judgement

7.10.1. 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.

7.10.2. 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.

7.10.3. 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.

7.10.4. 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. One then makes such assessment for classes of samples (*e.g.* grouped by type of matrix) and applies them 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 frequentistic 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 one is ready to study this 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.

7.10.5. 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" **[G.4]**. 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.

7.10.6. 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, by earlier experiments and observations.

7.10.7. This may appear to be a disadvantage, but need not lead in practice to worse estimates than those from repeated measurements 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.

7.10.8. A typical problem of this nature arises if long-term variability needs to be assessed when no round-robin 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.

7.10.9. 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

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, then 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.25] 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, (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 1.96σ. 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 not 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 $0.2/\sqrt{3} \approx 0.11$ 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, then 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.

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 uncertainty $u(y)$ of a value y and the uncertainty of the independent parameters x_1, x_2, ...x_n on which it depends is

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

where $y(x_1,x_2,..)$ is a function of several parameters $x_1,x_2...$, and 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. Each variable's contribution is just the 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:

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

where $\mathbf{s}(x,ik)$ is the covariance between x_i and x_k.and c_i and c_k the sensitivity coefficients as described and evaluated in **8.2.2.** In practice, the covariance is often related to the correlation coefficient r_{ik} using

$$\mathbf{s}(x,ik) = u(x_i).u(x_k).r_{ik}$$

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 result. In such cases, or when the uncertainty on a parameter is expressed directly in terms of its effect on *y*, the coefficient $\partial y/\partial x_i$ is equal to 1.0.

EXAMPLE

A result of 22 mg l^{-1} shows a measured standard deviation of 4.1 mg l^{-1}. The standard uncertainty u(ε) 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 the numerical equivalent must be employed. Appendix E gives details of a numerical method, suggested by Kragten, which makes effective use of standard spreadsheet software to provide a combined standard uncertainty from input standard uncertainties and a known measurement model **[G.5]**. It is recommended that this method 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.* $y=k(p+q+r+...)$ where *k* is a constant, the combined standard uncertainty $u_c(y)$ is given by

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

Rule 2

For models involving only a product or quotient, *e.g.* $y=k(pqr...)$, where k is a constant, the combined standard uncertainty $u_c(y)$ is given by

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

where $(u(p)/\bar{p})$ 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

$y = m.(p\text{-}q+r)$ The values are m=1, p=5.02, q=6.45 and r=9.04 with standard uncertainties deviations $u(p)$=0.13, $u(q)$=0.05 and $u(r)$= 0.22.

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

$$u(y) = 1 \times \sqrt{0.13^2 + 0.05^2 + 0.22^2} = 0.26$$

NOTE Since the value of y is only calculated to 2 decimal places then the final uncertainty value should not be quoted to more than 3 decimal places.

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 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 variance). 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 [E1] gives additional information on these approaches.

8.3. Combined 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; small samples may lead to optimistic estimates of expanded uncertainty.

8.3.3. For most purposes it is recommended that k is set to 2. However, this may be an under-estimate where the 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 27) gives a short list of values for t.

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 $\sqrt{0.01^2 + 0.08^2} = 0.081\,\text{mg}$. 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 combined expanded uncertainty U_c=2.8×0.081=0.23 mg.

8.3.5. The *Guide* **[G.1]** 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	2.5

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. At different levels of chemical measurement from primary reference material characterisation to routine testing, successively more of the information required may be available in the form of published reports, national or international standards, method documentation and test and calibration certificates. When the details of a measurement, including how the uncertainty was determined, depend on references to published documentation, it is imperative that these publications are 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 *Guide* **[G.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.

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 International Vocabulary of Basic and General terms in metrology, 2nd ed., ISO 1993 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 %w/w

Standard uncertainty: 0.07 %w/w *

*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 %w/w *

*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. Compliance against limits

9.6.1. 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. The following paragraphs give examples of common practice.

9.6.2. Assuming that limits were set with no allowance for uncertainty, four situations are apparent for the case of compliance with an upper limit (see figure 6.1):

i) The result exceeds the limit value plus the estimated uncertainty.

ii) The result exceeds the limiting value by less than the estimated uncertainty.

iii) The result is below the limiting value by less than the estimated uncertainty

iv) The result is less than the limiting value minus the estimated uncertainty.

Case i) is normally interpreted as demonstrating clear non-compliance. Case iv) is normally interpreted as demonstrating compliance. Cases ii) and iii) will normally require individual consideration in the light of any agreements with the user of the data. Analogous arguments apply in the case of compliance with a lower limit.

9.6.3. Where it is known or believed that limits have been set with some allowance for uncertainty, a judgement of compliance can reasonably be made only with knowledge of that allowance. An exception arises where compliance is set against a stated method operating in defined circumstances. Implicit in such a requirement is the assumption that the uncertainty, or at least reproducibility, of the stated method is small enough to ignore for practical purposes. In such a case, provided that appropriate quality control is in place, compliance is normally reported only on the value of the particular result. This will normally be stated in any standard taking this approach.

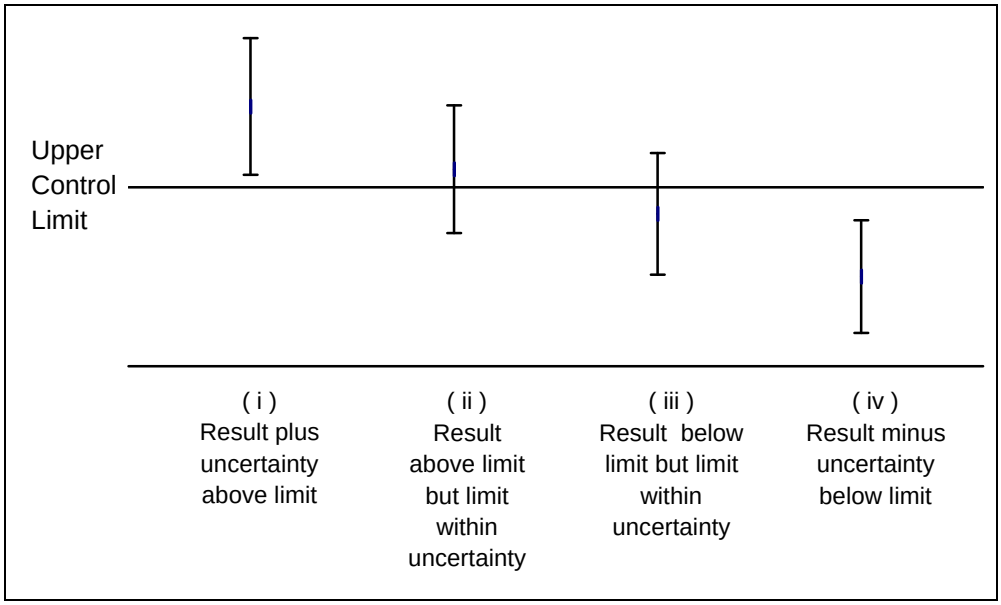


Figure 2: Uncertainty and compliance limits

Appendix A. Examples

Introduction

General introduction

These examples illustrate how the techniques for evaluating uncertainty, described in section 7, can be applied to some typical chemical analyses. They all follow the procedure shown in the flow diagram (Figure 1) The uncertainty sources are identified and set out on a cause and effect diagram (see appendix D). This helps to avoid double counting of sources and also assists in with the grouping together of components whose combined effect can be evaluated. Each example has an introductory summary page, giving an outline of the analytical method, a list of the uncertainty sources, the values derived for the uncertainty components and the combined uncertainty.

Examples 1-3 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. 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 1

Example 1 deals with the very simple case of the preparation of a calibration standard of Cadmium in HNO_3 for 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 2

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 1, but also examines the uncertainty associated with the end-point determination.

Example 3

This example expands on example 2 by including the standardisation of the NaOH against a titrimetric standard of KPH.

Example 4

This illustrates the use of in house validation data, as described in section 7.4., and shows how the data can be used to evaluated the uncertainty arising from combined effect of a number of sources. It also shows how to evaluate the uncertainty associated with method bias.

Example 5

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.-7.5.. 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.

Example 6

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 empirical. In this case, collaborative study data, in-house QA checks and literature study data were available, permitting the approach of section 7.3. 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 7

This gives a detailed description of the evaluation of uncertainty on the measurement of the lead content of a water sample using 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 7.10.(Use of judgement is a special case of Type B evaluation as described in the ISO *Guide* [G.2])

Example A1: Preparation of a calibration standard

Summary

Goal

Preparation of a 1000 $mg \cdot l^{-1}$ calibration standard a high purity metal (Cadmium).

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 show in the following flow chart.

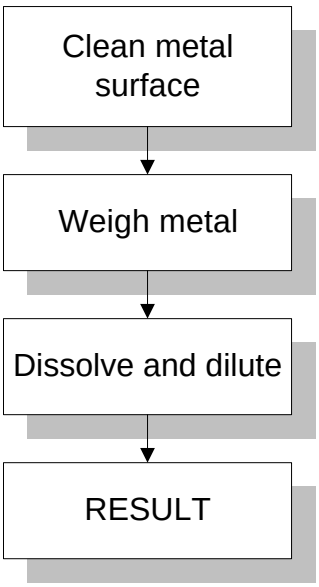


Figure A1.1: Preparation of Cadmium standard

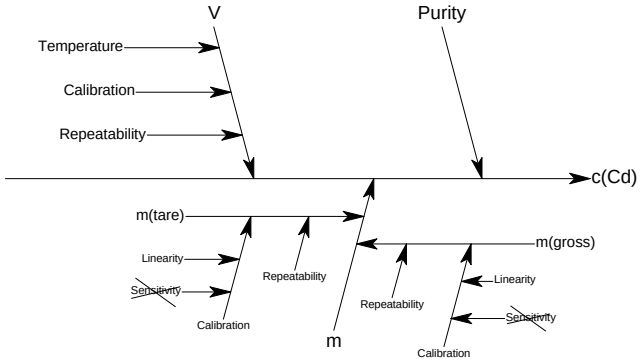
Measurand

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

where the parameters are those in Table A1.1 below. The factor of 1000 is a conversion factor from ml to l

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 Cd calibration standard is 0.9 mg/l

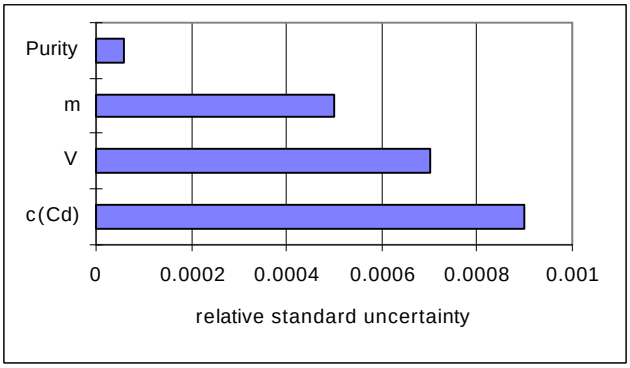
The different contributions are shown diagramatically in Figure A1.1.

Table A1.1: Values and uncertainties

	Description	Value	Standard uncertainty	Relative standard uncertainty*
P	Purity of the metal	0.9999	0.000058	0.000058
m	Weight 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	0.9 mg/l	0.0009

*$u(x)/x$

Figure A1.1: Uncertainty contributions in Cadmium standard preparation



Example A1: Detailed description

A1.1 Introduction

This introductory example discusses the preparation of a calibration standard for atomic absorption spectroscopy (AAS) from the corresponding high purity metal (in this example ≈ 1000 mg/l Cd in HNO_3 0.5 mol/l). 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

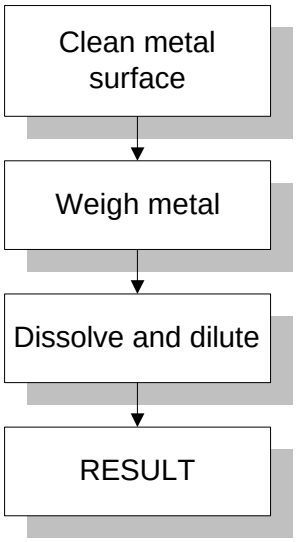


Figure A1.2: Preparation of Cadmium standard

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 manufacture of the metal and needs to be carried out to obtain the purity quoted on the certificate.

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

1 ml of nitric acid (65%) and 3 ml of ion-free water are added to the flask to dissolve the Cadmium (approximately 100 mg). Moderate heat is applied to speed up the dissolution process. 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

$$c_{Cd} = \frac{1000 \cdot m \cdot P}{V} \text{ [mg/l]}$$

where

c_{Cd} :concentration of the calibration standard [mg/l]

1000 :conversion factor from [ml] to [l]

m :weight 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 parameter which effect 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.

m

The second stage of the preparation involves weighing the high purity metal. A 100 ml quantity of a 1000 mg/l Cadmium solution is to be prepared.

The relevant weighings are:

container and high purity metal56.76325 g observed

container less high purity metal56.66297 g observed

high purity metal0.10028 g(calculated)

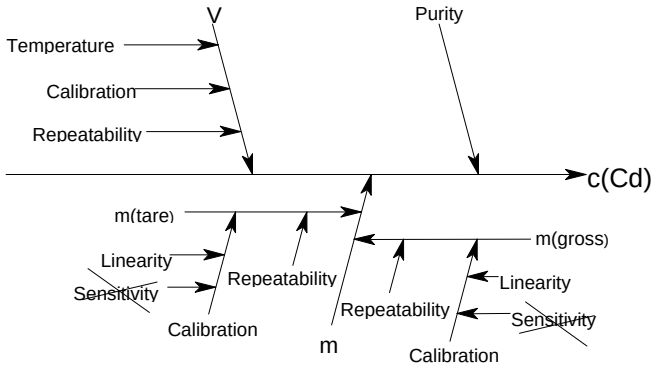
As these numbers show, the final weight is a weight by difference. The uncertainty sources for each of the two weighings are the run to run variability and the contribution due to the uncertainty in the calibration function of the scale. This calibration function has two potential uncertainty sources: The sensitivity of the balance and its linearity. The sensitivity can be neglected because the weight by difference is done on the same balance over a very narrow range.

V

- The volume of the solution contained in 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 A1.3 (see Appendix D for description).

Figure A1.3: 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 $\boldsymbol{u}(P)$ the value of 0.0001 has to be divided by $\sqrt{3}$ (see section ...)

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

m

The weight of the Cadmium is obtained from difference weighings which consist of two independent measurements. If the weighing procedure is performed on the same scale, in the same narrow weight region and within a short period of time, the sensitivity contribution to the calibration influence quantity cancels itself out.

Repeatability: The run to run variabilities are given in the manufacturer's handbook. The standard deviation (*s*) for weight measurements from 50 g to 200 g is quoted as 0.04 mg. According to the manufacture's information the value for the repeatability was determined by a series of always ten measurements of a tare and gross weight. The difference of each of the ten measurement pairs was calculated and then the standard deviation of these differences. The same

value for the standard deviation of the differences was obtained independently in the laboratory using check weights. The repeatability contribution has to be taken into account only once, because the standard deviation of the differences was directly determined in the given experimental design.

Linearity: The specification of the balance quotes that the difference from the actual weight on the scale pan and the reading of the scale is within the limits of ±0.03 mg. This value is only valid for a range of up to 10 g from the tare weight to the gross weight. According to the manufacturer's own uncertainty evaluation a rectangular distribution has to be assumed. Hence the value for the linearity contribution needs to be divided by $\sqrt{3}$ to give the component of uncertainty as a standard uncertainty

$$\frac{0.03\,\text{mg}}{\sqrt{3}} = 0.017\,\text{mg}$$

This component has to be taken into account twice because of the weight by difference.

Finally the two components, repeatability and linearity, are combined by taking the square root of the sum of their squares (see Appendix: 1) to give the standard uncertainty $\boldsymbol{u}(m)$

$$\boldsymbol{u}(m) = \sqrt{0.04^2 + 2\cdot(0.017)^2} = 0.05\,\text{mg}$$

Notes:-The given value for the repeatability reflect only pure repeatability conditions. Especially they do not account for differences between operators or measurement conditions.

-There is no need for a buoyancy correction, because the density of cadmium (8642 kg/m^3) and of the calibration weight (8006 kg/m^3 since 1997) are nearly the same.

<u>*V*</u>

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, therefore the appropriate standard uncertainty is calculated assuming a triangular distribution, since the actual volume is more likely to be at the centre than at the extremes of the range.

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

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 weight experiments on a typical 100 ml flask gave a standard uncertainty of 0.02 ml. This standard uncertainty can be used directly.

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, therefore only the former needs to be considered. The coefficient of volume expansion for water is $2.1\cdot10^{-4}$ °C^{-1}, which leads to a volume variation of

$$100\,\text{ml}\cdot\pm4^\circ\text{C}\cdot2.1\cdot10^{-4}{}^\circ\text{C}^{-1} = \pm0.084\,\text{ml}$$

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

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

The three contribution are combined to the standard uncertainty $\boldsymbol{u}(V)$ of the volume V

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

A1.5 Step 4: Calculating the combined standard uncertainty

c_{Cd} is given by

$$c_{Cd} = \frac{1000\cdot m\cdot P}{V} \qquad [\text{mg/l}]$$

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

$$c_{Cd} = \frac{1000\cdot100.28\cdot0.9999}{100.0} = 1002.7\,\text{mg}\cdot\text{l}^{-1}$$

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
Weight 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

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

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

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

$$= 0.0009$$

$$u_c(c_{Cd}) = c_{Cd} \cdot 0.0009 = 1002.7\,\mathrm{mg}\cdot\mathrm{l}^{-1} \cdot 0.0009$$

$$= 0.9\,\mathrm{mg}\cdot\mathrm{l}^{-1}$$

However it is preferable to derive the combined standard uncertainty ($\boldsymbol{u}_c(c_{Cd})$) using the spreadsheet method given in Appendix E, since this can be utilised even for complex expressions. The completed spreadsheet is show below. 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 relative contributions of the different parameters are shown Figure A1.1. The contribution of the uncertainty on the volume of the flask is the largest and that from the weighing procedure is similar, whereas the uncertainty on the purity of the Cadmium has virtually no influence on the overall uncertainty.

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

$$\boldsymbol{U}(c_{Cd}) = 2 \cdot 0.9\,\mathrm{mg}\cdot\mathrm{l}^{-1} = 1.8\,\mathrm{mg}\cdot\mathrm{l}^{-1}$$

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			0.05816	0.49995	-0.70140
11		0.74529	0.00338	0.24995	0.49196
12					
13	u(c(Cd))	0.9			

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).

Measurand:

$$c_{NaOH} = \frac{1000 \cdot m_{KHP} \cdot P_{KHP}}{F_{KHP} \cdot V_T} \qquad [\mathrm{mol \cdot l^{-1}}]$$

where

c_{NaOH} :concentration of the NaOH solution [mol·l^{-1}]

1000 :conversion factor [ml] to [l]

m_{KHP} :weight of the titrimetric standard KHP [g]

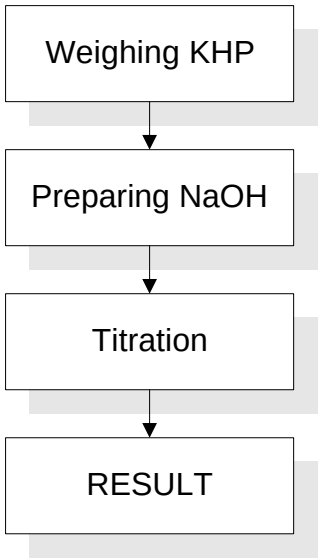
P_{KHP} :purity of the titrimetric standard given as mass fraction []

F_{KHP} :molecular weight of KHP [g·mol^{-1}]

V_T :titration volume of NaOH solution [ml]

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



Identification of the uncertainty sources:

The relevant uncertainty sources are shown as a cause and effect diagram in Figure A2.1.

Quantification of the uncertainty components

The different uncertainty contributions are given in Table A2.1, and shown diagramatically in Figure A2.2.

The combined standard uncertainty for the 0.10214 mol/l NaOH solution is 0.00009 mol/l

Table A2.1: Values and uncertainties in NaOH standardisation

	Description	Value *x*	Standard uncertainty *u*	Relative standard uncertainty*
m_{KHP}	weight of KHP	0.3888 g	0.00015 g	0.00036
P_{KHP}	Purity of KHP	1.0	0.00029	0.00029
F_{KHP}	Formula weight 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	0.015 ml	0.0008
c_{NaOH}	NaOH solution	0.10214 mol l^{-1}	0.00009 mol l^{-1}	0.00092

*$u(x)/x$

Figure A2.1: Cause and effect diagram for titration

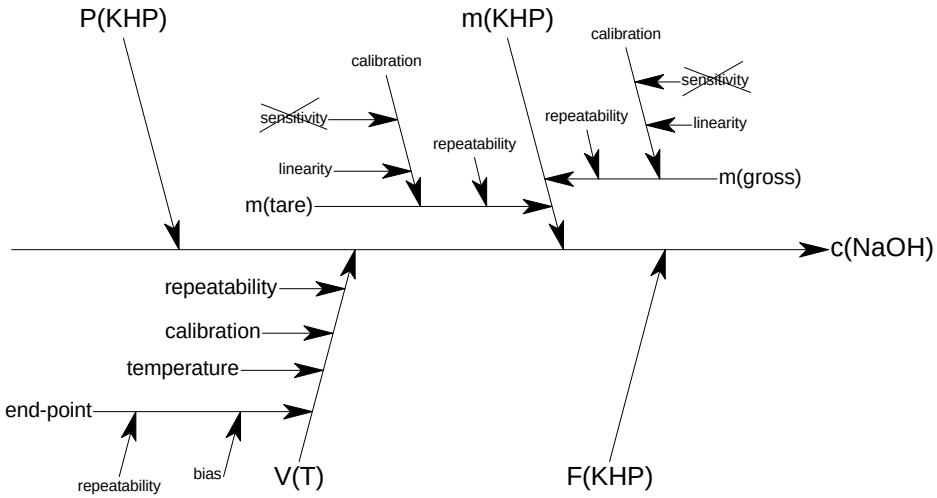
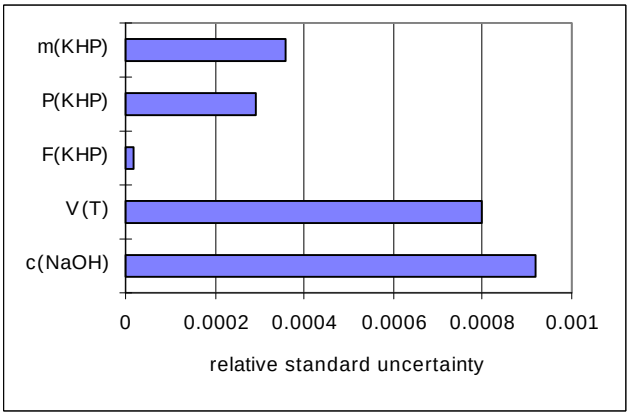


Figure A2.2: Contributions to Titration uncertainty



Example A2: Detailed description

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 $mol \cdot l^{-1}$. 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 titratable protons in relation to the overall number of molecules, provides traceability of the concentration of the NaOH solution to the SI units of measurement.

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.

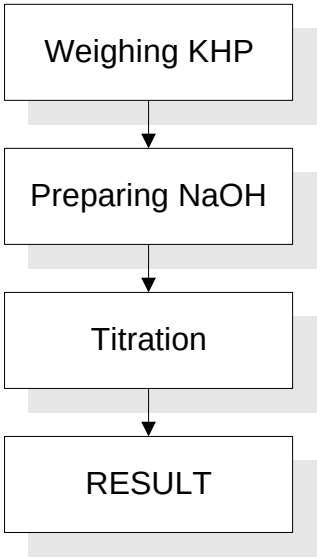


Figure A2.3: Standardisation of a solution of sodium hydroxide

The separate stages are:

i) The primary standard potassium hydrogen phthalate (KHP) is dried according to the description of the supplier. This description is given in the supplier's catalogue, which also states the purity of the titrimetric standard and its uncertainty. A titration volume of approximately 19 ml of 0.1 mol/l solution of NaOH entails weighing out an amount as close as possible to

$$\frac{294.2212 \cdot 0.1 \cdot 19}{1000 \cdot 1.0} = 0.388\,\text{g}$$

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

ii) A 0.1 mol/l solution of sodium hydroxide is prepared. In order to prepare 1 l of solution, it is necessary to weight 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 weight 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 weight of KHP, its purity, its molecular weight and the volume of NaOH at the end-point of the titration

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

where

c_{NaOH} :concentration of the NaOH solution [$mol \cdot l^{-1}$]

1000 :conversion factor [ml] to [l]

m_{KHP} :weight of the titrimetric standard KHP [g]

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

F_{KHP} :molecular weight 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

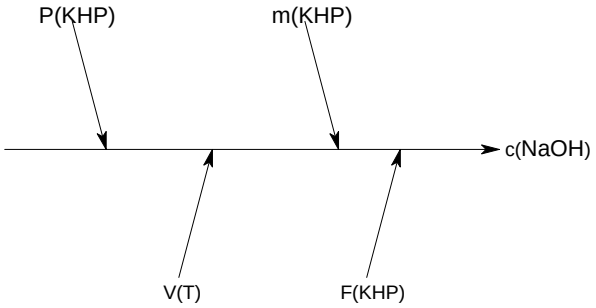


Figure A2.4: First step in setting up a cause and effect diagram

neglecting uncertainty sources on the one hand and an the other of double-counting them. The use of a cause and effect diagram (Appendix CE) 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.

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 .

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 suppliers specification.

F (KHP)

Potassium hydrogen phthalate (KHP) has the empirical formula

$$C_8H_5O_4K$$

The uncertainty in the formula weight of the compound can be determined by combining the

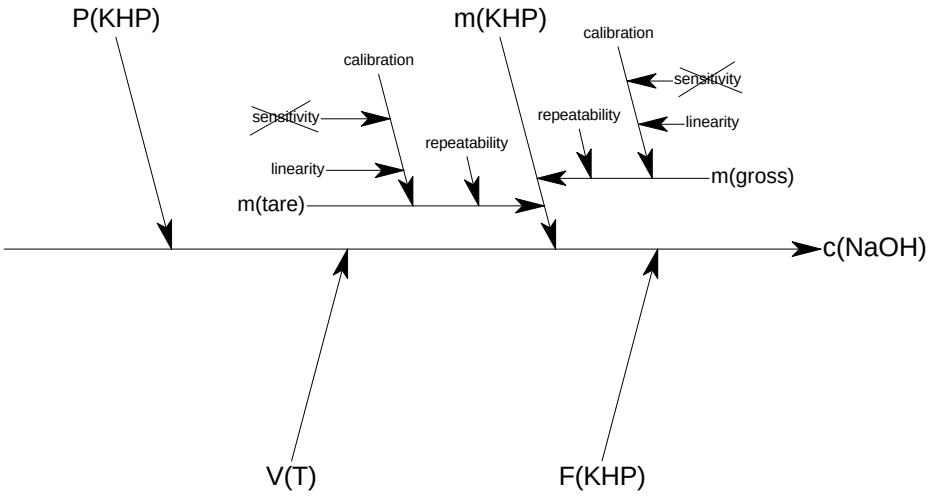


Figure A2.5:Cause and effect diagram with added uncertainty sources for the weighing procedure

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 formulae weight can be calculated directly from these; the cause and effect diagram (Figure A2.6) omits the individual atomic masses for clarity

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 completed cause and effect diagram shown in Figure A2.6.

A2.4 Step 3: Quantifying uncertainty components

In step 3 uncertainty from each source identified in step 2 has to be quantified and then converted to a standard uncertainty.

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)

1. *Repeatability*: The quality control log shows a standard uncertainty of 0.05 mg for check weighings of weights up to 100 g. This value for the repeatability was determined by a series of ten measurements of the tare and gross weight, followed by the calculation of the difference of each of the ten measurement pairs and the evaluation of the standard deviation of these differences. This repeatability contribution has to be taken into account only once, because the standard deviation of the differences was directly determined in the given experimental design.
2. *Calibration*/*Linearity*: The calibration certificate of the balance quotes ±0.15 mg for the linearity. This value is the maximum difference between the actual weight on the pan and the reading of the scale. The balance manufacture'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

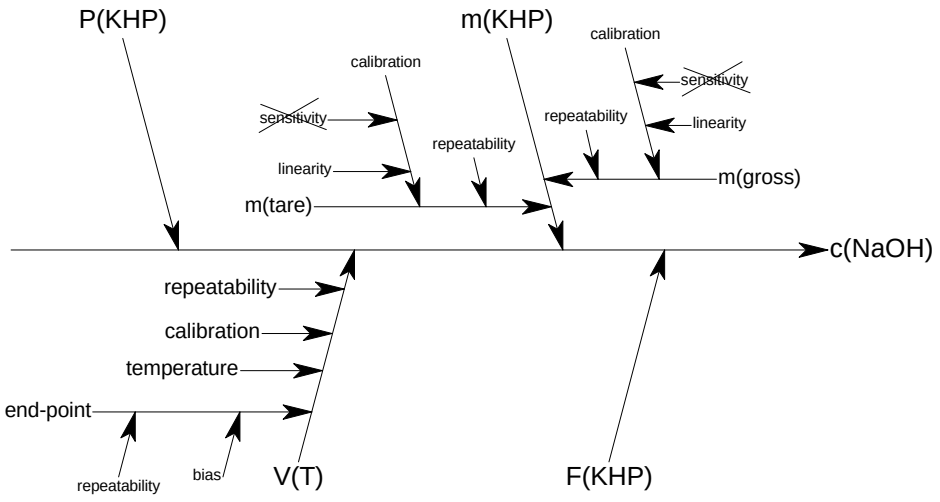


Figure A2.6: Final cause and effect diagram

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

This contribution has to be counted twice, once for the tare and once for the gross weight.

Combining the two contributions gives for the standard uncertainty $\boldsymbol{u}(m_{KHP})$ of the mass m_{KHP}, a value of

$$\boldsymbol{u}(m_{KHP}) = \sqrt{0.05^2 + 2\cdot(0.09^2)}$$
$$\Rightarrow \boldsymbol{u}(m_{KHP}) = 0.14\,\text{mg}$$

NOTE 1: Within the framework of titration experiments buoyancy corrections are very rarely made, because density differences between analysed samples are often smaller than 1000 kg/m^3. There is also no need for an additional uncertainty contribution due to neglecting the buoyancy correction, because its contribution is smaller than the repeatability or linearity contributions.

NOTE 2:-There are other difficulties 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 than 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. Therefore adsorption of some moisture is expected.

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 $\boldsymbol{u}(P_{KHP})$ is $0.0005/\sqrt{3} = 0.00029$.

F (KHP)

From the latest IUPAC table, the atomic weights and listed uncertainties 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 separate element contributions to the formula weight, 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 formula weight for KHP of

$$F_{KHP} = 96.0856 + 5.0397 + 63.9976 + 39.0983$$
$$= 204.2212\ \text{g}\cdot\text{mol}^{-1}$$

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

$$\boldsymbol{u}(F_{KHP}) = \sqrt{0.0037^2 + 0.0002^2 + 0.00068^2 + 0.000058^2}$$
$$\Rightarrow \boldsymbol{u}(F_{KHP}) = 0.0038\,\text{g}\cdot\text{mol}^{-1}$$

NOTE: Since the element contribution to KHP are simply the sum of the single atom contributions, it might be expected from the general rule for combing 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 , $u(F_C) = \sqrt{8\cdot 0.00037^2} = 0.001$. Recall, however, that this rule applies only to independent contributions, that is, contributions from separate determinations of the value. In this case, since the total contributions is obtained by multiplying the value from a single value by 8. Notice that the contributions from different elements are independent, and will therefore combine in the usual way.

$\underline{V(\mathrm{T})}$

1. *Repeatability of the volume delivery*: The use of a piston burette, unlike that of a volumetric flask, does not in general involve the complete delivery of its contents. When estimating the variability of the delivery it will be necessary to take into account the graduation marks between which discharge occurs. For a given burette, several such sets of delivery limits should be investigated and recorded; e.g. 0-10, 10-20, 5-15 etc. Similarly, it may be necessary to investigate the repeatability of different volumes delivered, such as 5, 10, 15 ml etc. In this example, the repeatability of the anticipated deliveries of 19 ml were checked, giving a sample standard deviation of 0.004 ml, used directly as a standard uncertainty.

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 $0.03/\sqrt{6} = 0.012\mathrm{ml}$.

   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. Therefore assuming unconditionally a rectangular distribution in all the cases where limits are given as its maximum bounds (without a confidence level) can hardly be justified.

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 $2.1\cdot10^{-4}$ °C^{-1} gives a value of

$$\frac{19\cdot 2.1\cdot 10^{-4}\cdot 3}{1.96} = 0.006\,\mathrm{ml}$$

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. *Repeatability of the end-point detection*: The repeatability of the end-point detection was thoroughly investigated during the method validation. Under the given conditions a standard uncertainty of 0.004 ml is appropriate.

5. *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 guidelines, that it is better to prevent any bias instead of correcting 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 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 four remaining contributions to the uncertainty $\boldsymbol{u}(V_T)$ of the volume V_T gives a value of

$$\boldsymbol{u}(V_T) = \sqrt{0.004^2 + 0.012^2 + 0.006^2 + 0.004^2}$$
$$\Rightarrow \boldsymbol{u}(V_T) = 0.015\,\mathrm{ml}$$

Table A2.1: Values and uncertainties for titration

	Description	Value x	Standard uncertainty $u(x)$	Relative standard uncertainty $u(x)/x$
m_{KHP}	weight of KHP	0.3888 g	0.00014 g	0.00036
P_{KHP}	Purity of KHP	1.0	0.00029	0.00029
F_{KHP}	Formula weight of KHP	204.2212 g/mol	0.0038 g/mol	0.000019
V_T	Volume of NaOH for KHP titration	18.64 ml	0.015 ml	0.0008

A2.5 Step 4: Calculating the combined standard uncertainty

c_{NaOH} is given by

$$c_{NaOH} = \frac{1000 \cdot m_{KHP} \cdot P_{KHP}}{F_{KHP} \cdot V_T} \qquad [\mathrm{mol \cdot l^{-1}}]$$

The values of the parameters in this equation, their standard uncertainties and their relative standard uncertainties are collected in table Table A2.1

Using the values given above:

$$c_{NaOH} = \frac{1000 \cdot 0.3888 \cdot 1.0}{204.2212 \cdot 18.64} = 0.10214\,\mathrm{mol \cdot l^{-1}}$$

In order to combine the uncertainties associated with each component of a multiplicative expression (as above) the standard uncertainties are used in the following way

$$\frac{\boldsymbol{u}_c(c_{NaOH})}{c_{NaOH}} = \sqrt{\left(\frac{u(m_{KHP})}{m_{KHP}}\right)^2 + \left(\frac{u(P_{KHP})}{P_{KHP}}\right)^2 + \left(\frac{u(F_{KHP})}{F_{KHP}}\right)^2 + \left(\frac{u(V_T)}{V_T}\right)^2}$$

$$= \sqrt{0.00036^2 + 0.00029^2 + 0.000019^2 + 0.0008^2}$$

$$= 0.00092$$

$$\Rightarrow \boldsymbol{u_c}(c_{NaOH}) = c_{NaOH} \cdot 0.00092 = 0.00009\,\mathrm{mol \cdot l^{-1}}$$

A standard spreadsheet is used to simplify the above calculation of the combined standard uncertainty. A comprehensive introduction into the method is given in Appendix E. The spreadsheet filled in with the appropriate values is shown as Table A2.2.

The values of the parameters are given in the second row from C2 to F2. Their standard uncertainties are entered in the row below (C3-F3). The spreadsheet copies the values from C2-F2 into the second column from B5 to B8. The result (c(NaOH)) using these values is given in B10. The C5 shows the value of m(KHP) from C2 plus its uncertainty given in C3. The result of the calculation using the values C5-C8 is given in C10. The columns D and F follow a similar procedure. The values shown in the row 11 (C11-F11) are the differences of the row (C10-F10) minus the value given in B10. In row 12 (C12-F12) the values of row 11 (C11-F11) are squared and summed to give the value shown in B12. B14 gives the combined standard uncertainty, which is the square root of B12.

At the end it is instructive to examine the relative contributions of the different parameters. The share of each contribution can easily be visualised using a histogram displaying the relative standard uncertainties:

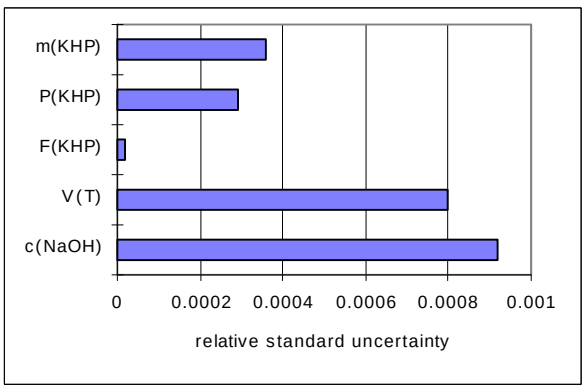


Figure A2.7: Uncertainty contributions in NaOH standardisation

The contribution of the uncertainty of the titration volume V_T is be far the largest. The weighing procedure and the purity of the titrimetric

Table A2.2: Spreadsheet calculation of titration uncertainty

	A	B	C	D	E	F
1			m(KHP)	P(KHP)	F(KHP)	V(T)
2		Value	0.3888	1.0	204.2212	18.64
3		Uncertainty	0.00014	0.00029	0.0038	0.015
4						
5	m(KHP)	0.3888	0.38894	0.3888	0.3888	0.3888
6	P(KHP)	1.0	1.0	1.00029	1.0	1.0
7	F(KHP)	204.2212	204.2212	204.2212	204.2250	204.2212
8	V(T)	18.64	18.64	18.64	18.64	18.655
9						
10	c(NaOH)	0.102136	0.102173	0.102166	0.102134	0.102054
11			0.000037	0.000030	-0.000002	-0.000082
12		9.0E-9	1.37E-9	9E-10	4E-12	6.724E-9
13						
14	u(c(NaOH))	0.000095				

standard show the same order of magnitude for their relative standard uncertainties, whereas the uncertainty in the formula weight is again nearly an order of magnitude smaller.

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

$$\boldsymbol{U}(c_{NaOH}) = 0.00009 \cdot 2 \cong 0.0002\ \mathrm{mol \cdot l^{-1}}$$

Thus the concentration of the NaOH solution is **0.1021 ±0.0002 mol·l^{-1}**.

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, which are 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 one. 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 A2.3. 1) According to the ISO Guide 4.3.9 Note 1: "For a normal distribution with expectation μ and standard deviation σ, the interval $\mu \pm 3\sigma$ 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, X_i can be assumed to be approximately normally distributed rather than there being no specific knowledge about X_i between the bounds as in 4.37, then $u^2(x_i) = a^2/9$. By comparison, the variance of a symmetric rectangular distribution of the half-width a is $a^2/3$ [equation (7)] and that of a symmetric triangular distribution of the half-width a is $a^2/6$ [equation (9b)]. The magnitudes of the variances of the three distributions are surprisingly similar in view of the differences 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 ($\boldsymbol{u}_c(c_{NaOH})$) and it is adequate to assume that it is triangular.

Table A2.3: Effect of different distribution asumptions

Distribution	factor	$u(V(T;cal))$ (ml)	$u(V(T))$ (ml)	$\boldsymbol{u}_c(c_{NaOH})$
rectangular	$\sqrt{3}$	0.017	0.019	0.00011 $mol \cdot l^{-1}$
triangular	$\sqrt{6}$	0.012	0.015	0.00009 $mol \cdot l^{-1}$
normal distribution[1]	$\sqrt{9}$	0.010	0.013	0.000085 $mol \cdot l^{-1}$

Example A3 - An acid/base titration

Summary

Goal

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

Measurement procedure

A solution of hydrochloride acid (HCl) is titrated against a solution of sodium hydroxide (NaOH), which before 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:

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

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

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

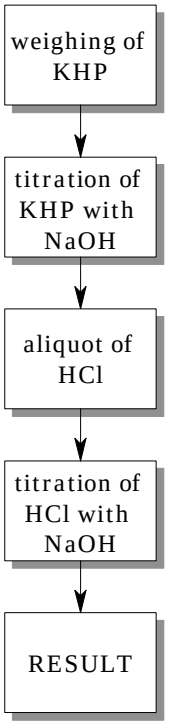


Figure A3.2: Cause and Effect diagram for acid-base titration

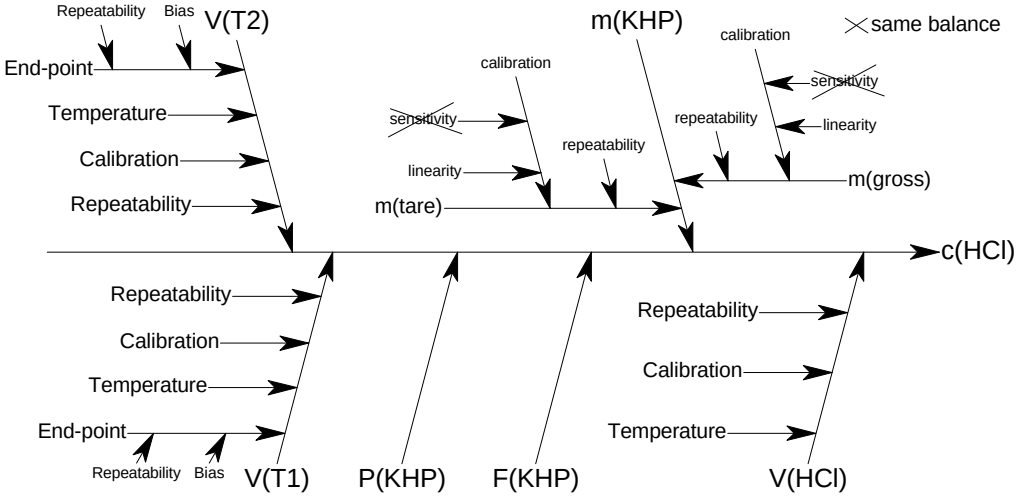
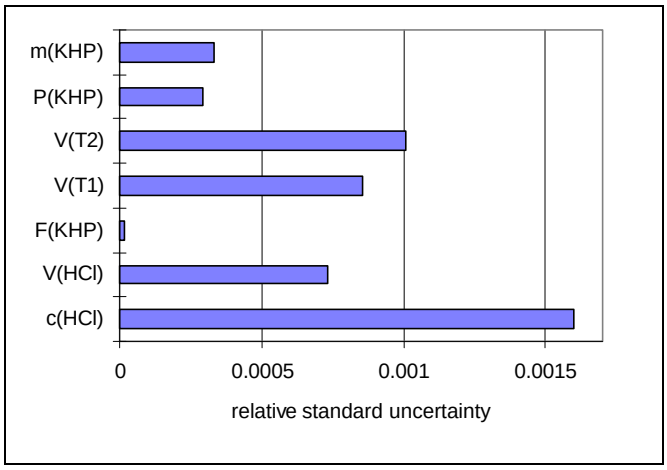


Table A3.1: Acid-base Titration values and uncertainties

	Description	Value x	Standard uncertainty $u(x)$	Relative standard uncertainty $u(x)/x$
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
F_{KHP}	Formula weight 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_{NaOH}	HCl solution	0.10139 mol l^{-1}	0.00016 mol l^{-1}	0.0016

Figure A3.3: Uncertainties in acid-base titration



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 hydrochloride 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 $mol \cdot l^{-1}$ 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 (Figure A3.4). The separate stages are:

i) The titrimetric standard potassium hydrogen phthalate (KHP) is dried to obtain the purity, which is quoted in the supplier's certificate. Afterwards approximately 0.388 g of the standard is 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 ion free water to have ≈ 50 ml solution in the titration vessel.

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

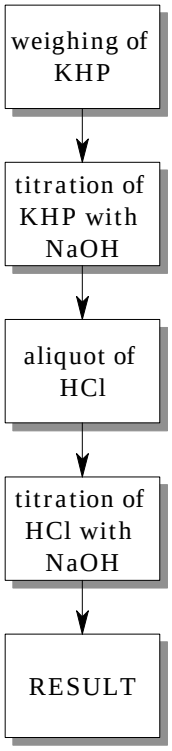


Figure A3.4: Determination of the concentration of a HCl solution

Calculation:

The measurand is the concentration of the HCl solution, given by

$$c_{HCl} = \frac{1000 \cdot m_{KHP} \cdot P_{KHP} \cdot V_{T2}}{V_{T1} \cdot F_{KHP} \cdot V_{HCl}} \qquad [\mathrm{mol} \cdot \mathrm{l}^{-1}]$$

where

c_{HCl} :concentration of the HCl solution [$mol \cdot l^{-1}$]

1000 :conversion factor [ml] to [l]

m_{KHP} :weight of KHP taken [g]

P_{KHP} :purity of KHP given as mass fraction []

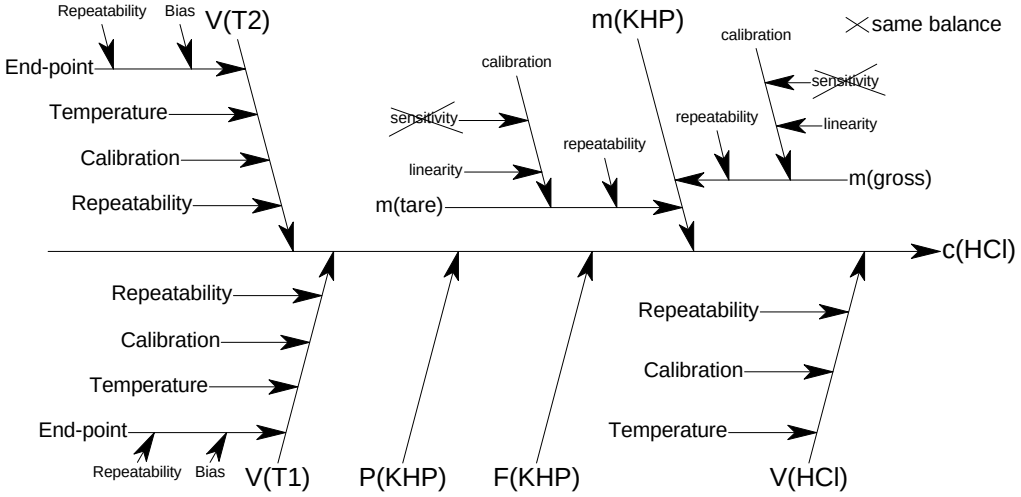
V_{T2} :volume of NaOH solution to titrate HCl [ml]

V_{T1} :volume of NaOH solution to titrate KHP [ml]

F_{KHP} : formula weight of KHP [$g \cdot mol^{-1}$]

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).

The cause and effect diagram will not be further simplified in the first part of the example. Such a simplification could be achieved by combining the different run to run variabilities to one overall repeatability contribution (cf. second part of this example). The influence quantities of the parameter V_{T2}, V_{T1}, m_{KHP}, P_{KHP} and F_{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.

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 form 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.

m(KHP)

1. *Repeatability*: The quality control log shows a standard uncertainty of 0.05 mg for check weighings in the range of the balance of 0 g up to 100 g. The employed check weights are in the same order of magnitude as the amount of the titrimetric standard.
2. *Calibration/linearity*: The balance manufacturer quotes ±0.15 mg for the linearity contribution. This value represents the maximum difference between the actual weight 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 weight.

Combining the two contributions to the standard uncertainty $\boldsymbol{u}\left(m_{KHP}\right)$ of the mass m_{KHP} gives a value of

$$\boldsymbol{u}(m_{KHP}) = \sqrt{0.05 + 2 \cdot (0.087)^2}$$
$$\Rightarrow \boldsymbol{u}(m_{KHP}) = 0.13\,\text{mg}$$

Note: There is no need for a buoyancy correction, because the density difference between the titrimetric standard and the HCl solution is only ≈600 kg m^{-3} leading to a correction or otherwise uncertainty contribution considerably smaller than the linearity component.

P(KHP)

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

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

V(T2)

1. *Repeatability of the volume delivery*: Sample standard deviation of 0.004 ml obtained from check weighing of the delivered volume.
2. *Calibration*: Figure given by the manufacturer (±0.03 ml) and approximated to a triangular distribution $0.03/\sqrt{6} = 0.012\,\text{ml}$.
3. *Temperature*: The possible temperature variation is within the limits of ±4°C and approximated to a rectangular distribution $15 \cdot 2.1 \cdot 10^{-4} \cdot 4/\sqrt{3} = 0.007\text{ml}$.
4. *Repeatability of the end-point detection*: Evaluations during the method validation provided a standard uncertainty of 0.004 ml.
5. *Bias of the end-point detection*: A bias between the determined end-point and the equivalence-point can be prevented by performing the titration under a layer of Argon gas.

V_{T2} is found to be 14.89 ml and combining the four contributions to the uncertainty $\boldsymbol{u}(V_{T2})$ of the volume V_{T2} gives a value of

$$\boldsymbol{u}(V_{T2}) = \sqrt{0.004^2 + 0.012^2 + 0.007^2 + 0.004^2}$$
$$\Rightarrow \boldsymbol{u}(V_{T2}) = 0.015\text{ml}$$

V(T1)

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

1. *Repeatability of the volume delivery*: 0.004 ml
2. *Calibration*: $0.03/\sqrt{6} = 0.012\text{ml}$
3. *Temperature*: The approximate volume for the titration of 0.3888 g KHP is 19 ml NaOH, therefore its uncertainty contribution is $19 \cdot 2.1 \cdot 10^{-4} \cdot 4/\sqrt{3} = 0.009\text{ml}$.
4. *Repeatability of the end-point detection*: 0.004 ml
5. *Bias*: Negligible

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

$$\boldsymbol{u}(V_{T1}) = \sqrt{0.004^2 + 0.012^2 + 0.009^2 + 0.004^2}$$
$$\Rightarrow \boldsymbol{u}(V_{T1}) = 0.016\text{ml}$$

F(KHP)

Atomic weights and listed uncertainties (from 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 formula weight F_{KHP} for KHP and its uncertainty $u(F_{KHP})$ are, respectively:

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

Note: The single atom contributions are not independent, therefore the uncertainty for the atom contribution is calculated by multiplying the number of atoms with the standard uncertainty of the single atom directly.

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

	Description	Value x	Standard Uncertainty $u(x)$	Relative standard uncertainty $u(x)/x$
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
F_{KHP}	Formula weight 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

V(HCl)

1. *Repeatability*: Standard uncertainty of 0.0037 ml obtained from a replicate weighing experiment of the delivered volume.
2. *Calibration*: Uncertainty stated by the manufacturer for a 15 ml pipette as ±0.02 ml and approximated with a triangular distribution: $0.02/\sqrt{6} = 0.008\text{ml}$.
3. *Temperature*: The temperature of the laboratory is within the limits of ±4°C. Using a rectangular temperature distribution gives a standard uncertainty of $15 \cdot 2.1 \cdot 10^{-4} \cdot 4/\sqrt{3}$ = 0.007 ml.

Combining those contributions to the standard uncertainty $\boldsymbol{u}(V_{HCl})$ give a figure of

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

A3.5 Step 4: Calculating the combined standard uncertainty

c_{HCl} is given by

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

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

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

The uncertainties associated with each component are combined accordingly:

$$\frac{\boldsymbol{u}_c(c_{HCl})}{c_{HCl}} = \sqrt{\left(\frac{u(m_{KHP})}{m_{KHP}}\right)^2 + \left(\frac{u(P_{KHP})}{P_{KHP}}\right)^2 + \left(\frac{u(V_{T2})}{V_{T2}}\right)^2 + \left(\frac{u(V_{T1})}{V_{T1}}\right)^2 + \left(\frac{u(F_{KHP})}{F_{KHP}}\right)^2 + \left(\frac{u(V_{HCl})}{V_{HCl}}\right)^2}$$
$$= \sqrt{0.00033^2 + 0.00029^2 + 0.001^2 + 0.00086^2 + 0.000019^2 + 0.00073^2}$$
$$= 0.0016$$
$$\Rightarrow \boldsymbol{u}_c(c_{HCl}) = c_{HCl} \cdot 0.0016 = 0.00016\,\text{mol} \cdot \text{l}^{-1}$$

A standard spreadsheet method is employed to simplify the above calculation of the combined standard uncertainty. A comprehensive introduction into the method is given in Appendix E. The spreadsheet filled in with the appropriate values is shown in Table A3.3.

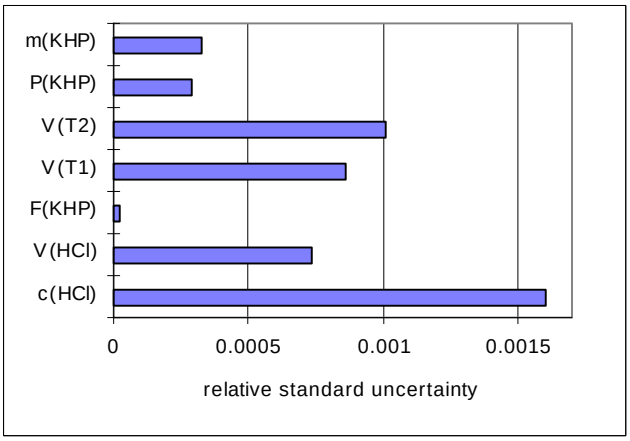
The values of the parameters are given in the second row from C2 to H2. Their standard uncertainties are entered in the row below (C3-H3). The spreadsheet copies the values from C2-H2 into the second column from B5 to B10. The result (c(HCl)) using these values is given in B12. The C5 shows the value of m(KHP) 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. The values shown in the row 13 (C13-H13) are the 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.

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

	A	B	C	D	E	F	G	H
1			m(KHP)	P(KHP)	V(T2)	V(T1)	F(KHP)	V(HCl)
2		value	0.3888	1.0	14.89	18.64	204.2212	15
3		Uncertainty	0.00013	0.00029	0.015	0.016	0.0038	0.011
4								
5	m(KHP)	0.3888	0.38893	0.3888	0.3888	0.3888	0.3888	0.3888
6	P(KHP)	1.0	1.0	1.00029	1.0	1.0	1.0	1.0
7	V(T2)	14.89	14.89	14.89	14.905	14.89	14.89	14.89
8	V(T1)	18.64	18.64	18.64	18.64	18.656	18.64	18.64
9	F(KHP)	204.2212	204.2212	204.2212	204.2212	204.2212	204.2250	204.2212
10	V(HCl)	15	15	15	15	15	15	15.011
11								
12	c(HCl)	0.101387	0.101421	0.101417	0.101489	0.101300	0.101385	0.101313
13			0.000034	0.000029	0.000102	-0.000087	-0.0000019	-0.000074
14		2.55E-8	1.1E-9	8.64E-10	1.043E-8	7.56E-9	3.56E-12	5.52E-9
15								
16	u(c(HCl))	0.00016						

The size of the different contributions can be best compared using a histogram showing their relative standard uncertainty (Figure A3.6).

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

$$U(c_{HCl}) = 0.0016 \cdot 2 = 0.0003\,\text{mol}\cdot\text{l}^{-1}$$

The concentration of the HCl solution is 0.1014 ±0.003 mol l^{-1}

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 quite 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

Analytical chemist rarely correct for the systematic effect of the current temperature in the laboratory on the volume. The question should one do so, as is explained in the rest of this section, or should the uncertainty for each of the volumes be increased accordingly. The volumetric measuring devices have been calibrated at a temperature of 20°C. But rarely does any analytical laboratory have a temperature controller to keep the room temperature that level. A mean room temperature of 25°C or even higher is more common at the bench during the summertime. Therefore the final result has to be calculated using the actual volumes and not the calibrated volumes at 20°C. A volume is corrected for the temperature effect according to

$$V' = V[1 - \mathsf{a}\,(T - 20^{\circ}\text{C})]$$

where

V' :actual volume at the mean temperature T

V :volume calibrated at 20°C

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

T :actual mean temperature in the laboratory [°C]

The equation of the measurand has to be rewritten in the following way:

$$c_{HCl} = \frac{1000 \cdot m_{KHP} \cdot P_{KHP}}{F_{KHP}} \cdot \frac{V'_{T2}}{V'_{T1} \cdot V'_{HCl}}$$
$$= \left(\frac{1000 \cdot m_{KHP} \cdot P_{KHP}}{F_{KHP}} \right)$$
$$\times \left(\frac{V_{T2}[1 - a\,(T - 20^{\circ}\mathrm{C})]}{V_{T1}[1 - a\,(T - 20^{\circ}\mathrm{C})] \cdot V_{HCl}[1 - a\,(T - 20^{\circ}\mathrm{C})]} \right)$$

This expression can be reduced making the assumption that the mean temperature T and the expansion coefficient of an aqueous solution a are the same for all three volumes

$$c_{HCl} = \left(\frac{1000 \cdot m_{KHP} \cdot P_{KHP}}{F_{KHP}} \right)$$
$$\times \left(\frac{V_{T2}}{V_{T1} \cdot V_{HCl} \cdot [1 - a\,(T - 20^{\circ}\mathrm{C})]} \right)$$

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

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

The figure is still within the range given by the combined standard uncertainty of the result at a mean temperature of 20°C. All the calculation and assumptions within the section have no influence on to the evaluation of the combined standard uncertainty because still a temperature variation of ±4°C at the mean room temperature of 25°C is assumed.

Visual end-point detection

A bias is introduced if the indicator phenolphthalein is used for a visual end-point detection instead of the automatic titration system extracting the equivalence-point out of the shape of the pH curve recorded with a combined pH-electrode. 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

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

where

$V_{T1;Ind}$: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

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

The standard uncertainties $u(V_{T2})$ and $u(V_{T1})$ 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.

$$u(V_{T1}) = u(V_{T1;Ind} - V_{Excess})$$
$$= \sqrt{0.004^2 + 0.012^2 + 0.009^2 + 0.03^2}$$
$$= 0.034 \mathrm{ml}$$

$$u(V_{T2}) = u(V_{T2;Ind} - V_{Excess})$$
$$= \sqrt{0.004^2 + 0.012^2 + 0.007^2 + 0.03^2}$$
$$= 0.033 \mathrm{ml}$$

The combined standard uncertainty

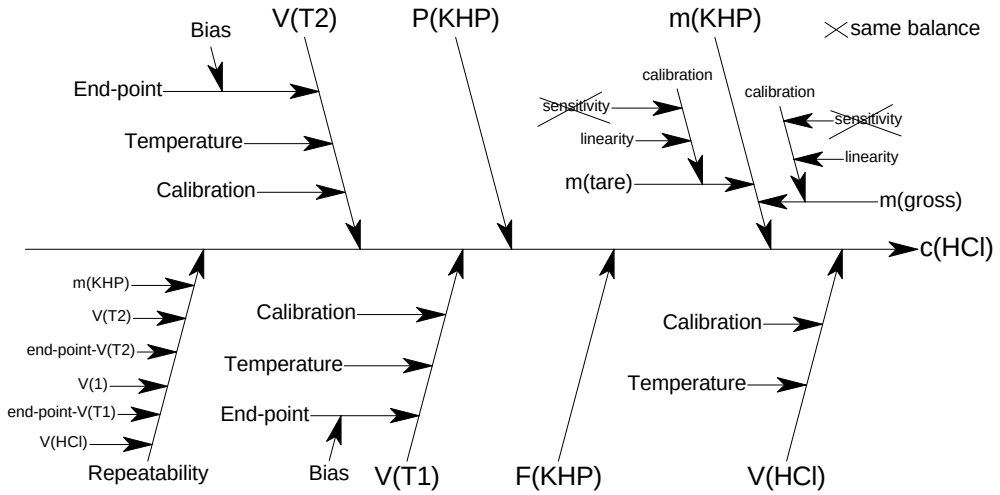
$$u_c(c_{HCl}) = 0.0003 \mathrm{mol} \cdot \mathrm{l}^{-1}$$

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 and the triple determination leads to the opportunity to calculate the overall repeatability of the experiment directly from the standard deviation in the final result. Therefore 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 A3.7).

Figure A3.7: Acid-base Titration values and uncertainties (repeatability grouped)



The uncertainty components are quantified in the following way:

<u>*m*(KHP)</u>

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

$\Rightarrow u(m_{KHP}) = \sqrt{2 \cdot 0.87^2} = 0.12\,\text{mg}$

<u>*P*(KHP)</u>

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

<u>*V*(T2)</u>

calibration: $0.03/\sqrt{6} = 0.012\,\text{ml}$

temperature: $15 \cdot 2.1 \cdot 10^{-4} \cdot 4/\sqrt{3} = 0.007\text{ml}$

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

<u>Repeatability</u>

The quality log of the triple determination shows a mean long term standard deviation of the experiment of $0.0006\,\text{mol}\cdot\text{l}^{-1}$. 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 $0.0006\,\text{mol}\cdot\text{l}^{-1}$ is divided by the square root of $\sqrt{3}$ to obtain the standard uncertainty of the triple determination. (Three independent measurements)

$$Rep = 0.0003/\sqrt{3} = 0.00017\,\text{mol}\cdot\text{l}^{-1}$$

<u>*V*(HCl)</u>

calibration: $0.02/\sqrt{6} = 0.008\,\text{ml}$

temperature: $15 \cdot 2.1 \cdot 10^{-4} \cdot 4/\sqrt{3} = 0.007\,\text{ml}$

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

<u>*F*(KHP)</u>

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

<u>*V*(T1)</u>

calibration: $0.03/\sqrt{6} = 0.02\text{ml}$

temperature:

$$19 \cdot 2.1 \cdot 10^{-4} \cdot 4/\sqrt{3} = 0.009\,\text{ml}$$

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

All the values of the uncertainty components are summarised in Table A3.4. The combined standard uncertainty is $0.00023\,\text{mol}\cdot\text{l}^{-1}$, which is not a significant reduction due to the triple determination. The comparison of the uncertainty contributions in the histogram, shown in Figure A3.8, highlights some of the reasons for that result.

Figure A3.8: Replicated Acid-base Titration values and uncertainties

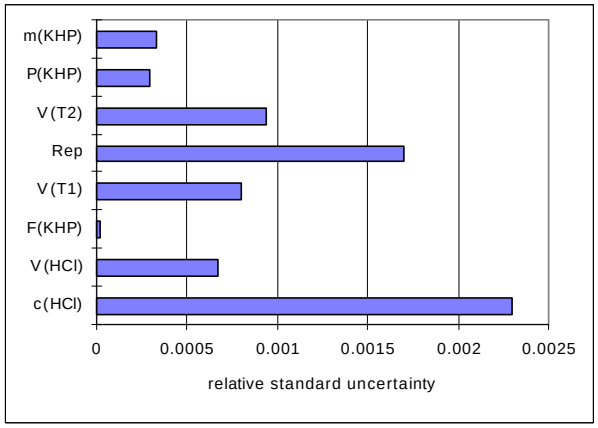


Table A3.4: Replicated Acid-base Titration values and uncertainties

	Description	approximate Value	Standard uncertainty	Relative standard uncertainty
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	14.90 ml	0.014 ml	0.00094
Rep	Repeatability of the determination	0.10140 mol l^{-1}	0.00017 mol l^{-1}	0.0017
V_{T1}	Volume of NaOH for KHP titration	18.65 ml	0.015 ml	0.0008
F_{KHP}	Formula weight 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 organophosorus pesticides residue in bread is determined employing an extraction and a GC-procedure.

Measurement procedure

The numerous stages needed to determine the amount of organophosorus pesticides residue are shown in Figure A4.1

Measurand:

$$P_{op} = \frac{I_{op} \cdot c_{ref} \cdot V_{op}}{I_{ref} \cdot R \cdot m_{sample}} \cdot F_{hom} \cdot 10^6 \text{ mg kg}^{-1}$$

where

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

I_{op} :Peak intensity of the sample extract

c_{ref} :Mass concentration of the reference standard [g ml^{-1}]

V_{op} :Final volume of the extract [ml]

10^6 :Conversion factor from [g/g] to [mg kg^{-1}]

I_{ref} :Peak intensity of the reference standard

Rec :Recovery

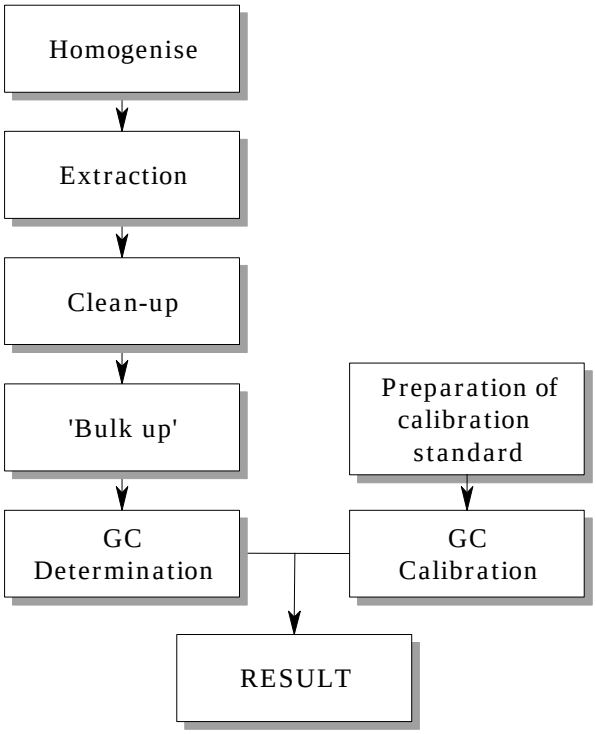
m_{sample}:Weight of the investigated sub-sample [g]

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.

Figure A4.1: Organophosphorus pesticides analysis



Quantification of the uncertainty components:

Based on in-house validation data, the three major contributions are listed in Table A4.1 and shown diagramatically in Figure A4.3.

Table A4.1: Uncertainties in pesticide analysis

Description	Value x	Standard uncertainty $u(x)$	Relative standard uncertainty $u(x)/x$	Remark
Repeatability(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
$u(P_{op})/P_{op}$	- -	- -	0.34	Relative standard uncertainty

Figure A4.2: Uncertainty sources in pesticide analysis

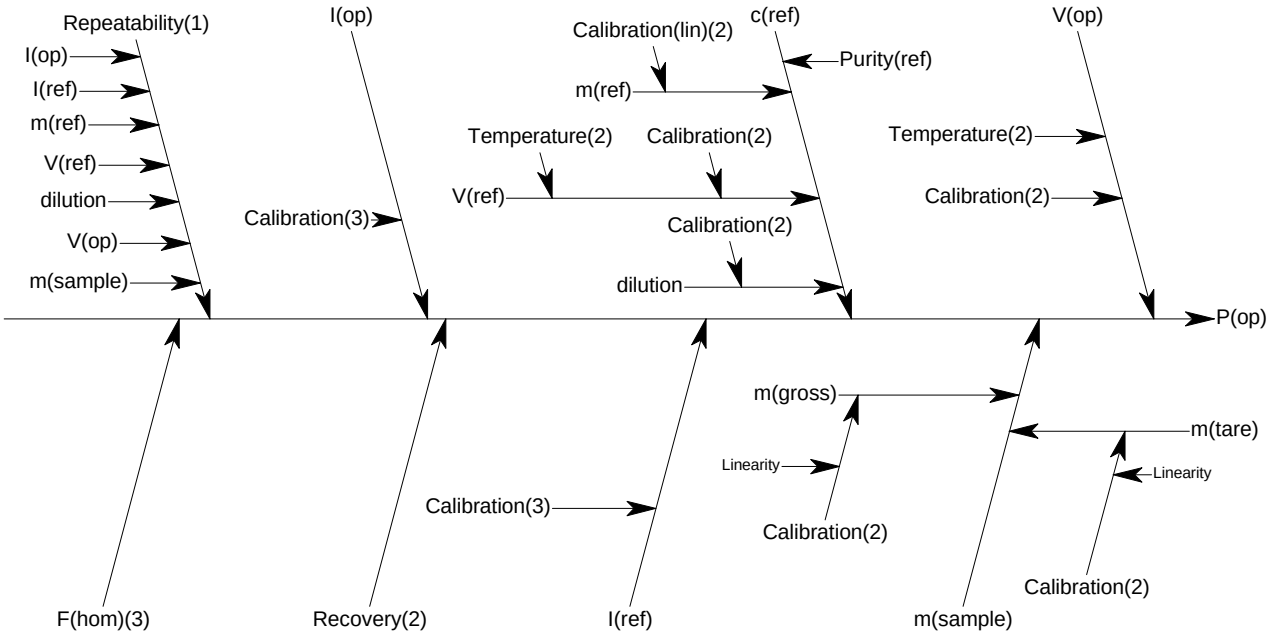
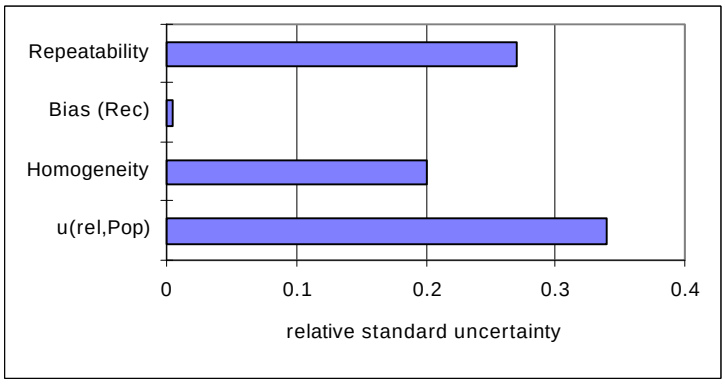


Figure A4.3: Uncertainties in pesticide analysis



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 traceability 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 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-sampling 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 Kedurna-Danish apparatus.

iv) Liquid-liquid extraction:
Acetonitrile/hexane liquid partition, washing the acetonitrile extract with hexane, drying the hexane layer through sodium sulphate column.

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

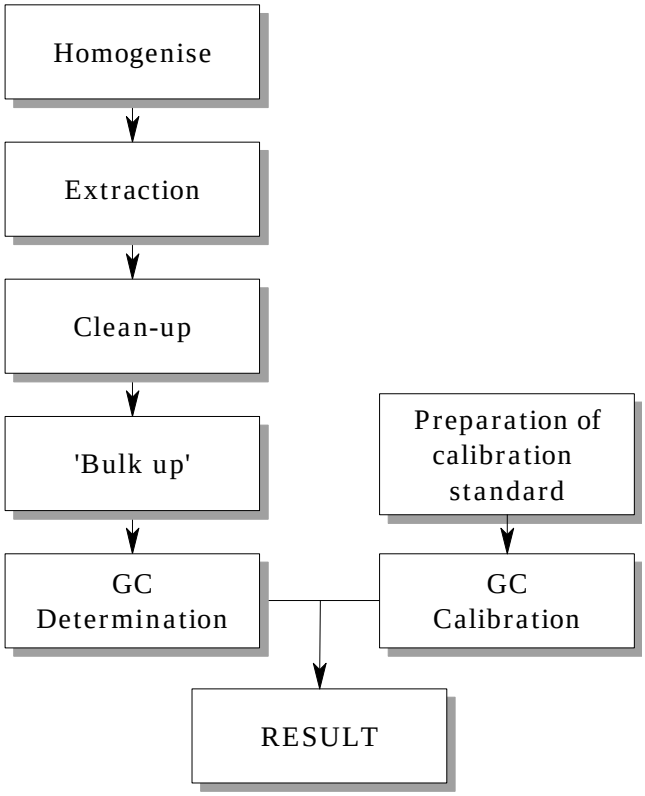
vi) Dilution to standard volume V_{op} (approx. 2 ml) in a graduated 10 tube.

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

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

ix) GC calibration using the standard prepared before 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 is given by

$$c_{op} = c_{ref} \cdot \frac{I_{op}}{I_{ref}} \qquad \text{g ml}^{-1}$$

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

$$P_{op} = \frac{c_{op} \cdot V_{op}}{Rec \cdot m_{sample}} \cdot 10^6 \qquad \text{mg kg}^{-1}$$

which leads to the comprehensive equation of the measurand

$$P_{op} = \frac{I_{op} \cdot c_{ref} \cdot V_{op}}{I_{ref} \cdot Rec \cdot m_{sample}} \cdot 10^6 \qquad \text{mg kg}^{-1}$$

where

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

I_{op} :Peak intensity of the sample extract

c_{ref} :Mass concentration of the reference standard [g ml^{-1}]

V_{op} :Final volume of the extract [ml]

10^6 :Conversion factor from [g/g] to [mg kg^{-1}]

I_{ref} :Peak intensity of the reference standard

Rec :Recovery

m_{sample}:Weight 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 kind of breads 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. All parameters of the equations of the measurand represent the main branches of the diagram. Afterwards any further factor is added to the diagram considering each step in the analytical procedure. (A 4.2) until the contributory factors become sufficiently remote. This leads to the following diagram:

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. Therefore a new main branch representing the sample inhomogeneity is added to the cause and effect diagram.

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

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

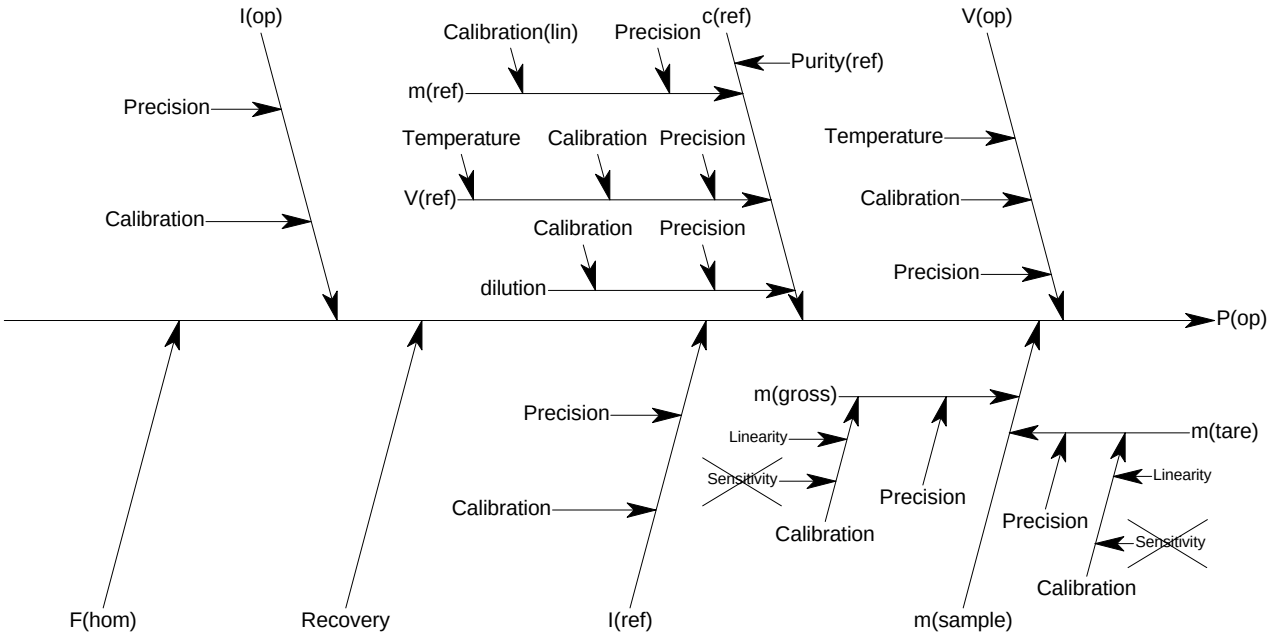
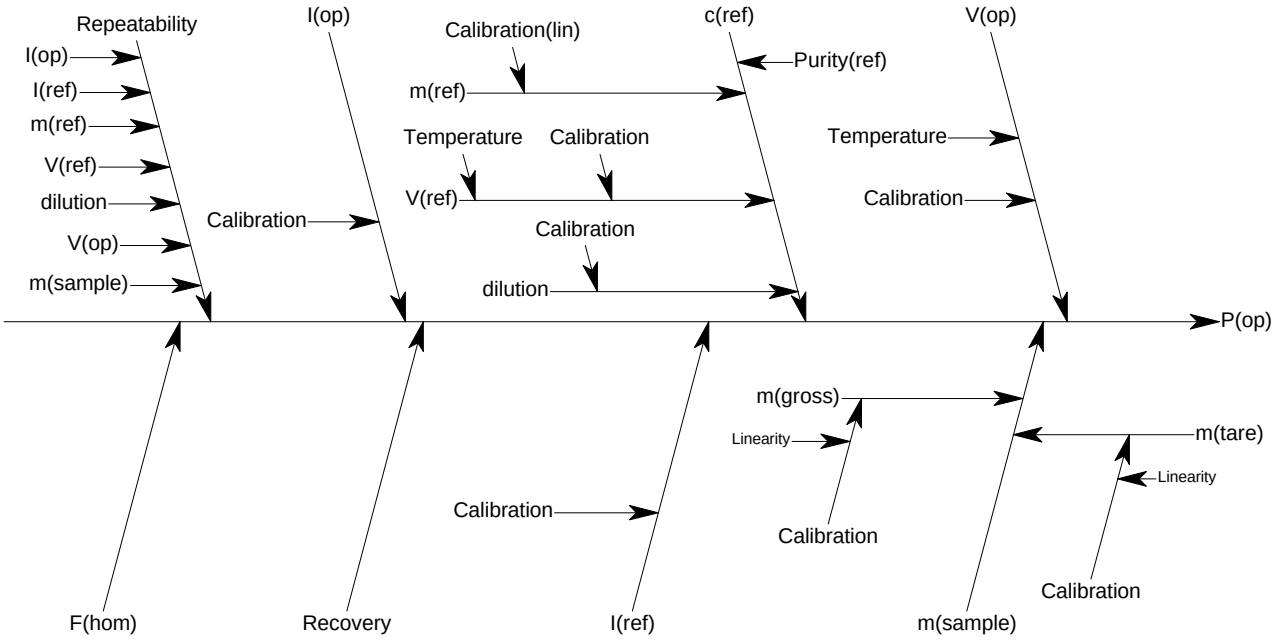


Figure A4.6: Cause and effect diagram after rearrangement to accommodate the data of the validation study



clearly, it is useful to write

$$P_{op} = F_{hom} \cdot \frac{I_{op} \cdot c_{ref} \cdot V_{op}}{I_{ref} \cdot Rec \cdot m_{sample}} \cdot 10^6 \qquad [\text{mg/kg}]$$

where from 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 to be 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

The quantification of the different uncertainty components utilises data from three major steps from the in-house development and validation studies:

The best feasible estimation 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 of influence quantities identified previous the cause and effect diagram (Figure A4.) has to be done to accommodate these three major steps.

1. Precision study

The overall run to run variation (precision) of the analytical procedure was performed with a number of duplicate tests for typical organophosphorus pesticides found in different bread samples. The results are collected in Table A4.2. The overall estimated standard deviation $s = 0.382$.

The normalised difference data (the difference divided by the mean) provides a measure of the overall run to run variability. 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

Table A4.2: Results of duplicate pesticide analysis

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

standard uncertainty due to run to run variation of the overall analytical process 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 and sample levels for a representative selection of typical organophosphorus pesticides. This is done in the most efficient way by duplicate tests on many materials, providing (for the repeatability estimate) approximately one degree of freedom for each material studied in duplicate.

2. Bias study

The bias of the analytical procedure was investigated during the in-house validation study using spiked samples. 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 $u(\overline{Rec}) = 0.28/\sqrt{42} = 0.0432$. There are three possible cases arising for the value of the recovery $\overline{Rec}$

1) $\overline{Rec}$ taking into account $u(\overline{Rec})$ is not significantly different from 1 so no correction is applied.

2) $\overline{Rec}$ taking into account $u(\overline{Rec})$ is significantly different from 1 and a correction is applied.

3) $\overline{Rec}$ taking into account $u(\overline{Rec})$ is significantly different from 1 but a correction is not applied.

A significance test is used to determine whether the recovery is significantly different from 1 . The test statistic t is calculated using the following equation

$$t = \frac{\left|1 - \overline{Rec}\right|}{u(\overline{Rec})} = \frac{(1 - 0.9)}{0.0432} = 2.315$$

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

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.

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 have to be examined and eventually considered in the calculation of the measurement uncertainty.

(1) Considered during the variability investigation 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.

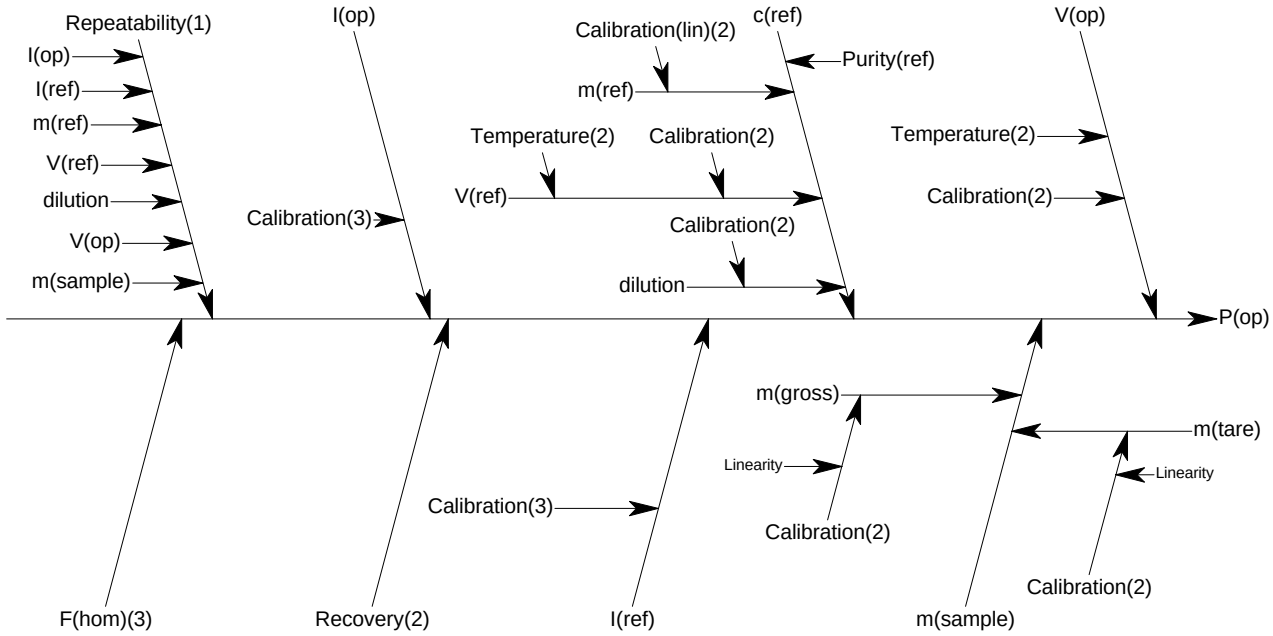
All balances and the important volumetric measuring devices are under regular control. The bias study takes into account the influence of the calibration of the different volumetric measuring devices because during the investigation various volumetric flasks and pipettes have been used. The extensive variability studies, which lasted for more than half a year, determined also the influence of the environmental temperature onto the result.

The purity of the reference standard is given by the manufacturer as 99.53% ±0.06%. The purity is a potential additional other uncertainty source with a standard uncertainty of $0.0006/\sqrt{3} = 0.00035$ (Rectangular distribution). But its value is much too small to be considered any further as an essential uncertainty contribution.

Another feasible influence quantity is the nonlinearity of the signal of the examined organophosphorus pesticides within the given concentration range. 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. Therefore its contribution has been estimated on the basis of the sampling method used.

Figure A4.7: Evaluation of other sources of uncertainty



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

- Residue distributed on the top surface only: 0.58.
- Residue distributed evenly over the surface only: 0.20.
- 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: It would arise in the case of decorative additions (whole grains) added to one 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 repeatability, the bias and all other feasible uncertainty sources had been thoroughly investigated. Their values and uncertainties are collected in Table A4.4.

Only the relative value of the combined standard uncertainty can be calculated because the uncertainty contribution for the entire range of the analyte is evaluated.

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

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

The standard spreadsheet for this case (Table A4.5) takes the form shown in Table A4.5.

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. 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-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

Table A4.4: Uncertainties in pesticide analysis

Description	Value x	Standard uncertainty $u(x)$	Relative standard uncertainty $u(x)$	Remark
Repeatability(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
$u(P_{op})/P_{op}$	- -	- -	0.34	Relative standard uncertainty

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 size of the three different contributions can be compared by employing a histogram (Figure A4.8) showing their relative standard uncertainties.

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

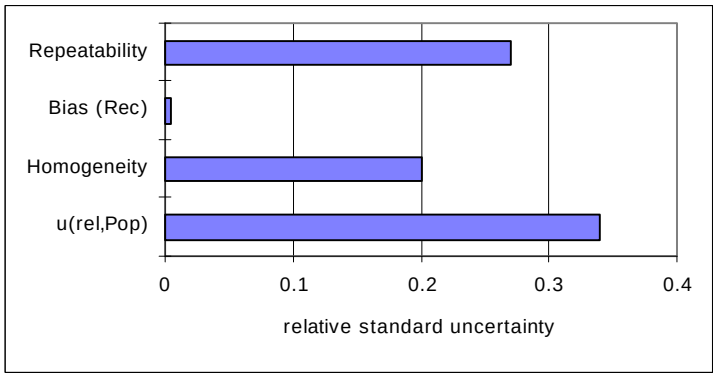
The expanded uncertainty $U(P_{op})$ is calculated by multiplying the combined standard uncertainty with a coverage factor of 2 to give:

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

Table A4.5: Uncertainties in pesticide analysis

	A	B	C	D	E
1			Repeatability	Bias	Homogeneity
2		value	1.0	0.9	1.0
3		uncertainty	0.27	0.043	0.2
4					
5	Repeatability	1.0	1.27	1.0	1.0
6	Bias	0.9	0.9	0.9043	0.9
7	Homogeneity	1.0	1.0	1.0	1.2
8					
9		1.1111	1.4111	1.1058	1.333
10			0.30	-0.0053	0.222
11		0.1394	0.09	0.000028	0.04938
12					
13	$u_r(P_{op})$	0.37			

Figure A4.8: Uncertainties in pesticide analysis



A4.6 Special aspect:

Modelling inhomogeneity for organophosphorus pesticide uncertainty

Assuming that all of the material of interest 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 of the substance of interest. A more general, but closely related, case is that in which two levels, say L_1 and L_2 of the material 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

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

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

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

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 meet 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

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

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

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

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

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

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

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

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

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:

$$\mathsf{m} = 15 \cdot 0.63 \cdot l_1 = 9.5 \cdot l_1$$

$$\mathsf{s}^2 = 15 \cdot 0.63 \cdot (1 - 0.63) \cdot l_1^2 = 3.5 \cdot l_1^2$$

$$\Rightarrow \mathsf{s} = \sqrt{3.5 \cdot l_1^2} = 1.87 \cdot l_1$$

$$\Rightarrow RSD = \frac{\mathsf{s}}{\mathsf{m}} = 0.2$$

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

$$\mathsf{m} = 15 \cdot 0.37 \cdot l_1 = 5.6 \cdot l_1$$

$$\mathsf{s}^2 = 15 \cdot 0.37 \cdot (1 - 0.37) \cdot l_1^2 = 3.5 \cdot l_1^2$$

$$\Rightarrow \mathsf{s} = \sqrt{3.5 \cdot l_1^2} = 1.87 \cdot l_1$$

$$\Rightarrow RSD = \frac{\mathsf{s}}{\mathsf{m}} = 0.33$$

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

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

$$\begin{aligned}\mathsf{m} &= 15 \cdot 0.37 \cdot (l_1 - l_2) + 15 \cdot l_2 \\ &= 15 \cdot 0.37 \cdot l_2 + 15 \cdot l_2 = 20.6 \cdot l_2\end{aligned}$$

$$\mathsf{s}^2 = 15 \cdot 0.37 \cdot (1 - 0.37) \cdot (l_1 - l_2)^2 = 3.5 l_2^2$$

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 s/m 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, then the contribution to uncertainty will not exceed any already calculated in the scenarios above.

Example 5 -Empirical method: Determination of cadmium release from ceramic ware by atomic absorption spectrometry

Summary

Goal

The amount of released cadmium from ceramic ware is determined using atomic absorption spectrometry. The employed procedure is the empirical method BS 6748.

Measurement procedure

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

Measurand:

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

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 size of the different contributions is given in Table A5.1 and shown diagrammatically in Figure A5.2

Figure A5.1: Extractable metal procedure

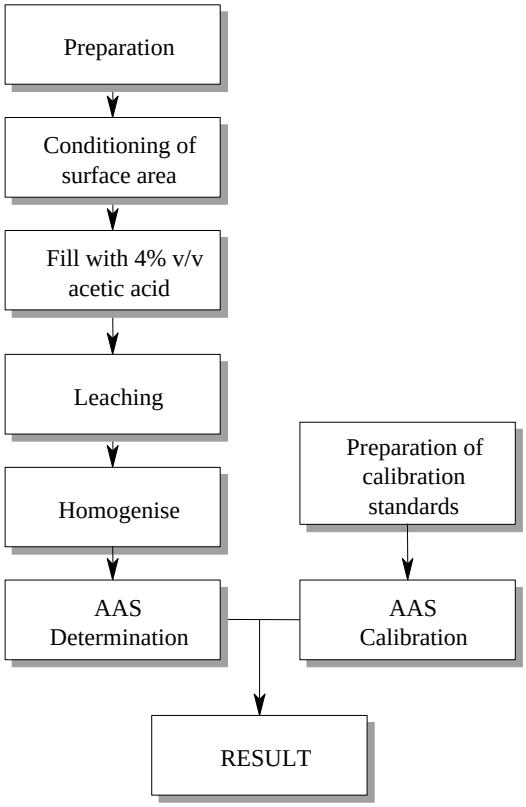


Table A5.1: Uncertainties in extractable cadmium determination

	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	0.018 mg/l	0.064
V_L	Volume of the leachate	0.332 l	0.0018 l	0.0056
a_V	Surface area of the vessel	2.37 dm^2	0.06 dm^2	0.025
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	0.036 mg/dm^2	0.0033 mg/dm^2	0.09

Figure A5.2: Uncertainty sources in leachable Cadmium determination

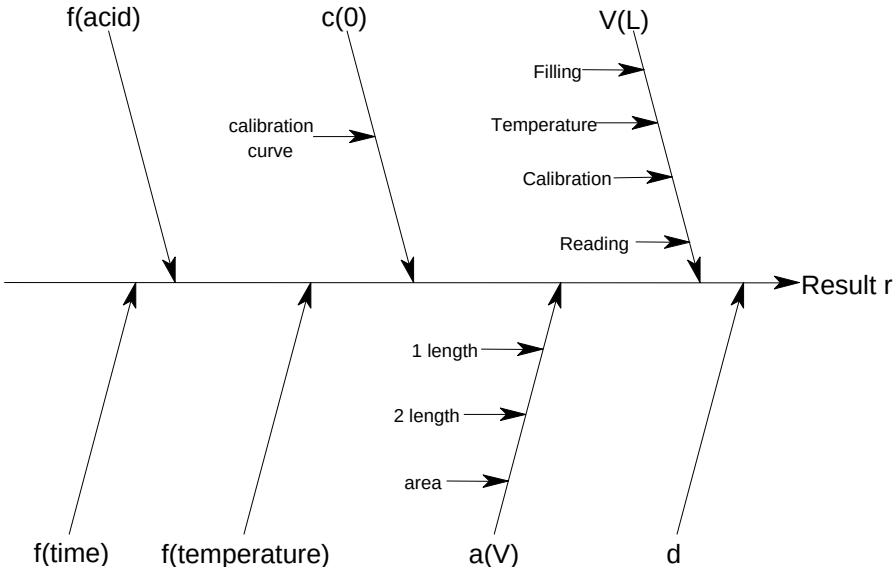
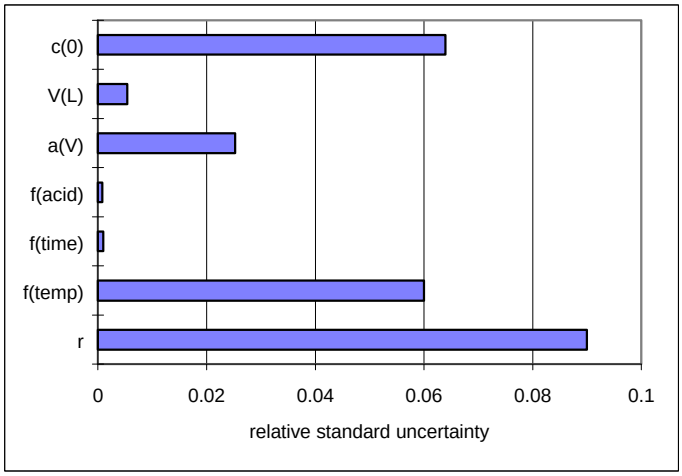


Figure A5.3: Uncertainties in leachable Cd determination



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 empirical method, in this case (BS 6748) limits of metal release form ceramic ware, glassware, glass-ceramic ware and vitreous enamel ware. 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 procedure (empirical method) are only traceable within the boundaries of its specifications. There is no traceability to the SI-units.

A5.2 Step 1: Specification

The following extract from BS 6748:1986 "Limits of metal release from ceramic ware, glass ware, glass ceramic ware and vitreous enamel ware" forms the specification for the measurand.

A5.2.1 Reagents

Water, complying with the requirements of BS 3978.

Acetic acid CH_3COOH, glacial.

Acetic acid solution 4% *v*/*v* 40 ml of glacial acetic acid is added to 500 ml of water and made up to 1 litre. The solution is freshly prepared prior to use.

Standard metal solutions

1000 ±1 mg Pb in 1 l at 4% (*v*/*v*) acetic acid.

500 ±0.5 mg Cd in 1 l of 4% (*v*/*v*) acetic acid.

A5.2.2 Apparatus

Atomic absorption spectrophotometer, with a detection limit of at least 0.2 mg/l Pb (in 4% *v*/*v* acetic acid) and 0.02 mg/l Cd (in 4% *v*/*v* acetic acid).

Laboratory glassware, volumetric glassware of at least class B of Borosilicate glass incapable of releasing detectable levels of lead or cadmium into 4% acetic acid during the test procedure.

A5.2.3 Preparation of samples

Samples are to be washed at 40 ±5°C in an aqueous solution containing 1 mg/l of domestic liquid detergent, rinsed with water (as specified above), drained and wiped dry with clean filter paper. Areas of the samples, which do not contact foodstuffs in normal use, are covered after washing and drying with a suitable protective coating.

A5.2.4 Procedure

The analytical procedure is illustrated schematically in Figure A5.4. The different steps are:

The sample is conditioned to 22 ±2°C . Where appropriate ('category 1' articles the surface area of the article is determined.

The conditioned sample is filled with 4% *v*/*v* acid solution at 22 ±2°C to a level no more than 1 mm from the overflow point, measured from the upper rim of the sample, and to no more than 6 mm from the extreme edge of a sample with a flat or sloping rim.

The quantity of 4% *v*/*v* acetic acid required or

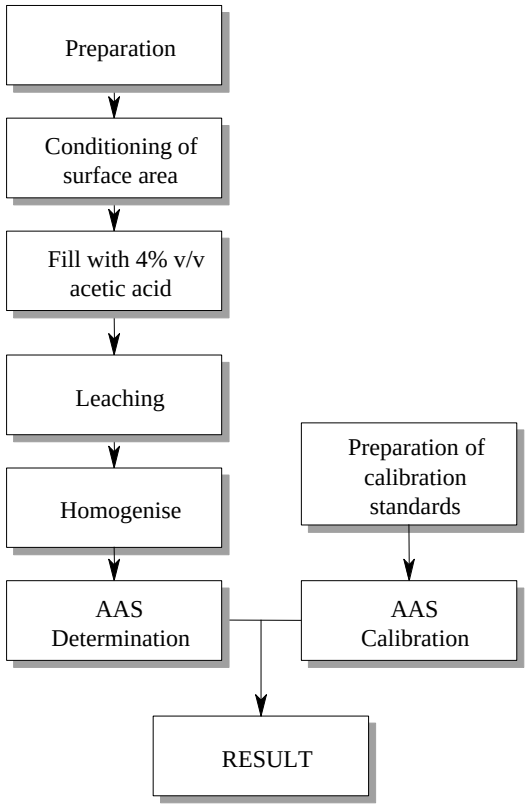


Figure A5.4: Extractable metal procedure

used is recorded to an accuracy of ±2%.

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.

The extract solution is homogenised, by stirring, or by other means, without loss of solution or abrasion of the surface being tested and a portion taken for analysis by AAS.

A5.2.5 Analysis

The AAS instrument is set up according to the manufacturer's instruction using wavelengths of 217.0 nm for lead determination and 228.8 nm for cadmium determination with appropriate correction for background absorption effects.

Provided that absorbance values of the dilute standard metal solutions an of the 4% *v*/*v* acetic acid solution indicate minimal drift, the result may be calculated from a manually prepared calibration curve (below), or by using the calibration bracketing technique.

A5.2.6 Calculation of results from a manually prepared calibration curve

The lead or cadmium content, c_0 expressed in mg/l at the extraction solution, is given by the equation:

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

where

c_0 :content of lead or cadmium of the extraction solution [mg/l]

A_0 :absorbance of the lead or cadmium in the extraction solution

B_1 :slope of the manually prepared calibration curve

B_0 :intercept of the manually prepared calibration curve [mg/l]

d :the factor by which the sample was diluted

NOTE: The calibration curve should be chosen to have absorbance values within the range of that of the sample extract or diluted sample extract.

A5.2.7 Test report

The test report is to include, *inter alia*:

- the nature of the article under test.
- the surface area or volume, as appropriate, of the article.
- the amount of lead and/or cadmium in the total quantity of the extracting solution expressed as milligrams of Pb or Cd per square decimetre of surface area for category 1 articles or milligrams of Pb or Cd per litre of the volume for category 2 and 3 articles.

NOTE: This extract from BS 6748:1986 is reproduced with the permission of BSI. Complete copies can be obtained by post from BSI customer services, 389 Chiswick Leigh Road, London W4 4AL England J 0181 996 7000.

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 with the control of method parameters influencing the result. Such influence quantities are time, temperature, mass and volumes, etc.

The concentration of lead or cadmium in the acetic acid is determined by atomic absorption spectrometry. For vessels that cannot be filled completely the empirical method call for the result to be expressed as mass r of Pb or Cd leached per unit area. r is given by

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

where

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 vessel [dm^2]

c_0 :content of lead or cadmium in the extraction solution [mg l^{-1}]

A_0 :absorbance of the metal in the sample extract

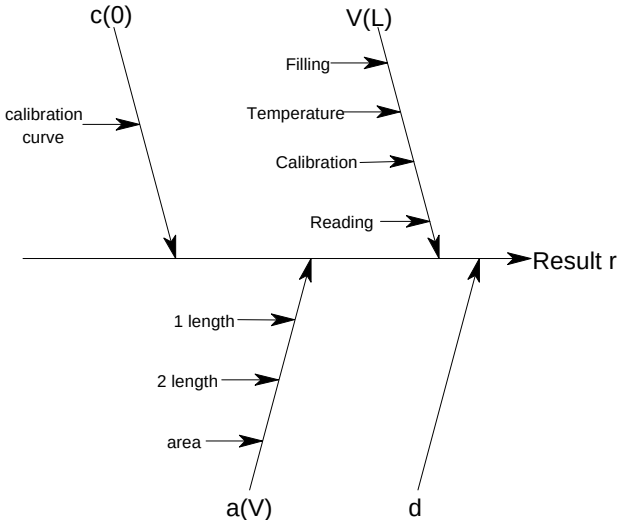
B_0 :intercept of the calibration curve

B_1 :slope of the calibration curve

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 and which would help to assess the laboratory performance. Hence all the feasible influence quantities such as temperature, time of the leaching process and acid concentration 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).

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 descried as bias as they represent results obtained in accordance with the specification.

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 be either using experimental data or well based assumptions.

Dilution factor d

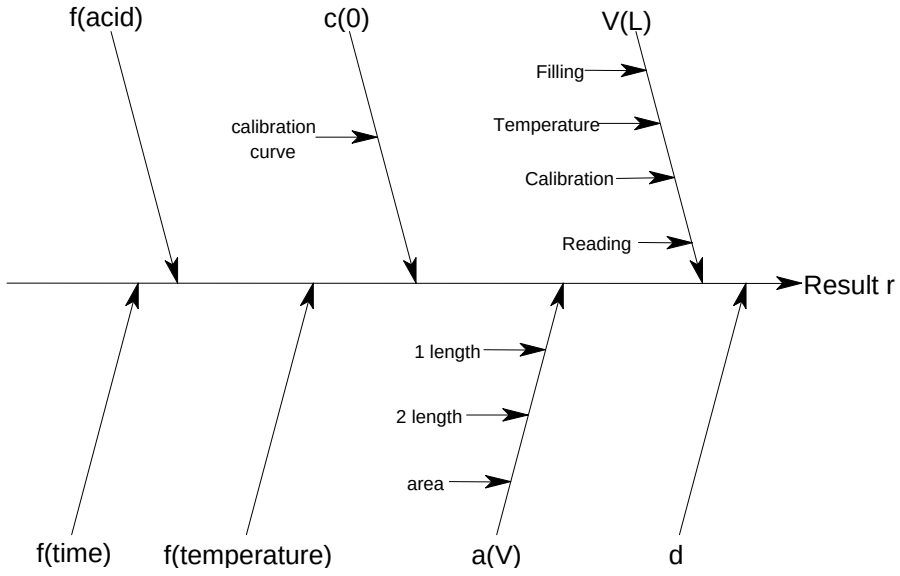
For the current example no dilution of the leaching solution is necessary, therefore no uncertainty contribution have to be accounted for.

V_L

Filling: The empirical method requires the vessel to be filled 'to within 1 mm from the brim'. For a typical 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

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



liquid compared with the vessel. The standard uncertainty assuming a rectangular temperature distribution is

$$\frac{2.1\cdot 10^{-4}\cdot 322\cdot 2}{\sqrt{3}} = 0.08\,\text{ml}$$

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. $0.01\cdot V_L$). 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 used and the four uncertainty components are combined accordingly

$$u(V_L) = \sqrt{\left(\frac{0.005\cdot 332}{\sqrt{6}}\right)^2 + (0.08)^2 + \left(\frac{0.01\cdot 332}{\sqrt{6}}\right)^2 + \left(\frac{2.5}{\sqrt{6}}\right)^2} = 1.83\,\text{ml}$$

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, 0.3 mg/l, 0.5 mg/l, 0.7 mg/l and 0.9 mg/l, were prepared from a 500 ±0.5 mg/l cadmium reference standard. The linear least square fitting procedure used assumes that the uncertainties of the values of the abscissa are considerable smaller than the uncertainty on the values of the ordinate. Therefore the usual uncertainty calculation procedures for c_0 only reflect the uncertainty in the absorbance and not the uncertainty of the calibration standards. In this case the uncertainty of the calibration standards is sufficiently small for this procedure to be used.

The five calibration standards were measured three times each providing the following results.

Concentration [mg/l]	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 calibration curve is given by

$$A_j = c_i \cdot B_1 + B_0$$

where

A_j :j^{th} measurement of the absorbance of the i^{th} calibration standard

c_i :concentration of the i^{th} calibration standard

B_1 :slope

B_0 :intercept

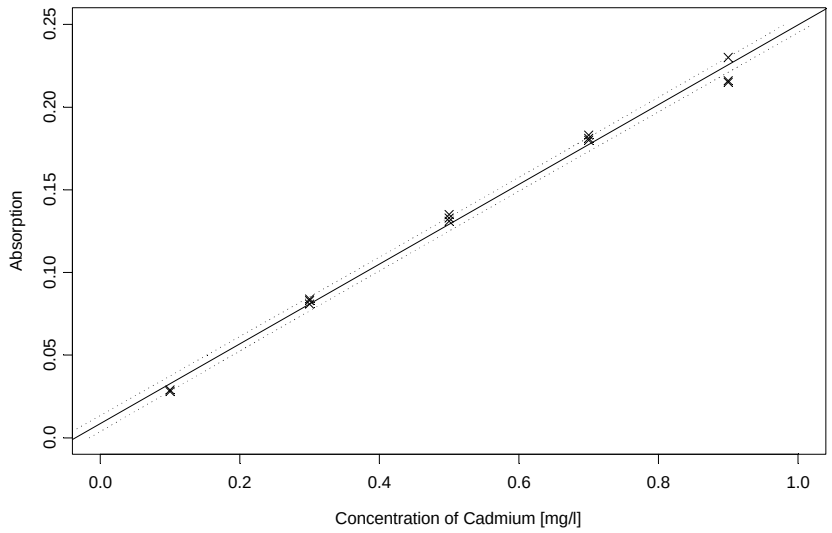
and the results of the linear least square fit are

	value	uncertainty
B_1	0.2410	0.0050
B_0	0.0087	0.0029

with a multiple *R*-squared of 0.9944 and the residual standard error is 0.005486. *R* is the correlation coefficient for the linear least square fit.

The actual leach solution was measured twice leading to a concentration c_0 of 0.26 mg/l. The calculation of the uncertainty $u(c_0)$ of the linear least square fitting procedure is described thoroughly in Appendix E3. Therefore only a short description of the different calculation steps is given here.

Figure A5.7:Linear least square fit and uncertainty interval for duplicate determinations



$u(c_0)$ is given by

$$u(c_0) = \frac{s}{B_1}\sqrt{\frac{1}{p}+\frac{1}{n}+\frac{(c_0-\bar{c})^2}{S_{xx}}}$$

$$= \frac{0.005486}{0.241}\sqrt{\frac{1}{2}+\frac{1}{15}+\frac{(0.26-0.5)^2}{1.2}}$$

$$\Rightarrow u(c_0) = 0.018\,\text{mg}\cdot\text{l}^{-1}$$

with the residual standard deviation *s* given by

$$s = \frac{\sum_{j=1}^{n}[A_j-(B_0+B_1\cdot c_j)]^2}{n-2} = 0.005486$$

and

$$S_{xx} = \sum_{j=1}^{n}(c_j-\bar{c})^2 = 1.2$$

where

B_1 :slope

p :number of measurement to determine c_0

n :number of measurement 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: The total surface area of the sample vessel was calculated, from measured dimensions, to be 2.37 dm^2. Since the item is approximately cylindrical but not perfectly regular, measurements are estimated to be within 2 mm at 95% confidence. Typical dimensions are between 1.0 dm and 2.0 dm leading to an estimated dimensional measurement uncertainty of 1 mm (after dividing the 95% figure by 1.96). Area measurements typically require two length measurements height and width respectively (i.e. 1.45 dm and 1.64 dm)

Area: 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.

The uncertainty contribution of the length measurement and area itself are combined in the usual way.

$$u(a_V) = \sqrt{0.01^2+0.01^2+\left(\frac{0.05\cdot 2.38}{1.96}\right)^2}$$

$$\Rightarrow u(a_V) = 0.06\,\text{dm}^2$$

<u>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 has given an indication of effects in the range of an 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. 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:

$$u(f_{temp}) = 0.1/\sqrt{3} = 0.06$$

f_{time}

For a relative 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 6 hours of leaching was approximately 1.8 mg/l in 86, that is, about 0.3%/h. For a time of 24 ±0.5h 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

$$u(f_{time}) = 0.0015/\sqrt{3} \cong 0.001.$$

f_{acid}

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, 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 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 A5.2. Employing those values

$$r = \frac{0.26 \cdot 0.332}{2.37} \cdot 1.0 \cdot 1.0 \cdot 1.0 = 0.036\,\text{mg} \cdot \text{dm}^{-2}$$

In order to calculate the combined standard uncertainty of a multiplicative expression (as above) the standard uncertainties of each

Table A5.2: Intermediate values and uncertainties for leachable Cadmium analysis

	Description	Value	Standard uncertainty	Relative standard uncertainty
c_0	Content of cadmium in the extraction solution	0.26 mg/l	0.018 mg/l	0.064
V_L	Volume of the leachate	0.332 l	0.0018 l	0.0056
a_V	Surface area of the vessel	2.37 dm^2	0.06 dm^2	0.025
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

component is used in the following way.

$$\frac{u_c(r)}{r} = \sqrt{\left(\frac{u(c_0)}{c_0}\right)^2 + \left(\frac{u(V_L)}{V_L}\right)^2 + \left(\frac{u(a_V)}{a_V}\right)^2 + \left(\frac{u(f_{acid})}{f_{acid}}\right)^2 + \left(\frac{u(f_{time})}{f_{time}}\right)^2 + \left(\frac{u(f_{temp})}{f_{temp}}\right)^2}$$

$$= \sqrt{0.064^2 + 0.0056^2 + 0.025^2 + 0.0008^2 + 0.001^2 + 0.06^2} = 0.09$$

$$\Rightarrow u_c(r) = r \cdot 0.09 = 0.0033\,\mathrm{mg \cdot dm^{-2}}$$

The simpler spreadsheet approach to calculate the combined standard uncertainty is shown below. A comprehensive introduction to the method is given in Appendix E.

The values of the parameters are entered in the second row from C2 to H2. Their standard uncertainties are in the row below (C3-H3). The spreadsheet copies the values from C2-H2 into the second column from B5 to B10. The result (r) using these values is given in B12. The 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. The values shown in the row 13 (C13-H13) are the differences of the row (C13-H13) 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 B11.

The contributions of the different parameters and influence quantities to the measurement uncertainty are illustrated in Figure A5.8, comparing their relative standard uncertainties.

The expanded uncertainty $U(r)$ is obtained by applying a coverage factor of 2

$$U(r) = 0.0033 \cdot 2 = 0.007\,\mathrm{mg \cdot dm^{-2}}$$

Thus the amount of released cadmium measured according to BS 6748:1986

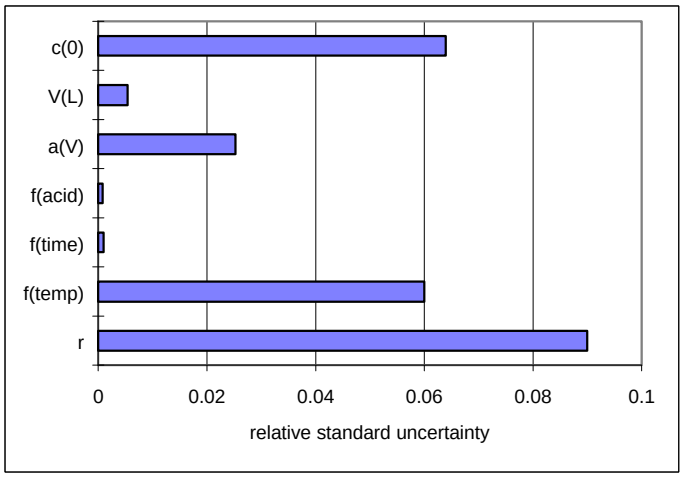
$$0.036 \pm 0.007 \text{ mg dm}^{-2}$$

where the stated uncertainty is calculated using a coverage factor of 2.

Table A5.3: 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	0.322	2.37	1.0	1.0	1.0
3		uncertainty	0.018	0.0018	0.06	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	2.37	2.37	2.37	2.43	2.37	2.37	2.37
8	f_{acid}	1.0	1.0	1.0	1.0	1.0008	1.0	1.0
9	f_{time}	1.0	1.0	1.0	1.0	1.0	1.001	1.0
10	f_{temp}	1.0	1.0	1.0	1.0	1.0	1.0	1.06
11								
12		0.036422	0.038943	0.036619	0.035523	0.036451	0.036458	0.038607
13			0.002521	0.000197	-0.000899	0.000029	0.000036	0.002185
14		1.199 E-5	6.36 E-6	3.90 E-8	8.09 E-7	8.49 E-10	1.33 E-9	4.78 E-6
15								
16	$u_c(r)$	0.0034						

Figure A5.8: Uncertainties in leachable Cd determination



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)

Example A6.The Determination of Crude Fibre in Animal Feeding Stuffs

Summary

Goal

The determination of crude fibre ("Dietary 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:

$$C_{fibre} = \frac{(b-c)\times 100}{a}$$

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 in the detailed discussion 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.

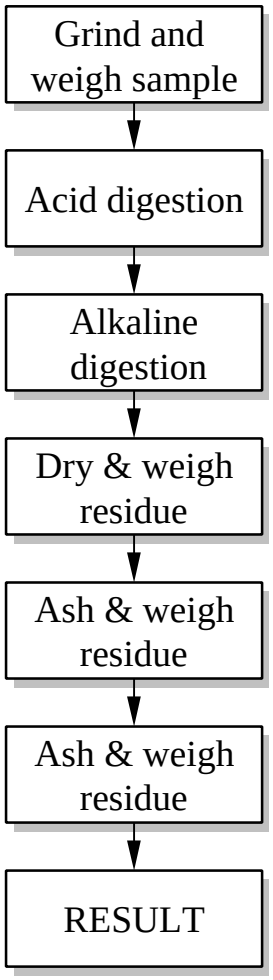


Table A6.1: Combined standard uncertainties

Fibre content (%w/w)	Standard uncertainty $u(C_{fibre})$ (%w/w)	Standard uncertainty as CV(%)
2·5	$\sqrt{0.29^2 + 0.115^2} = 0.31$	12
5	0·4	8
10	0·6	6

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 empirical method.

This is a statutory method for which collaborative trial data (repeatability and reproducibility) were available. 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:

$$C_{fibre} = \frac{(b-c)\times 100}{a}$$

Where:

a is the mass (g) of the sample. Approximately 1 g of sample is taken for analysis;

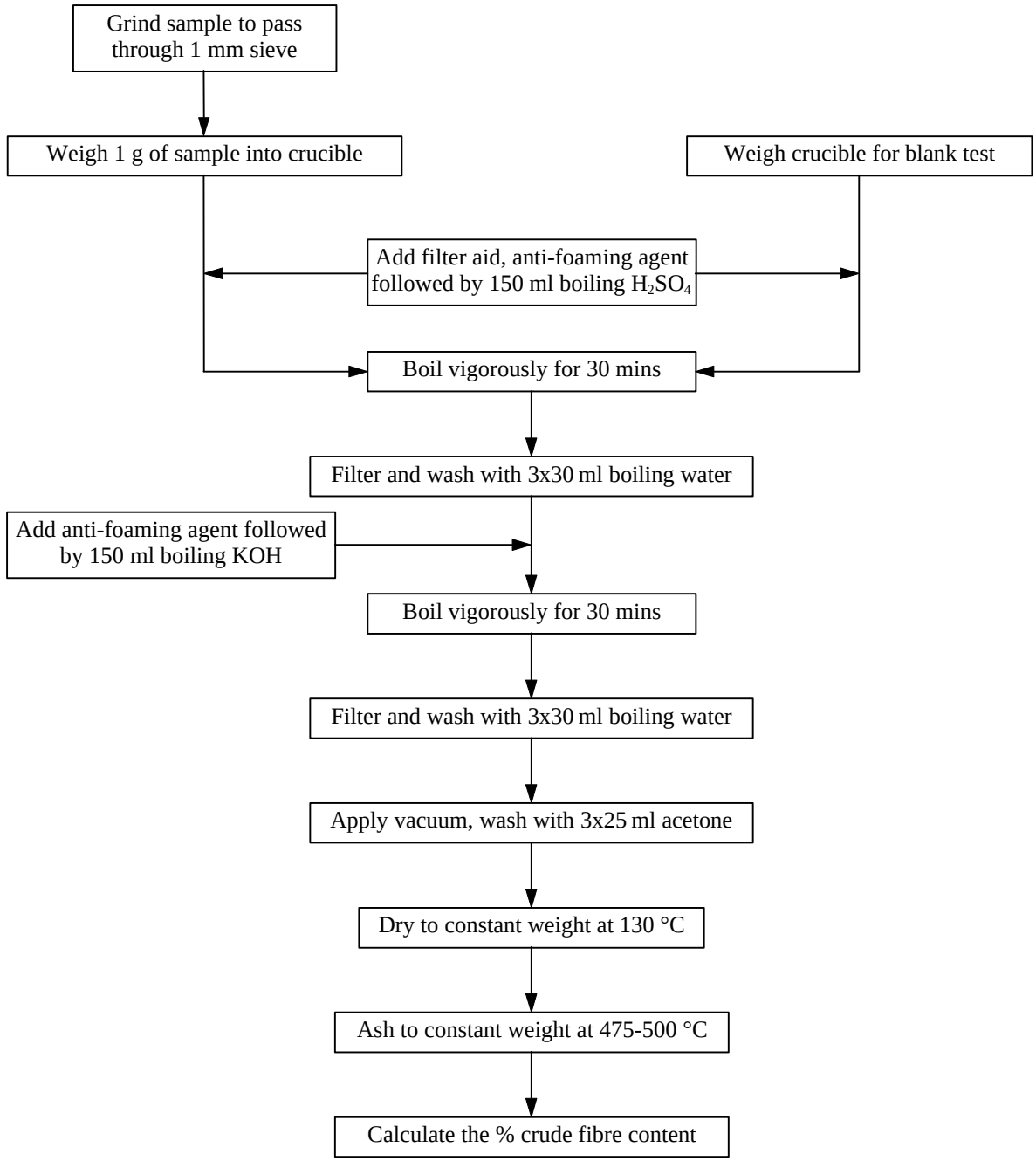
b is the loss of mass (g) after ashing during the determination;

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 were 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, leads to the simplified cause and effect diagram in Figure A6.10.

Figure A6.2: Flow diagram illustrating the stages in the regulatory method for the determination of fibre in animal feeding stuffs



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.

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

Table A6.2: Summary of results from collaborative trial of the method and in-house repeatability check

Sample	Fibre content (% w/w)			
	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

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.

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. These 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 %(w/w) fibre is 0·293 %(w/w). The uncertainty associated with variations in the acid digestion time is estimated as 0·021 %(w/w) (i.e., $2{\cdot}3 \times 0{\cdot}009$). We can therefore safely neglect the uncertainties associated with variations in these method parameters.

Table A6.3: Uncertainties associated with method parameters

Parameter	Sensitivity coefficient[(1)]	Uncertainty in parameter	Uncertainty in final result as RSD[(4)]
acid concentration	0·23 $(mol/l)^{-1}$	0·0013 mol/l[(2)]	0·00030
alkali concentration	0·21 $(mol/l)^{-1}$	0·0023 mol/l	0·00048
acid digestion time	0·0031 min^{-1}	2·89 mins[(3)]	0·0090
alkali digestion time	0·0025 min^{-1}	2·89 mins	0·0072

NOTES ON Table A6.3

(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.

(2) The standard uncertainties in the concentrations of the acid and alkali solutions were calculated from estimates of the precision and accuracy of the volumetric glassware used in their preparation, temperature effects *etc.* (see workshops 2 and 5-7 for further examples of calculating uncertainties for the concentrations of solutions).

(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 $\sqrt{3}$.

(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.

Drying temperature and time

No prior data were available. The method states that the sample should be dried at 130 °C to "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 was less than 2 mg. This was therefore taken as the worst case estimate of the uncertainty in the weight change on drying. ±2 mg is 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 %(w/w) 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 %(w/w) 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, as discussed above, the effects of variations in this parameter are likely to be small.

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 comparable to that predicted by the collaborative trial. It is therefore appropriate to use the s_R values from the collaborative trial. The discussion presented in Step 3leads to the conclusion that, with the exception of the effect of drying conditions at low fibre concentrations, the other sources of uncertainty identified are all small in comparison to s_R. The performance of the laboratory producing the uncertainty estimate is therefore comparable to that of the laboratories which took part in the trial. 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 %(w/w), 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 below:

Expanded uncertainty

Typical expanded uncertainties are given in the table below. These were calculated using a coverage factor of 2 which gives a level of confidence of approximately 95%.

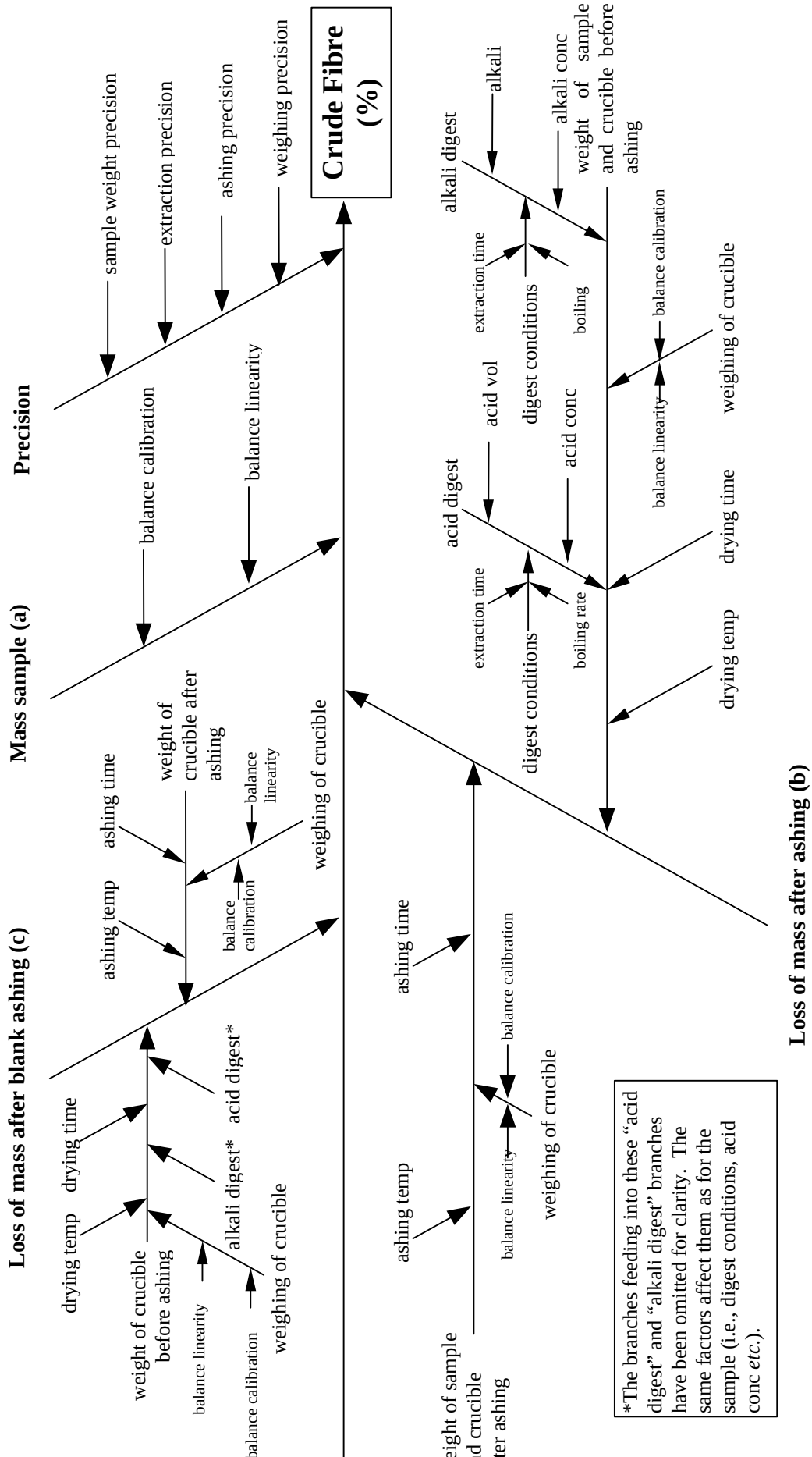
Table A6.4: Combined standard uncertainties

Fibre content (%w/w)	Standard uncertainty $u(C_{fibre})$ (%w/w)	Standard uncertainty as CV(%)
2·5	$\sqrt{0.29^2 + 0.115^2} = 0.31$	12
5	0·4	8
10	0·6	6

Table A6.5: Expanded uncertainties

Fibre content (%w/w)	Expanded uncertainty $U(C_{fibre})$ (%w/w)	Expanded uncertainty as CV (%)
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



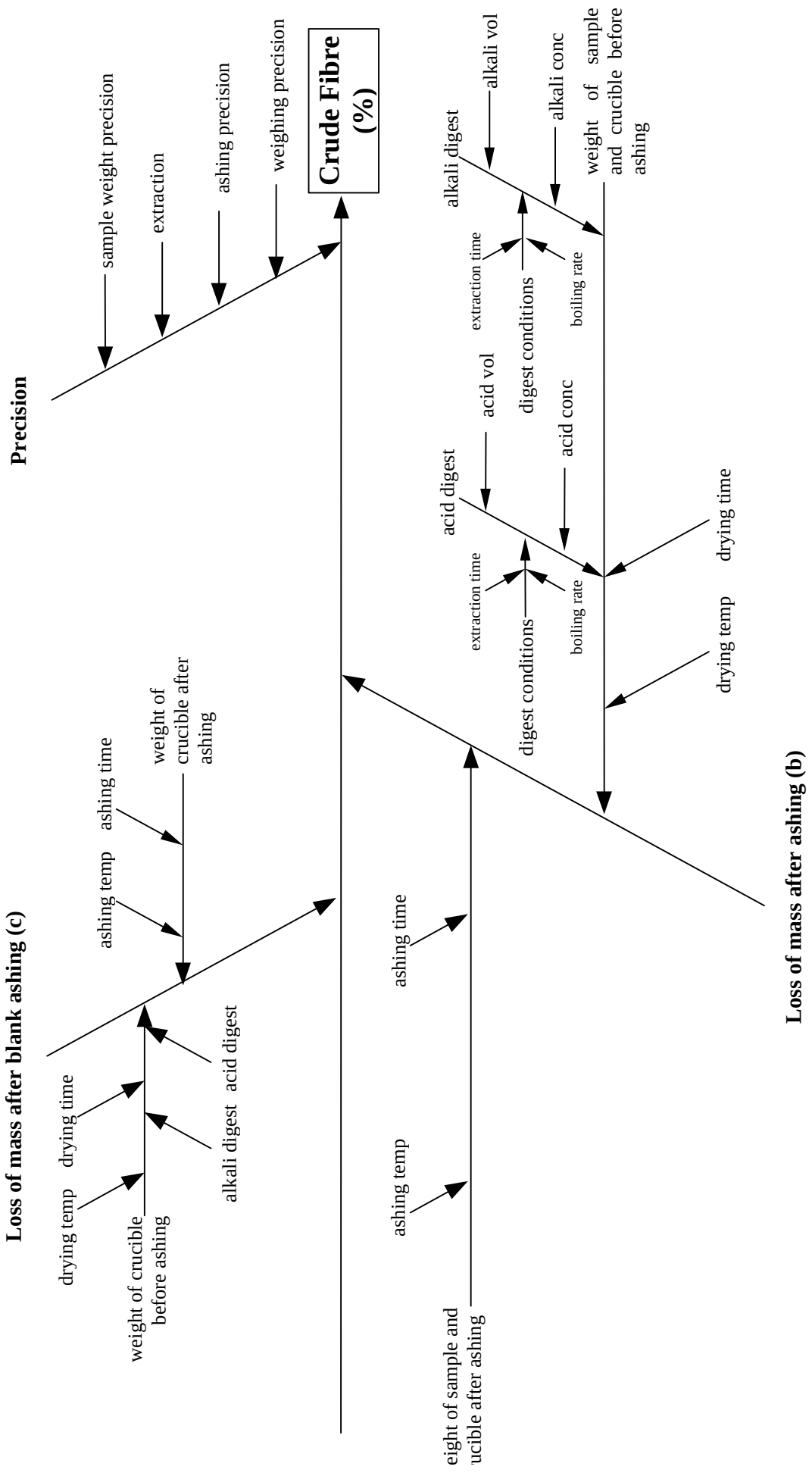


Figure A6.10: Simplified cause and effect diagram

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 will illustrate how the uncertainty concept can be applied to a measurement of the amount of lead in a water sample using Double Isotope Dilution Mass Spectrometry (IDMS) and ICP-MS.

General introduction to Double IDMS

IDMS is one of the techniques that is recognised by 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, to measure the amount of an element present in a sample, the expression takes this form:

$$c_x = c_y \cdot \frac{m_y}{m_x} \cdot \frac{K_{y1} \cdot R_{y1} - K_b \cdot R_b}{K_b \cdot R_b - K_{x1} \cdot R_{x1}} \cdot \frac{\sum_i (K_{xi} \cdot R_{xi})}{\sum_i (K_{yi} \cdot R_{yi})} \qquad (1)$$

where c_x and c_y are the amount of the element in the sample and the spike respectively. The symbol c is used instead of k for the amount to avoid confusion with K-factors. 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 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 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 ratio, in sample, spike and blend respectively. The K-factors are calculated using a certified isotopic reference material according to eqn. (2).

$$K = K_0 + K_{bias};\ \text{where}\ K_0 = \frac{R_{certified}}{R_{observed}} \qquad (2)$$

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. The K_{bias} can also include other possible sources of biases like multiplier dead time correction and background correction. $R_{certified}$ is the certified isotope amount ratio taken from the certificate of an isotopic reference material and $R_{observed}$ is the measured isotope ratio of this isotopic reference material. In IDMS experiments, using Inductively Coupled Plasma Mass Spectrometry (ICP-MS), mass fractionation will vary with time which leads to eqn. (1) where all isotope amount ratios need to be individually corrected for mass discrimination.

The availability of certified material enriched in a specific isotope is often very scarce. To overcome this 'double' IDMS is frequently used. The idea here is to use a material of natural isotopic composition as primary assay standard. To perform double IDMS we need to make another blend, here called blend b'. Blend b is the blend between sample and spike from eqn. (1). This time, for blend b', we use the well characterised primary assay standard with the amount content c_z. This gives us a similar expression to eqn. (1):

$$c_z = c_y \cdot \frac{m'_y}{m_z} \cdot \frac{K_{y1} \cdot R_{y1} - K'_b \cdot R'_b}{K'_b \cdot R'_b - K_{z1} \cdot R_{z1}} \cdot \frac{\sum_i (K_{zi} \cdot R_{zi})}{\sum_i (K_{yi} \cdot R_{yi})} \qquad (3)$$

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 thus represents the assay standard. Equation (1) and (3) are similar and in order to eliminate c_y from the expressions we divide equation (1) with equation (3):

$$\frac{c_x}{c_z} = \frac{c_y \cdot \frac{m_y}{m_x} \cdot \frac{K_{y1} \cdot R_{y1} - K_b \cdot R_b}{K_b \cdot R_b - K_{x1} \cdot R_{x1}} \cdot \frac{\sum_i (K_{xi} \cdot R_{xi})}{\sum_i (K_{yi} \cdot R_{yi})}}{c_y \cdot \frac{m'_y}{m_z} \cdot \frac{K_{y1} \cdot R_{y1} - K'_b \cdot R'_b}{K'_b \cdot R'_b - K_{z1} \cdot R_{z1}} \cdot \frac{\sum_i (K_{zi} \cdot R_{zi})}{\sum_i (K_{yi} \cdot R_{yi})}} \qquad (4)$$

Simplifying this equation we get:

$$c_x = c_z \cdot \frac{m_y}{m_x} \cdot \frac{m_z}{m'_y} \cdot \frac{K_{y1} \cdot R_{y1} - K_b \cdot R_b}{K_b \cdot R_b - K_{x1} \cdot R_{x1}} \times \frac{K'_b \cdot R'_b - K_{z1} \cdot R_{z1}}{K_{y1} \cdot R_{y1} - K'_b \cdot R'_b} \cdot \frac{\sum_i (K_{xi} \cdot R_{xi})}{\sum_i (K_{zi} \cdot R_{zi})} \quad (5)$$

This is the final equation. For reference, the parameters are summarised in Table A7.1.

A7.2 Step 1: Specification

Calculation procedure for the amount content c_x

For this determination of lead in water, four blends each of the 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. The number of digits displayed for the parameters in the calculations will sometimes be more than what would be appropriate, but this is to minimise rounding off errors.

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 already 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:

$$A_r(\mathrm{E}) = \frac{\sum_{i=1}^{p} R_i M_i}{\sum_{i=1}^{p} R_i} \quad (6)$$

where R_i are all true isotope amount ratios for the element E and M_i are the tabulated nuclide masses.

Note that the isotope amount ratios in eqn. (7) have to be absolute ratios, that is, they have to be corrected for mass discrimination. With the use of proper indexes this gives equation (8). For the calculation, nuclide masses, M_i, were taken from

Table A7.1. Summary of IDMS parameters

Param.	Description
c_z	Amount content of the primary assay standard
m_x	Mass of sample in blend b
m_y	Mass of enriched spike in blend b
$m`_y$	Mass of enriched spike in blend b'
m_z	Mass of primary assay standard in blend b'
R_b	Measured ratio of blend b
$R`_b$	Measured ratio of blend b'
R_{x1}	Measured ratio of the enriched isotope to the reference isotope. Here in the sample
R_{y1}	As above but in the enriched spike
R_{z1}	As above but in the primary assay standard
K_b	Mass bias correction of R_b
$K`_b$	Mass bias correction of R'_b
K_{y1}	Mass bias correction of R_{y1}
K_{zi}	Mass bias correction factors for all ratios of a particular element, correcting for mass discrimination in the measured ratios of the primary assay standard. An element with 3 isotopes would give K_{z1}, K_{z2} and K_{z3}.
K_{xi}	As above but for the sample
R_{zi}	All ratios in the primary assay standard, R_{z1}, R_{z2} etc.
R_{xi}	All ratios in the sample

G. Audi and A.H. Wapstra, Nuclear Physics, A565 (1993) while Ratios, R_{zi}, and *K*-factors, K_{zi}, were measured and taken from Table A7.8.

$$M(\text{Pb}, Assay1) = \frac{\sum_{i=1}^{p} K_{z\,i} R_{z\,i} M_{z\,i}}{\sum_{i=1}^{p} K_{z\,i} R_{z\,i}} = 207.21036 g \cdot mol^{-1} \quad (7)$$

Measurement of K-factors and isotope amount ratios

To correct for mass discrimination, a correction factor, *K*, is used, see equation (2). The *K*-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*-factor. The *K*-factor is measured before and after the ratio it will correct. A typical sample sequence could be: 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 counts were 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*-factors and masses are summarised in Table A7.8 together with the calculated amount content of lead in the primary assay standard, *Assay* 2.

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 mass percent. The two pieces came from the same batch of high purity lead. The pieces were dissolved in about 10mL of 1:3 w/w HNO_3:water with the aid of heat and then further diluted. The values from one of the produced standard assays will be displayed.

0.36544g lead, m_1, was dissolved and diluted to a total of d_1=196.14 g 0.5M HNO_3, this solution is named *Assay 1*. A more diluted solution was needed and m_2=1.0292g of *Assay 1*, was diluted to a total mass of d_2=99.931g 0.5 M HNO_3. This solution is named *Assay 2*. The amount content of Pb in *Assay 2*, c_z, is then calculated according to eqn. (8)

$$c_z = \frac{m_2}{d_2} \cdot \frac{m_1 \cdot w}{d_1} \cdot \frac{1}{M(\text{Pb}, Assay1)} = 9.2606 \cdot 10^{-8} \left(\text{mol} \cdot \text{g}^{-1}\right) = 0.092606 \text{ mol} \cdot \text{g}^{-1} \quad (8)$$

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. In Table A7.3 are 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

Calculation of the unknown amount content c_x

Inserting the measured and calculated data, see Table A7.8., into equation (5) gives c_x=0.0537377 µmol·g^{-1}. The results from all four replicates are given in Table A7.4.

Table A7.4

	c_x (µmol·g^{-1})
Replicate 1 (our example)	0.0537377
Replicate 2	0.0536208
Replicate 3	0.0536101
Replicate 4	0.0538223
Average	**0.05370**
Experimental standard deviation (s)	**0.00011**

A7.3 Steps 2 and 3: Identifying and Quantifying uncertainty sources

Strategy for the uncertainty calculation

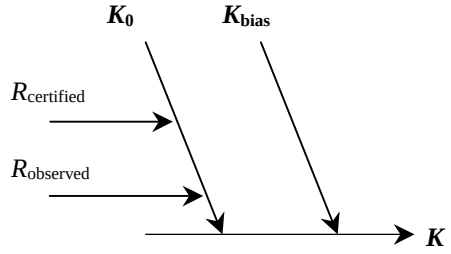
If eqn. (2,6,7) were to be included in the final IDMS eqn. (5), the sheer number of parameters would make the equation almost impossible to handle. To keep it simpler, *K*-factors and amount

content of the standard assay solution and their associated uncertainties can be 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 determinations, as described in A7.2, will be used. The combined uncertainty of c_x will be calculated using the spreadsheet method. This method is described in Appendix E.

Uncertainty on the K-factors

A cause and effect diagram is constructed below for the 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:

$$K_{0(x1)} = \frac{R_{\text{certified}}}{R_{\text{observed}}} = \frac{2.1681}{2.1699} = 0.99917 \quad (10)$$

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 the certified ratio a standard uncertainty of $u(R_{\text{certified}})$=0.0004. The observed amount ratio, $R_{\text{observed}}=n(^{208}\text{Pb})/n(^{206}\text{Pb})$, had a relative standard uncertainty, (RSu), of 0.25%. For the *K*-factor, the combined uncertainty can be calculated, following Appendix D.5, as:

$$u_c(K_{0(x1)}) = 0.99917 \cdot \sqrt{\left(\frac{0.0004}{2.1681}\right)^2 + (0.0025)^2} = 0.002505 \quad (11)$$

This clearly points out that the uncertainty contribution 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 compensate for drifts in the value of the mass discrimination factor. As can be seen in the cause and effect diagram above, and in eqn.(2), 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 eqn. (12), using an excerpt from eqn. (5) and the parameters K_{y1} and R_{y1} to demonstrate this principle.

$$c_x = \ldots . \frac{\left(K_0(y1) + K_{\text{bias}}(y1)\right) \cdot R_{y1} - \ldots}{\ldots\ldots} \cdot \ldots . \quad (12)$$

The drawback with this approach is that this would increase the number of parameters and would make the uncertainty calculation less manageable. Therefore the type B, bias uncertainties, are included later in the spreadsheet calculation as an additional contribution in the spreadsheet equation, see eqn. (14). In this example the uncertainty from a bias has been estimated as a fraction of the type A contribution of that particular R_{observed}. Note that the bias is NOT, in any way, a function of the standard deviation of R_{observed}, it just gives a convenient base for the estimation of the variation in a possible bias.

To explain how the bias uncertainty is implemented let us look at eqn. (13) which is the general equation used when applying the spreadsheet model. The square of the combined uncertainty is the sum of the uncertainty contributions from the different parameters.

$$u_c^2(y) = \sum \left(f(x_i + u(x_i)) - f(x_i) \right)^2 \quad (13)$$

In our case the uncertainties in the biases were estimated to be 20% of the type A contributions of R_{observed} and hence 20% of K_0. An example of how it is applied in the generic case using eqn. (13) is seen below:

$$u_c^2(c_x) = \ldots + \left[f(K_0 + u_c(K_0)) - f(K_0) \right]^2 + (20\%) \cdot \left[f(K_0 + u_c(K_0)) - f(K_0) \right]^2 + \ldots \quad (14)$$

In eqn. (14), the first term is the uncertainty associated with variability; the second (20%…) is an *estimate* of the uncertainty associated with systematic effects which have not been observed. Eqn (14) is used to calculate the experimental combined uncertainty by combining the experimental standard uncertainties and the estimated standard uncertainties of the parameters in eqn (5). These uncertainties are given in Table A7.8 columns 3 and 4.

In Table A7.8 there is a summary of the parameters, their value and their experimental standard uncertainties. For the calculation of the experimental combined uncertainty the concept described in eqn. (14) was applied. In the next step, the calculation of the final combined uncertainty, the number of measurements of every parameter needs to be taken into account. In this example every ratio was measured eight times and every type A uncertainty has to be divided by √8. Implementing this, in eqn. (14), gives:

$$u_c^2(c_x) = \ldots + \frac{\left[f(K_0 + u_c(K_0)) - f(K_0)\right]^2}{8} + (20\%)\cdot\left[f(K_0 + u_c(K_0)) - f(K_0)\right]^2 + \ldots \quad (15)$$

In the last two columns in Table A7.8 the contribution of the type A and type B uncertainties to the final uncertainty can be seen. The value at the bottom of these two columns is the final combined uncertainty for the measurand, c_x, calculated using eqn. (15).

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.

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 following parameters are known or have been measured:

Table A7.5

	Value	**Standard Uncertainty**	**Type[1]**
R_{z1}	2.1429	0.0054	A
K_{z1}	0.9989	0.0025	A
K_{z2}	1	0	A
K_{z3}	0.9993	0.0035	A
K_{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

[1] Type A (statistical evaluation) or Type B (other)

The equation used to calculate the molar mass is given by eqn (16):

$$M(\mathrm{Pb}, Assay1) = \frac{K_{z1}\cdot R_{z1}\cdot M_1 + K_{z2}\cdot R_{z2}\cdot M_2 + K_{z3}\cdot R_{z3}\cdot M_3 + K_{z4}\cdot R_{z4}\cdot M_4}{K_{z1}\cdot R_{z1} + K_{z2}\cdot R_{z2} + K_{z3}\cdot R_{z3} + K_{z4}\cdot R_{z4}} \quad (16)$$

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*-factor. This gave a molar mass of $M(\mathrm{Pb}, Assay\ 1) = (207.21036 \pm 0.00085)\ \mathrm{g\cdot mol^{-1}}$. The uncertainty was calculated according to the concept outlined in A7.3.5

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 A7.2.1 and equation (8) will be used. The uncertainties were taken from the weighing certificates, see A7.3.3. All parameters used in equation (8) are given with their uncertainties in Table A7.6.

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* ($g \cdot mol^{-1}$)	207.21036	0.00085

The amount content, c_z, was calculated using equation (7). Following Appendix D.5 the combined standard uncertainty in c_z, is calculated according to: $u_c(c_z)=0.000028$. This gives $c_z=(0.092606\pm0.000028)$ $\mu mol \cdot g^{-1}$ and a $RSu_c(c_z)=0.03\%$

To calculate $u_c(c_x)$, for replicate 1, the spreadsheet model was applied, see 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.

A7.4 Step 4: Calculating the combined standard uncertainty

In Table A7.7 the average and the experimental standard deviation of the four replicates are displayed. 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	$\mu mol \cdot g^{-1}$
$u_c(c_x)$=	0.00019	s^1=	0.00011	$\mu mol \cdot g^{-1}$

[1] Note, this is the experimental standard uncertainty and not the standard deviation of the mean.

We can now compare the type A contribution from the experimental combined *uncertainty*, which is 83% of 0.00043 $\mu mol \cdot g^{-1}$, see Table A7.8, with the experimental standard *deviation* of the four replicates, which is 0.00011 $\mu mol \cdot g^{-1}$, see Table A7.7. The experimental combined uncertainty, is larger than the obtained experimental standard deviation of the four replicates. This indicates that the experimental standard deviation is fully explained by the type A contributions and that no further type A contribution, due to the making 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 covered by the bias associated with the *K*-factors. The amount content of lead in the water sample is then:

$$\boldsymbol{c_x=(0.05370\pm0.00038)\ \mu mol \cdot g^{-1}}$$

The result is presented with an expanded uncertainty using a coverage factor of 2.

Table A7.8

Parameter	value	Experimental standard uncertainty	Estimated standard uncertainty	Contribution to experimental u_c (%)		Contribution to final u_c (%)	
		A	B	A	B	A	B
c_z	0.092606		0.000028		0.2		0.7
m_x	1.0440		0.0002		0.1		0.3
m_y	1.1360		0.0002		0.1		0.3
$m`_y$	1.0654		0.0002		0.1		0.3
m_z	1.1029		0.0002		0.1		0.3
R_b	0.29360	0.00073		14.3		8.6	
$R`_b$	0.5050	0.0013		18.1		10.9	
R_{x1}	2.1402	0.0054		4.3		2.6	
R_{y1}	0.000640	0.000040		0.0		0.0	
R_{z1}	2.1429	0.0054		6.6		3.9	
K_b	0.9987	0.0025		14.3	2.9	8.6	13.8
$K`_b$	0.9983	0.0025		18.1	3.6	10.9	17.4
K_{x1}	0.9992	0.0025		4.3	0.9	2.6	4.1
K_{y1}	0.9999	0.0025		0.0	0.0	0.0	0.0
K_{z1}	0.9989	0.0025		6.6	1.3	3.9	6.3
K_{x2}	1			0.0	0.0	0.0	0.0
K_{x3}	1.0004	0.0035		1.0	0.2	0.6	1.0
K_{x4}	1.0010	0.0060		0.0	0.0	0.0	0.0
K_{z2}	1			0.0	0.0	0.0	0.0
K_{z3}	0.9993	0.0035		1.0	0.2	0.6	1.0
K_{z4}	1.0000	0.0060		0.0	0.0	0.0	0.0
Rx_2	1			0.0		0.0	
Rx_3	0.9142	0.0032		1.0		0.6	
Rx_4	0.05901	0.00035		0.0		0.0	
Rz_2	1			0.0		0.0	
Rz_3	0.9147	0.0032		1.0		0.6	
Rz_4	0.05870	0.00035		0.0		0.0	
c_x=	0.05374	µmol·g^{-1}		90.6%	9.4%	**54.5%**	**45.5%**
			$u_c(c_x)$=	**0.00042**	µmol·g^{-1}	0.00019	µmol·g^{-1}

Appendix B - Definitions

General

B.1 Accuracy of measurement

The closeness of the agreement between the result of a measurement and a *true value* of the measurand [**G.4,** 3.5].

NOTE 1 "Accuracy" is a qualitative concept.

NOTE 2 The term "precision" should not be used for "accuracy".

B.2 Precision

The closeness of agreement between independent test results obtained under stipulated conditions. [3534-1, 3.14]

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 and reproducibility conditions are particular sets of extreme stipulated conditions.

B.3 True value

Value consistent with the definition of a given particular quantity [**G.4,** 1.19].

NOTE 1 This is a value that would be obtained by a perfect measurement.

NOTE 2 True values are by nature indeterminate.

NOTE 3 The indefinite article "a" rather than the definite article "the" is used in conjunction with "true value" because there may be many values consistent with the definition of a given particular quantity.

B.4 Conventional true value

Value attributed to a particular quantity and accepted, sometimes by convention, as having an uncertainty appropriate for a given purpose. [**G.4,** 1.20].

EXAMPLES

a) at a given location, the value assigned to the quantity realised by a reference standard may be taken as a conventional true value.

b) the CODATA (1986) recommended value for the Avogadro constant, N_A: 6.0221367×10^{23} mol^{-1}

NOTE 1 "Conventional true value" is sometimes called *assigned value*, *best estimate* of the value, *conventional value* or *reference value*.

NOTE 2 Frequently, a number of results of measurements of a quantity is used to establish a conventional true value.

B.5 Influence quantity

A quantity that is not the measurand but that affects the result of the measurement [**G.4,** 2.7].

EXAMPLES

1. Temperature of a micrometer used to measure length;

2. Frequency in the measurement of an alternating electric potential difference;

3. Bilirubin concentration in the measurement of haemoglobin concentration in human blood plasma.

Measurement

B.6 Measurand

Particular quantity subject to *measurement.* [**G.4,** 2.6]

NOTE The specification of a measurand may require statements about quantities such as time, temperature and pressure..

B.7 Measurement

Set of operations having the object of determining a value of a quantity [**G.4,** 2.1].

B.8 Measurement Procedure

Set of operations, described specifically, used in the performance of measurements according to a given method [**G.4,** 2.5].

NOTE A measurement procedure is usually recorded in a document that is sometimes itself 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.9 Method of measurement

A logical sequence of operations, described generically, used in the performance of measurements [**G.4,** 2.4].

NOTE Methods of measurement may be qualified in various ways such as:

- substitution method
- differential method
- null method

B.10 Result of a measurement

Value attributed to a measurand**,** obtained by measurement [**G.4,** 3.1].

NOTE 1 When the term "result of a measurement" is used, it should be made clear whether it refers to:

- The indication.
- The uncorrected result.
- The corrected result.

and whether several values are averaged.

NOTE 2 A complete statement of the result of a measurement includes information about the uncertainty of measurement.

Uncertainty

B.11 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 [**G.4,** 3.9]**.**

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.12 Traceability

"the property of the result of a measurement or the value of a standard whereby it can be related to stated references, usually national or international standards, through an unbroken chain of comparisons all having a stated uncertainties" [VIM G.4**]**

B.13 Standard uncertainty

$u(x_i)$ uncertainty of the result x_i of a measurement expressed as a standard deviation. [**G.2**, 2.3.1]

B.15 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. [**G.2**, 2.3.4].

B.16 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. [**G.2**, 2.3.5]

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.17 Coverage factor

k numerical factor used as a multiplier of the combined standard uncertainty in order to obtain an expanded uncertainty [**G.2**, 2.3.6].

NOTE A coverage factor is typically in the range 2 to 3.

B.18 Type A evaluation (of uncertainty)

Method of evaluation of uncertainty by the statistical analysis of series of observations [**G.2**, 2.3.2].

B.19 Type B evaluation (of uncertainty)

Method of evaluation of uncertainty by means other than the statistical analysis of series of observations [**G.2**, 2.3.3]

Error

B.20 Error (of measurement)

The result of a measurement minus a true value of the measurand [**G.4,** 3.10].

NOTE 1 Since a true value cannot be determined, in practice a conventional true value is used.

B.21 Random error

Result of a measurement minus the mean that would result from an infinite number of measurements of the same measurand carried out under repeatability conditions [**G.4,** 3.13].

NOTE 1 Random error is equal to error minus systematic error.

NOTE 2 Because only a finite number of measurements can be made, it is possible to determine only an estimate of random error.

B.22 Systematic error

Mean that would result from an infinite number of measurements of the same measurand carried out under repeatability conditions minus a true value of the measurand. [**G.4,** 3.14].

NOTE 1: Systematic error is equal to error minus random error.

NOTE 2: Like true value, systematic error and its causes cannot be known.

Statistical terms

B.23 Arithmetic mean

$\bar{x}$ arithmetic mean value of a sample of n results.

$$\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n}$$

B.24 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.25 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.26 Relative Standard Deviation (RSD)

RSD an estimate of the standard deviation of a population from a sample of n results divided by the mean of that sample. Often known as coefficient of variation (CV). Also frequently stated as a percentage.

$$\mathbf{RSD} = \frac{s}{\bar{x}}$$

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.
   - 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 [G.11]), 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) [G.12]

- 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

$$d(EtOH)=(M_{gross} - M_{tare})/V$$

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

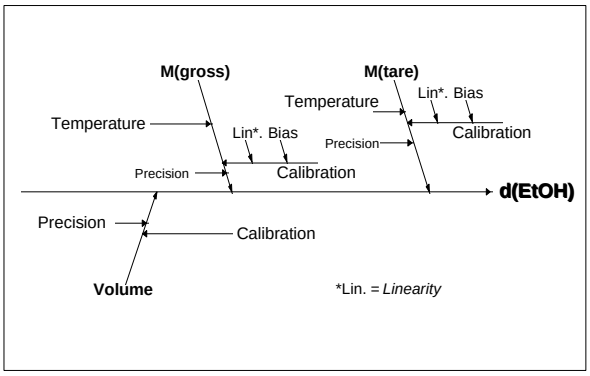


Figure D2: Combination of similar effects

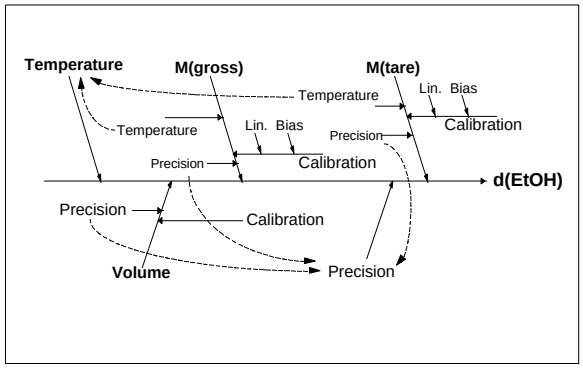
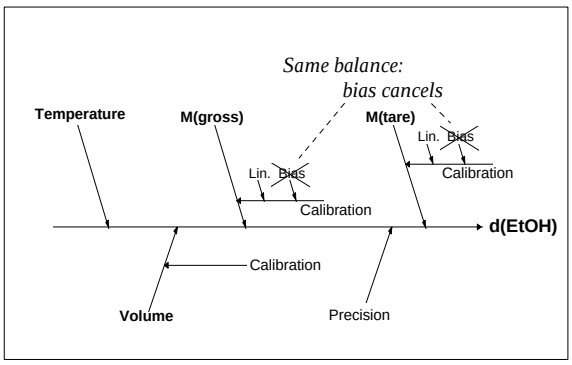


Figure D3: Cancellation



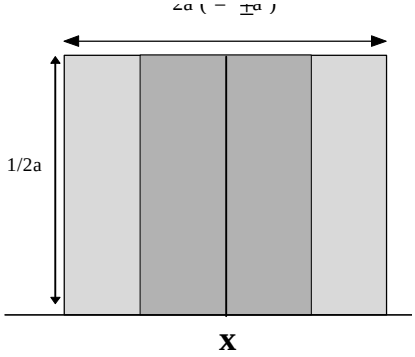
Appendix E - Useful Statistical Procedures

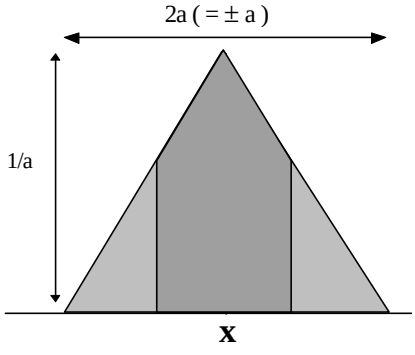
E.1 Distribution functions

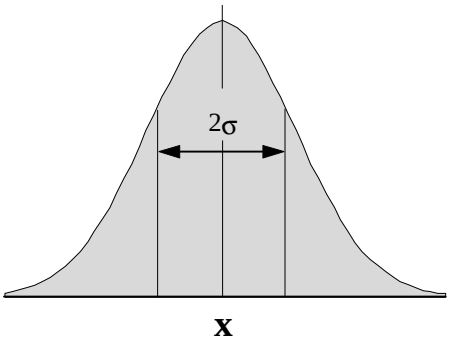
The following table shows how to calculate a standard uncertainty from the parameters of the two most important distribution functions, and gives an indication of the circumstances in which each should be used .

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. 25ml ± 0.05ml) • 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 coefficient of variance CV% without specifying the distribution. • An uncertainty is given in the form of a 95% (or other) confidence interval **I** without specifying the distribution.	$u(x) = s$ $u(x) = s$ $u(x)=x.(s/\bar{x})$ $u(x)=\frac{CV\%}{100}\cdot x$ $u(x) = \mathbf{I}/2$ (for **I** at 95%)

E.2 Spreadsheet method for uncertainty calculation

E.2.1 A standard spreadsheet 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 **[G.9]**.

E.2.2 In the expression for $u(y(x_1, x_2...x_n))$

$$\sqrt{\sum_{i=1,n}\left(\frac{\partial y}{\partial x_i}\cdot u(x_i)\right)^2+\sum_{i,k=1,n}\left(\frac{\partial y}{\partial x_i}\cdot\frac{\partial y}{\partial x_k}\cdot s(x,ik)\right)}$$

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

$$u(y,x_i) \approx y(x_1,x_2,..(xi+u(x_i))..x_n)-y(x_1,x_2,..x_i..x_n)$$

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 G.9 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

$$u(y,p)=f(p+u(p), q, r ..) - f(p,q,r ..) \text{ etc.}$$

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 contributions 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 $2\times r(p,q) \cdot u(y,p) \cdot u(y,q)$ 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 Uncertainties from Linear Least Squares Calibration

E.3.1 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.:

$$y = b_0 + b_1 x$$

The concentration x_{obs} of the analyte from a sample which produces an observed response y_{obs} is then given by:-

$$x_{obs} = (y_{obs} - c)/b_1$$

It is usual to determine the constants b_1 and b_0 by least squares regression on a set of n values (x_i, y_i).

E.3.2 There are four main sources of uncertainty to consider in arriving at an uncertainty on the estimated concentration x_{obs}:

- 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 e.g. 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.3.3 The uncertainty $u(x_{obs}, y)$ in a predicted value x_{obs} 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) is given by

$$\text{var}(x) = \frac{\text{var}(y) + x^2 \cdot \text{var}(b_1) + 2 \cdot x \cdot \text{covar}(b_0, b_1) + \text{var}(b_0)}{b_1^2}$$

and the corresponding uncertainty $u(x_{obs}, y)$ is $\sqrt{\text{var}(x)}$.

From the RMS error or the variance of residuals S.

var(x) is approximately equal to S^2 / b_1^2, where S^2 is the variance of the y values about the fitted line:

$$S^2 = \frac{\sum (y_i - y)^2}{n-2}$$

and $(y_i - y_i)$ is the residual for the i^{th} point. S can also be calculated from the RMS error using

$$\text{RMS error} = \sqrt{\frac{\sum (y_i - y)^2}{n}}$$

It follows that S is given by

$$S^2 = (\text{RMS error})^2 \cdot \frac{n}{n-2}$$

From the correlation coefficient r

The correlation coefficient r together with the range R(y) of the y values can be used to obtain an approximate estimate of S using

$$S^2 = \text{R}(y)^2 \cdot \frac{1 - r^2}{12}$$

If using this value of S shows that var(x) is not significant compared with the other components of the uncertainty, then it is not necessary to obtain a better estimate of it. However if it is significant then a better estimate will be required.

From the calibration data

Given a set of data (x_i,y_i), the uncertainty $u(x_{obs}, y)$ in x_{obs} arising from random variability in y values is given by

$$u(x_{obs},y) = \sqrt{\frac{\sum (y_i - y_i)^2}{b_1^2 \cdot (n-2)} \cdot \left(1 + \frac{1}{n} + \frac{(y_{obs} - \bar{y})^2}{b_1^2 (\sum (x_i^2) - (\sum x_i)^2 / n)}\right)}$$

where $(y_i - y_i)$ 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, and $(y_{obs} - \bar{y})$ the

difference between y_{obs} and the mean $\bar{y}$ of the y_i values.

Other methods

Some software gives 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) since :-

$$\text{var}(x) = [\, s(y_c) / b_1 \,]^2$$

If $s(y_c)$ is not given then it can be calculated from:-

$$s(y_c)^2 = S^2 \cdot \left[1 + \frac{1}{n} + \frac{n \cdot (x - \bar{x})^2}{D} \right]$$

where S^2 is the variance of the y values about the fitted line defined above and

$$D = n \cdot \sum (x_i^{\,2}) - \left(\sum x_i \right)^2$$

In most cases it is sufficient to use an estimate of D obtained from the range R of the n values of x used in the calibration and then:-

$$s(y_c)^2 = S^2 \cdot \left[1 + \frac{1}{n} + \frac{(x - \bar{x})^2 \cdot 12}{n \cdot R^2} \right]$$

and at the extreme of the calibration range

$$s(y_c)^2 = S^2 \cdot \left(1 + \frac{4}{n} \right)$$

This is a sufficient approximation for most cases where the var(x) is not a dominant component of the final uncertainty.

E.3.4 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 $u(x_{obs}, x_i)$ in a predicted value x_{obs} due to uncertainties in x_i is

$$u(x_{obs}, x_i) \approx u(x_i)/n$$

where n is the number of x_i values used in the calibration. This expression can be used to check the significance of $u(x_{obs}, x_i)$.

E.3.5 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.3.6 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_{obs} is given by:-

$$u(x_{obs})^2 =$$

$$u(x,\text{const})^2 + (u(y,\text{const})/b_1)^2 + \text{var}(x)$$

E.3.7 The overall uncertainty arising from calculation from a linear calibration can then be calculated in the normal way from the four components above.

Appendix E4: Documenting uncertainty dependent on analyte level

E4.1 Introduction

E4.1.1 It is often observed in chemical measurement that, over a large range of analyte 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, for example, coefficient of variation (%CV). However, at low levels, other effects dominate and the proportionality is lost (for example, direct proportionality leads to zero estimated uncertainties as the observed levels approach zero). In these circumstances, or where a relatively narrow range of analyte level is involved, it is sensible to quote an absolute value for the uncertainty. This section sets out a general approach to recording uncertainty information where variation of uncertainty with analyte level is an issue.

E4.2 Basis of approach

E4.2.1 To allow for both proportionality of uncertainty and the possibility of an essentially constant value with level, the following general expression is used:

$$u(x)=\sqrt{s_0^2+(x \cdot s_1)^2} \qquad \textbf{[1]}$$

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 ($x.s_1$) proportional to the result. Figure E4.1 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 $u(x) \approx s'_0 + x.s'_1$

E4.2.2 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 $x.s_1$, and some curvature is visible.

C: The term $x.s_1$ dominates; the uncertainty rises approximately linearly with increasing x and is close to $x.s_1$.

E4.2.3 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.

E4.3 Documenting level-dependent uncertainty data

E4.3.1 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 are documented in the

* An important example of non-linear dependence is the effect of instrument noise on absorbance

form of values for a constant term represented by s_0 and a variable term represented by s_1.

E4.4. Special cases

E4.4.1. Uncertainty not dependent on level of analyte (s_0 dominant)

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 E4.1
- 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.

E4.4.2. 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 $x.s_1$ dominates (see Region **C** in Figure E4.1). 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.

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 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.

E4.4.3. Intermediate dependence

In intermediate cases, and in particular where the situation corresponds to region **B** in figure 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 E4.2.

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.

E4.5. Determining s_0 and s_1

E4.5.1. 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.

E4.5.1. 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:

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 $u(x)^2 = m.x^2 + c$
4. Calculate s_0 and s_1 from $s_0 = \sqrt{c}$, $s_1 = \sqrt{m}$
5. Record s_0 and s_1

E4.6.Reporting

E4.6.1. 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.

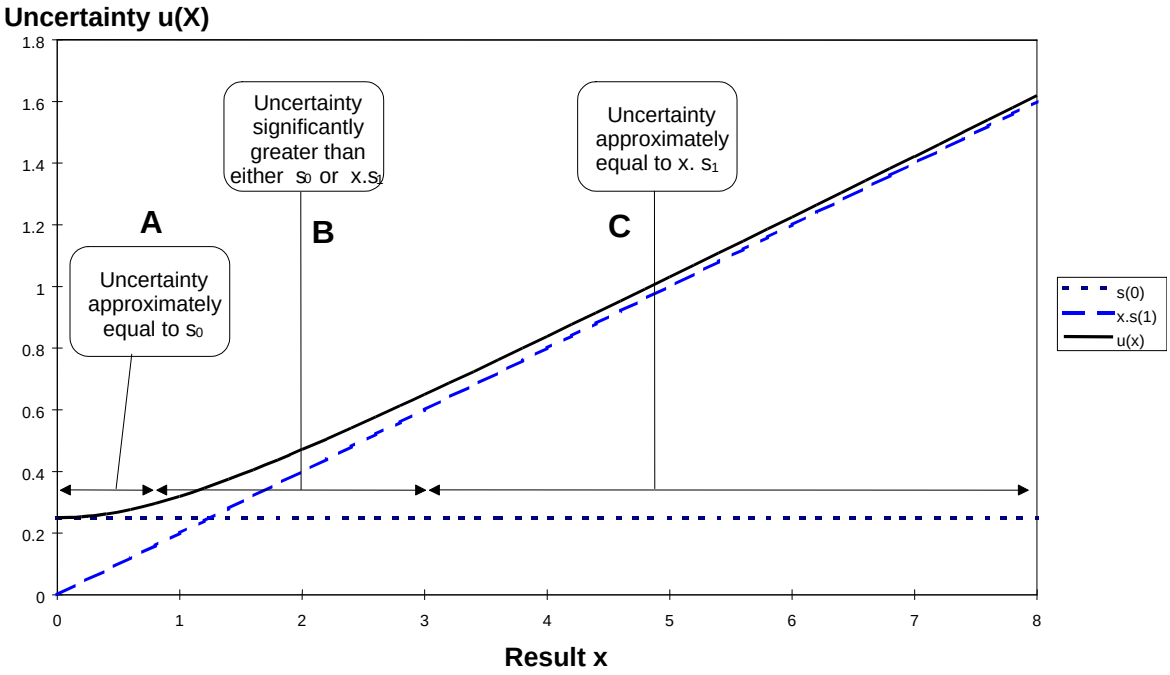
E4.6.1. Table E4.2 gives some examples. The uncertainty figures for a list of different analytes may usefully be tabulated following similar principles.

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 E4.2: Summarising uncertainty for several samples

Situation	Dominant term	Reporting example(s)
Uncertainty essentially constant across all results	s_0 or fixed approximation (sections E4.4.1. or E4.4.3.a)	Standard deviation: expanded uncertainty; 95% confidence interval
Uncertainty generally proportional to level	$x.s_1$ (see section E4.4.2.)	relative standard deviation; coefficient of variance (%cv)
Mixture of proportionality and lower limiting value for uncertainty	Intermediate case (section E4.4.3.)	quote %cv or rsd together with lower limit as standard deviation.

Figure E4.1: Variation of uncertainty with observed result



Appendix F - Common sources and values of uncertainty

The following tables summarise typical examples of uncertainty components from among those found in the EURACHEM document. 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 summarise the examples and to indicate general methods of estimating uncertainties in analysis. 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 (absolute)	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
	Daily drift	Various	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.
	TOTAL specification uncertainty	Combination of above	Combine above as standard deviations	4-figure balance	0.5 mg
	Calibration weight/sample density mismatch	The mismatch causes a difference in the effect of atmospheric buoyancy.	To correct, calculate atmospheric buoyancy effect and subtract buoyancy effect on calibration weight.	100 g water 10 g Nickel	+0.1g < 1 mg

Determination	Uncertainty Components	Cause	Method of determination	Typical values	
				Example	Value
Mass (by difference)	Run to run variation	Various	Standard deviation of successive sample or check weighings	2-Figure Balance	10g check weight: **s**=0.03g
	Balance Linearity		i) Experiment, with range of certified weights ii) Manufacturer's specification	4-figure balance	QC shows **s**=0.07mg for range of weights
	TOTAL:	Combination of above	Combine above as standard deviations	4-figure balance: Run-to-run: s= 0.07mg. Spec = ±0.1mg at 95% confidence	$\sqrt{0.07^2 + \left(\frac{0.1}{1.96}\right)^2}$ =0.087mg

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.	$\Delta T.\alpha/2.\sqrt{3}$ 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 x 10^{-4} K^{-1} for water and 1 x 10^{-3} K^{-1} for organic liquids.	100 ml water	0.03 ml for operating within 3°C of the stated operating temperature
	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
Reference material concentration	Purity	Impurities reduce the amount of reference material present. Reactive impurities may interfere with the measurement.	Stated on manufacturer's certificate. Reference certificates usually 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)	Certified uncertainty in reference material concentration.	Stated on manufacturer's certificate. Reference certificates usually 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 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 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 distribu

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 AA 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) Estimated standard deviation.	Sampling from bread of assumed two-valued inhomogeneity	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 comparable 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%	$28/\sqrt{42}$= 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.	0.31 as RSD. See text for calculation.

Appendix G - Bibliography

G.1. ISO/IEC Guide 25:1990 (3rd Edition). General Requirements for the Competence of Calibration and Testing Laboratories. ISO, Geneva (1990)

G.2. Guide To The Expression Of Uncertainty In Measurement. ISO, Geneva, Switzerland 1993. (ISBN 92-67-10188-9)

G.3. EURACHEM, Quantifying Uncertainty in Analytical Measurement. Laboratory of the Government Chemist, London 1995. ISBN 0-948926-08-2

G.4. International Vocabulary of basic and general standard terms in Metrology. ISO, Geneva, Switzerland 1993 (ISBN 92-67-10175-1)

G.5. ISO 3534:1993. Statistics - Vocabulary and Symbols. ISO, Geneva, Switzerland 1993

G.6. EURACHEM, The Fitness for Purpose of Analytical Methods. 1998 (ISBN 0-948926-12-0)

G.7. ISO/IEC Guide 33:1989, “Uses of Certified Reference Materials”. ISO, Geneva 1989

G.8. I.J.Good, "Degree of Belief" 1982, in Encyclopaedia of Statistical Sciences, Vol. 2, Wiley, New York .

G.9. J. Kragten, “Calculating standard deviations and confidence intervals with a universally applicable spreadsheet technique”, *Analyst*, 119, 2161-2166 (1994)

G.10. British Standard BS 6748:1986. Limits of metal release from ceramic ware, glassware, glass ceramic ware and vitreous enamel ware.

G.11.S L R Ellison, V Barwick. Accred. Qual. Assur. (1998) **3** 101-105.

G.12.ISO 9004-4:1993, Total Quality Management. Part 2. Guidelines for quality improvement, (ISO 1993)